

Journal of Scientific & Industrial Research



J. sci. industr. Res. Vol. 25 No. 3 Pp. 89-138

March 1966

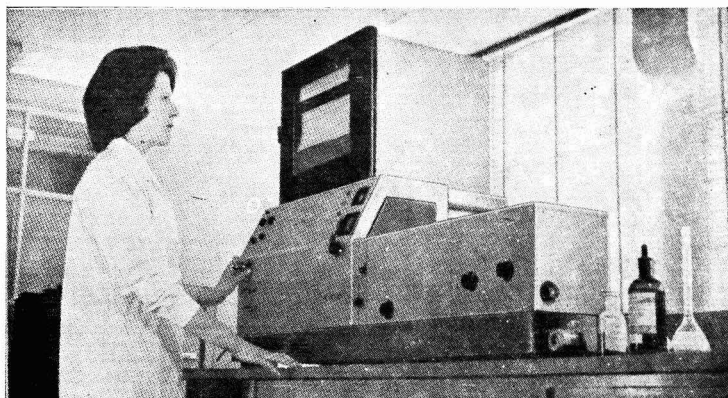
Published by the Council of Scientific & Industrial Research, New Delhi

Sole Distributors Outside India: Pergamon Press

Oxford London Paris Frankfurt New York

UNICAM

Recording Spectrophotometer



HIGH PERFORMANCE TO-COST RATIO

The **Unicam SP700 Double-Beam Recording Spectrophotometer**, with its wide wavelength/wavenumber coverage (186μ to 3.6μ), prism and grating optics, choice of operating conditions and ease and speed of operation, offers outstanding performance at a keenly competitive price. This powerful instrument is improving results and cutting costs in many laboratories throughout the world. It has also achieved distinction for the high quality of its work and its reliability in arduous conditions.

PERFORMANCE

Easily selected synthetic silica prism or 1500 lines/inch grating gives quality performance over the whole operating range.

RESOLUTION

Typical values at 50000 cm^{-1} ($200 \text{ m } \mu$)	20 cm^{-1} (0.8 \AA)
at 10000 cm^{-1} (1μ)	50 cm^{-1} (50 \AA)
at 3333 cm^{-1} (3μ)	3 cm^{-1} (26 \AA)
Photometric accuracy	$\pm 0.5\%$ transmission

RELIABILITY

Built-in features include a multipotentiometer for accurate beam balance over long working periods. Monitor points on all plug-in chassis for quick check of electronic systems.

SERVICE

Every SP700 is backed by the skills and experience of the worldwide Unicam service organization — including regular contract maintenance, if required.

For details please write to:

SOLE DISTRIBUTORS

THE SCIENTIFIC INSTRUMENT COMPANY LIMITED

ALLAHABAD BOMBAY CALCUTTA MADRAS NEW DELHI

Head Office: 6 Tej Bahadur Sapru Road, Allahabad



NOTICE

It is proposed to appoint a Chief Editor for the Publications & Information Directorate of the Council of Scientific & Industrial Research in the scale of Rs 1600-100-1900. A higher scale of pay may be considered in the case of an exceptionally qualified person.

The main duties of the post are: (a) editing the "Journal of Scientific & Industrial Research" and the associated scientific journals; (b) building up the background information for and publication of the "Wealth of India" series; and (c) publication of monographs, reports of seminars and conferences and other scientific and technical literature.

The Chief Editor will be responsible for the maintenance of a high standard of excellence in the editing and production of the journals and the literature published.

Good basic qualifications in Science will be preferred. In addition, sound knowledge of English and experience in publication, production, documentation and editorial work of high standard and experience in management will be considered essential.

There is no standard form of application. Those who wish to be considered are invited to send a statement of their curriculum vitae by name to the Director-General, Scientific & Industrial Research. Others who may wish to send a nomination for the above post may also kindly forward their proposals to the Director-General.

All communications will be treated as *confidential*. Statements and proposals should be received by the Director-General on or before 15 May 1966 at the office of the Council of Scientific & Industrial Research, Rafi Marg, New Delhi 1.

FORM IV

(See Rule 8)

- | | |
|--|--|
| 1. Place of publication | NEW DELHI 12 |
| 2. Periodicity of its publication | MONTHLY |
| 3. Printer's Name | A. KRISHNAMURTHI |
| Nationality | INDIAN |
| Address | PUBLICATIONS & INFORMATION
DIRECTORATE
HILLSIDE ROAD, NEW DELHI 12 |
| 4. Publisher's Name | A. KRISHNAMURTHI |
| Nationality | INDIAN |
| Address | PUBLICATIONS & INFORMATION
DIRECTORATE
HILLSIDE ROAD, NEW DELHI 12 |
| 5. Editor's Name | A. KRISHNAMURTHI |
| Nationality | INDIAN |
| Address | PUBLICATIONS & INFORMATION
DIRECTORATE
HILLSIDE ROAD, NEW DELHI 12 |
| 6. Names and addresses of individuals who own the newspaper and partners or shareholders holding more than one per cent of the total capital | — |

I, A. Krishnamurthi, hereby declare that the particulars given above are true to the best of my knowledge and belief.

(Sd) A. KRISHNAMURTHI

Signature of Publisher

4 March 1966

EDITORIAL BOARD

DR S. HUSAIN ZAHEER, Director-General, Scientific & Industrial Research (*ex officio* Chairman), New Delhi

DR VIKRAM A. SARABHAI, Physical Research Laboratory, Ahmedabad

DR K. VENKATARAMAN, National Chemical Laboratory, Poona

PROF. S. R. PALIT, Indian Association for the Cultivation of Science, Calcutta

PROF. P. MAHESHWARI, Delhi University, Delhi

PROF. B. R. SESHACHAR, Delhi University, Delhi

DR M. S. KRISHNAN, Osmania University, Hyderabad

PROF. N. R. KULLOOR, Indian Institute of Science, Bangalore

SHRI S. B. DESHAPRABHU, *ex officio* Secretary

SHRI A. KRISHNAMURTHI, Editor

EDITORIAL STAFF

Editor: A. Krishnamurthi

Assistant Editors: R. N. Sharma, D. S. Sastry, S. S. Saksena, P. D. Gujral & K. Satyanarayana

Technical Assistants: K. S. Rangarajan, A. K. Sen, S. Arunachalam, R. K. Gupta, Kuldip Chand, D. R. Anandakumar, R. P. Grover & D. S. R. Murty

Production Officer: S. B. Deshaprabhu

The Journal of Scientific & Industrial Research is issued monthly.

The Council of Scientific & Industrial Research assumes no responsibility for the statements and opinions advanced by contributors. The Editorial Board in its work of examining papers received for publication is assisted, in an honorary capacity, by a large number of distinguished scientists working in various parts of India.

Communications regarding contributions for publication in the Journal, books for review, subscriptions and advertisements should be addressed to the Editor, Journal of Scientific & Industrial Research, Publications & Information Directorate, Hillside Road, New Delhi 12.

Annual Subscription

A: For Libraries, Government Departments and Industry Rs 15.00 (inland); £ 3.10.0 or \$ 10.00 (foreign)

B: For individuals Rs 11.25 (inland); £ 2.5.0 or \$ 6.50 (foreign)

Single Copy

Rs 2.00 (inland); 6s. or \$ 1.50 (foreign)

Payments in respect of subscriptions and advertisements may be sent by cheque, bank draft, money order or postal order marked payable to Publications & Information Directorate, Hillside Road, New Delhi 12.

© 1966 THE COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH, NEW DELHI

Sole Distributors Outside India

PERGAMON PRESS

Oxford London Paris Frankfurt New York

Journal of Scientific & Industrial Research

VOLUME 25

NUMBER 3

MARCH 1966

CONTENTS

CURRENT TOPICS

Softlanding on the Moon	89
Second Exhibition of Scientific & Technical Publications ...	90
Sixth International Conference on Soil Mechanics & Foundation Engineering	91
DINESH MOHAN	
Spectroscopic Methods of Structure Determination: Applications to Natural Phenolic Pigments	97
K. VENKATARAMAN	
Lipid Metabolism of Insects	119
S. SRIDHARA	
Reviews	128
Nonlinear Partial Differential Equations in Engineering; Elementary Particle and High Energy Physics; Analysis of Essential Nuclear Reactor Material; Oriented Nuclei: Polarized Targets and Beams; Advances in Heterocyclic Chemistry: Vol. 4; Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry; Organometallic Synthesis: Vol. 1—Transition Metal Compounds; General Chemistry; Sillimanite; The Anatomy of the Laboratory Mouse	
Notes & News	132

New absolute temperature scale for the range 4-14°K.; First observation of Hall effect in superconductors; Raman laser excited by an ordinary laser; Synthesis of cubane, a new box-like compound; Disulphide interchange and the three-dimensional structure of proteins; Role of labile sulphide in metalloflavoprotein catalysis; Estrogen action and ribonucleic acid; Mechanism of action of aldolases; Order of induction and deinduction of the enzymes of the lactose operon in *Escherichia coli*; Weizmann Institute of Science, Israel; Dr L. B. Singh; *Brain Research*

For Index to Advertisers, see page A21

Towards Self Sufficiency in Instrumentation

A wide range of quality instruments are now manufactured in India
by TOSHNIWALS, thereby saving foreign exchange

GENERAL LABORATORY INSTRUMENTS

Gas Plants, Sieve Shakers & Vibrators, Water Baths, Centrifuge, Multimantles,
Flask Shakers, Water Stills, Vacuum Pumps, etc.

CHEMICAL LABORATORY INSTRUMENTS

pH Meter, Polarograph, Electrophoresis Apparatus, Titration Potentiometer,
Conductivity Bridge, Thin Layer Chromatograph, Universal Moisture Meter,
Ovens, etc.

PHYSICS LABORATORY INSTRUMENTS

Kelvin Bridge, Wheatstone Bridge, Potentiometer, Regavolts, Rheostats,
Galvanometer, Standard Cell, Electrical Indicating Instruments like Ammeters,
Voltmeters, etc.

ELECTRONIC INSTRUMENTS

Double-beam Oscilloscope, Universal AC Meter Tester, Distortion Factor Meter,
Pulse Generator, Q Meter, Universal Impedance Bridge, Audio Oscillator,
Strobometer, Output Power Meter, VTVM, etc.

INDUSTRIAL INSTRUMENTS

Process Control Instruments like Temperature Indicating Controller & Recorder,
Moisture in Timber Meter, Hand Tachometer, Thermocouples, Insulation &
Continuity Tester. Textile Testing Instruments like Wrap Reel, Fibre Fineness
Tester, Yarn Twist Tester, etc.

OIL AND PETROLEUM TESTING INSTRUMENTS

Redwood Viscometers, Flash Point Apparatus, Cloud & Pourpoint Apparatus, etc.



SOLE SELLING AGENTS

TOSHNIWAL BROTHERS PRIVATE LIMITED

198 JAMSHEDJI TATA ROAD, BOMBAY 1

Branches:

Kacheri Road
AJMER

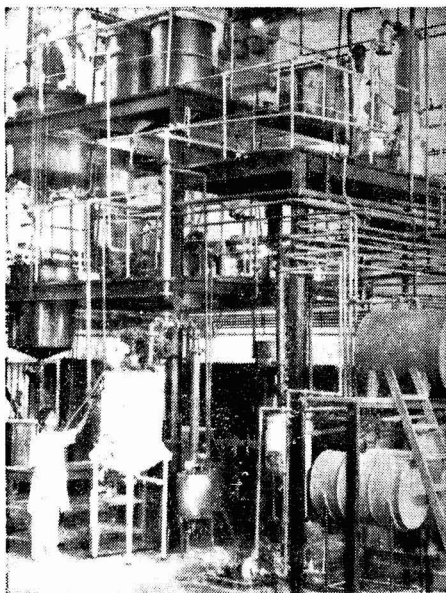
85A Sarat Bose Road
CALCUTTA 26

3E/8 Jhandewalan Extension
NEW DELHI 1

Round Tana, Mount Road
MADRAS 2

B.I. CHEMICALS

It is long since Bengal Immunity started manufacturing chemicals for pharmaceutical and laboratory use. To-day the manifold activities of the Company are based upon its accumulated experience and continued expansion and as such it is in a position to offer a wide range of chemical products —well known for their reliability and purity.



A SECTION OF ISONIAZID PLANT



BENGAL IMMUNITY CO., LTD.
153, Dharamtala Street, Calcutta-13

- AMINACRINE HYDROCHLORIDE I.P.
- BEEF EXTRACT
- CALCIUM LACTOBIONATE
- CHLOROQUINE PHOSPHATE U.S.P.
- CHOLIC ACID
- DEHYDROCHOLIC ACID U.S.P.
- DI-iodoHYDROXYQUIN U.S.P.
- HYDROPROTEIN POWDER
- ISONIAZID B.P.
- MAGNESIUM ALUMINIUM SILICATE
- MAGNESIUM HYDROXIDE CAKE
- MAGNESIUM TRISILICATE U.S.P.
- MILD SILVER PROTEIN I.P.
- OX BILE EXTRACT N.F.
- PEPTONE POWDER
- PHENFORMIN HYDROCHLORIDE
- PROTEOLYSED LIVER I.P.
- PROTEOSE PEPTONE
- SULPHANILYLBENZAMIDE
- THIAcETAZONE B.P.C.
- TRYPTONE

Matthey SPECTROGRAPHICALLY STANDARDISED SUBSTANCES

GROUP 0	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII	GROUP VIII
	H							
He	Li	Be	B	C	N	O	F	
Ne	Na	Mg	Al	Si	P	S	Cl	
Ar	K	Ca	Sc	Ti	V	Cr	Mn	Fe Co Ni
	Cu	Zn	Ga	Ge	As	Se	Br	
Kr	Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru Rh Pd
	Ag	Cd	In	Sn	Sb	Te	I	
Xe	Cs	Ba	La Ce Pr Nd Pm Sm Eu Gd Tb					
	Dy Ho Er Tm Yb Lu		Hf	Ta	W	Re	Os Ir Pt	
	Au	Hg	Tl	Pb	Bi	Po	At	
Rn	Fr	Ra	Ac	Th	Pa	U		

70

ELEMENTS

The Johnson Matthey range of spectrographically standardised substances is wide and now embraces metals and compounds representing 70 elements. All the materials are of the highest practicable degree of purity and are subjected to stringent analysis. The report that accompanies every batch states the amount of each metallic impurity in parts per million and also the method of determination.

Where the highest purity is not needed, many of the elements can be supplied as metals and compounds of a lower degree of purity.

Johnson  Matthey

Available through:

The Andhra Scientific Co. Ltd.,
4, Blackers Road, Mount Road,
MADRAS-2.

Arora-Matthey Limited
166, Netaji Subhas Chandra Bose Road,
Tollygunge, CALCUTTA-40

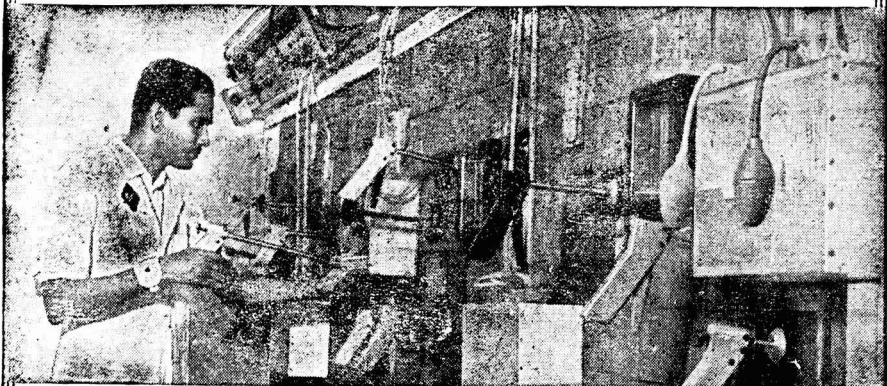
Associated Instrument Manufacturers (India)
Private Limited,
Sunlight Insurance Buildings,
26-27 Asaf Ali Road, NEW DELHI

Raj-Der-Kar & Co.,
Sadhana Rayon House,
Dr. D. Nacroji Road,
BOMBAY-1

The Scientific Instrument Company Ltd.,
6, Tej Bahadur Sapru Road,
ALLAHABAD-1

JOHNSON, MATTHEY & CO., LIMITED, HATTON GARDEN LONDON ENGLAND

Gansons ISOTOPES HANDLING EQUIPMENT



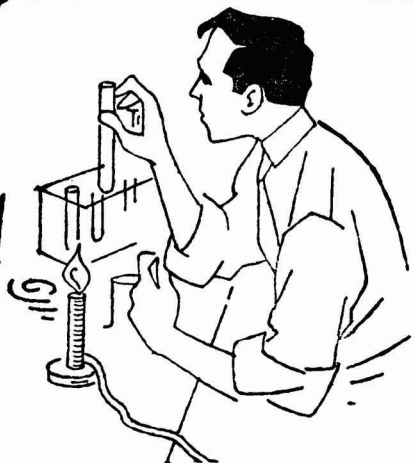
Production Plant for Iodine-131 at the Atomic Energy Establishment, Trombay



GANSONS PRIVATE LIMITED, POST BOX NO. 5576, BOMBAY-14.

RB/GS/20

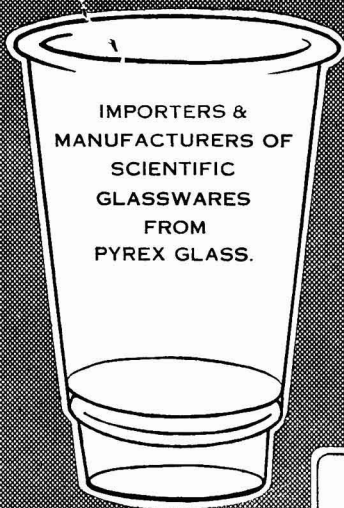
**FOR CHEMICAL
EXPERIMENTS
AND ANALYSIS
USE ANALYTICAL
REAGENTS
MANUFACTURED
BY...**



**THE INTERNATIONAL
CHEMICAL INDUSTRIES**

103-B, UPPER CIRCULAR ROAD
(ACHARYA PRAFULLA CHANDRA ROAD)
CALCUTTA-9





IMPORTERS &
MANUFACTURERS OF
SCIENTIFIC
GLASSWARES
FROM
PYREX GLASS.

SPECIALISTS
in

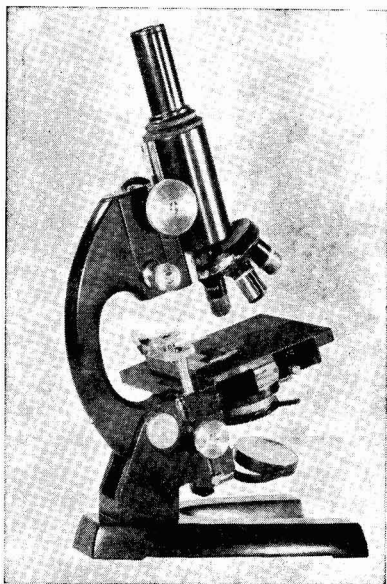
- GAS ANALYSIS APPARATUS
- VOLUMETRIC GLASSWARES
- EXTRACTION APPARATUS
- SINTERED GLASSWARES
- CHEMICAL APPARATUS

K **SCIENTIFIC
INDUSTRIES**
11, ULTADANGA ROAD, CALCUTTA - 4

SCIENTIFIC INSTRUMENTS & EQUIPMENTS

for

Education, Research & Industry



RESEARCH MICROSCOPE

RESEARCH, STUDENT & DISSECTING MICROSCOPES
● MICROTOMES ● pH METERS ● PHOTOELECTRIC
COLORIMETERS ● OVENS ● INCUBATORS ● WATER
BATHS ● ANALYTICAL BALANCES ● LABORATORY
GLASSWARE, PORCELAINWARE & SILICAWARE
● PHYSICS INSTRUMENTS

**INTERNATIONAL
AGENCIES**

79 GHOGA STREET, FORT, BOMBAY I

Gram: 'SCIENAPP'

Phone: 253753


Cauvery Brand



STABLE BLEACHING POWDER

for

- textile bleaching,
- water purification,
- environmental sanitation



THE METTUR CHEMICAL & INDUSTRIAL CORPORATION LIMITED

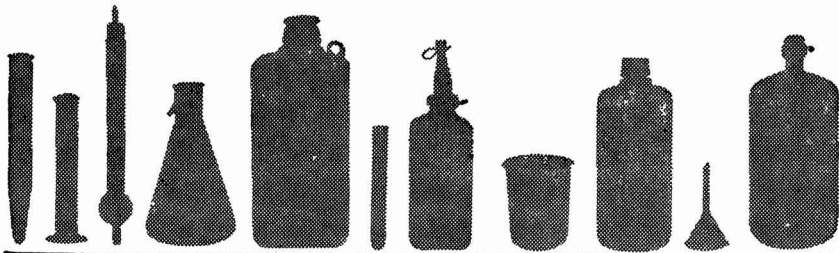
Mettur Dam R. S. Salem Dist.

Managing Agents:

SESHASAYEE BROTHERS PRIVATE LIMITED

CRITERION-MC. 370

PLASTIC LABORATORY WARE



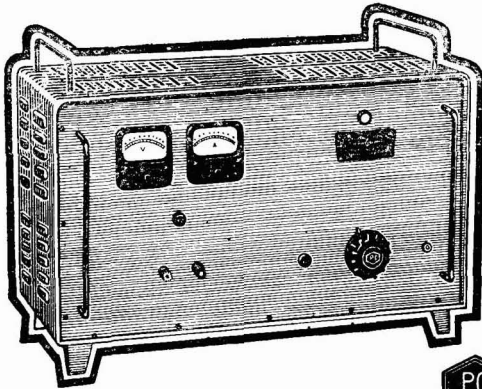
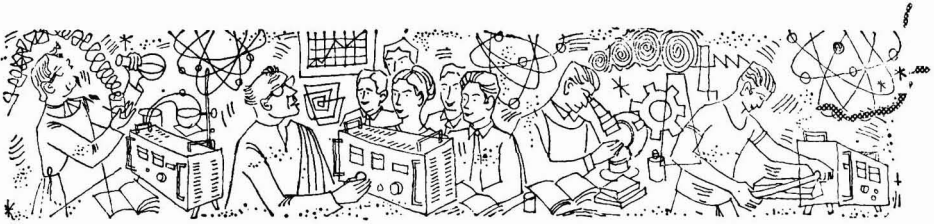
MADE OF POLYETHYLENE, POLYPROPYLENE, POLYCARBONATE

Nalgene[®]

THE QUALITY
STANDARD OF
PLASTIC LABWARE

The NALGE CO., Inc. Rochester 2. N.Y.

AGENTS: J. T. JAGTIANI, NATIONAL HOUSE, TULLOCH RD., APOLLO BUNDER, BOMBAY - 1



ELECTRONIC INSTRUMENTS

L.T., H.T. & E.H.T. Power Supplies, Transistorised Power Supplies, Temperature Controllers, Timers, Constant Current Regulators, Electromagnetic Demonstration Apparatus, Photo Electronic Devices, Shortwave Demonstration Apparatus, E/M Demonstration Apparatus (with Cathode-ray tubes), Valve Characteristic Apparatus.

for electronics, science and technology

POLYTRONIC CORPORATION

20A, Shakti Sadan, Junction of Tardeo Bridge & Lamington Road, Bombay-7 WB.

mass-pc-5

for laboratory and hospital equipment

LABORATORY GLASSWARE, ETC.: Pyrex, Quickfit, Westglass Hygrometers, Hydrometers, Micro Cover Glasses and Slides, Vitreosil Silicaware (Crucibles, Basins, etc.), Sintered Glasswares (Crucibles, Filters, Funnels, etc.), Porcelainware (Crucibles and Basins), etc.

LABORATORY THERMOMETERS: Chemical Thermometers, all types, Long Stem Thermometers by G. H. Zeal (London), etc.

FILTER PAPERS: Whatman Filter Papers and Specialities, Qualitative Grades, Folded Grades, Single and Double Acid Washed, Hardened Grades, Papers for Chromatography and Electrophoresis, Papers in Reels, Sterimat Pads, etc.

LABORATORY APPARATUS AND INSTRUMENTS: Analytical Balances and Weights, Centrifuges, Hand and Electrical, Colorimeters, Comparators and Colour Discs, Haemometers, Haemocytometers, Incubators and Ovens, Microscopes, Microtomes, Photocolorimeters, Test Sieves, Stoplocks and Stopwatches, etc.

MEDICAL AND SURGICAL APPARATUS: BP Blades and Handles, Blood Pressure Apparatus, Diagnostic Sets, Stethoscopes, Hypodermic Syringes and Needles, etc.

PLEASE CONTACT:

B. PATEL & COMPANY

DIRECT IMPORTERS & STOCKISTS OF SURGICAL & SCIENTIFIC GOODS

27/29 POPATWADI, KALBADEVI ROAD, BOMBAY 2

Phones : 38689 & 39702 · Grams : GLASALSORT

Current Topics

Softlanding on the Moon

ONE more vision of the science fiction writer has been transformed into fact with the successful softlanding, on the moon, of the 1.5 ton spacecraft Luna-9 by Russian space scientists at 21.45 hrs Moscow time on 3 February 1966. The historic importance and the technological achievement of the event rank only with such landmarks as the launching into space of the first artificial earth satellite, man's first space-walk by Leonov and the Gemini space rendezvous. This marvellous feat after a series of initial failures since 1959 marks another vital phase in the 'step by step ascent of the stairway to the moon'.

Any vehicle freely travelling towards the moon will be accelerated to a speed of at least 6000 miles/hr at touchdown. A hit at this speed will disintegrate all equipment aboard. Hence softlanding of the instrument-equipped craft is necessary for any useful observations of the 'moonscape'. Thus the main problem is to decelerate the craft to a 'pedestrian' speed just before touchdown. The required braking cannot be effected by parachutes as in the case of the earth due to the absence of any atmosphere on the moon; retrorockets have to be used to slow down the vehicle. Luna-9 consisted essentially of three parts: (i) a space station provided with a shock-absorbing system, the antennae opening on touchdown; (ii) an engine system; and (iii) a compartment housing the guidance systems. The landing programme of Luna-9 involved five stages. In the first stage, Luna-9 was launched as an earth satellite (on 31 January 1966). In the second stage, the engine was speeded up to put it on a lunar trajectory. In the third stage, the flight trajectory was corrected (on 1 February 1966) to ensure its landing in the pre-chosen area of the Ocean of Storms about 100 km. to the east of the big crater Cavelerious. In the fourth stage, the retrorockets were fired on command from earth and the speed was reduced from 6000 miles/hr to about 15 miles/hr and the craft brought to a smooth landing; the television transmitting payload was then detached from its rocket engines to one side well away from the area disturbed by the fiery blasts of the descending retrorockets. In the fifth stage, the station started scanning the moon's surface at 0150 GMT on 4 February 1966, about 7 hr after softlanding. Throughout the period of its mission, reliable radio contact with the station was established on a frequency of 183.538 Mc/s. Television photographs were also received on earth in a 3 hr 20 min. radio session with Luna-9.

The Luna-9 pictures differed considerably from those taken by the American Ranger-9. The Luna-9 photographs showed more rocky outcrops than the

Ranger-9 indicated and the surface to be much more firm than imagined. The Luna-9 pictures indicated almost a level ground but with an uneven structure consisting of small mounds and depressions, resembling volcanic rock with a sponge-like structure. This leads to the belief that the terrain may be lava which had cooled quickly. The evidence provided by Luna-9 pictures dispels the 'meteoric theory' of the origin of lunar craters visualizing a thick layer of dust on the surface considered to be resulting from intense destructive impact from meteors over the ages. On the other hand, the clear view of considerable portions of parts like antennae of the space station in the television pictures transmitted shows that the surface, though spongy in structure, is rigid enough to support heavy structures like a lunar spacecraft. This lends support to the 'volcanic theory'. It now appears almost certain that the porous pumice-like surface resulted from the explosive expansion of gases in the lava as it emerged on to the moon's airless surface.

Luna-9 has achieved two things definitely: (i) perfecting multipurpose remote-controlled guidance systems requiring split-second accuracy, and (ii) providing the first real and closer view of the lunar surface. It is believed that during its mission Luna-9 might have had a certain directed mobility. Such manoeuvres are essential hereafter in the exploration by unmanned vehicles, from different areas of the moon, to know more about the actual conditions in the inhospitable moon environment. Attempts are being made to design a variety of cross-country vehicles for moon reconnaissance. One vehicle with flexible wheels is already being tested by Soviet scientists under field conditions. Preliminary results on this vehicle indicate that it is capable of smooth movement over every terrain irregularity, softly bending along recesses and rolling over hillocks and stones without jerks.

Some special problems which arise as a result of the absence of an atmosphere on the moon are: (i) large difference between day and night temperatures, (ii) colour distinction in the moon's topographic features being difficult because the light is not scattered as on earth, and (iii) hazards of cosmic radiation and meteoric impact. All these and many more problems have to be successfully solved before man can explore *in situ* on the moon. Finally, returning the cosmonauts safely to the earth poses an even bigger problem than keeping them on the moon for a short period. It appears that this should await the practical realization of huge space platforms assembled bit by bit, say by docking 5 or 6 Proton rockets in an earth orbit. The huge platform could then serve as an intermediate launching pad to spacecraft to and from the moon.

The growing cooperation and feedback of space technological know-how between the USSR and USA augurs well for making the long-cherished goal of landing a man on the moon a reality by 1970.

Second Exhibition of Scientific & Technical Publications

IN order to assess the progress made in scientific and technical publishing activity in the country during 1960-65, the Publications & Information Directorate of the Council of Scientific & Industrial Research organized an exhibition of scientific and technical books, and serial publications brought out in English and in the Indian languages. The exhibition, inaugurated by Dr Zakir Husain, Vice-President of India, on 15 February 1966, comprised 4492 books and 768 serial publications. As on the occasion of the first exhibition held in 1960, an attractive and useful bibliography* has been brought out. Entries in this bibliography are numbered serially and arranged alphabetically by title, author or publisher, irrespective of the language. An alphabetical index of the serial publications displayed is also provided. A list of Indian publishers along with their addresses is also included.

Scientific and technical books published during 1960-65 show a phenomenal increase. As indicated in the bibliography published in 1960, the number of books published up to the end of 1959, covering a period of few decades, was 4801, whereas during the short period of five years covered by the exhibition the number published is 4492. Prior to 1960, the number of books brought out in the Indian languages and in English was 1893 and 2908 respectively; the corresponding figures for the period 1960-65 are 1920 and 2572 respectively. Text-books appear to form a substantial proportion and number about 1450.

Books dealing with pure sciences account for 45 per cent (2018) of the total; the remainder (55

per cent; 2474) are concerned with applied sciences. In the latter category, publications dealing with medicine, agriculture and engineering account for nearly 80 per cent of the books published. It is gratifying to note that in the field of medicine alone the Indian language publications (509) are twice as many as those in English; books in Hindi number 131. Books in general sciences in the Indian languages (104) are nearly 3 times as many as those in English. It is also of interest to note that the number of publications in Indian languages in some of the modern branches of science and technology is increasing; 40 books have been published in nuclear physics and 81 in aeronautics and space sciences.

Of the serial publications exhibited, new ones which started publication from 1960 are 137; of these only 6 are in the Indian languages. Thus the publication of periodicals in Indian languages has not kept up the same tempo as in the case of books and very few books are published to keep the non-English-speaking public informed of developments in science and technology. Vigorous attempts are called for to fill this lacuna.

Dr Husain Zaheer, Director-General, Council of Scientific & Industrial Research, has pointed out in his foreword to the bibliography that there is a great paucity of scientific and technical books and periodicals, both in English and in the Indian languages, catering to the needs of children. Similar is the case in respect of popular science publications as well as science fiction. Special efforts are necessary to meet these needs.

In presenting a graphic picture of the current status of scientific and technical publication activity in the country, the exhibition has indeed served a useful purpose. It has indicated the serious lacunae that exist in respect of books and periodicals covering different branches of science and technology as well as in respect of those catering to different types and levels of readership. It is hoped that the next exhibition will be as great a success as the present one and will receive the same generous cooperation of various agencies so that it will be more exhaustive in its coverage and content.

**Indian Scientific & Technical Publications, 1960-65 — A Bibliography* (Council of Scientific & Industrial Research, New Delhi), 1966, Pp. 284. Price Rs 18 or \$ 6.00.

Sixth International Conference on Soil Mechanics & Foundation Engineering

DINESH MOHAN

Central Building Research Institute, Roorkee

THE Sixth International Conference on Soil Mechanics and Foundation Engineering was held at Montreal (Canada) during 8-15 September 1965. The conference was attended by about 1000 delegates from several countries; about half of the delegates were from Canada and USA alone. India was represented by Dinesh Mohan and Pais Cuddou. A few of the Indian students studying in USA and Canada also attended the conference.

Opening Session

The opening session started with an appreciation of services of Dr Terzaghi in the field of soil engineering and naming the latest Mission dam in Canada after him.

The subject of the presidential address delivered by Prof. Arthur Casagrande was 'Literature information service in soil mechanics—Search for new ways'. In this lecture he explained the need for an efficient abstracting and documentation service and suggested an interesting system of rating the usefulness of various publications.

The technical sessions covered various subjects under the heads: (1) General soil properties, (2) Shear strength and consolidation, (3) Deep foundations, (4) Shallow foundations and pavements, (5) Earth and rock pressures, and (6) Earth and rock dams, slopes and open excavations.

General Soil Properties

An interesting paper on soil exploration and soil sampling by Andresen (Norway) reported a new type of thin-walled fixed-piston sampler, operated by the release of gas charge generated by igniting a rocket propellant which drives the sampling tube forward into the soil. The sample tube is about 50 per cent longer than that recommended by Hvorslev, but good samples are claimed. Though the instrument has been devised for use on the sea floor it can also find application in boreholes filled with water. Bergemann's (Netherlands) paper dealt with a new application of the Dutch deep-sounding apparatus, modified to include a local friction-measuring sleeve. The ratio of cone resistance to local friction depends upon the nature of the material penetrated and clear differences are recorded for sands and clays. Tests have shown that the ratio may be used to differentiate between small differences in grading. This allows a soil profile to be drawn from the results of a single deep-sounding test, thus obviating the need for a separate adjacent borehole simply for this purpose. This is an important development in a device with wide application. In their paper, Lundstrom and Stenberg (Sweden) dealt with the important problem of deciding whether a drill is penetrating solid country rock or merely intersecting large boulders or detached masses of the

rock. An underwater microphone is set up in an adjacent borehole, up to 100 m. distance and the noise made by the drill is noted. Analysis of the noise spectrum allows deduction of the type of material being drilled. It is possible to distinguish soil, boulders, loose rock, fractured rock and solid rock.

A paper by Rethate (Hungary) dealt with the problem of additional settlements of loaded foundations due to collapse of grain structure after wetting of the subsoil. This problem has previously been associated with soils of a loess type, but according to the author the same phenomenon may occur with normal clean sand, even when loads are negligible. A method of estimating the probable subsidence, based on void ratio changes with changes in degree of saturation, was described.

Ranganathan and Satyanarayana (India) described a novel method of estimating the potential expansiveness of compacted clay soils. The method, based on determination of the shrinkage index of the disturbed soil, has been developed mathematically, starting from a relation for swelling potential proposed by Seed; it represents an advance on earlier methods for estimating potential expansiveness of soils.

A paper by Dos Santos and de Castro (Portugal) dealt with the assessment of the erodability of soils. The authors reviewed many previous attempts made to lay down criteria for defining soils liable to erosion, and, from a consideration of soils of known field behaviour, concluded that the percentage swell and grain size factors are the most important parameters in judging the erodability of a soil.

Shear Strength and Consolidation of Soils

There were a number of papers describing novel techniques for testing the shear strength of soils. The versatility of the triaxial cell permits the control of consolidation and of pore pressures, coupled with the possibility of reaching a state of complete saturation with the help of back pressures. This has led recently to the encasing of other laboratory testing devices, e.g. vane, shear ring, in the triaxial cells for studying the implications of the results obtained.

Laboratory measurement of pore pressures—Pore-pressure measurement has become a routine operation in triaxial testing. Its use is also being extended to other types of tests, such as direct shear and consolidation. Consequently, the development of simple devices is important in order that the common errors resulting from inadequate air expulsion from tubes and pipes, leakage of pore water, and uncontrolled volume change may be avoided. A paper by Leussink and Prange (Germany) described the development of an electronically operated pore-pressure transducer actuated by short

waves; in this instrument, any connection between transducer and receiver is avoided.

Studies on pore pressures in soil tests have shown that the propagation of this pressure through the soil mass is not instantaneous, even if soils are fully saturated by the application of high back pressures. Because of this time lag, triaxial tests with pore-pressure measurements cannot be run as fast tests, but require an increase in the deviator stress over approximately 8-12 hr for samples of clay. Observations made on earth dams have shown the same behaviour. Systematic studies of this peculiar hydraulic behaviour of the soil water may prove helpful and have an important bearing on the design of the drawdown state of earth dams. Barberis (Italy) described the results of such a study involving the measurement of time lag for pore pressure in a triaxial cell using partially saturated soil samples. It has been found that time lag is a function of soil permeability, more permeable soils needing less time for pore-pressure propagation.

Anisotropic state of stress—Lorenz (Germany) reported the development of a triaxial apparatus in which the three principal stresses can be varied at will by separately loading the face of a cube-shaped specimen. Tests under plane strain conditions of stress were carried out on dry sand in loose, medium and dense state. It has been found that Poisson's ratio is independent of relative density and E decreases linearly with deviator stress. A paper by Shibata and Karube (Japan) dealt with the relative behaviour of normally consolidated remoulded clay under standard triaxial and other stress conditions. A triaxial cell was adapted to hold prismatic specimens to whose faces stresses could be applied in such a way as to vary at will the three principal stresses. It has been found that anisotropic stressing does not essentially influence shear strength. It does, however, significantly influence the stress-strain relationship and the development of pore pressure during shear.

Time effects—A paper by Borowicka (Austria) reported a new test technique designed to distinguish between soils that are highly influenced by loads of long duration from those that are not or are only slightly affected. It has been found that, depending on the colloidal content of the clay and other still unknown properties, angle of internal friction (ϕ) for ultimate shear strength may vary between its value on first loading and a value less than 10° .

High confining pressures—In view of the increasing heights to which earth and rockfill dams are being built, need is being felt for a better insight into the deformations accompanying high confining pressures. The realization that in stiff clays, point resistances developed by piles may be very much higher than were previously predicted has also prompted studies on the subject. Intensive studies are, therefore, in progress on the behaviour of both compacted and natural soils under elevated cell pressures. Bishop *et al.* (UK) described an apparatus suitable for basic research on the behaviour of soils under high pressure. The results of a multiple-stage undrained test performed with constant effective pressure ($\sigma - \mu$) and two widely varied

total pressures, one in the low range and the other in the high range, have shown that the principle of effective stress holds true for the higher confining stress used (about 70 kg./sq. cm.).

Extensions of Terzaghi's theory of consolidation—Three papers from Netherlands, Portugal and USSR referred to extension of Terzaghi's theory of one-dimensional consolidation. By changing the assumptions about soil parameters made by Terzaghi, mathematical solutions for various situations in one-, two- and three-dimensional consolidations have been obtained.

In Terzaghi's original theory, even if field conditions approximate the fundamental theoretical hypothesis, the comparisons between computations and measurements are not always satisfactory, because, among other reasons, the hypothesis ignores secondary time effects. With preconsolidated clays, for example, it usually leaves much to be desired, often predicting magnitudes of settlements several times larger and rates of settlements much slower than those actually observed. To obtain better results a search is being made for new theories. A paper by Marsal (Mexico) reported an attempt to develop a new theory based on the analysis of the properties and behaviour of soil grain skeleton, without taking into consideration the action of either the gas or the liquid phase of the material.

Preconsolidation loads—In settlement problems, it is important to correctly predict the preconsolidation load of clay deposits. A paper by Togrol (Turkey) reported an attempt to find a more reliable procedure. Only slightly over-consolidated soils, which do not dilate in triaxial shear, were used. It has been found that the ultimate pore pressure at failure for a given soil is a unique function of water content and over-consolidation ratio.

Secondary consolidation—A paper by Christie (UK) reported the results of experimental verification of various modifications of Terzaghi's theory by making long duration oedometer tests in which the effect of secondary consolidations is emphasized. A paper by Thompson (USA) dealt with studies on a remoulded saturated silty clay tested in the oedometer and also in the triaxial cell.

Penetration tests—Schultze and Melzer (Germany) reported the results of studies on the comparison of sand compressibility as determined in confined compression tests with the static cone penetration resistance and with the dynamic standard penetration test for various overburden pressures.

Deep Foundations

Behaviour of single piles—Salas and Belzunce (Spain) reported the results of computer studies on the behaviour of single piles making use of Mindlin's solution for stresses due to a point load in the interior of a semi-infinite half-space. Cambefort (France) dealt with the analysis of the loading diagrams of piles pressed into sand, and, on the basis of simple assumptions, presented methods for their interpretation. His statement about the unequal distribution of surface friction on some sections of the pile shaft during pile penetration was reasonable and interesting. Berezaintzov (USSR) contributed an interesting paper on the methods adopted for

predicting the bearing capacity of piles. In his opinion the settlement should be the determining factor in predicting the bearing capacity of soil.

Williams and Colman (UK) described large-scale field tests carried out in the highly over-consolidated and stiff-fissured London clays for investigating the load-carrying capacity of piles with enlarged bases. They have found that the resistance acting along the pile shaft is fully mobilized at small values of settlement. On additional loading, only the point resistance continues to increase. These tests confirm that the initial resistance to deformation is predominantly from surface friction, but for larger settlements of the piles, as ultimate load is approached, point resistance becomes the dominant factor in the total resistance. Shashkoy (USSR) described a novel method of sounding for estimating the load-bearing capacity of piles. Two rods, one with a wide cone and the other with a smooth point, are driven into the ground. The rods are then tested under a static vertical load. The conclusion that the specific resistances of piles to vertical loads depend upon the properties of the soil surrounding the pile and not upon the diameter of the pile very likely holds to a certain extent but only for specific soil conditions.

Begemann (Netherlands) proposed the use of a friction jacket cone for determining the maximum pulling force on a single tension pile. The adhesion jacket cone measures both the cone resistance and the local friction in various layers. The ratio of these is related to the type of soil encountered.

Pile foundations and pile groups — All the papers presented on this subject were concerned with case histories and semi-empirical methods. Dinesh Mohan *et al.* (India) reported on the settlement behaviour of a large six-storeyed structure founded on cast-in-place reinforced concrete Franki piles. Consolidation of the silty clay stratum underneath the pile tips with only the dead load of the structure (hardly 50 per cent of the total design load) gave rise to excessive settlements and cracks developed in the lower floors. To partially relieve the extra stresses, preloading at the periphery was performed. Uniform settlement under the extra live load was assured by excavating a stepped basement in the centre. This study and the one conducted by Yu and others (China) covering the settlement records of 14 structures founded on piles in Shanghai prove the classical thesis that relatively short piles have very limited value in minimizing settlements.

Lateral loads on piles — Davisson and Robinson (USA) reported the results of a theoretical investigation on the determination of lateral loads on piles, based on classical assumptions. Heyman (Netherlands), presenting the results of field tests on laterally loaded piles, emphasized that measurement of horizontal displacements is very expedient in order to calculate the bending moments in the pile. These measurements may also assist in investigating the validity of an assumed equation relating horizontal force with displacement. Van Milaan and Lonsberg (Netherlands) described a novel instrument for measuring these displacements. A light ray in the

pile and an interference pattern serve to measure the deformations.

Johannessen and Bjerrum (Norway) dealt with vertical forces caused by the compression of the soil surrounding the pile, i.e. by negative skin friction. The authors computed average stress in the pile from the observed compression of the pile. They assumed that the adhesion was proportional to the effective stresses in the surrounding soil.

Van der Veen (Netherlands) described a novel type of pile, 'Cuff pile', which is claimed to be advantageous when the surface friction on the upper part is small or negligible or when prevention of negative friction is intended. The pile is a precast RC pile enlarged a little away from the tip along the shaft in the centres of two successive soil strata, thereby increasing the bearing capacity. The idea is somewhat analogous to the concept of double under-reamed piles developed for black cotton soils at the Central Building Research Institute, Roorkee.

Construction problems — Wu (China) developed the theory of vibro-sinking, applying it to precast reinforced pipe piles. The author began with the measured values of acceleration, average sinking velocity and stress in the reinforcing bars of the pile wall. The model that serves to set up the governing differential equation is rather complicated and the solution requires extensive mathematical treatment. The author evaluated the highly mathematical solution in practical applications and furnished the soil constants that make the calculation of vibro-sinking possible.

Szechy (Hungary) reported a method of avoiding damage to the heads of precast concrete piles during driving caused by insufficient strength of the concrete, or by overdriving. The method is a graphical one using the ratio of the elastic penetration and the set of the pile.

A paper by Trofimenkov and Mariupskii (USSR) dealt with the bearing capacity of screw piles. From comprehensive testing programme, formulae and design diagrams for a screw pile have been developed. The design diagrams take into consideration both the bearing capacity of the surrounding soil and the allowable deformation of the pile. It is of interest that the loading diagram never goes over to a vertical tangent, not even in the case of pulled out piles.

Shallow Foundations and Pavements

Stability of foundations — Theoretical solutions — An interesting paper by Gorbunov-Possadov (USSR) tried to establish a statically correct and kinematically more acceptable solution for the stability of a sand bed. For cohesive soils an approximate solution for rupture surface due to inclined loadings was presented by Bent Hansen (Denmark). Several participants from France, USSR, Israel and USA discussed theoretical solutions taking into account more intricate factors which may influence the ultimate bearing capacity, such as parallel strip footings, wedge-shaped footings, limitation of the thickness of the bearing layer and dynamic loading. Kany (Germany) discussed the determination of the most economical foundation of a building, taking

into consideration depth, size and weight of the foundation.

Penman and Watson (UK) reported the improvement of a tank foundation by the weight of its own test load. Pore pressure and settlement observations were made to control the test loading and the tank was successfully filled without any detrimental settlements in a much shorter time than had been predicted. In another interesting paper Mehra and Natrajan (India) reported on the foundation treatment of an ore handling yard by preloading and sand drains.

Settlements estimation — In the estimation of settlements, the crucial problem is not so much to have a correct theory as to introduce in the formulae the correct deformation parameters. For sands and over-consolidated clays, the results of normally performed oedometer tests cannot give the answer. To correctly interpret the results of laboratory tests performed on undisturbed samples, it is necessary to know the change introduced in the stress field in taking the samples. Knight and Blight (S. Africa) showed that this change depends on the sample and also on the geological history of the layer. Bachelier and Parez (France) described a semi-empirical method for estimating the modulus of compressibility in silty and clayey soils from static penetration tests. It is a follow-up of Buisman's work.

Measures taken to prevent excessive settlements were touched upon by two authors. Costa Nunes and Porto (Brazil) described the compaction of foundations of a reservoir by driven sand piles and Peynircioglu (Turkey) reported on the use of adjustable box foundation to counteract excessive settlements and tilting. After estimating the amount of probable settlement and tilt the building was constructed with a reverse inclination. In addition, niches for hydraulic jacks, tubes for mortar injection and reaction units were placed under the rigid box.

Pressure distribution under footings — The problem of distribution of soil reactions under footings depends to a large extent on the assumed soil deformation characteristics. The simplified assumptions of modulus of soil reaction and constant modulus of compressibility very often do not cover the real soil characteristics. Therefore, it is necessary to establish theories for more intricate soil deformation characteristics. This has been done for a rigid foundation by Zaretsky and Tsytoich (USSR). The distribution of soil reactions depends also on the rigidity of the superstructure. A method in which its influence is taken into account was described by Sommer (Germany). Ogorov (USSR) reported a formula for the distribution of soil reactions under rigid ring footings. Lee (Australia) showed by experimental data that the distribution of soil reactions depends on the stress history. That the intensity and frequency of load repetitions also have an influence was reported by Chae *et al.* (USA).

Pavements — A criterion for permissible deformation in the design of pavements was reported by Ivanov *et al.* (USSR). The authors pointed out the importance of tensile stresses in layers with flexural strength and that of shearing stresses in

other cases. Boromisza and Gaspar (Hungary) described methods for determining the thickness and composition of flexible pavements in terms of the characteristics of subgrade and the intensity of traffic. In another approach adopted by Mitchell and Marx (Rhodesia), charts have been used for designing flexible airfield pavements based on data from plate bearing tests. Yamanouchi (Japan) reported on the sandwich layer system used for increasing the rigidity of a pavement on a volcanic ash subgrade of low bearing capacity by placing a relatively rigid layer of soil-cement directly on the subgrade.

The problem of the settlement of soft layers under embankments and their preconsolidation was treated in three reports from India (Henkel), USSR (Shakhunyant) and USA (Halton *et al.*). In the last report preconsolidation has been obtained by lowering the water table to create a vacuum under the compressible layer combined with the use of sand drains.

Earth Dams, Slopes and Open Excavations

Embankments and earthfill dams — Gilg (Switzerland) dealt with some problems of compaction met with in the construction of a 150 m. high earth dam and its subsequent deformations. The upstream slope was founded on silts and peat lenses which were stabilized by the provision of sand drains. Interesting data on the laboratory and field compaction and properties of materials in various zones were given. Little (UK) gave an interesting account of the performance of some earth dams during construction. The values of pore pressures, shear parameters and other soil properties on samples extracted from the fill have been used for checking the stability of the dams during and after construction. Vargas *et al.* (Brazil) gave an account of the use of residual clays (from weathering of gneiss, basalt and sandstone) in seven earth dams constructed or under construction in Brazil.

Rockfill and hydraulic fill dams — Anagnosti (Yugoslavia) described the field performance of a 90 m. high rockfill dam. Useful data on the change of stresses with time and their distribution, and settlements in the clay core which could be used with advantage in the design of larger dams with a wide clay core placed in a steep canyon were reported. Donaldson (South Africa) discussed the use of the residue of crushed ore of the gold mining industry in South Africa as a construction material for hydraulic fill dams. The effect of drying on the consolidation and shear characteristics of the material has been determined and it has been found that, although heavy over-consolidation of the material occurs during drying, leading to increase in shear strength, its lasting effect is insignificant because of the frequent rewetting and capillary effect. Hølestad *et al.* (Norway) described the placing of a tunnel spoil rockfill dams in Norway and measurements of its subsequent settlement. The paper gave interesting details of construction in summer and winter seasons, and their effect on settlements. Reynaud and Rosset (France) described field tests for measuring and controlling the densities in the 20 million cu. yd Mon Cenis dam in France with a sloping

clay core, a rockfill downstream slope and a compacted earth upstream slope. Sowers *et al.* (USA) reported the results of an interesting study of the average rate of settlement in fourteen rockfill dams and compared them with laboratory consolidation test data on broken rocks. The authors have found that settlement-log time curves in respect of the actual dams approximated straight lines similar to secondary compression of soils. Laboratory consolidation tests of broken rock exhibited similar curves and the settlement rates were comparable, indicating that the prediction of rockfill settlements from such tests is feasible. Laboratory tests have shown that the settlement rate is accelerated by both shock and wetting of the rock, and the gradation changes are ample proof that settlement results from crushing of the point of contact between rock fragments.

Cores and filters — Silveira (Brazil) put forward a new approach to the design of filters based on void size distribution curves, primarily Cedergren's design criteria, which take into account the carrying capacity of filters. Expressions derived for the determination of thickness of filters have been verified experimentally. Wolski (Poland) described model studies on the effect of seepage erosion in the silty clay core of earth dams making use of X-ray technique. Pore water pressure measurement and visual examination of the sample, before and after seepage, give a fair indication of the performance. It has been observed that seepage erosion depends upon the hydraulic gradient and duration of percolation. A definite design criterion has been established for impervious cores and filters for earth and rockfill dams.

Stability analysis — Fyedorov (USSR) repudiated the hypothesis of cylindrical slip surface for stability analysis of hydraulic fill dams and put forward the concept of two flat sliding surfaces, one being the internal surface of the side fill and the other an unknown sliding surface inclined at a critical angle to the bed plane. The analysis has been carried out under various boundary conditions. Stability charts have been developed which make the design simple. Langejan (Netherlands) pointed out that the factor of safety value at present used in stability analysis of earth structures is arbitrary and suggested that a better approach would be to use the theory of probability. Londe and Sterenberg (France) discussed the definition of the factor of safety. The conventional factor of safety which is the ratio of force mobilized and force applied is, according to the authors, inconsistent when the geometry of the slope is such that no failure occurs. From a critical study of the existing methods they concluded that the knowledge of the distribution of stresses along the slip surface would be of great importance in zoned embankments, and further advance in this field could be made by the use of conditions of elastoplastic equilibrium.

Nonveiller (Yugoslavia) pointed out that the moment equilibrium of a single circular slice, considered by Janbu, in a slip surface does not necessarily imply that the moment of equilibrium of the entire sliding element is assured, whatever be its shape. Considering the moment equilibrium of the

entire sliding element, an implicit expression for the factor of safety has been derived which could be programmed for use with a digital computer. The expression coincides with Bishop's expression when the slip is circular. Saito (Japan) reported a method of predicting the time of failure of a slope after initial movement is visible. An expression has been developed relating the rate of strain and the rupture life due to creep. It was claimed that the expression can be used universally for predicting the time of failure by measuring the surface strains of slopes.

Stafanoff and Christow (Bulgaria) reported a graphical method for the rapid design of slopes. The method assumes that Coloumb's law is valid and that the normal stress at any point is the product of density and depth (γh). In actual practice, it is best to start with a factor of safety of one and then apply more exacting method to find the actual factor of safety.

Embankment and slope failures — Aitchison and Wood (Australia) reported an interesting analysis of failure of small earth dams based on erosion of soil due to deflocculation. Dispersion of soil by sodium ion being absorbed by exchange complex and low electrolyte concentration cause outward flow of suspended clay through pores of the soil. The authors recommend that satisfactory performance of an earth dam built from a susceptible soil-water combination can be achieved either by chemical additions or by raising the standard of construction control to ensure a field permeability from 10^{-5} to 10^{-7} cm./sec. The preferred compaction moisture content is at or on the wet side of the optimum. Fukuoka and Yamamura (Japan) described the results of full scale model tests of instrumented river embankments carried out at a number of sites. The *in situ* shear strength and compaction properties have been found to be different from those obtained in the laboratory. The authors advocated suitable modifications to the design of embankments in the light of these results.

Bolognesi *et al.* (Argentina) presented data on piezometric observations in a tunnel below a dam in Argentina. It has been found that pressure grouting in rock reduces seepage, but does not always reduce uplift pressures. The inefficiency of indiscriminate grouting was brought out and it was suggested that a grout cut off should be used only when a thoroughly competent and detailed analysis shows that it would be efficient and fully effective.

Miscellaneous studies — Grandclement and Langa (France) described the use of nuclear tracer technique in soil and subsoil water investigations using ^{135}I . The method can also be used for mapping the variations in soil profile.

Nash *et al.* (UK) discussed the various factors which require consideration, besides economic factors, in the construction of a soil-cement dam. These include: anisotropic permeability, sandwich layers of uncemented materials, transverse cracking, brittleness and long-term stability. They recommended that a proper evaluation of current design methods should also involve field trials, if possible, on a coffer dam, before soil-cement is used in actual dam construction.

Piaskowski and Kowalski (Poland) described the excavation of a cut without strutting, the lateral support being provided by the hydrostatic pressure of clay slurry. The specific gravity of the slurry was related to the depth of the cut, ground water conditions and soil type. The authors have also tried activation of clay to increase its thixotropic properties. They claim that though activated clay may be costlier, the additional cost may be justified because of the lesser quantity of the material required.

Ter-Stepanion (USSR) described the open and closed well methods of observing creep deformations. It is claimed that these observations would make it possible to measure two rheological properties — coefficient of mobilized shear and coefficient of flow — which are related to the time of plastic flow and viscosity by simple expressions. These field studies are free from limitations that beset laboratory tests.

Concluding Remarks

Considerable advance has been made since the last international conference at Paris in 1961 in a number of fields of soil engineering.

In the realm of shear strength, laboratory techniques have continued to improve and new developments have been reported in the design of both triaxial and direct shear apparatuses. Analysis of particulars of testing such as the effects of non-uniformity of distribution of both effective stresses and pore pressures, and the influences of stress history, stress paths, and rate of strain have been made by various testing techniques. Design of apparatuses for testing soils under high pressures, new devices for pore-pressure measurements, and methods for testing under anisotropic states of stress have received attention. Significant progress has been made in the design and construction of triaxial equipment to handle very large samples under pressures comparable to those existing in high earth and rockfill dams. A method has been developed for eliminating friction at the end caps restraining the samples in triaxial tests.

Fundamental studies have continued on the shear strength behaviour of soils. The aim of some workers is to develop a general theory which would give the mechanism of ultimate shear strength behaviour, both for remoulded clays and sands. Undisturbed soils have seldom been included in these investigations. Other studies pertain to methods of obtaining the components of shear strength of clays, either based on Hvorslev's criteria or supported by new ideas. Investigations have

been made of the frictional characteristics of soil minerals and of the shear strength of chemically purified clays, minerals, etc.

The effect of varying the stress path, and especially that produced by reaching failure under a so-called anisotropic state of stress, with particular reference to plane strain, has engaged the attention of many investigators. Shear strength behaviour of soils and rock fragments under high confining pressures has been one of the favourite subjects of research. The results obtained have led to the construction of many high pressure cells, so that most good soil mechanics laboratories are now provided with at least one such device for testing fine-grained soils.

Considerable progress has been achieved in the study of long-term strength of over-consolidated fissured clays. Very little work seems to have been done, by contrast, on the strength behaviour of non-saturated soils, an outstanding problem in everyday soil mechanics practice, about which present knowledge is scanty. The few papers published on the subject refer mainly to the application of the principle of effective stress to such soils, but practically no progress has been made on representative testing in relation to field soil behaviour.

In contrast to the large number of papers presented on shear strength, the small number of contributions on soil deformations leaves one with the impression that solutions for settlements are making slower progress. Renewed and vigorous examination of the problem, therefore, appears to be necessary.

On the determination of the behaviour and bearing capacity of single piles, the papers presented pointed out the right path for research, at least as regards the methods used. No mention was made of the pseudo-theoretical pile-driving formulae.

For India, which is building two large rockfill dams in the north, one on the Ramganga in UP and the other on the Beas in Panjab, the papers on similar dams being constructed abroad should be of great interest. Large-scale instrumentation and use of bigger laboratory test equipment has given more rational results for the design of earth and rockfill dams. There is also a need for using some of the more advanced techniques of soil exploration, design and construction, involving the use of electronic computers, and radioactive tracer techniques. The use of bentonite slurry for stabilizing the sides of a narrow open cut has established itself and this technique may be usefully employed in many of our foundation engineering and earth construction projects.

Spectroscopic Methods of Structure Determination: Applications to Natural Phenolic Pigments*

K. VENKATARAMAN

National Chemical Laboratory, Poona 8

AT the outset I should like to thank the Vice-Chancellor and Prof. R. C. Paul for inviting me to give the Professor Bawa Kartar Singh Memorial Lectures. Prof. Singh was a very old and valued friend, for whom I had a high regard as one of the leading personalities in our chemical life.

Professor Bawa Kartar Singh was a versatile organic chemist who worked in such widely different areas as synthesis, carbohydrates, fats and oils, and stereochemistry, but he is best known as a pioneer in the study of the dependence of optical rotatory power on chemical constitution. When I was a student of chemistry in the early twenties, I remember that in many issues of the *Journal of the Chemical Society* there were papers from B. K. Singh's school. As early as 1930, he resolved several quaternary ammonium iodides and embarked on an extensive study of the relation between optical activity and chemical constitution with special reference to camphor derivatives. He was one of the first to realize the limitations of studying optical rotation only in sodium light or at a few other wavelengths, and he attempted to extend his observations to a wider range of wavelength, but unfortunately facilities for scanning in the UV and near-visible regions were not available at the time. There is no doubt that if a modern spectropolarimeter had been accessible to him, he would have made far-reaching contributions to optical rotatory dispersion (optical rotation as a function of wavelength), which is now playing so important a part in determining the structure, conformation and configuration of terpenes, steroids and other natural products¹. Notwithstanding the lack of instrumental facilities, he was able to do very useful work on optical activity in relation to the nature, number and location of various groups and to show that the individual contribution of a group is modified by its environment. One part of his work which I used to follow with interest was the study of optically active dyes derived from camphor and the photochemistry of camphor derivatives. In view of B. K. Singh's major interest, it seemed appropriate to choose as the topic of my lectures the application of spectroscopic methods for structure determination, using phenolic pigments of some Indian plants as examples.

The determination of organic structures by physical methods is by no means new², but in recent years the scope of physical methods in determining

the structures of complex natural products has been greatly extended by mass spectra, nuclear magnetic resonance (NMR) spectra and optical rotatory dispersion^{3,4} apart from new techniques of separation of natural compounds and their degradation products such as gas chromatography and thin layer chromatography. It is to be hoped that our universities will soon find it possible to include these methods⁵ in a course for postgraduate students before they proceed to Ph.D. research.

Ultraviolet Spectra

Scott's very recent and excellent book⁶ gives a complete account of definitions and concepts, characteristic absorptions of single and conjugated chromophores, applications to the analysis of natural products, investigation of gross molecular structure, and interpretation of the UV spectra of some complex molecules; an appendix describes applications to the analysis of steroids, supported by numerous tables^{7,8}.

The famous Woodward rules have now been modified and extended by Fieser and others, and Scott gives the following tabulation of rules of enone absorption (Table 1). Such rules and correlations

TABLE 1 — RULES FOR ENONE ABSORPTION

		λ	
		m μ	
6-Membered and acyclic parent enone		215	
5-Membered parent enone		202	
Increments for			
double bond extending conjugation		30	
alkyl group, ring residue			
α		10	
β		12	
γ and higher		18	
Polar groupings	—OH	35	
		α	30
		β	50
		δ	6
	—OAc	α, β, δ	35
	—OMe	α	30
		β	17
		γ	31
		δ	85
		β	15
	α	12	
	β	25	
	α	30	
	β	95	
	β	5	
Exo double bond		5	
Homodiene component		39	
Solvent correction			

*Based on the Bawa Kartar Singh Memorial Lectures delivered under the auspices of the Panjab University, Chandigarh, on 16-18 November 1964. Since there has been considerable delay in preparing the manuscript for publication, the material has been brought up to date as far as possible.

$$\lambda_{\text{calc.}}^{\text{EtOH}} = \text{Total}$$

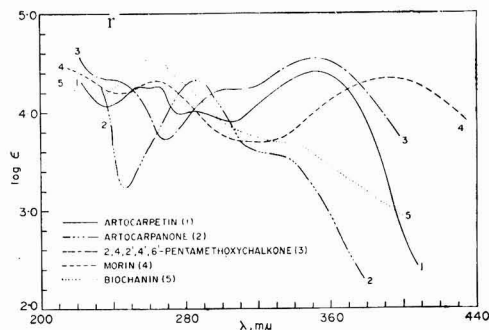


Fig. 1—UV absorption spectra in ethanol of a chalcone, flavanone, flavone, flavonol and isoflavone

are useful in the investigation of terpenes and a few other types of natural products, but the interpretation of the UV spectra of phenolic pigments containing complex chromophores has to be based on more specific data. Thus Birkinshaw⁹ examined the UV spectra of a series of hydroxy-anthraquinones to locate hydroxyl groups in natural anthraquinone pigments. UV spectra are of value in the preliminary identification of a flavonoid class as well as the determination of finer structural features¹⁰. Fig. 1 illustrates the UV spectra of a chalcone, flavanone, flavone, flavonol, and isoflavone. Further information can be obtained from the bathochromic or hypsochromic shifts produced by the addition of aluminium chloride, sodium acetate, or sodium ethoxide. In combination with chromatographic separation procedures Harborne has provided comprehensive UV-visible spectral data for the characterization of anthocyanins^{6,10}. UV and IR spectra may sometimes be of little help even for identifying functional groups in a complex molecule such as terramycin, but the spectra of the degradation products may be much more useful¹¹.

Infrared Spectra

IR spectra are well established as the physical measurements most commonly used by the organic chemist^{12,13}. However, progress in physical methods (and also in natural products chemistry) has been so rapid that Cole's¹⁴ statement in 1956 that "there is no doubt today that of absorption studies in all parts of the electromagnetic spectrum, the IR measurements are generally the most useful for structural determinations" is not strictly true at the present time.

As stated by Nakanishi in his most valuable book¹³, "for the effective usage of IR spectroscopy in qualitative problems it is important to become acquainted with the appearance as well as the range of absorption of the various characteristic absorption bands. The simplest way to achieve this is naturally to become familiar with actual curves." His book accomplishes this purpose by presenting a wide variety of curves to be analysed in addition to data tables and a description of the factors that influence band positions and intensities. The two main uses of IR spectroscopy in the present context

are for the identification of functional groups and for proof of identity (for instance, of a natural and synthetic product) by the superposability of the spectra, especially in the 'fingerprint region', the low frequency end of the spectrum (< 1400 cm^{-1}). In the interpretation of a spectrum regarding functional groups it is to be remembered that the frequencies of the vibrations are also affected by the environment of the bond under consideration in the molecule as a whole.

The IR spectra of steroids have been extensively studied in great detail and used effectively for structure determination because the numerous members are built on practically one molecular skeleton¹⁵. So far as the flavonoids are concerned, IR spectra are of limited value, because the carbonyl frequencies in flavones, flavanones, and isoflavones occur within a narrow range^{16,17}. This is also largely true of the naturally occurring xanthenes and anthraquinones; but some applications of IR data to the pigments we have studied are mentioned later.

Nuclear Magnetic Resonance Spectra

No spectroscopic technique, including IR spectroscopy, is more widely used today by the organic chemist working on the structures of natural products than nuclear magnetic resonance (NMR) spectroscopy¹⁸⁻²⁰. After its discovery in 1946 and its first application to structural organic chemistry in 1953, the organic chemist has more or less taken it out of the hands of the physicist because of its tremendous potentialities for structure determination²¹. It does not displace UV or IR spectroscopy, but is complementary to them; however, it often gives a straight answer to specific structural problems more readily. The organic chemist normally deals with proton magnetic resonance and uses the term NMR as equivalent to PMR.

NMR spectra are commonly used for integrating the total number of protons in a molecule, for characterizing the types of protons (as in a methyl, methylene, methoxyl or hydroxyl group) in terms of the chemical shift from the signal of a standard substance such as tetramethylsilane (TMS), and for locating a proton or group of protons in the neighbourhood in terms of the spin coupling. NMR spectroscopy provides a method of confirming the molecular formula based on the proton count; it gives detailed information on the different kinds of hydrogen atoms in the molecule even when the molecule has as many as 30 or 40; for estimating such groups as methoxyl and C-methyl it is often simpler and more dependable than chemical analysis; and the spin coupling patterns (the multiplicity and the coupling constant J), analysed if necessary with the aid of a computer, reveal the number and nature of the bonds separating one proton from another as well as their spatial relationship.

For NMR spectroscopy, unlike IR or mass spectroscopy, we need quantities of the order of 25-50 mg., although special techniques are now available for handling 1 mg. or less. Further, the compound has always to be in solution, preferably in an aprotic solvent such as carbon tetrachloride or deuterated chloroform (CDCl_3) or deuterium oxide (D_2O). A

greater flexibility in solvents can be obtained by using pyridine for the non-aromatic region and acetone or dimethyl sulphoxide (DMSO) for the aromatic or low field region, but solvent effects on some of the proton signals must be borne in mind.

In the present discussion chemical shifts are cited on the τ scale in which the TMS signal is marked at 10.00.

NMR spectroscopy is particularly valuable for investigating the structures of flavonoids, anthraquinone derivatives and other naturally occurring phenolic pigments because, with exceptions such as morellin, they contain a relatively small number of protons in groups which have different electronic environments as a result of the oxygen functions which are present. The side-chains are in general isoprenoid in type and are also amenable to characterization from NMR data. We have examined a series of anthraquinone derivatives from this point of view; the results will be published elsewhere, but a few applications are mentioned later in connection with some anthraquinone pigments recently examined by us. The flavonoids, benzofurans, 2,2-dimethylchromenes and the rotenoids have been extensively studied and we have a wealth of data available for use in determining the structures of new natural products.

Waiss *et al.*²² have reported preliminary results on the usefulness of trimethylsilyl ethers in determining the structures of flavonoids. Optically active dihydroquercetin was silylated and hydrolysed back to the parent compound with retention of optical activity. The silyl ethers are thermally stable and volatile so that it is possible to purify them by gas-liquid chromatography and submit them to mass spectrometry.

Following Sweeley's report²³ on the preparation of trimethylsilyl ethers of carbohydrates, Mabry *et al.*^{24,25} have examined the utility of these derivatives for the NMR analysis of flavonoids. They have in particular analysed the NMR spectral patterns of the sugar components of flavonoid glycosides. They have drawn attention to the fact that tabular presentation of NMR data does not provide all the available evidence and the actual NMR spectra are of much greater value for structural studies. Trimethylsilyl ethers are readily prepared by treatment of the phenol in pyridine with hexamethyldisilazane and trimethylchlorosilane. These ethers have the advantage that in general they are readily soluble in carbon tetrachloride, thus avoiding the use of deuterated solvents which involve high cost or tedious preparation. The signals for trimethylsilyl groups occur well out of the absorption region of the protons of the flavonoid or glycoside moieties. Further, the trimethylsilyl groups are hydrolysed quantitatively under mild conditions.

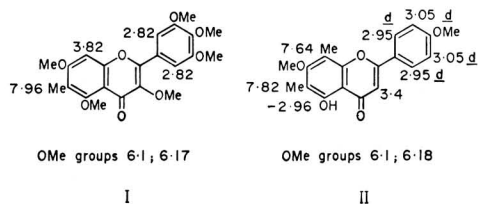
While our work in the course of which the NMR spectra of many flavones and isoflavones have been determined in this area have been reported. Massicot and Marthe^{26,27} have examined the NMR spectra in CDCl_3 of a series of flavones and isoflavones, flavanones and dihydrochalcones as the methyl or ethyl ethers or the acetate.

Batterham and Highet²⁸ have examined the NMR spectra of aromatic, olefinic, and hydroxyl protons of 41 flavonoids in deuterated DMSO. The use of this solvent instead of CDCl_3 made it possible to examine polyhydroxyflavonoids. From their results they were able to arrive at useful correlations concerning the pattern of oxygen substitution in the A and B rings as well as the state of oxidation of the C ring. Chart 1 shows the chemical shifts of the protons in a few typical flavonoids.

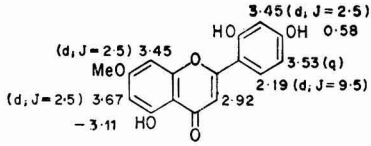
Two good examples of flavones, the structures of which were determined by tedious chemical methods and which could have been derived very simply from the NMR spectra (Radhakrishnan, P. V. & Rama Rao, A. V., unpublished data), are calycopterin and pinomyricetin¹⁶. The NMR spectrum of calycopterin (Fig. 2) in DMSO and pyridine shows a total proton count of 18, four methoxyl groups at 5.86-5.98, a chelated hydroxyl at -2.58, a second hydroxyl at -0.36, and four aromatic protons appearing as two doublets at 1.96 and 2.98 ($J = 9.5$ cps). The A_2B_2 pattern of signals in the aromatic region can arise only from the B ring; the A ring is therefore fully substituted and the 3-position is also occupied as further confirmed by the absence of the 3-proton signal at about 3.2. Since a 5-hydroxyl has been located, the only problem which remains is the placement of the second hydroxyl group; the chemical shift (-0.36) shows that the choice is only between the 7- and 4'-positions. The spectrum of calycopterin diacetate in DMSO shows the A_2B_2 pattern of two bands at 1.84 and 2.58; the shift of 0.4 in the signal representing the 3'- and 5'-protons can only be the result of the deshielding produced by acetylation of a 4'-hydroxyl. These data immediately lead to the indicated structure for calycopterin.

Pinomyricetin does not contain any methoxyl group and it forms a hexamethyl ether as shown by the NMR spectrum. The NMR spectrum of the ether also indicates a nuclear C-methyl group at 7.96, probably sandwiched between two methoxyl groups. The absence of a signal at about 3.2 shows that the 3-position is occupied by a methoxyl group, and the singlet single-proton absorption at 3.52 represents the 8-proton. The two-proton singlet at 2.82 corresponds to the 2',6'-protons in the B ring, which is substituted by methoxyl groups in the 3',4',5'-positions. These data lead to structure (I) for pinomyricetin hexamethyl ether, pinomyricetin therefore being the corresponding hexahydroxyflavone.

The structure of eucalyptin (II), found in small amounts in the leaf wax of *Eucalyptus globulus*, *E. cinerea* and *E. risdoni*, was readily determined from its NMR spectrum²⁹.

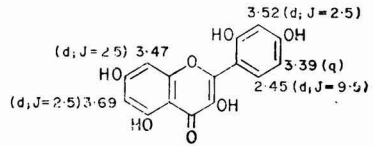


Flavone (Artocarpetin)



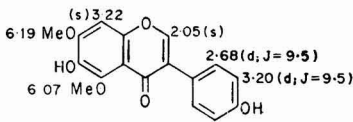
Solvent DMSO

Flavonol (Morin)



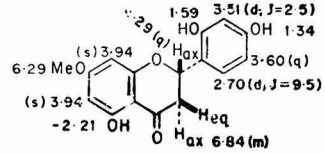
Solvent: Acetone

Isoflavone (Muningir.)



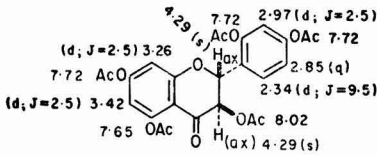
Solvent: CDCl₃

Flavanone (Artocarpانون)



Solvent: Acetone, Pyridine

Flavanonol (Dihydromorin pentacetate)



Solvent: CDCl₃

J = 2 (ax) 3ax = 12.5 cps
 = 3 (ax) 3(eq) = 17. cps
 = 2 (ax) 3(eq) = 4.0 cps

s = singlet
 d = doublet
 q = quadruplet
 m = multiplet

Chart 1 — Chemical shifts of protons in some flavonoids

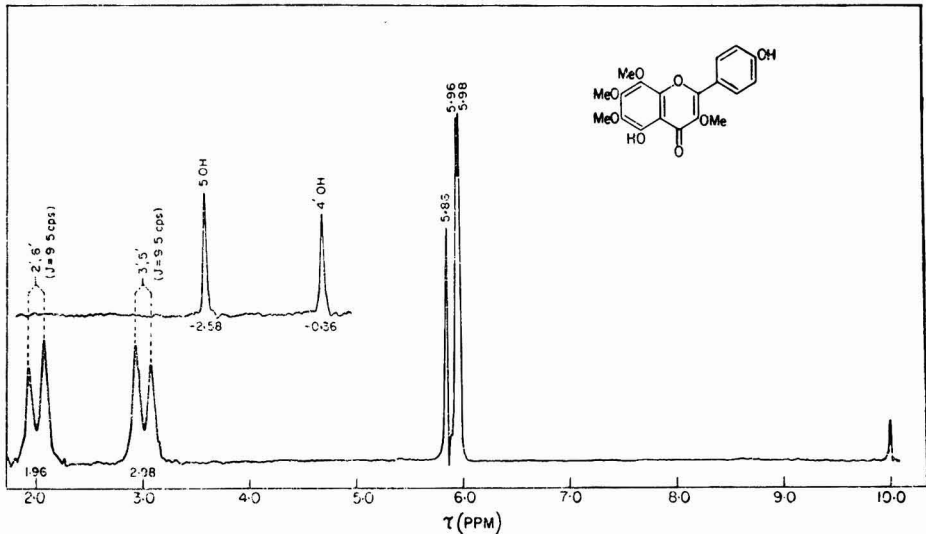


Fig. 2 — NMR spectrum of calycopterin

Mass Spectrometry

Mass spectrometry³⁰ was first used for the qualitative analysis of organic compounds by Beynon³¹ in 1950, and it is now recognized to be a valuable addition to the armoury of the organic chemist³²⁻³⁴. Starting with Ryhage's work on phthiocerol, a waxy constituent of the tubercle bacillus, its possibilities in natural products chemistry have become increasingly clear from Biemann's work on the indole alkaloids and Djerassi's numerous publications on alkaloids and ketosteroids³⁵.

In the mass spectrometer the molecules of a compound in the gas phase are bombarded with low energy electrons (about 70 eV.). An electron is thus ejected from the molecule which then breaks up into a series of fragments consisting of positively and negatively charged ions, radicals, and neutral molecules. In the commercial mass spectrometers, the positively charged ions are separated according to their mass to charge ratio (m/e) by a magnetic analyser (single focusing) or a combination of electrostatic and magnetic fields (double focusing) and the mass spectrum is recorded photographically or by other means. The intense peak of highest mass number normally represents M^+ , the ion formed by removal of one electron from the molecule, and therefore the molecular weight; anomalous molecular weights are obtained if M^+ has broken up before reaching the collector or if two fragments undergo recombination to form an ion of higher mass than M^+ . From the cracking pattern useful information for deducing the structure of the molecule may be obtained, but a mass spectrum is at present far more difficult to interpret than an IR or NMR spectrum. In the equations of organic chemistry an arrow represents two-electron shifts; in Djerassi's mass spectroscopic convention³⁶ (which has not been generally adopted) a fish hook is used to show that only one of the bonding electrons is removed. Structural conclusions concerning the fragmentation of complex products have to be accepted with caution. It is only when a series of compounds of a given type have been studied that the fragmentation patterns can be applied to the determination of the structure of a similar natural product.

In a recent book³⁶ the fragmentation processes occurring in organic molecules are discussed, commencing with compounds containing simple functional groups such as CO, OH and NH₂. Thus the fragmentation of anthraquinone probably proceeds³¹ as shown in Chart 2.

Fragmentation mechanisms may be postulated in the light of our general knowledge of reaction mechanism, but direct and dependable methods based on isotope (mainly deuterium) labelling and on high resolution mass spectrometry (using instruments giving a resolution of 1 in 30,000 or more), in which two species of the same nominal mass may be distinguished if they differ by a small fraction of the mass unit, are being developed³⁷.

In UV, IR and NMR spectroscopy the compound may be recovered after investigation, but in mass spectrometry it undergoes degradation and is irrecoverable; this is not a disadvantage because the

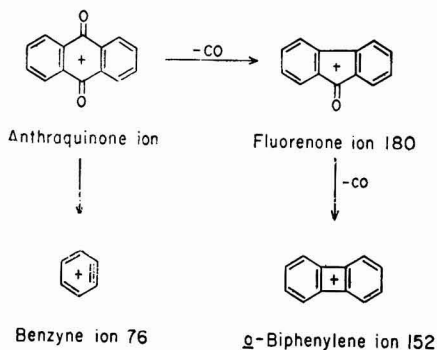


Chart 2 — Mass spectral fragmentation of anthraquinone

quantity required is about 1 mg. or less. Another important characteristic of mass spectrometry is that mass spectra can only be obtained when the compound is present as a gas, but the minimum vapour pressure required is so small (about 10^{-5} mm. Hg) that most organic compounds can now be submitted to mass spectrometry. Compounds with insufficient vapour pressure to be introduced through the inlet system heated to about 350° may be directly fed into the ion source, but thermal stability in either procedure is a prerequisite.

Our own use of mass spectrometry has mainly been for the elementary purpose of determining molecular weights, but the NCL hopes to acquire a CEC type 21-110B mass spectrometer shortly and to study in detail the fragmentation processes in a wide range of natural products.

X-ray Diffraction

The X-ray diffraction method³⁸, with the important advances of isomorphous substitution, the introduction of a heavy atom, and the use of crystals solvated by a liquid containing a heavy atom³⁹, has the tremendous advantage of directly yielding the three-dimensional structure of a molecule in a crystal, but from the point of view of the organic chemist it is the last resort for several reasons. The X-ray crystallographer, even if he has access to a high speed digital computer, will not spend a few months or more in determining the structure of an organic compound unless it has special attraction such as intractability by chemical and other physical methods or unusual complexity or biological properties (e.g. vitamin B₁₂), although he has sometimes dealt with compounds (limonin, clerodin, aflatoxin) whose structures were completely determined by chemists.

Quoting Mathieson⁴⁰ the final step of the "determination of absolute configuration is a relatively minor step experimentally, but it has taken a rather long time to arrive at what is now obvious—a fairly persistent pattern in research". Using ZrK α radiation for effecting a phase advance in the rays refracted by rubidium, the absolute configuration of NaRb (+)-tartrate was determined by Bijvoet in 1951. The absolute configuration of

strychnine hydrobromide was confirmed by using $\text{CuK}\alpha$ radiation (Peerdeman, 1955), and this technique was used by Mathieson to define the absolute configuration of himbacine hydrobromide and jacobine bromohydrin⁴⁰.

Referring to Bijvoet's X-ray work on absolute configuration, Cahn *et al.*⁴¹ have stated: "methods are known by which the structure of an optically active molecule can be so determined as to require its representation, without need or room for convention, by a unique three-dimensional model. We are therefore required to describe such a model uniquely, that is, to specify absolute configuration."

Fortunately X-ray crystallography will never put the organic chemist out of business or keep him in the subsidiary and inglorious role of providing the X-ray crystallographer with suitable crystals. Otherwise the contribution of the organic chemist to structure determination will be like that of a patient with a new and complex disease to medical research. Investigation of the structures of natural products by chemical methods and instrumental techniques (other than X-ray crystallography), which the organic chemist can handle and interpret for himself, continues to be exciting and profitable because of the interest in structure-spectra correlations, degradation methods, synthetic reactions, and biogenesis. A good example is morellin, a fascinating molecule which we will discuss in some detail later. We had to resort ultimately to X-ray crystallography for the complete determination of its structure; but the extensive work on UV, IR and NMR spectroscopy has not been unrewarding. It led to a biosynthetic scheme which found support in the isolation of several related pigments, whose structures were rapidly unravelled in the light of our knowledge of morellin; there are structural and mechanistic problems concerning some of the products obtained from morellin which remain to be solved; and the synthesis of morellin is a challenging task.

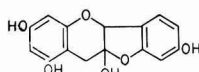
The modern X-ray crystallographer often prefers not to have any chemical evidence placed before him and he makes what Mathieson⁴⁰ has called an all-or-none approach; on the other hand, Stout *et al.*⁴² in their study of the structure of celebixanthone used detailed crystallographic analysis concurrently with chemical investigations "for providing confirmation of the deductions leading to a proposed structure, the role classically assigned to synthesis. This analysis is no less independently certain in its results for having begun with a postulated structure than is a synthesis, and it may reasonably be argued that the mass of data to be fitted and the difference in kind between the deductive arguments and the diffraction evidence makes this approach the more certain".

Pigments of *Artocarpus heterophyllus*

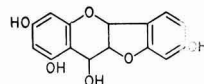
Perkin and Cope (1895) reported the isolation of cyanomaclurin and morin from the heartwood of *Artocarpus heterophyllus* (formerly *A. integrifolia*). We have now isolated six other flavonoids from *A. heterophyllus*, and the presence of dihydromorin (not found by us) has been reported by other workers⁴³. They all have the unique β -resorcylic

acid orientation of hydroxyl groups in the B ring not present in any other flavonoids excepting the isoflavones, ferreirin and homoferreirin. Chart 3 presents a biosynthetic scheme for the nine flavonoids of *A. heterophyllus* in which the hydroxylation pattern of both the A and B rings is fixed at the chalcone stage.

In 1935 Appel and Robinson proposed structure (III), the hemiketal form of 5,7,2',4'-tetrahydroxy-3-ketoflavan, for cyanomaclurin. Later Robinson himself⁴⁴ considered the possibility that cyanomaclurin may be a derivative of flavan-3,4-diol, and 'the same idea has been bandied about in several personal communications'. In 1960 Freudenburg⁴⁵ in a paper on catechins and hydroxyflavandiol discussed structures (III) and (IV) for cyanomaclurin. Accepting the suggestion that NMR data were not consistent with the presence of three consecutive aliphatic CH groups in the molecule, he favoured structure (III), which was supported by the observation that the compound did not lose any hydroxyl group by catalytic reduction. Clark-Lewis⁴⁶ also discussed the two structures, and he expressed the view that all the available evidence is consistent with (IV). Chakravarty and Seshadri⁴³ showed on the basis of NMR data that cyanomaclurin does not have a benzylic methylene group as required by (III), and they supported structure (IV).



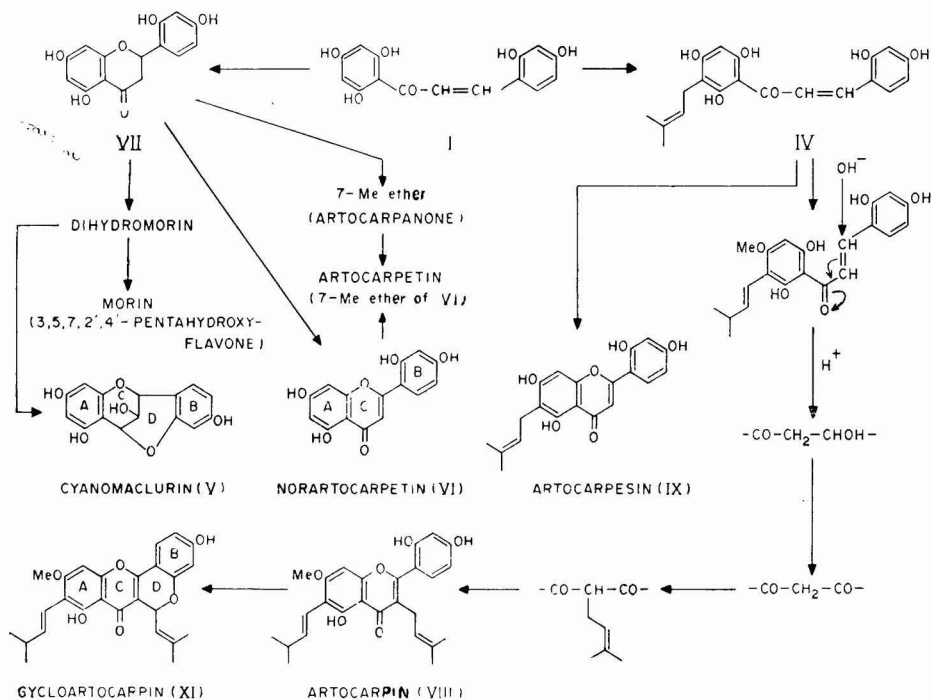
III



IV

Since the behaviour of cyanomaclurin in catalytic hydrogenation and in oxidation with manganese dioxide or chromic anhydride would be anomalous if structure (IV) is correct, we proposed structure (V), which was readily confirmed by the paramagnetic shift suffered by the proton α to the hydroxyl group in cyanomaclurin trimethyl ether when it was acetylated⁴⁷. The trimethyl ether showed single proton absorptions at 4.61, 4.88 and 5.67. The first two were readily assigned to the two benzylic and the third to the non-benzylic $-\text{CH}-\text{O}-$ groups; between the two benzylic protons the higher signal at 4.88 is assigned to the 4-H in view of the contiguity to the phloroglucinol nucleus. In the acetate spectrum all three protons showed absorptions in the region 4.4-4.7.

Corey *et al.*⁴⁸ have stated that "the stereochemistry of substituted flavan derivatives has been a more subtle and elusive matter than similar problems with saturated carbocyclic ring systems. This is mainly because the usual generalizations correlating reactivity and stereochemistry are not very reliable for a ring system containing a heteroatom and also aromatic unsaturation, features which conspire to produce unique complications." They have reported the application of NMR to the clarification of the molecular configurations of the two known flavan-3,4-diols prepared from flavan-3-ol-4-one, illustrating the power of NMR as a tool


 Chart 3 — Biosynthesis of *Artocarpus* flavonoids

in stereochemical analysis in which a single measurement defined clearly the interrelationship of three asymmetric centres⁴⁹. The *trans* or *cis* relationship of the hydroxyl groups in the two diols has been confirmed by IR data⁵⁰.

On the model of cyclohexene a half-chair conformation has been suggested for the catechins⁵¹. Philbin and Wheeler have also considered the sofa or cyclohexenone type in which only the C atom in the 2-position of chroman lies out of the plane of the benzene ring. The flavonoid configuration of lowest energy has the 2-phenyl in an equatorial position, but as mentioned by Clark-Lewis we must regard conformational inversion of flavan derivatives as a mobile equilibrium. In cyanomaclurin the stereochemistry is restricted (and also complicated) by the additional dihydropyran ring. The H atoms in the 2- and 4-positions are equatorial, and the H atom in the 3-position can be axial or equatorial. Although they have not specified the precise flavan-3,4-diols which were examined, Clark-Lewis and Jackman⁵² have observed that the 3,4-coupling constants for 3(*ax*)4(*eq*) and 3(*eq*)4(*eq*) are about 6.6 and 0.1-0 cps respectively. A coupling constant of the order of 6-7 cps was not observed in the spectrum of cyanomaclurin trimethyl ether; 3-H is therefore equatorial, and is *trans* to both 2-H and 4-H. However, when the dihydropyran ring D fused to the B ring is considered, 3-H and 4-H have to be *ax-eq*; but the expected J 6-7 is absent. This anomaly in the stereochemistry of

cyanomaclurin and its absolute stereochemistry are under investigation.

On the basis of chemical evidence, artocarpetin and artocarpinone were shown to be the 7-methyl ethers of (VI) and (VII) respectively; artocarpin was assigned structure (VIII), and these have now received synthetic support. Norartocarpetin and artocarpesin were readily identified as (VI) and (IX) respectively from our knowledge of artocarpetin and artocarpin⁵³.

The structure of artocarpin was confirmed by the NMR spectra of the pigment and its derivatives. The spectrum (Fig. 3) of the dimethyl ether showed a chelated hydroxyl (-3.5), an isopropyl [(CH₃)₂ 8.93; CH 7.6] and two methyl groups (8.45; 8.63) on double bonds. A total of seven aromatic and vinyl protons as well as one allylic methylene (7.1; J = 7 cps) which is not attached to an aromatic ring, but probably to the 3-position of the flavone nucleus, were also indicated. Three of the vinyl protons disappeared in the tetrahydro-derivative, simultaneously with diamagnetic shifts of the methyl groups and the isopropyl group on double bonds as would be expected from structure (VIII). The lone proton of the phloroglucinol moiety of tetrahydroartocarpin dimethyl ether absorbed at 3.87, and the 1,2,4-substitution pattern of the B ring was indicated by the nature of the absorptions of the 3'-, 5'- and 6'-protons [3'-H 3.6 d (J = 2.5 cps); 5'-H 3.57 q; 6'-H 2.92 d (J = 9.5 cps)]. One feature of the NMR spectra of artocarpin and its derivatives

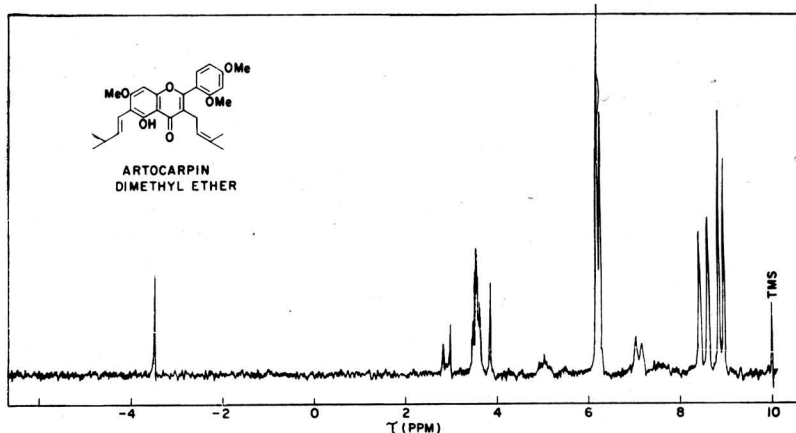
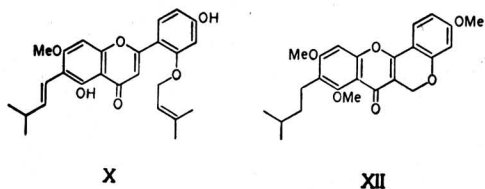


Fig. 3 — NMR spectrum of artocarpin dimethyl ether

deserves special mention. The 6'-proton absorbs at about 2.92, 0.6-0.7 upfield from the absorption position of the corresponding proton of the other flavonoids of this series, because of the steric effect of the 3-substituent in artocarpin which rotates the B ring out of conjugation with the chromone system.

Cycloartocarpin was originally designated isoartocarpin since it was considered to be an isomer of artocarpin, and structure (X) was proposed as the result of degradative evidence, the interpretation of which concerning one structural feature later proved to be erroneous. When the NMR spectrum of isoartocarpin was determined, it became clear that the prenyl ether group was not present. The mass spectra of cycloartocarpin and its dimethyl ether, kindly determined by Dr J. S. Shannon, gave the molecular weight 434 and 462 respectively, confirming structure (XI) for cycloartocarpin⁵⁴. By the action of halogen acids on dihydroisoartocarpin and tetrahydroisoartocarpin, followed by methylation, Telang⁵⁵ obtained a compound, m.p. 191°, which he considered to be 6-isoamyl-5,7,2',4'-tetramethoxyflavone, although he recorded the fact that the Zeisel methoxyl value corresponded to 3 and not 4 methoxyl groups. We have repeated the two reactions on tetrahydroisoartocarpin and found that the product is a mixture containing the compound, m.p. 191°, as shown by thin layer chromatography and NMR spectrum. This compound shows three methoxyl groups (6.13, 6.18 and 6.24) and an allylic OCH₂ at 4.83 (singlet) in the NMR spectrum, and the mass spectral molecular weight is 410.



These facts are in agreement with structure (XII), but we are at present unable to explain the fission of the C-C bond involved in the conversion of (XI) to (XII).

The UV spectra of artocarpetin, artocarpin and cycloartocarpin in the long wavelength region (Fig. 4) show the steric effect of alkyl substitution in the 3-position. Artocarpetin and cycloartocarpin have high intensity cinnamoyl bands at 355 and 370 m μ (ϵ 26300; 27000) respectively, but for artocarpin this band is of relatively low intensity (ϵ 12300) and appears at 324 m μ . The corresponding absorptions of chrysin and 3-isopropylchrysin are at 313 m μ (ϵ 12000) and 300 m μ (ϵ 9700) respectively. The large hyperchromic red shift of 42 m μ shown by artocarpetin as compared with chrysin is attributable to the electron donor action of the hydroxyl groups in the *ortho* and *para* positions in the cinnamoyl moiety. This shift is largely eliminated in artocarpin in which the 3- γ -dimethylallyl substituent may be expected to prevent the 2-phenyl and the chromone ring systems from being coplanar.

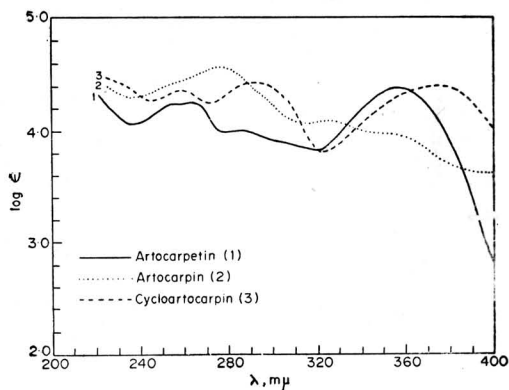


Fig. 4 — UV absorption spectra in ethanol of artocarpetin, artocarpin and cycloartocarpin

in the double bond region, and this was assigned to a chelated carbonyl group as in a 5-hydroxy-chromanone. Bands at 875 cm^{-1} and in the 1030-1124 cm^{-1} region probably indicated a tetra-hydropyran ring.

Isomorellin, into which morellin is converted by several mild treatments, and morellin had very similar spectra, except that the band at 1668 cm^{-1} of morellin appeared at 1685 cm^{-1} , suggesting a change in the conjugation of the $\alpha\beta$ - $\alpha'\beta'$ -unsaturated carbonyl group postulated in morellin.

Ultraviolet spectra—Morellin had three maxima at 236, 288.5 and 360 μ . Dihydromorellin had a more complex spectrum with two additional maxima in the 260-280 μ region. Tetrahydromorellin and the products of further reduction, including the Mozingo reduction product, had similar spectra with a peak in the 300-306 μ region and a weak broad band near 345 μ . This spectrum was typical of a compound in which phloroglucinol and carbonyl chromophores were conjugated as in C-methyl-phloracetophenone and 5,7-dihydroxychromanone. Comparison of the spectra of the reduction products of morellin with those of dihydrotoxicarol and hexahydro-osajin showed that the latter compounds had bands in about the same region, but of much higher intensity. This was attributed to the substituted phenyl groups in the 3-position of the chromanone nucleus in dihydrotoxicarol and hexahydro-osajin. A 3-phenyl group appeared therefore to be absent in morellin.

The UV spectra of morellin and some of its derivatives in comparison with related compounds which provided useful structural evidence are shown in Figs. 5 and 6.

An important difference in the absorption spectra of morellin and its reduction products was the intensity of the morellin band in the 360 μ region, which virtually disappeared on hydrogenation. A similar high intensity band at about 360 μ occurs in the spectrum of rottlerin, and it has been attributed to a cinnamoyl group conjugated with a chromene nucleus⁶¹.

Evidence for an aldehyde group—Although the IR spectra gave some indication of an aldehyde group, Bringi rejected it because morellin and octahydromorellin did not form a dimedone derivative and did not respond to other tests for aldehydes. On the other hand, the formation of a diol⁶² from morellin, which only contains one hydroxyl group, suggested the possibility of an aldehyde group capable of enolization. The IR spectra of tetrahydromorellin and octahydromorellin were therefore reinvestigated by Jose and Bhat⁶², looking particularly for the presence of overtone or combination bands in the near-IR region in the light of the recent work of King and Vig⁶³. They measured the near-IR spectra of more than 30 aldehydes and found that all of them showed two bands (around 4500 and 4400 cm^{-1}) expected from the combination of the C-O stretching mode (around 1700 cm^{-1}) with each of the formyl C-H bands (around 2800 and 2700 cm^{-1}). In their recent work on the structure of helminthosporal, the toxin from *Helminthosporium sativum*, De Mayo⁶⁴ found a band at 4480 cm^{-1} in the near-IR region useful for characterizing a conjugated aldehyde group. Jose determined the spectra in carbon tetrachloride on a Perkin-Elmer 221 spectrophotometer with a sodium chloride prism in the region 4500-650 cm^{-1} . Using NaCl prism-grating interchange, the band at 1668 cm^{-1} in morellin earlier reported by Bringi was found to be at 1680 cm^{-1} , and it disappeared in the Mozingo reduction products. The band at 1680 cm^{-1} could well be assigned to an $\alpha\beta$ -unsaturated aldehyde group; however, since the C=O stretching vibration for conjugated aldehydes is in the range 1705-1680 cm^{-1} , additional evidence was necessary. There were C-H bands at 2740 and 2770 cm^{-1} , which may arise from a formyl group, and the sum of each of these with the 1680 frequency should appear respectively at 4420 and 4450 cm^{-1} . Although two distinct peaks were not noticed, a broad band at 4400-4480 cm^{-1} provided

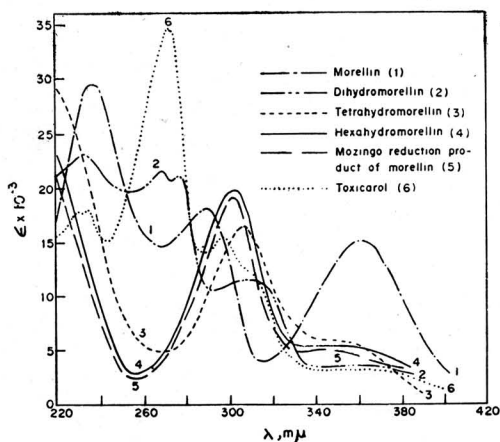


Fig. 5 — UV absorption spectra in ethanol of morellin and some of its derivatives

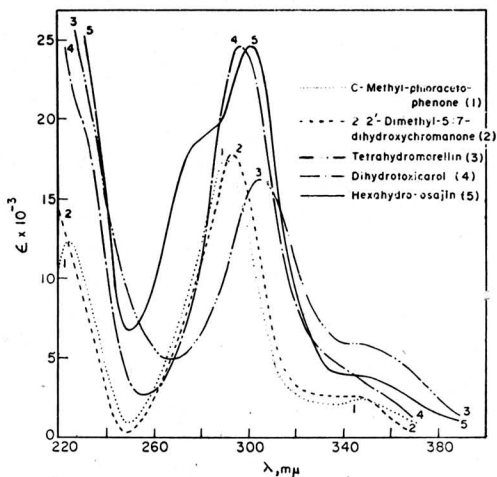
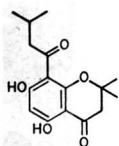


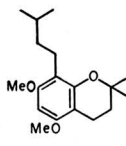
Fig. 6 — UV absorption spectra of compounds related to morellin

clear evidence of an aldehyde group. The observed $\text{C}=\text{O}$, formyl C-H and near-IR frequencies for tetrahydromorellin were as follows: 1685, 2725 and 2739 cm^{-1} and broad absorption around 4400 cm^{-1} , confirming the presence of an aldehyde group in tetrahydromorellin. Drastic ozonization of morellin gave acetone, formaldehyde, acetaldehyde, and p-ruvic aldehyde, the last indicating the group =C-CHO.

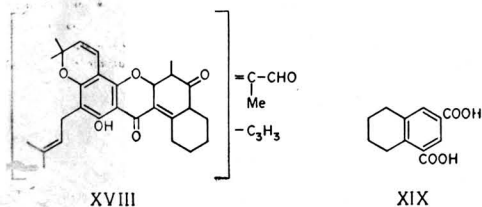
Me
A partial structure for morellin — From the products of alkali fusion of octahydromorellin, Vijayaraghavan⁶⁵ isolated a phenol, $\text{C}_{16}\text{H}_{24}\text{O}_4$, which was also obtained synthetically as the most readily crystallizable constituent of the products of Clemmensen reduction of 5,7-dihydroxy-8-isovaleroyl-2,2-dimethylchromanone (XVI). Since this phenol obviously came from the phloroglucinol part of the morellin molecule, in which one hydroxyl had undergone cyclization to a 2,2-dimethylchromene as shown by the formation of acetone by mild alkaline hydrolysis, it was clear that the phloroglucinol nucleus carried a second C_5 residue. This was confirmed by the fact that the $\text{C}_{16}\text{H}_{24}\text{O}_4$ phenol was accompanied in the products of alkali fusion by a $\text{C}_{16}\text{H}_{24}\text{O}_3$ phenol, which was isolated as its crystalline dimethyl ether identical with a product of the authentic structure (XVII) prepared by synthesis. These results, taken in conjunction with the UV spectral data discussed earlier, led to the part structure (XVIII). The partially hydrogenated naphthalene ring was postulated in (XVIII), because a product of the Mozingo reduction of morellin with ten times its weight of Raney nickel in cyclohexanol gave on alkali fusion the $\text{C}_{16}\text{H}_{24}\text{O}_4$ phenol and a dicarboxylic acid, $\text{C}_{12}\text{H}_{12}\text{O}_4$, identified as tetrahydronaphthalene-5,7-dicarboxylic acid (XIX) by synthesis.



XVI



XVII



XVIII

XIX

NMR spectra — The first few spectra were kindly determined for us by Dr M. Caserio of the California Institute of Technology, Dr A. Melera of Varian Associates, Zürich, and Dr C. S. Barnes of CSIRO, Melbourne. Subsequently, all the NMR spectra were determined on our own instrument, a Varian A-60. The spectra were taken in CDCl_3 solution using tetramethylsilane as internal standard. Figs. 7, 8 and

9 represent the spectra of morellin, isomorellin and morellin diacetate. The molecular formula $\text{C}_{33}\text{H}_{36}\text{O}_7$ is confirmed by the total proton count of 36. A single-proton singlet at -2.77 corresponds to the strongly chelated hydroxyl group in (XVIII). The sharp singlet at 0.37 in Fig. 7 is a clear proof of an aldehyde group attached to a carbon atom which does not carry a proton; the aldehyde group is, therefore, tertiary or $\alpha\beta$ -unsaturated with an α -substituent. The vinyl doublet at 3.35 and 4.43 ($J = 10$ cps), in conjunction with the two Me groups at 8.47, indicates a 2,2-dimethylchromene system^{66,67}. The vinyl triplet at 4.75 and the Me signals at 8.2 and 8.3 may be assigned to a $\gamma\gamma$ -dimethylallyl group; if this is attached to an aromatic ring, the allylic methylene will be benzylic also, and one would expect it to absorb in the 6.6-6.7 region, giving a doublet⁶⁷. In morellin itself the absorption in this region shows more than two protons in a multiplet structure; however, there is a strong suggestion of a 2-proton doublet. In isomorellin, which has a very similar spectrum, the position is more satisfactory, the 2-proton doublet at 6.77 being well resolved. Together with the chelated hydroxyl signal at -2.77 , the part structure (XVIII) or the linear isomer, without the hydrogenated naphthalene ring, may be formulated.

The vinyl doublet at 2.4 ($J = 7$ cps) shows that the proton is attached to a double bond linked to a strongly electron-withdrawing group. This can be the β -proton of an $\alpha\beta$ -unsaturated ketone, which has a planar *S-cis* conformation⁶⁸ or is part of a cyclopentenone ring. The absorption of morellin in the near-UV region (λ_{max} 360 $\text{m}\mu$) requires that there should be a double bond in conjugation with the chromanone CO in (XVIII) (or the linear isomer), and the proton under consideration can well be on this double bond. It is spin coupled to a lone proton on an adjacent carbon. If this carbon is part of a ring, the magnitude of the coupling constant implies that the dihedral angle between them is close to zero. In tetrahydromorellin the chromene doublets and the doublet at 2.4 disappear.

The triplet absorption at 3.9 in the morellin spectrum indicates another proton with an adjacent CH_2 and on a double bond attached to an electron-withdrawing group. This is shifted to 3.63 in tetrahydromorellin. The shift may be associated with destruction of cross-conjugation, removal of anisotropic shielding, or isomerization during hydrogenation. For isomorellin the corresponding absorption is at 3.68. It is also suggestive that, whereas the morellin aldehyde signal is at 0.37, the corresponding signals of tetrahydromorellin and isomorellin appear at 0.68 and 0.73 respectively. It is more than likely that isomerization has taken place during hydrogenation.

In the spectrum of morellin diacetate the vinyl triplet at 3.9 and two protons, probably belonging to a CH_2 , coming in the region of 6.2-7.15 in the morellin spectrum, as well as the aldehyde signal, have disappeared; and three new vinyl protons appear at 2.80, 3.52 and 4.84. The first gives a singlet absorption, and the remaining two constitute a pair of *trans* vinyl protons (doublets, $J = 15.5$ cps). The first appears to be the α -proton of a vinyl

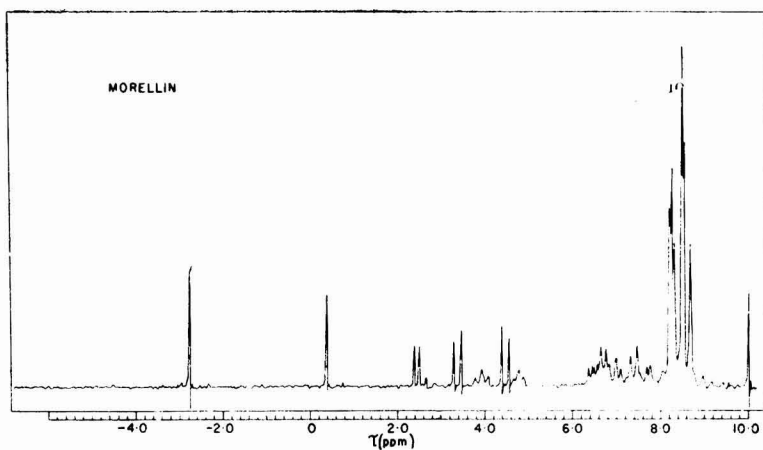


Fig. 7 -- NMR spectrum of morellin

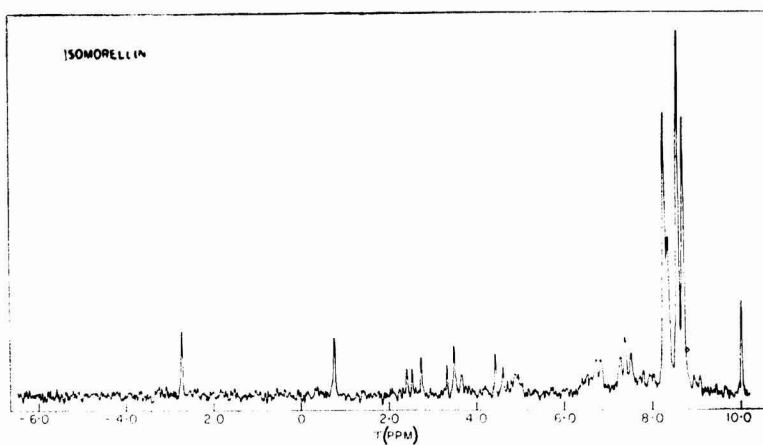


Fig. 8 -- NMR spectrum of isomorellin

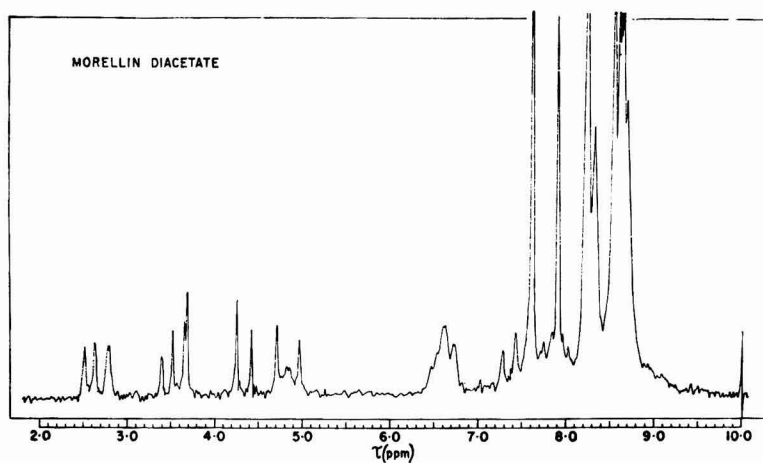


Fig. 9 -- NMR spectrum of morellin diacetate

acetate formed by acylation of the hydroxymethyl-tautomer of morellin. If morellin has an angular aldehyde group, the formation of a pair of *trans*-vinyl hydrogens at the acetylation of morellin will involve the opening of a ring. Alternatively, if the aldehyde group is $\alpha\beta$ -unsaturated and is present in the side-chain, the observed changes will be consistent with the presence of a $-\text{CH}_2-\text{CH}=\text{C}-\text{CHO}$ group in morellin, converted to $-\text{CH}=\text{CH}-\text{C}=\text{CH}-\text{OAc}$ in the diacetate, the γ - and δ -protons giving rise to the doublet absorptions at 3.52 and 4.84. The formation of pyruvic aldehyde in the ozonolysis of morellin suggests that the α -substituent here is a methyl group. If this is correct, the methyl resonance seen at 8.67 may be assigned to it; this is the methyl group involved in the isomerization to isomorellin which is discussed later.

Fig. 7 shows the presence of seven methyl groups. The molecular formula $\text{C}_{33}\text{H}_{36}\text{O}_7$ is confirmed by the total proton count of 36. The 3-proton peaks at 8.25 and 8.52 in the spectrum of morellin occur in the expected region for Me groups on C attached to O. In isomorellin these Me groups are in similar environments and lead to a single sharp resonance at 8.67, suggesting that they may form a *gem*-dimethyl system. In morellin itself they are probably differentiated by magnetic anisotropy of the double bonds in their neighbourhood.

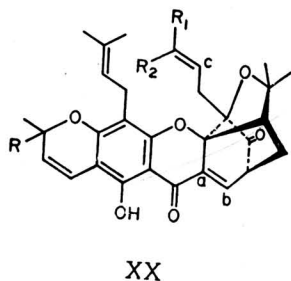
Summarizing the NMR results they are in excellent agreement with the part structure (XVIII) or its linear isomer. They do not distinguish between the angular or linear attachment of the chromene ring and they leave unexplained the formation of a tetrahydronaphthalene derivative in the alkali fusion of a reduction product of morellin. At least three structures for the entire morellin molecule can be reconciled with the NMR data. We were at this stage seeking other approaches which might have led to the complete and correct structure for morellin.

X-ray crystallography—Almost from the commencement of our work on morellin it was realized that X-ray crystallography may prove to be the only technique by which the complete structure of morellin can be proved. Prof. J. D. Bernal, during a visit in 1955, was attracted by the size and beauty of the crystals of morellin and he took with him a sample for X-ray study. Morellin was investigated by Dr Carlisle and Dr Dayal in Birkbeck College, London, and subsequently by the latter in Banaras Hindu University. Although Dr Dayal had no contact with the workers on morellin in my laboratory since 1955, he and S. C. Mathur have referred in a recent note⁶⁹ to the preliminary chemical data provided to him casually. Dayal and Mathur have included in their note a 'provisional chemical formula' in which a double bond in a benzene ring is omitted and a double bond in the hydrogenated naphthalene ring is in a wrong position. They finally state: "Trials of atomic positions based on the formula have shown reasonable agreement between calculated and observed structure factors; but, owing to the complexity of the molecule and its awkward projections on (001) and (010), are not sensitive to further refinement. It is clear that

the analysis needs to be carried out in three dimensions and this is now being done."

It was observed by Dr L. M. Pant at the NCL that the main difficulty in the X-ray crystallographic study of morellin by two-dimensional methods is that there is too much overlapping of peaks in the Fourier maps and the molecule therefore cannot be identified. It was not possible to use three-dimensional methods, because the necessary computing facilities were not available.

Prof. G. N. Ramachandran, who has been in touch with us continuously, and Dr G. Kartha have carried out an X-ray crystallographic study of the *p*-bromobenzenesulphonyl ester of morellin. The first structure suggested by them was similar to (XX; $\text{R} = \text{R}_2 = \text{Me}$; $\text{R}_1 = \text{CHO}$), but in place of the aldehydic oxygen there was a methylene group. This was not compatible with the presence of seven oxygen atoms in morellin, one of them as an aldehyde group as shown by NMR evidence and confirmed by the formation of pyruvic aldehyde on ozonolysis. The X-ray structure was therefore modified to (XX; $\text{R} = \text{R}_1 = \text{Me}$; $\text{R}_2 = \text{CHO}$), which was then confirmed by X-ray data⁷⁰. Full details of the X-ray work will be published by Kartha and Ramachandran.



XX

Structure (XX; $\text{R} = \text{R}_1 = \text{Me}$; $\text{R}_2 = \text{CHO}$) is in excellent agreement with the NMR data discussed earlier. The part structure (XVIII) arrived at from NMR and chemical data forms the left half of the X-ray structure. As expected, there is a double bond in conjugation with a CO attached to an aromatic ring, and the proton on this double bond has been correctly placed as the one that gives rise to the absorption at 2.4 in the morellin spectrum. It was deduced with the help of the spectrum of morellin diacetate that there is one proton in morellin (showing up at 6.4) which is either on a carbon bond attached to oxygen or α to a keto group and allylic at the same time. The proton at the bicyclo-octenone bridgehead has the latter type of environment. Since the signal concerned overlaps with that of the benzylic CH_2 , the splitting is not observable. The probable presence of a C_5 side-chain like $\text{CH}_3-\text{C}=\text{CH}-\text{CH}_2$ was indicated

$$\text{CHO}$$

by the NMR spectrum of the diacetate and has now been firmly established. The cage system formed by the bicyclo-octenone and oxide ring of the right part of the molecule could of

course^{1,2,3,4} be inferred from the NMR or other chemical data available. However, the disposition of remaining groups with respect to each other revealed by the X-ray structure makes possible a rationalization of the more obscure features of the NMR spectra of morellin and its derivatives. The unsymmetrical quartet (centred at 7.35) representing two protons in the spectrum of morellin corresponds to the methylene group of the bicyclic system. The proton in the bicyclo-octenone ring α to the *gem*-dimethyl group seems to be coupled to only one of the methylene protons; it gives a doublet at 7.7 ($J = 4.5$ cps) in the spectrum of morellin.

Isomorellin⁷¹

The *p*-bromobenzenesulphonyl ester of morellin supplied to Prof. Ramachandran was prepared by refluxing morellin and *p*-bromobenzenesulphonyl chloride in acetone in presence of potassium carbonate. Since isomerization to isomorellin is a possibility, the fact that structure (XX; $R = R_1 = \text{Me}$; $R_2 = \text{CHO}$) is that of morellin, and not isomorellin, was confirmed by the preparation of the *p*-bromobenzenesulphonyl ester of isomorellin and comparison of the NMR spectra in the aldehyde region.

Morellin and isomorellin have been shown to differ only in the configuration of the groups round the double bond of the side-chain carrying the aldehyde group; in morellin the methyl and methylene groups are *trans* and in isomorellin *cis*. In the NMR spectra the vinyl and aldehyde protons of this side-chain in morellin are at 3.87 and 0.37, and they are at 3.52 and 0.73 in isomorellin. In the aldehyde signal there is a diamagnetic shift of 0.36 p.p.m. for isomorellin, which is apparently the result of long-range shielding by neighbouring groups. Although Δ for the vinyl proton in morellin and isomorellin is smaller than in other $\alpha\beta$ -unsaturated *cis-trans* pairs such as methyl crotonate and isocrotonate, or methyl angelate and tiglate, the relative stabilities of morellin and isomorellin, isomerization of morellin to isomorellin being very facile, confirm that morellin and isomorellin have the structures (XX; $R = R_1 = \text{Me}$; $R_2 = \text{CHO}$) and (XX; $R = R_2 = \text{Me}$; $R_1 = \text{CHO}$) respectively. There are very interesting differences in the methyl spectra of morellin and isomorellin, which are discussed in detail elsewhere⁷¹.

Desoxymorellin and Dihydroisomorellin

From the mother liquor after the crystallization of morellin from the ethanol extract of the pericarp of the seeds of *Garcinia morella*, three pigments were isolated⁷². Two, designated desoxymorellin and dihydroisomorellin, have the structures (XX; $R = R_1 = R_2 = \text{Me}$) and (XX; $R = R_2 = \text{Me}$; $R_1 = \text{CHO}$; single bond at C_a-C_b). In the light of the established structure of morellin, the NMR spectra gave enough evidence for the structures, but additional UV, IR and chemical evidence were also obtained. In the NMR spectrum of desoxymorellin the total proton count is 38 corresponding to the replacement of the aldehyde group of morellin by a methyl group; the signal of the vinyl proton *c* at 3.90 and 3.68 (triplets, $J = 7.5$ cps) in the morellin

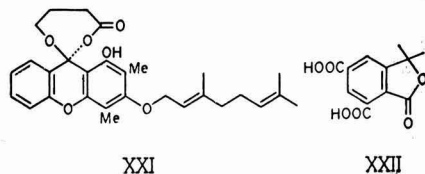
and isomorellin spectra has shifted upfield to 5.58, which is considerably higher than the expected value for the corresponding proton in desoxymorellin. The abnormality is presumably caused by the magnetic anisotropy of the neighbouring benzene ring. The aldehyde signal of morellin disappears and the methyl region shows the additional methyl group. Except for minor differences, which are discussed in detail elsewhere, the spectra of desoxymorellin and morellin are very similar. The NMR spectrum of dihydroisomorellin has all the main features of the spectra of morellin and isomorellin, except that the doublet absorption of the vinyl proton *b* in isomorellin is missing in dihydroisomorellin, showing that the C_a-C_b double bond is saturated in the new pigment. This is confirmed by the number of protons (11) absorbing in the region 6.0-8.0 in comparison with 8 for morellin and isomorellin. The triplet absorption at 3.17 corresponding to the vinyl proton *c* suggests that the side-chain bearing the aldehyde group is the same as in isomorellin.

The third pigment is an artefact arising from the addition of ethanol to morellin during crystallization. The NMR spectrum resembles that of dihydroisomorellin very closely, but shows an additional *C*-methyl group which appears as a triplet ($J = 6.5$ cps) superposed on the tertiary methyl signals at 8.86. The triplet methyl absorption shows that the new pigment has an ethyl group. In the region 6.0-8.0 there are additional protons. As in the spectrum of dihydroisomorellin, the signal of the vinyl proton *b* in morellin or isomorellin has disappeared, and a new single-proton doublet appears at 5.55 ($J = 5$ cps). From these data, confirmed by other evidence, it was clear that the pigment has the structure ethoxydihydroisomorellin (XX; $R = R_2 = \text{Me}$; $R_1 = \text{CHO}$; single bond at C_a-C_b ; OEt at *b*).

Gambogic Acid

Gambogic acid is the main pigment of gamboge, the latex of *Garcinia hanburryi*; its presence in the latex of *Garcinia morella* has been mentioned, but we have not been able so far to isolate it from this material obtained from Mangalore. The probable explanation is that *G. hanburryi* was formerly regarded as a variety of *G. morella*. Gambogic acid has not yet been obtained crystalline, but it forms a crystalline pyridine salt, acetate, and dimethyl ether-ester; until recently very little was known about the structure of gambogic acid. The complexity of the gambogic acid problem can be imagined from the fact that in a 1962 paper⁷³ the molecular formula $C_{29}H_{34}O_6$ and the structure (XXI) were suggested.

Dyson and Rigby⁷⁴ assigned the molecular formula $C_{38}H_{44}O_8$ to gambogic acid without mentioning how this was established. Alkali fusion of gambogic



acid gave phloroglucinol, homophthalic acid, isovaleric acid, methyl succinic acid, methyl heptenol [$\text{Me}_2\text{C}=\text{CH}-\text{CH}_2\text{CH}_2\text{CHOHMe}$] and a mixture of amphiphilic acids. Oxidation of the last gave a dicarboxylic acid identified as the phthalide (XXII). From its plant origin and its yielding nearly the same alkali fusion products as morellin and other properties we suspected during the progress of our work on morellin that gambogic acid and morellin must have a close structural relationship. Simultaneously with the complete elucidation of the structure of morellin, Dyson and Rigby gave gambogic acid the molecular formula $\text{C}_{38}\text{H}_{44}\text{O}_8$. The difference in the molecular formulae of gambogic acid and morellin was therefore a C_6H_8 residue containing an ethylenic bond, since gambogic acid and morellin respectively gave decahydro and octahydro derivatives by catalytic hydrogenation. The attachment of an isopentenyl chain to morellin and the replacement of CHO by COOH could well represent the structure of gambogic acid, but an examination in particular of the NMR spectrum of gambogic acid acetate was essential. Unfortunately as stated earlier, the sample of *Garcinia morella* latex available to us contained no gambogic acid and it was several months before we were able to procure *Garcinia hanburryi* gamboge from Bangkok with the kind assistance of Dr V. V. Bhatt of the Asian Development Institute. Meanwhile the note of Yates *et al.*⁷⁵ appeared in which the structure (XX; $\text{R}=\text{CH}_2\text{CH}_2\text{CH}=\text{CMe}_2$; R_1 or $\text{R}_2 = \text{Me}$ or COOH) was suggested for gambogic acid on the basis of our structure for morellin. Very recently Ollis *et al.* also arrived at the same structure for gambogic acid⁷⁶. They state that they were unable to use their chemical and other evidence effectively until the paper on the constitution of morellin appeared. An interesting reaction discovered by Ollis *et al.* which provides valuable confirmation of the structure of gambogic acid is a cyclization involving the new C_5 residue attached to the chromene system.

Morellic Acids

Two new pigments which C. G. Karanjgaokar very recently isolated from the latex (gamboge) of *Garcinia morella* are morellic and isomorellic acids. They were isolated as the crystalline acetates whose mass spectral molecular weight (602) and the NMR spectra showed very readily that they are the carboxylic acids (XX; $\text{R}=\text{R}_1 = \text{Me}$; $\text{R}_2 = \text{COOH}$) and (XX; $\text{R}=\text{R}_2 = \text{Me}$; $\text{R}_1 = \text{COOH}$) corresponding to morellin and isomorellin respectively.

Biogenesis of *Garcinia* Pigments

We have suggested⁷⁰ a biosynthetic scheme for morellin in which it is derived from one molecule of 1,3,6-trihydroxyxanthone and four molecules of 'active isoprene' by a series of biochemically acceptable transformations. An intermediate in the biosynthetic scheme is dihydrodesoxymorellin (XX; $\text{R}=\text{R}_1 = \text{R}_2 = \text{Me}$; single bond at C_a-C_b), for which powerful support has now been obtained by the isolation of desoxymorellin, dihydroisomorellin, and the morellic acids from *Garcinia morella*. The addition of a further C_5 unit to morellic acid

will lead to gambogic acid. Among the xanthones and flavonoids, morellin and its analogues are unique in possessing a carbocyclic ring derived from an isoprenoid side-chain; they are also among the few natural products containing the bicyclo[2.2.2]octane ring system.

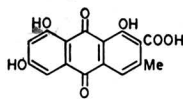
Endocrocin

Endocrocin (XXIII), which occurs in a lichen, as a metabolite of *Aspergillus* and *Penicillium* species, and in ergot, is of special interest because in the acetate hypothesis for the biosynthesis of anthraquinones, intramolecular condensation of the heptaketopalmitic acid (XXIV) yields endocrocin-anthrone. Endocrocin is a good example of the relative advantages of two physical methods for structure determination. The fact that in the carbonyl region the IR spectrum of endocrocin has a band at 1718 cm^{-1} , which is too high a frequency for an aromatic carboxylic acid (Table 2), led us to consider (XXV) as a possible structure. It is obvious that (XXIV) can yield (XXV) as readily as (XXIII). We therefore synthesized (XXV), but found that its tetramethyl ether-ester was different from the ether-ester of endocrocin. Ultimately, the ether-ester of (XXIII) was synthesized by two methods and found to be identical with endocrocin ether-ester⁷⁷.

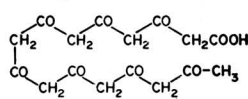
If we had determined the NMR spectrum of endocrocin ether-ester earlier, structure (XXV) would have been ruled out immediately, but as a compensation for the trouble taken in synthesizing (XXV), it is being used for the synthesis of

TABLE 2 — INFRARED SPECTRA (1600-1750 CM^{-1} REGION) OF ANTHRAQUINONE CARBOXYLIC ACIDS IN PARAFFIN MULL

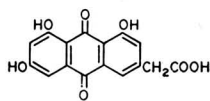
Substitution in anthraquinone	C=O stretching vibrations
1-COOH	1668
2-COOH	1685
1,3-(OH) ₂ -2-COOH (munjistin)	1669; 1620
1,8-(OH) ₂ -3-COOH (rhein)	1692; 1634
1,6-(OH) ₂ -2-COOH	1685; 1650
1,6,8-(OH) ₃ -3-COOH (emodic acid)	1704; 1616
1-OH-3-Me-2-COOH	1670; 1633
2-CH ₃ COOH	1708; 1680
1-OH-2-CH ₃ COOH	1729; 1668; 1639
1,6,8-(OH) ₃ -3-CH ₂ COOH (XXV)	1721; 1670; 1616
Endocrocin	1718; 1666; 1615



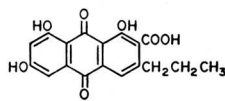
XXIII



XXIV



XXV

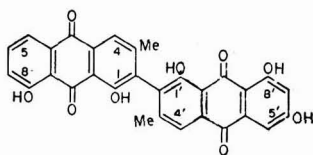


XXVI

nalginensin. The NMR spectrum in CDCl_3 of the ether-ester of endocrocin is in complete agreement with the structure (XXIII): three aromatic protons (4,5,7-positions) at 2.12 (singlet), 2.62 and 3.20 (doublets; $J = 2.5$ cps); four methoxyls at 6.02-6.03; and nuclear methyl at 7.60. Powell and Sutherland (private communication) recently isolated ptilometric acid from a crinoid and assigned to it the structure (XXVI), a homologue of endocrocin. They compared the NMR spectra of the tetramethyl ether-ester of ptilometric acid and of (XXIII) and observed that the agreement was satisfactory.

Cassiamin

From the root bark of *Cassia siamea* Lam. we have isolated a new pigment, cassiamin, the first bianthraquinonyl occurring in a plant and the first 2,2'-bianthraquinonyl isolated from any natural source⁷⁸. The structure (XXVII) of cassiamin was mainly based on UV, IR, and NMR data. The Rast method did not give a dependable molecular weight, but a consideration of the NMR data on cassiamin and its derivatives and of other physical and chemical properties suggested the molecular formula, $\text{C}_{30}\text{H}_{18}\text{O}_9$, confirmed by the mass spectrum kindly determined by Dr Shannon.



XXVII

On methylation with dimethyl sulphate and potassium carbonate in acetone cassiamin gave an orange-yellow ether, m.p. 295-6°, which had no active hydrogen. Acetylation of cassiamin with acetic anhydride catalysed by perchloric acid yielded a yellow acetate, m.p. 182-3°. The NMR spectrum obtained in CDCl_3 solution of the methyl ether shows three methoxyl signals at 6.01, 6.05 and 6.3 and one signal at 7.85 with the intensity ratio of 1:2:2:2. In the spectrum of the acetate there are no signals in the region 4-7, showing the absence of protons on carbon bound to oxygen; four signals at 7.6, 7.66, 7.84 and 7.93 are shown with an intensity ratio of 2:1:2:2. From a comparison of the spectra of the methyl ether and the acetate it is evident that the group of signals at 6.01, 6.05 and 6.3 of the methyl ether is replaced in the acetate spectrum by signals at 7.6, 7.66 and 7.93. The one to one correspondence between the methoxyl and acetoxy signals indicates that the weaker signals at 6.01 and 7.66 in the two spectra represent one methoxyl and acetoxy group respectively, and the other signals represent two methoxyl and two acetoxy groups respectively. Cassiamin therefore has five phenolic hydroxyl groups; the spectrum of the parent compound in dioxane shows four distinct signals in the chelated hydroxyl region (-2.29 to -1.83); and a fifth hydroxyl at higher

field (0.75) is readily recognized as an unchelated phenolic hydroxyl group. The 6-proton signals at 7.85 and 7.84 in the spectra of the ether and the acetate indicate two methyl groups on aromatic rings. The spectra further give an aromatic proton count of seven. A total of 14 substitutable positions for the aromatic skeleton (5 hydroxyls, 2 methyls, and 7 aromatic protons) pointed to a bianthraquinonyl structure. The relative positions of the hydroxyl groups were demonstrated by a further consideration of the NMR data, supported by UV and IR data. The hydroxyl signals indicate four α -hydroxyls and one β . The spectrum of cassiamin pentamethyl ether shows a single proton absorption at 3.25 which is characteristic of an anthraquinone proton having methoxyls in both *ortho* positions⁷⁹; and the doublet character of this absorption ($J = 2.5$ cps) indicates the presence of a resorcinol nucleus as in structure (XXVII).

From the NMR spectrum of cassiamin pentamethyl ether, the low field part of which is shown in Fig. 10, the resorcinol nucleus of (XXVII) has already been deduced. The α -proton of this nucleus is expected⁷⁹ to absorb at about 2.7 and shows up at 2.67. Since the absorptions of β -protons that have methoxyls *meta* to them also suffer small but definite diamagnetic shifts in methoxyanthraquinones, all the β -protons of cassiamin methyl ether may be expected to absorb above 2.2, the absorption for the β -protons of unsubstituted anthraquinone. The spectrum of cassiamin methyl ether indicates three protons absorbing below 2.25, which are therefore to be assigned to α -positions, giving a total of four α -protons in the molecule. It follows that the anthraquinone moieties of cassiamin are coupled β - β .

The ABC spectrum of the 7-, 6- and 5-protons of the chrysophanol part in (XXVII) is traceable in the spectrum of the pentamethyl ether: 7H 2.73, quartet; 6H 2.39, triplet; 5H 2.17, quartet. In the spectra of the pentamethyl ether and penta-acetate the signals of the methoxyl, methyl and acetoxy groups attached to the 1-, 3-, 1'- and 3'-positions show

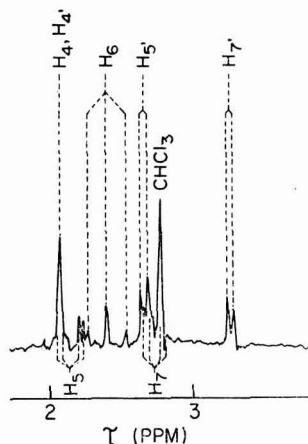


Fig. 10 — NMR spectrum of cassiamin pentamethyl ether (aromatic region)

shifts of 0.2-0.3 p.p.m. from their normal positions in simple anthraquinones. Cassiamin is optically active; the penta-acetate has $[\alpha]_{589}^{30} = -169.5^{\circ}$ ($c = 0.1$ g./100 ml., chloroform).

In a recent paper Chatterjee and Bhattacharjee⁸⁰ have described the isolation of three pigments, cassianin, siameanin and siameadin, from the trunk bark of *Cassia siamea*. The first may be identical with cassiamin, but the structure proposed is that of a 1,1'-bianthraquinonyl, the two anthraquinone moieties otherwise being substituted as in our structure (XXVII); further, the melting points of the two penta-acetates are very different. These authors have merely stated that the NMR spectrum of cassianin penta-acetate confirms its structure, but the actual data have not been presented.

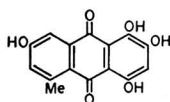
Lac Pigments

The lac insect, *Coccus laccae* (*Laccifer lacca* Kerr.), thrives on certain Indian trees. Stick lac, a resinous secretion, was at one time valued for the dye content, but now shellac resin is a commercial product with a large export market and the dye (in quantities of perhaps 100,000 kg.) is rejected in the water washings. The Indian Lac Cess Committee asked us to suggest possible uses for lac dye and, since preliminary experiments showed that 'laccic acid' was a complex mixture and that the structure assigned to 'laccic acid' in text-books on the basis of Dimroth and Goldschmidt's work in 1913 was incorrect, we undertook a complete examination of the chemistry of lac dye. Lac dye, purified by the procedure outlined later, fulfils the normal requirements of a food colour, except that the chronic toxicity tests carried out by the Central Drug Research Institute appear to show that it is not completely free from toxicity. If further purification makes it comparable to cochineal in its freedom from toxicity, it may find a market as a food colour, but probably only to a very limited extent in this country because of its insect origin, although cochineal, also produced from the dried bodies of an insect, *Dactylopius coccus* Costa. and other *D.* species, continues to be used by devout vegetarians for colouring ice-cream and other food products. In the light of our present knowledge of the structures of the main constituents, there are also possibilities of using lac dye, after appropriate chemical modification, for dyeing wool and silk as acid-mordant colours and for dyeing cellulose acetate and polyester fibre as disperse dyes.

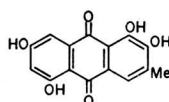
Erythrolaccin

When stick lac is freed completely from the water-soluble dye, the residue (seed lac) contains a yellow pigment, erythrolaccin, $C_{15}H_{10}O_6$, which was isolated by Tschirch and Lüdy⁸¹ in 1923, who stated that it had the "entire behaviour of a tetrahydroxy-methylanthraquinone of the tentative structure (XXVIII)". On the basis of the IR spectra, colour reactions, and the formation of 2-methylanthracene by zinc dust distillation, we suggested structure (XXIX)⁸². A projected synthesis of the tetramethyl ether of (XXIX) unexpectedly yielded an isomeric compound, the tetramethyl ether (XXXI) of alaternin isolated by Briggs from the

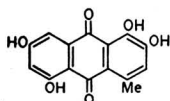
bark of *Rhamnus alaternus*⁸³. In connection with our work on lac dye and other anthraquinone pigments we happened at this stage to determine the NMR spectra of a series of anthraquinone derivatives, including erythrolaccin and its tetramethyl ether⁸⁴. We then realized our fatal mistake in not doing this earlier because they proved immediately the incorrectness of structure (XXIX) and the need for modifying it to (XXX), in which the methyl group is shifted to the adjacent α -position.



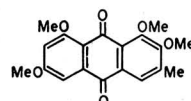
XXVIII



XXIX



XXX



XXXI

The NMR spectrum of erythrolaccin in DMSO shows 3 protons in the aromatic ring: a singlet at 3.17 and two doublets ($J = 2.5$ cps) at 3.08 and 3.58. The doublets can obviously be assigned to the resorcinol nucleus, but the singlet absorption at 3.17 is too high for an α -proton in anthraquinone. The spectrum of the tetramethyl ether in $CDCl_3$ shows a single-proton absorption at 3.30, characteristic of an anthraquinone proton which is flanked by two methoxyl groups; the doublet character ($J = 2.5$ cps) in conjunction with a doublet at 2.76 with the same coupling constant indicates that the substituents in one ring are two methoxyl groups in 1,3-positions. A third aromatic proton appears as a singlet at 3.03, which is too high for an α -proton in anthraquinone, but is in the right position for a β -proton flanked by a methyl and a methoxyl group. In the spectrum of emodin trimethyl ether (1,6,8-trimethoxy-3-methylanthraquinone) the corresponding β -proton appears at 2.95. The spectrum of (XXXI) in $CDCl_3$ exhibits two doublets ($J = 2.5$ cps) at 3.28 and 2.61 and a singlet at 2.12 in complete agreement with the aromatic proton substitution in (XXXI).

The methyl signals in the NMR spectra of erythrolaccin tetramethyl ether, (XXXI) and emodin trimethyl ether confirm structure (XXX) for erythrolaccin. In erythrolaccin itself (solvent pyridine) the methyl signal is at 7.28; in erythrolaccin tetramethyl ether it occurs at 7.25. The β -methyl groups in (XXXI) and emodin trimethyl ether appear at 7.63 and 7.58; in 1,3-dimethylanthraquinone the α -methyl is at 7.22 and the β at 7.55.

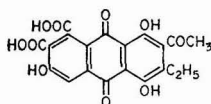
Polyhydroxyanthraquinones occurring in plants carry a methyl group or other carbon side-chain in a β -position, but the insect pigments, kermesic acid and carminic acid, are α -methylanthraquinone derivatives.

The isolation of 2-methylanthracene from the zinc dust distillation of erythrolaccin must be

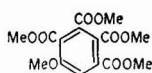
ascribed to methyl migration to form a thermodynamically stabler product. It has been observed that zinc dust distillation of 1,8-dihydroxy-3-hydroxymethyl-4-methylnaphthalene yields 1,3-dimethylnaphthalene⁸⁵. The behaviour of various polyhydroxyanthraquinones containing a methyl group in the 1-position towards zinc dust distillation is being studied. After our note⁸⁴ on the revised structure (XXX) was communicated, we saw that Yates *et al.*⁸⁶ had arrived at the same conclusion; but their synthetic proof is not entirely unambiguous. Very recently N. S. Bhide (unpublished work) has confirmed (XXX) by an improved method of synthesis, starting from 3-chlorophthalic anhydride and 4-hydroxy-3-methoxytoluene.

Lac Dye

Dimroth and Goldschmidt⁸⁷ carried out an extensive investigation of 'laccic acid', the crystalline pigment isolated by them from lac dye and considered to be the main constituent. They did not propose a structure for 'laccic acid', $C_{20}H_{14}O_{10}$, but concluded their paper with the statement: "It would be far more fruitful to resort to still other degradative methods for the elucidation of the constitution of laccic acid, and research in this direction is already in progress. Already in the present state of researches on this dye a considerable fragment of the molecule has been structurally established with certainty." After summarizing Dimroth and Goldschmidt's results, Mayer and Cook⁸⁸ suggested structure (XXXII). At a very early stage of the present work in which we have been engaged for a much longer period than we care to think, it was observed that Dimroth's laccic acid was a complex mixture containing about 2 per cent nitrogen, which Dimroth curiously enough had missed. Colour reactions showed that 'laccic acid' is a purpurin derivative and there was no evidence for a *C*-acetyl or *C*-ethyl group as in (XXXII). However, chemical evidence for the presence of a 3-hydroxyanthraquinone-1,2-dicarboxylic acid nucleus in one or more of the laccic acids was obtained by oxidation of water-crystallized lac dye with alkaline hydrogen peroxide. It is well known that purpurin (XXXIV) breaks down under these conditions to phthalic acid. Methylation of the product of acidification led to the tetramethyl ester (XXXIII) of anisole-2,3,4,5-tetracarboxylic acid, the structure of which was proved by synthesis. The left half of structure (XXXII) for Dimroth's laccic acid proves therefore to be correct.



XXXII



XXXIII

The isolation of even one major constituent of lac dye as a pure homogeneous compound proved to be the toughest problem in the separation of natural products in our experience. Without going

into all the details of the separation methods which were examined, it may be stated that thin layer chromatography on silica gel impregnated with oxalic acid reveals seven or more pigments, of which three are major constituents. By column chromatography of a solution in butanolic hydrochloric acid on polycaprolactam a nitrogen-containing pigment, which is apparently homogeneous, and a non-nitrogenous pigment (a mixture of two products) were separated, as well as other minor constituents. However, the NMR data, on the basis of which we are able to suggest a structure for a laccic acid, were obtained on the ether-esters of the crystalline product, consisting of the mixed pigments as indicated above, which was prepared by passing the water washings of stick lac through a cation-exchange resin from cashew-nut shell liquid. The percolate gave two spots on filter paper, one of which constituted the major part separable by a second passage through the same resin or more rapidly through Dowex-50.

Nitrogen Content of Laccic Acid

Laccic acid prepared by the Dimroth and Goldschmidt method contains about 2 per cent nitrogen, and the nitrogen content of the water-crystallized lac dye varied from 0.5 to 1.4 per cent. Since the nitrogen contents of several samples determined by the Dumas and Kjeldahl methods were more or less identical with the values obtained by van Slyke estimation, it was considered at one stage that nitrogen in the laccic acids is present as a primary amino group. This conclusion has now proved to be erroneous, and the van Slyke value is anomalous. It has been shown by Kainz and Huber⁸⁹ that such anomalous van Slyke values are obtained by phenols interacting with nitrous acid. From chemical tests, IR, and NMR data, we have now found that the nitrogen-containing laccic acid isolated by us does not carry a primary amino group. The present evidence indicates that it is present as a secondary amino group.

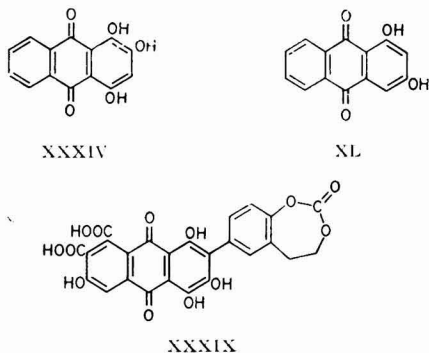
Methylation of Lac Dye

The ether-esters obtained by methylation of water-crystallized lac dye with dimethyl sulphate and potassium carbonate in acetone were separated by chromatography of a benzene solution on a silica gel column, using thin layer chromatography for following the progress of the separation. Five ether-esters designated MLA (methyl laccic acid) I, II, III, IV and V were thus separated as homogeneous crystalline compounds with sharp melting points. Except MLA-IV which was obtained in very small amount, the ether-esters were characterized by mass spectral molecular weights, UV, IR and NMR spectra, and chemical properties. Only one of them, MLA-III, contained nitrogen.

The NMR spectra of the methylated laccic acids clearly demonstrate that structure (XXXII) for laccic acid is untenable. Important features of the NMR spectra of all the methylated laccic acids are the absence of ethyl and acetyl groups, the presence of a group of the type $ArCH_2CH_2O-$, and the presence of four aromatic protons.

Xantholaccic Acids

A very old and well-known reaction of purpurin (XXXIV) is its reduction to xanthopurpurin (XL) by treatment with aqueous sodium hydroxide and dithionite.



When lac dye was treated with aqueous alkaline sodium dithionite or submitted to catalytic reduction in alkaline solution, it underwent the purpurin \rightarrow xanthopurpurin change. The product, called xantholaccic acids, was also a mixture of several constituents. Methylation by methyl iodide and silver oxide in dimethylformamide at room temperature, followed by chromatography on silica gel, led to three ether-esters, designated MXLA (methylated xantholaccic acid) I, II, and III. Only MXLA-III contained nitrogen (2.4 per cent). Mainly from NMR data obtained on MXLA-I and the product of its hydrolysis and decarboxylation it is possible to assign structure (XXXV) to MXLA-I.

The mol. wt of MXLA-I is 578 (mass spectrum) corresponding to structure (XXXV) with which the NMR data (Fig. 11) are broadly in agreement. The methylene protons of the group CH_2OMe are not seen clearly because of methoxyl protons in the same region. The appearance of a new aromatic

proton as a singlet in MXLA-I (in comparison with MLA-I or V) is in accordance with the purpurin \rightarrow xanthopurpurin change in which a nuclear hydroxyl group is replaced by hydrogen. A methoxyl at 6-7 corresponds to the methoxyl in $\text{CH}_2\text{CH}_2\text{OMe}$. The NMR spectra point to a probable relationship between MLA-V and MXLA-I. The three-proton multiplet at 2.82-3.22 corresponds to three protons in 1,2,4-positions in the benzene ring.

Hydrolysis of MXLA-I with methanolic potassium hydroxide gave a crystalline carboxylic acid and decarboxylation by copper bronze in boiling diphenyl oxide and a few drops of quinoline gave a compound with the molecular formula $\text{C}_{27}\text{H}_{26}\text{O}_7$ (mol. wt by the mass spectral method 462). The NMR spectrum in CCl_4 is shown in Fig. 12.

Only the central peak of the triplet corresponding to the indicated CH_2 in $\text{CH}_2\text{CH}_2\text{OMe}$ is visible at 6.63 and the remainder is submerged below the methoxyl signals at 6.76 and 6.53. The doublet at 2.0 and the coupling constant suggest that it occupies the α -position in an anthraquinone nucleus and is coupled to an *o*-proton. All the available data are in agreement with the structure (XXXVI). Additional support for the CH_2OMe group was obtained by the fact that treatment with boron fluoride-etherate and acetic anhydride at room temperature⁹⁰ gave a product which in its NMR spectrum (Fig. 13) and other properties agreed with (XXXVII). Since the replacement of OMe by OAc in CH_2OMe has resulted in a paramagnetic shift of about 0.8, the methylene triplet is now clearly visible.

MLA-III and MXLA-III correspond to each other. They have respectively the molecular formulae $\text{C}_{31}\text{H}_{27}\text{NO}_{11}$ and $\text{C}_{32}\text{H}_{31}\text{NO}_{11}$. Both of them contain a C-methyl group (Pandhare, E. D. & Rama Rao, A. V., unpublished work).

Structure of a Laccic Acid

The NMR spectrum of MLA-V (Fig. 14), which has a molecular weight of 652 (mass spectrum), shows a proton count of 32, eight OMe groups, two methylene triplets corresponding to $\text{ArCH}_2\text{CH}_2\text{OCOR}$,

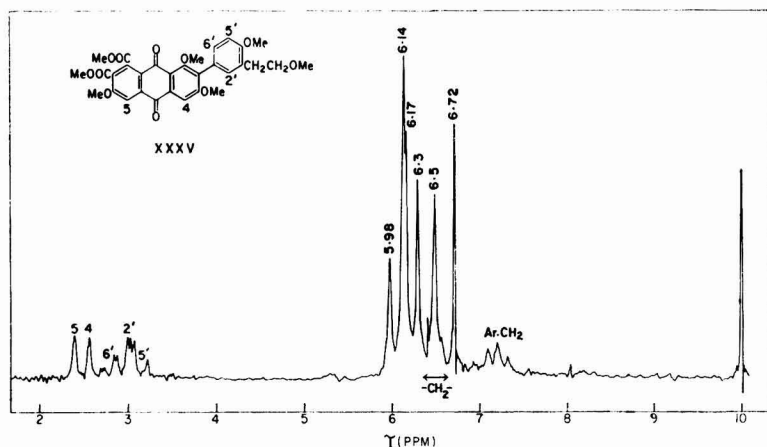


Fig. 11 — NMR spectrum of methylated xantholaccic acid (MXLA-I)

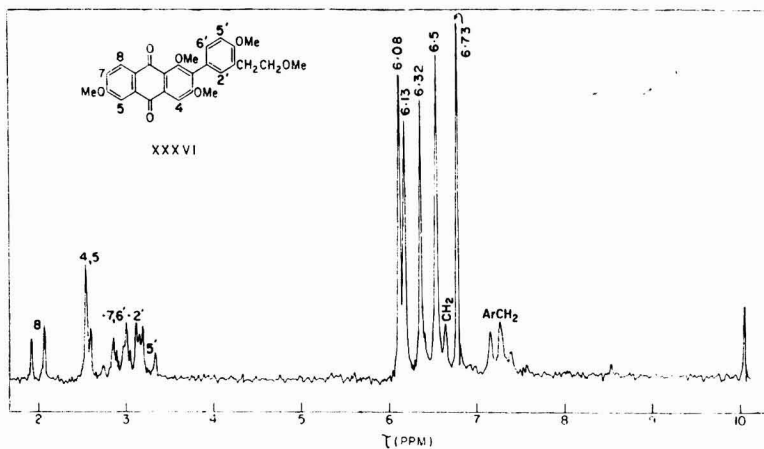


Fig. 12 — NMR spectrum of compound (XXXVI)

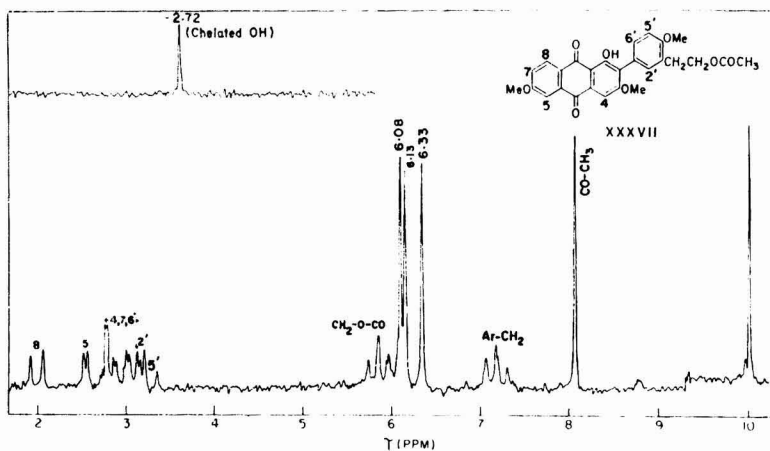


Fig. 13 — NMR spectrum of compound (XXXVII)

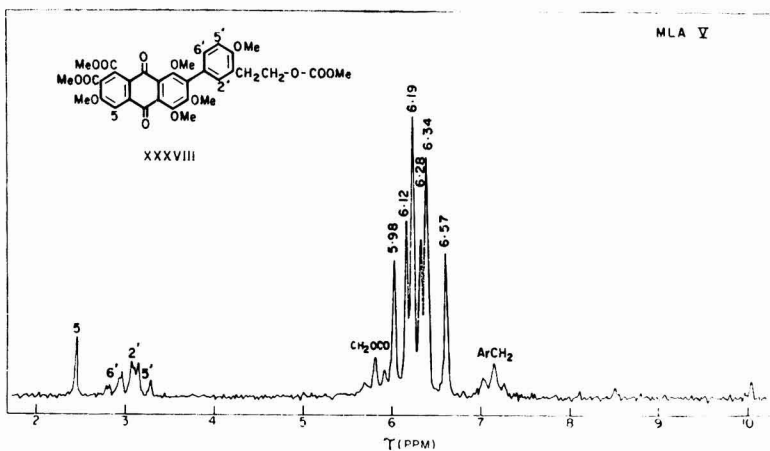
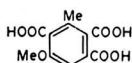


Fig. 14 — NMR spectrum of MLA-V

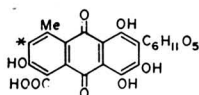
one aromatic proton (singlet) corresponding to the unsubstituted position in the anthraquinone nucleus of (XXXVIII), and three aromatic protons as a multiplet indicating 1,2,4-substitution in a benzene ring. All the data are in agreement with structure (XXXVIII) for MLA-V, and structure (XXXIX) may therefore be deduced for the corresponding laccaic acid.

The Position of the Carboxyl Group in Carminic Acid

In the course of the synthesis of tetramethyl anisole-2,3,4,5-tetracarboxylate (XXXIII) required in connection with our study of lac pigments we obtained a tricarboxylic acid (XLI), the trimethyl ester of which proved to be identical with the product of methylation of the phenolic acid obtained by oxidation of carminic acid, the pigment of cochineal, by alkaline hydrogen peroxide. The Dimroth and Kämmerer structure (XLII) for carminic acid⁹¹



XLI



XLII

has, therefore, to be modified by shifting the carboxyl group to the indicated β -position. This is confirmed by the NMR spectrum of carminic acid in DMSO; a single aromatic proton appears at 2-33 which must therefore be an α - and not a β -proton in the anthraquinone nucleus⁹².

Acknowledgement

I am deeply indebted to the collaborators whose names I have mentioned in the references, and in particular to Dr P. Madhavan Nair and Dr A. V. Rama Rao for stimulating discussions and for valuable assistance in preparing this review.

References

1. DJERASSI, C., *Optical rotatory dispersion* (McGraw-Hill Book Co. Inc., New York), 1960.
2. BRAUDE, E. A. & NACHOD, F. C., *Determination of organic structures by physical methods*, Vol. 1 (Academic Press Inc., New York), 1955.
3. NACHOD, F. C. & PHILLIPS, W. D., *Determination of organic structures by physical methods*, Vol. 2 (Academic Press Inc., New York), 1962.
4. *Elucidation of structures by physical and chemical methods: Technique of organic chemistry*, edited by A. Weissberger, Vol. XI, Part II (Interscience Publishers Inc., New York), 1963.
5. SILVERSTEIN, R. M. & BASSLER, G. C., *Spectrometric identification of organic compounds* (John Wiley & Sons, New York), 1963.
6. SCOTT, A. I., *Interpretation of the ultraviolet spectra of natural products* (Pergamon Press, Oxford), 1964; see also 7 and 8.
7. JAFFE, H. H. & ORCHIN, M., *Theory and applications of ultraviolet spectroscopy* (John Wiley & Sons, New York), 1961.
8. RAO, C. N. R., *Ultraviolet and visible spectroscopy: Chemical applications* (Butterworth's Scientific Publications, Washington), 1961.
9. BIRKINSHAW, J. H., *Biochem. J.*, **59** (1955), 485.
10. JURD, L., in *The chemistry of flavonoid compounds*, edited by T. A. Geissman (Pergamon Press, Oxford), 1962.

11. HOCHSTEIN, F. A., STEPHENS, C. R., CONOVER, L. H., REGNA, P. P., PASTERNAK, R., GORDON, P. N., PILGRIM, F. J., BRUNINGS, K. J. & WOODWARD, R. B., *J. Amer. chem. Soc.*, **75** (1953), 5455.
12. BELLAMY, L. J., *Infrared spectra of complex molecules* (Methuen & Co. Ltd, London), 1958.
13. NAKANISHI, K., *Infrared absorption spectroscopy—Practical* (Holden-Day Inc., San Francisco), 1962.
14. COLE, A. R. H., in *Progress in the chemistry of organic natural products*, edited by L. Zechmeister, Vol. XIII (Springer-Verlag, Vienna), 1956.
15. JONES, R. N. & DOBRINER, K., *Vitam. & Horm.*, **7** (1949), 293; and numerous other papers of Jones and his collaborators.
16. VENKATARAMAN, K., in *Progress in the chemistry of organic natural products*, edited by L. Zechmeister, Vol. XVII (Springer-Verlag, Vienna), 1959.
17. LOOKER, J. H. & HANNEMAN, W. W., *J. org. Chem.*, **27** (1962), 381.
18. JACKMAN, L. M., *Applications of nuclear magnetic resonance spectroscopy in organic chemistry* (Pergamon Press, London), 1959.
19. ROBERTS, J. D., *Nuclear magnetic resonance applications to organic chemistry* (McGraw-Hill Book Co. Inc., New York), 1959.
20. POPE, J. A., SCHNEIDER, W. G. & BERNSTEIN, H. J., *High resolution nuclear magnetic resonance* (McGraw-Hill Book Co. Inc., New York), 1959.
21. BHACCA, N. S. & WILLIAMS, D. H., *Applications of NMR spectroscopy in organic chemistry* (Holden-Day Inc., San Francisco), 1964.
22. WAISS (JR), A. C., LUNDIN, R. E. & STERN, D. J., *Tetrahedron Lett.*, No. 10 (1964), 513.
23. SWEETLEY, G. C., BENTLEY, R., MAKITA, M. & WELLS, W. W., *J. Amer. chem. Soc.*, **85** (1963), 2497.
24. MABRY, T. J., KAGAN, J. & RÖSLER, H., *Nuclear magnetic resonance analysis of flavonoids* (University of Texas, Texas), 1964.
25. MABRY, T. J., KAGAN, J. & RÖSLER, H., *Phytochem.*, **4** (1965), 177.
26. MASSICOT, J. & MARTHE, J. P., *Bull. soc. chim. Fr.*, (1962), 1962.
27. MASSICOT, J. & MARTHE, J. P., *Bull. soc. chim. Fr.*, (1962), 2712.
28. BATTERHAM, T. J. & HIGHET, R. J., *Aust. J. Chem.*, **17** (1964), 428.
29. HORN, D. H. S. & LAMBERTON, J. A., *Chem. & Ind.*, (1963), 691.
30. McDOWELL, C. A., Editor, *Mass spectrometry* (McGraw-Hill Book Co. Inc., New York), 1963.
31. BEYNON, J. A., *Mass spectrometry and its applications to organic chemistry* (Elsevier Publishing Co., Amsterdam), 1960.
32. BIEMANN, K., *Mass spectrometry—Organic chemical applications* (McGraw-Hill Book Co. Inc., New York), 1962.
33. McLAFFERTY, F. W., *Mass spectrometry of organic ions* (Academic Press Inc., New York), 1963.
34. McLAFFERTY, F. W., *Mass spectral correlations* (American Chemical Society, Washington), 1963.
35. BUDZIKIEWICZ, H., DJERASSI, C. & WILLIAMS, D. H., *Structure elucidation of natural products by mass spectrometry*, Vol. I: Alkaloids; Vol. II: Steroids, terpenoids, sugars; and miscellaneous classes (Holden-Day Inc., San Francisco), 1964.
36. BUDZIKIEWICZ, H., DJERASSI, C. & WILLIAMS, D. H., *Interpretation of mass spectra of organic compounds* (Holden-Day Inc., San Francisco), 1964.
37. DJERASSI, C. & BIEMANN, K., *Pure & appl. Chem.*, **9** (1964), 159, 95.
38. NYBURG, S. C., *X-ray analysis of organic structures* (Academic Press Inc., New York), 1961.
39. ROBERTSON, J. M., *Pure & appl. Chem.*, **9** (1964), 179.
40. MATHIESON, A. M., *Pure & appl. Chem.*, **2** (1961), 505.
41. CAHN, R. S., INGOLD, C. K. & PRELOG, V., *Experientia*, **12** (1956), 81.
42. STOUT, G. H., STOUT, V. F. & WELSH, M. J., *Tetrahedron*, **19** (1963), 667.
43. CHAKRAVARTY, G. & SESHADRI, T. R., *Tetrahedron Lett.*, (1962), 787.
44. ROBINSON, R., *Chem. & Ind.*, (1963), 108.
45. FREUDENBURG, K., *Experientia*, **16** (1960), 101.

46. CLARK-LEWIS, J. W., *The chemistry of flavonoid compounds* (Pergamon Press, Oxford), 1962, 220, 228.
47. MADHAVAN NAIR, P. & VENKATARAMAN, K., *Tetrahedron Lett.*, No. 5 (1963), 317.
48. COREY, E. J., PHILBIN, E. M. & WHEELER, T. S., *Tetrahedron Lett.*, No. 13 (1961), 429.
49. See also LILLYA, C. P., DREWES, S. E. & ROUX, D. G., *Chem. & Ind.*, (1963), 783.
50. PHILBIN, E. M., WHEELER, T. S., BRUTCHER, F. V. & BAUER, W., *J. org. Chem.*, **27** (1962), 4114.
51. CLARK-LEWIS, J. W., *Rev. pure & appl. Chem.*, **12** (1962), 96.
52. CLARK-LEWIS, J. W. & JACKMAN, L. M., *Proc. chem. Soc.*, (1961), 165; see also CLARK-LEWIS, J. W., JACKMAN, L. M. & WILLIAMS, L. R., *J. chem. Soc.*, (1962), 3858.
53. RADHAKRISHNAN, P. V., RAMA RAO, A. V. & VENKATARAMAN, K., *Tetrahedron Lett.*, No. 11 (1965), 663.
54. NAIR, P. M., RAMA RAO, A. V. & VENKATARAMAN, K., *Tetrahedron Lett.*, No. 3 (1964), 125.
55. TELANG, S. A., *Some naturally occurring flavones and related synthetical experiments*, Ph.D. thesis, University of Bombay, 1961.
56. DUTTA, N. L., *J. Indian chem. Soc.*, **33** (1956), 716.
57. BARNES, C. S., OCCOLOWITZ, J. L., DUTTA, N. L., MADHAVAN NAIR, P., PHADKE, P. S. & VENKATARAMAN, K., *Tetrahedron Lett.*, No. 5 (1963), 281.
58. BURROWS, B. F., FINCH, N., OLLIS, W. D. & SUTHERLAND, I. O., *Proc. chem. Soc.*, (1959), 150.
59. BRINGI, N. V., SHAH, K. H. & VENKATARAMAN, K., *J. sci. industr. Res.*, **14B** (1955), 135.
60. BRINGI, N. V., PADHYE, M. R. & VENKATARAMAN, K., *J. sci. industr. Res.*, **15B** (1956), 128.
61. MORTON, R. A. & SAWIRES, Z., *J. chem. Soc.*, (1940), 1052.
62. BHAT, H. B., *Constitution of the pigments of Garcinia morella*, Ph.D. thesis, University of Poona, 1963.
63. KING, J. F. & VIG, B., *Canad. J. Chem.*, **40** (1962), 1023.
64. DE MAYO, P., SPENCER, E. Y. & WHITE, R. W., *Canad. J. Chem.*, **39** (1961), 1608.
65. VIJAYARAGHAVAN, V. K., *Constitution of morellin*, Ph.D. thesis, University of Bombay, 1960.
66. BURROWS, B. F., OLLIS, W. D. & JACKMAN, L. M., *Proc. chem. Soc.*, (1960), 177.
67. CROMBIE, L. & LOWN, J. W., *J. chem. Soc.*, (1962), 775.
68. JACKMAN, L. M., *Applications of NMR spectroscopy in organic chemistry* (Pergamon Press, London), 1959, 122.
69. DAYAL, B. & MATHUR, S. C., *Acta Cryst.*, **13** (1960), 269.
70. KARTHA, G., RAMACHANDRAN, G. N., BHAT, H. B., NAIR, P. MADHAVAN, RAGHAVAN, V. K. V. & VENKATARAMAN, K., *Tetrahedron Lett.*, (1963), 459.
71. NAIR, P. MADHAVAN & VENKATARAMAN, K., *Indian J. Chem.*, **2** (1964), 402.
72. BHAT, H. B., NAIR, P. MADHAVAN & VENKATARAMAN, K., *Indian J. Chem.*, **2** (1964), 405.
73. AUTERHOFF, H., FRAUDENDORF, H., LIESENKLAS, W. & SCHWANDT, C., *Angew. Chem. Internat. Ed.*, **1** (1962), 455.
74. DYSON, N. H. & RIGBY, W., *J. chem. Soc.*, (1963), 1858.
75. YATES, P., KARMARKAR, S. S., ROSENTHAL, D., STOUT, G. H. & STOUT, V. F., *Tetrahedron Lett.*, No. 24 (1963), 1623.
76. OLLIS, W. D., RAMSAY, M. V. J., SUTHERLAND, I. O. & MONGKOLSUK, S., *Tetrahedron*, **21** (1965), 1453.
77. JOSHI, B. S., RAMANATHAN, S. & VENKATARAMAN, K., *Tetrahedron Lett.*, No. 21 (1962), 951.
78. DUTTA, N. L., GHOSH, A. C., NAIR, P. M. & VENKATARAMAN, K., *Tetrahedron Lett.*, (1964), 3023.
79. GHOSH, A. C., Ph.D. thesis, University of Poona, 1963.
80. CHATTERJEE, A. & BHATTACHARJEE, S. R., *J. Indian chem. Soc.*, **41** (1964), 415.
81. TSCHIRCH, A. & LÜDY, F., *Helv. chim. Acta*, **6** (1923), 994.
82. DAVE, K. G., JOSHI, B. S., PATWARDHAN, A. V. & VENKATARAMAN, K., *Tetrahedron Lett.*, (1959), 22.
83. BRIGGS, L. H., JACOMBS, F. E. & NICHOLLS, G. A., *J. chem. Soc.*, (1953), 3069.
84. BHIDE, N. S., RAMA RAO, A. V. & VENKATARAMAN, K., *Tetrahedron Lett.*, (1965), 33.
85. HOCHSTEIN, F. A. *et al.*, *J. Amer. chem. Soc.*, **75** (1953), 5455.
86. YATES, P., MACKAY, A. C., PANDE, L. M. & AMIN, M., *Chem. & Ind.*, (1964), 1991.
87. DIMROTH, O. & GOLDSCHMIDT, G., *Liebigs Ann.*, **399** (1913), 62.
88. MAYER, F. & COOK, A. H., *Chemistry of natural colouring matters* (Reinhold Publishing Co., New York), 1943, 147.
89. KAINZ, G. & HUBER, H., *Mikrochim. Acta*, (1959), 891.
90. NARAYANAN, C. R. & IYER, K. N., *Tetrahedron Lett.*, (1964), 759; YOUSSEFYEH, R. D. & MAZUR, M., *Tetrahedron Lett.*, (1963), 1287.
91. DIMROTH, O. & KÄMMERER, W., *Ber. dtsh. chem. Ges.*, **53** (1920), 471.
92. BHATIA, S. B. & VENKATARAMAN, K., *Indian J. Chem.*, **3** (1965), 92.

Lipid Metabolism of Insects

S. SRIDHARA*

Fermentation Technology Laboratory, Indian Institute of Science, Bangalore 12

AS in the case of higher animals, more work has been forthcoming on protein and carbohydrate metabolism than on lipid metabolism in insects. This is to be attributed to the difficulty in bringing together the water-insoluble (in some cases slowly diffusible) lipid substrate with the enzyme in its aqueous milieu as also to the lack of suitable micro-analytical techniques. From the present status of our knowledge, significant differences in lipid metabolism can be discerned between animals and insects. The demonstration of sterols as essential nutrients for insects and the non-essentiality of the fat soluble vitamins are suggestive of differences in the metabolism of other lipid components as well. The two terms fat and lipid are used synonymously. In this review, attempts have been made to provide an organized account of the information gathered on the silkworm, and also published work on other insects, mainly on the free fatty acids and their esters, sterols (which have been reviewed recently)¹ and other unsaponifiable constituents are briefly dealt with. While an integrated and coherent pattern has not emerged from this in respect of all aspects of lipid metabolism in insects, it has no doubt helped in clearly indicating the large gaps still awaiting to be filled in our knowledge and in providing a working hypothesis to stimulate future investigations.

Composition

Hilditch², Gilmour³ and Gilby⁴ have compiled the data available on the composition of insect lipids. Considering these, one can safely say that no broad generalizations can be drawn, and it is not certain whether the pattern of distribution of fatty acids fits into the phylogenetic scale and follows a classification of species on morphological grounds as found in other cases⁵. This situation is due not only to the limitation on the number of insects studied, but also to the limited work done on any single insect. However, certain patterns in the distribution of fatty acids in insects are emerging out. Strong's work on aphids⁶ showed that their fatty acid composition may be a characteristic of species rather than a manifestation of diet. Similarly, it appears that palmitoleic acid and cephalin may be the most abundant of fatty acids and phospholipids respectively, and may be characteristic of *Diptera*.

The fat content itself has been shown to vary from as low as 2 per cent to as high as 50 per cent of body dry weight⁷. There exists some controversy as to whether lipid content increases or decreases during metamorphosis, and whether females of any particular species store more lipid than males. The former view has been arrived at by finding higher percentage

of lipid in the pupae than in the larvae. In the silkworm (*Bombyx mori* L.) there is an apparent increase (about 10 per cent) in the lipid content of the pupae as 100 g. wet weight of larvae (dry weight 20 per cent) on transformation to pupae weigh 50 g. (dry weight 25 per cent). Since the loss of lipid in the cocoon is negligible, the amount of lipid found in the pupae is the same as that expected in the absence of any utilization and synthesis or a steady state during metamorphosis^{7,8}. Similarly, Domrose and Gilbert⁹ found that the amount of lipid on a fresh weight basis would show an increase from the 20th day of development to the 2nd day of adult life in *Hyalophora cecropia*, whereas in absolute amounts the lipid content was the same. On the other hand, Villeneuve and Lemonde¹⁰ and Barlow¹¹ showed a loss of 76 and 7.5 per cent respectively during metamorphosis in the insects they studied. Fawzi *et al.*¹² showed that the absolute fat content of the female of *Locusta migratoria* was higher than that of the male, but the reverse was true when this was considered as percentage of body weight, since the female was twice as heavy as the male. Barlow¹¹ found the same percentage of lipid in both the males and females of *Agria affinis* and *B. mori*⁸. Some of the available data is tabulated in Table I.

A number of workers have found a decrease in lipid content during pupal development¹³⁻¹⁵. Lambremont and Blum¹⁶ showed about 50 per cent decrease in the total lipids from the beginning of the pupal stage to emergence as adults in bollweevils. Similar observations have been made on *Popillia japonica*¹⁷ and *Musca domestica*¹⁸. It is suggested that this is due to the fact that fat supplies the energy needed at this stage¹¹. Moran¹⁹ and Villeneuve and Lemonde¹⁰ claimed free lipids to be the source of energy for change in the pupae. At this stage also there seems to be differences between males and females. Males of *H. cecropia* were found to store lipids and not to utilize them during pupal development, while females utilized them⁹. In *B. mori* no reduction in the lipid content was seen during pupal development⁸. The use of lipid as an energy source has more importance than apparent here and will be discussed further later.

Several qualitative and quantitative studies suggested an increase in the degree of saturation of lipids during pupation in many insects^{13,20,21}. Herodek and Farkas²² noted that the fat of *B. mori* males exhibited a tendency towards saturation, while that of female were less saturated. Niemierko²³ and Sridhara and Bhat⁸ have shown that in the silkworm, the iodine value of fat increases during metamorphosis. Lambremont and Blum¹⁶ observed little change in the lipids of bollweevils by the gas chromatographic technique. Thus it is obvious that the data on lipid content of insects and the changes it undergoes during its life-cycle vary widely, that unified concepts

*Present address: Department of Biological Chemistry, School of Medicine, University of California, Los Angeles, California.

TABLE 1 — AMOUNT OF FAT AND FATTY ACID COMPOSITION OF SOME INSECTS*

	Larva				Pupa				Moths			
	1	2	3	4	1	2	3	4	1	2	3	4
Fat (dry wt), %	21.7	—	17	8.6	16.2	—	27	13	13.2	—	26	6.2
Total fatty acids, %	19.9	22	20	36.5	15.4	26	16	24	16	22	16	33
Palmitic acid	5.5	2	18		5.0	2	11	13	4.7	4	10	2.6
Stearic acid	24.3	16	—	63.4	23.0	16	—	7	17.6	12	—	6.6
Palmitoleic acid	31.2	29	26		30.0	26	35	26	26.7	28	36	33.4
Oleic acid	11	13	12	13	11	12	12	22.0	25	11	13.8	
Linoleic acid	—	—	26	—	—	26	6	—	—	25	7.4	
Linolenic acid	—	—	—	—	—	—	—	—	—	—	—	—

(1) *A. affinis*¹¹, (2) *C. tarsalis*^{15,14}, (3) *B. mori*⁸, and (4) *A. grandis*^{6*}.

*Some values have been calculated and approximated.

are not readily apparent and can be summed up, as Barlow¹¹ has done, that the qualitative and quantitative changes in insect fats during pupation differ with species, sex and age.

Some doubts are still expressed that the high proportion of free fatty acids (FFA) found in insects may be an artifact formed during the extraction of lipids by the action of lipases^{4,24}. However, the same view was held by many in the case of FFA found in higher animals till Dole²⁵ showed its importance in normal lipid metabolism. Thus numerous observations on the presence of FFA in various insects and their tissues^{8,10,26-28} cannot be ruled out as of no significance. In fact, more attention to the composition and metabolism of FFA at different stages of development of insects is urgently needed. The composition of the FFA was found to differ from that of neutral fat both in aphids and the silkworm^{6,8}.

In their total fatty acid composition, insects follow a pattern similar to that in higher animals in that the majority of fatty acids present are of chain length C₁₂ to C₁₈. One of the unusual features is the presence of C₁₈ polyunsaturated fatty acids in large amounts^{13,26,29,30}. *Diptera* seem to contain more palmitoleic acid and it has been suggested that it plays some important role as yet unexplained^{31,32}. Gilby⁴, on the basis of the data available, concluded that since the fat reserve of insects is mainly in the fat body, extracts of whole body lipids tend to approximate the composition of the lipid in the fat body and also that the major fatty acids, after saponification of crude lipids, will be similar to the fatty acids of the triglyceride fraction. These conclusions are not valid. Fawzi *et al.*¹² showed that some phospholipids found in the total lipid was absent in the fat body. Similarly, in the silkworm only about 17 per cent of the phospholipid present in the total lipid was found in the fat body. Further there was no correlation between the total fat content and its composition between the fat body and the whole insect⁸. In both the cases mentioned there was a difference in the fatty acid composition also (Table 2). These differences become accentuated as analysis of other tissues are carried out⁸.

The interest in the chemistry of insect phospholipids is increasing with the realization of the importance and participation of phospholipids in almost all aspects of normal metabolism^{33,34}. A cursory examination

TABLE 2 — COMPOSITION OF TOTAL FAT AND FAT BODY FAT OF *B. mori* AND *L. migratoria*

	Total fat		Fat body	
	<i>B. mori</i> ⁸	<i>L. migratoria</i> ¹²	<i>B. mori</i>	<i>L. migratoria</i>
Fat, %	17	—	32	—
Phospholipid, %	23	15.2	3.0	0.0
Total fatty acids, %	—	—	—	—
Palmitic acid	20	25	27	30.0
Stearic acid	18	5.8	6	3.4
Palmitoleic acid	—	—	—	—
Oleic acid	26	47.6	21	52.9
Linoleic acid	12	13.1	15	8.6
Linolenic acid	26	6.9	28	4.6

of the published data shows that the variation is much more pronounced than in other lipid fractions. The percentage of phospholipids in the total fat varies from 0 to 40 per cent^{12,13,28,35-38} and up to 60 per cent in some tissues. Fawzi *et al.*¹² and Schmidt and Osman³⁹ found some phospholipids which do not contain any nitrogen. Further fractionation of the total phospholipids into individual components revealed large variations, from the non-existence of a single component³⁶ to the whole phospholipid being made up of one or two components^{40,41}. With all this variation the principal constituents of the phospholipids remain essentially the same as found in higher animals, viz phosphatidylcholine, phosphatidylserine and ethanolamine, phosphatidyl inositol, sphingosine and plasmalogens^{8,42} (Table 3). An exception is the finding of sphingomyelin in the housefly, *M. domestica*, whose choline is replaced by ethanolamine⁴³.

Although Chaudhary and Lemonde⁴⁴ observed the changes in phospholipids during the life-cycles of some insects to be so variable that they were unable to draw any generalizations, Beiber *et al.*³⁶ working with modern techniques showed the phospholipid pattern of *Phormia regina* to be same at all stages of development of the insect. Taylor and Hodgson⁴⁵ found the phospholipid profile of mitochondria, microsomes, the soluble fraction and the residue, not to differ significantly from that of the whole organism. They concluded that phospholipids

TABLE 3 PHOSPHOLIPID COMPOSITION OF SOME INSECTS

	<i>B. mori</i> ⁸	<i>M. domestica</i> ³³	<i>P. regina</i> ³⁶	<i>A. acypti</i> ³⁷
Phospholipid* (mg./g. wet wt)	8.6	18.75	8.01	5.82
Phosphatidyl choline	62	17	<25	28
Phosphatidyl ethanolamine	3	65	} >60	62
Phosphatidyl serine	8	3.5		
Sphingosine	16	3.5	—	10
Inositide	—	3	—	—
Plasmalogen	—	1.3	—	—

*Calculated on phosphorus basis.

containing ethanolamine can carry out the functions performed in other organisms by phospholipids containing choline. This might be the reason for cephalin being the most abundant component of the phospholipids of *Diptera*. In a series of studies Newburgh and his associates⁴⁶⁻⁴⁸ have shown the ability of *P. regina* to replace choline in phospholipids by β -methyl choline, 2,2-dimethyl isoamino-isopropanol, dimethyl amino methanol and monomethyl amino methanol and also ethanolamine, by the last two when grown on synthetic diets. Similar ability for replacements was found in *M. domestica* whose phospholipid profile is very similar to that of *P. regina*^{43,49}. Sridhara and Bhat⁸ found a very rigidly bound protein and polysaccharide with the phospholipid. The vast differences found in the distribution of phospholipids in various organs of the silkworm was taken to mean that the distribution was according to the metabolic status of each tissue. The earlier observations of Gupta and Kamboj⁵⁰ on the relationship between the lipids and the ability of the silkglands for protein synthesis and secretion was thought to be due to the role of phospholipids in protein synthesis and secretion⁸. A similar distribution between neutral lipids and phospholipids was reported by Fast and Brown³⁷. In the silkworm also the pattern was almost similar, but for the higher stearic acid content. Typical of all animal phospholipids, the silkworm phospholipid contained more of stearic acid than palmitic acid⁸. Snake venoms released considerable FFA from phospholipids and lecithins *in vitro* but conclusion as to whether only unsaturated fatty acids were released could not be reached⁸. The venoms on injection into silkworms (approximately 1 mg. per larva) were not only lethal within about 10 min. but also raised the FFA concentration fivefold. The fatty acid composition of this FFA, taking into consideration the FFA originally present, was mainly unsaturated fatty acids and was in keeping with the fatty acid composition of FFA released by these venoms *in vitro* on phospholipids⁸ (also unpublished data).

The sterols of insects have been extensively reviewed^{1,4,51-53}. Previous reviews on insect lipids have not taken into consideration other lipid components, generally classified as unsaponifiables. This is due to the negligible amount of work done on these.

Now that the role of coenzyme Q in mitochondrial metabolism and its biosynthesis, probably via ubiquinomenol, and that of vitamin E in reproduction have been fairly well established, it will be worth while to start investigating these compounds and their role in insect metabolism, at least to prove or disprove the present assumption that fat-soluble vitamins are not required by insects. Coenzyme Q, ubiquinomenol and vitamin E were shown to be present in the silkworm. Although adequate data were not available for comparison, large differences were seen among the insects studied^{8,54-56}. Till the recent isolation of vitamin A from the eyes of the housefly and the honeybee^{57,58}, and the demonstration of its possible role in their vision, the status of vitamin A in insects was open to question. Even now answers to such questions as to how far vitamin A participates in the vision of insects, and if so, what percentage of vision is mediated by it compared to other eye pigments, will have to await more work.

Nutrition

Studies on the qualitative and quantitative lipid requirements of insects have not been extensive. Those made on a few insects have emphasized the similarity between insects and higher animals regarding the necessity of supplying polyunsaturated fatty acids from external sources for their normal growth and development⁵⁹⁻⁶⁴ and also on the effect of dietary fat on depot fat. The first view has been confirmed both directly and indirectly while the second one is still debatable^{65,66}. Gilmour³ concluded that generally high fat diets influence body fat composition to a greater extent than low fat diets. In the midge, *T. lewis*, the adults contained polyenoic acids very similar to those in its diet⁶⁷. In the case of bollweevils, feeding on both natural and artificial diets, it was shown that the fatty acids stored in the body fat were an exact duplication of those in the food⁶⁸. Generally, though the fatty acid pattern of the depot fat is same as that found in the diet, considerable differences are observed in their proportion and also in the manner they are stored in the body. In this respect it is also important to note that differences are seen between insects of the same species grown on synthetic diets with added fat and those on natural food with added fat. After an extensive survey of these effects Lambremont *et al.*⁶⁸ concluded that although numerous examples are cited in the literature to illustrate the influence of dietary fat on the fatty acid content of depot fat, this relationship is not easily correlated in insects feeding at random in the field or on a large section of a plant (or any natural food) in the laboratory. This argument holds good for some other insects as well^{6,13,32}. In the silkworm, feeding of various fatty acids from C₄ to C₁₈ chain length as sodium salts at neutral pH along with its natural food, the mulberry leaf, did not affect the body lipid composition significantly. However, the growth of the insect was inhibited in a particular fashion and the fat content showed a decrease⁶⁹. On the other hand, feeding of the same fatty acids as triglycerides under the same conditions again did not affect the lipid composition, but favoured growth and caused an increase in the lipid content. In the former case there was a

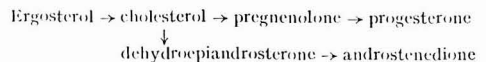
generalized decrease in the activity of lipase in the gut, while there was an increase in the latter. These and other results were interpreted to mean that the absorption of lipids in the insect was controlled by the lipase level of the gut, whose activity was in turn controlled by the FFA concentration⁷. That other insects can also metabolize extraneous fatty acids has been amply demonstrated^{32,70,71}.

Feeding of glucose had no effect on the lipid composition of the silkworm larvae and the absence of conversion of carbohydrate to fat was further confirmed by radioactive studies with labelled glucose⁷². Conversion of fat to carbohydrate was claimed by Hsuen and Tang⁷³ but was not confirmed by studies with labelled fatty acids⁷². Clements⁷⁴ also showed the non-conversion of fat to carbohydrate in the locust fat body. Dadd⁷⁵ found that various fatty substances were totally ineffective as substitutes for the large proportion of carbohydrates required in the diet of locusts. Although a definite and important relationship between sterols and fatty acids in the nutrition of higher animals has been shown to exist^{76,77} and considerable controversy exists as to the relationship of sterol content and atherosclerosis⁷⁸, no attempt has been made to examine this correlation in insects except in the silkworm where neither the fatty acids (both in the free and esterified form) nor the sterols had any effect on each other when included in the diet^{7,79}.

Supplementation of sterols from external sources for the normal development of insects is well known⁸⁰⁻⁸². Differences do exist in the ability of various insects to utilize sterols supplied in the diet. Levinson^{51,52} has shown an evolutionary tree in this respect depending on the number of sterols that an insect can utilize. The theory of Clark and Bloch⁸⁴ that some essential role played by cholesterol (requiring only minute quantities) cannot be achieved by replacing it with other sterols while all other functions can be seems to be gaining support from the studies of Kaplanis *et al.*⁸⁵. There is also sufficient evidence regarding the ability of these insects to effect these conversions⁸⁶⁻⁸⁸. Levinson's⁵² demonstration of the ability of *M. domestica* to convert β -sitosterol to cholesterol could not be confirmed by Kaplanis *et al.*⁸⁵. That the insect's gut may be the site of these conversions was deduced by considering the various theories on the mode of absorption of sterols in higher animals as applied to insects and by comparing the substrate specificity of the sterol esterifying enzyme⁸⁹. This enzyme, which had been earlier demonstrated in the tissues of the cockroach⁹⁰, and the one capable of hydrolysing sterol esters were shown to be present in the silkworm. The esterifying enzyme showed almost the same pattern of specificity towards sterols and particularly fatty acids as that found in higher animals^{91,92}. This specificity and other considerations led the author to conclude that esterification might be a prior necessity for sterol absorption as suggested by Noland⁹³ and that this determines the differential ability of insects to utilize more or less number of sterols depending on the rate at which they can be esterified and absorbed⁸⁹. Ishii *et al.*⁹⁴ found high concentrations of esterified sterol in the fat body, wings and alimentary tract of *Periplaneta americana* and suggested that esterifica-

tion may be important in sterol storage. In the silkworm, however, the presence of esterifying enzyme at higher levels than the hydrolytic enzyme, while most of the sterol was in the free form, led to the assumption that sterol esters may participate in some transesterification reactions.

Nutritional studies in insects have also shown that certain insects can selectively accumulate cholesterol from the diet in the presence of even a greater concentration of other sterols⁹⁵⁻⁹⁸. Why and how this is brought about are fascinating problems to tackle. One possible explanation is that the sterol esterifying enzyme in the gut, having a very high specificity to cholesterol, favours cholesterol absorption at a much faster rate than others, if esterification is necessary for absorption, as discussed before. Recently, Sissman *et al.*⁹⁹ have shown the ingested sterol to be degraded and excreted as follows in *Tribolium confusum*:



There are a number of reports indicating that farnesol and sterols are implicated in hormonal activities¹⁰⁰⁻¹⁰⁴. Some doubts have also been expressed as to whether these activities are due to contaminants^{105,106}. With the demonstration of farnesol as an insect sex attractant¹⁰⁷, some sterols as phagostimulants and a triterpene as a possible food attractant¹⁰⁸, and the known vitamin nature of cholesterol, it appears as if these various functions are intricately knit together. This problem is further complicated by the absence of any significant effect on larval moulting, pupation or adult development by extraneously administered sterols, even at levels where accumulation occurs in all tissues of the insect^{79,109}.

In almost all the cases studied, insects have been shown to require choline³. Inositol was also found to be indispensable¹¹⁰⁻¹¹³. Thorsteinson and Nayar¹¹³ have demonstrated that phosphatidyl choline and phosphatidyl-inositol isolated from wheat germ oil evoked feeding activity from old larvae and adults of two species of grasshoppers. The sterol of the mulberry leaf, now identified as β -sitosterol (cholesterol has also been found in plants¹¹⁴) was shown to be a specific attractant for the silkworm^{115,116}. These various observations bring to the fore the complications that arise due to very closely related compounds acting as vitamins, hormones, attractants, etc., and point to the difficulty of working on these. One is at a loss to determine where one function ceases and where the next one commences.

Whether all insects require β -carotene in their diet as an essential nutrient is still doubtful. Dadd⁷⁵ showed that β -carotene was necessary at least for normal pigmentation in *Schistocerca* and *Locusta*, and possibly for growth also. Murthy¹¹⁷ had shown that the silkworm required a plentiful supply of β -carotene along with other vitamins for good growth. Till very recently it was believed that β -carotene cannot be converted to vitamin A and the presence of the latter itself was open to question. But with the demonstration of vitamin A in the eyes of the housefly and the honeybee^{57,58,118}, one has to be careful while putting

forward any hypothesis. If these insects are only exceptions or if the amount of vitamin A present is far below the optimum, it is tempting to correlate the inability of insects to synthesize sterols and the absence of vitamin A. In higher animals it is believed that vitamin A is involved in isoprenoid metabolism, particularly at the stage of cyclization of squalene¹¹⁹⁻¹²¹. It is at this stage only the block is supposed to exist in the biosynthetic pathway of sterol in insects. Therefore, this may be explained by the absence of vitamin A or its presence in suboptimal levels. However, this correlation has to await further work for confirmation not only in insects but also in higher animals where some workers think that there may not exist any such correlation at the metabolic level^{122,123}.

Oxidation

The lack of unequivocal data on the ability of insects to oxidize fatty acids seems to be a major stumbling block for a clear understanding of their lipid metabolism. Studies on the energy requirements of insects have shown fat to be as good a source as carbohydrate¹²⁴⁻¹²⁷. Discussing the various data on the RQ measurements in insects, Zebe¹²⁸ concluded that flight muscles of insects can be divided into three types: exclusive carbohydrate users, exclusive fat users, and those that use both. It is to the last category that the majority of insects belong. In spite of these indications, almost all attempts to demonstrate fatty acid oxidation *in vitro* in sarcosome preparations have failed¹²⁹⁻¹³¹. Meyer *et al.*¹³² demonstrated the ability of mitochondria from *Schistocerca gregaria* to oxidize fatty acids under certain specialized conditions. Fatty acid activating enzymes were found in the fat body of the cockroach¹³³ and several species of Lepidoptera¹³⁴. The condensing enzyme and β -ketoacylthiolase were demonstrated in the mitochondria from locust flight muscle¹³⁵. The most convincing demonstration of fatty acid oxidation *in vitro* was by Domrose and Gilbert⁹ in the flight muscle mitochondria of *H. cecropia* silkworms. Their system showed many similarities in its requirements to the fatty acid oxidation system of rat liver. On the other hand, silkworm larval intestinal mitochondria could not oxidize fatty acids *in vitro*¹³⁶. None of these observations can be generalized till data on many more insects are available. However, that the two results are not incompatible will be shown later. The inability of silkworm larvae and pupae to utilize fatty acids to any great extent was shown by *in vivo* studies. It was found that almost 70 per cent of the administered radioactive fatty acids were recoverable in the lipid fraction even after a week¹³⁷. Similar results were recorded by Chino and Gilbert¹³⁸.

The maximum divergence of opinion is on the utilization of fat as an energy source. As mentioned earlier, any apparent reduction in the fat content during pupation and adult development is taken to mean that fat is utilized. Gilbert and Schneiderman¹³⁹ considered carbohydrate to be the main energy source for *cecropia* male pupa. This is also the case for the larvae and pupae of the silkworm⁷².

Another possibility that the carbohydrate may be converted to neutral fat and then utilized was also

ruled out. However, this does not mean that insects belonging to other classes follow the same pattern.

The detailed analysis of Domrose and Gilbert⁹ of the lipid content with respect to growth and development of *H. cecropia* moths showed that the male uses non-lipid components for energy during adult development and lipids during adult life, while the female utilized fat at both the stages. Since lipid catabolism in the male could be significantly reduced even during adult life by preventing flight activity, they concluded that there was some correlation between flying activity and lipid utilization. This result suggests a plausible reason for the silkworm's inability to utilize fat, that is, the fatty acid oxidation system is totally inactive due to disuse since the silkworm is essentially a non-flier. Another possibility is that the various insects, including the silkworm, may be able to utilize fatty acids at a particular stage of development as in males of *H. cecropia* and cannot do so at other stages. It will, therefore, be of great interest and importance if it could be shown either that *B. mori* can oxidize fatty acids as an adult (at which stage it has not been experimented with) or that *H. cecropia* cannot oxidize fatty acids at the larval stage. If this proves right, it will open up new avenues for the investigations on the evolutionary significance and the nature of control of the energy utilization processes, and will be another major departure from the pattern of lipid metabolism of higher animals. That this assumption has some basis is shown in the next section. Continuing the argument further, one wonders whether there exists any correlation between α -glycerophosphate dehydrogenase activity and lipid utilization, since a direct correlation exists between this enzymic activity and flying behaviour¹⁴⁰. All these results and hypotheses can be summed up by the observation of Krebs¹⁴¹ that the number of acetyl CoA molecules originating from fatty acids in an intact animal is dependent upon and is limited by the nature of the physico-chemical environment and the type of enzymatic equipment within its specific structure. In turn, intracellular concentration of available substrates and the degree of cellular organization are greatly influenced by the nutrition and endocrine status of the whole organism.

Biosynthesis

It is only recently comprehensive studies on the ability of insects to synthesize fatty acids, to esterify them and to store them have been undertaken. With the demonstration of Zebe and McShan¹⁴² and Teitz¹⁴³ that the nature of the biosynthetic set-up for fatty acids in the fat body of *Prodenia* and *Locusta* is essentially similar to that found in *in vitro* systems of higher animals, attempts have been made to determine the extent of these processes *in vivo*^{66,144-146}. The first outcome of these studies was that no insect studied could synthesize sterols *de novo* from [1-¹⁴C] acetate. A couple of reports to that effect were later realized to be due to the activity of gut flora^{90,147,148}. The second generalization that can be drawn is that with a few exceptions most of the insects studied cannot synthesize polyunsaturated fatty acids. Kilby²⁴, discussing the *in vitro* studies of Zebe and McShan¹⁴² and Teitz¹⁴³ argued that the very low incorporation of acetate into polyunsaturated fatty

acids might be due to the short duration of incubation in these experiments and that desaturation might be a slower process. However, long time experiments of Sridhara and Bhat¹⁴⁹ did not show any incorporation of radioactivity into linoleic and linolenic acids. Long duration studies with labelled stearic acid also showed that desaturation stopped at oleic acid¹³⁷. The large number of reports appearing in recent years on the essentiality of polyunsaturated fatty acids for most of the insects studied indicate that insects in general are incapable of synthesizing polyenoic fatty acids.

Though the experimental conditions are not strictly comparable in many cases, there does not seem to be any correlation between one insect and another regarding the extent of fatty acid synthesis. Considerable differences have been observed in the percentage incorporation of radioactivity between silkworm and higher animals and between the silkworm and other insects^{66,149}. These differences become clearer as the lipid is fractionated. However, a rough comparison of the distribution of the radioactivity of the saponifiable fraction only among individual fatty acids shows that in some insects at least palmitic acid is the one that picks up maximum activity^{66,142,143}. In some other insects maximum activity is found in unsaturated fatty acids like palmitoleic or oleic acids^{146,150}. Any conclusion as to whether this is due to the fact that sufficient time has been allowed for the palmitic acid synthesized to be desaturated or desaturated after elongation or whether their synthesis follows a different pathway as suggested by Sedee¹⁴⁶ will have to await further work. These data are tabulated in Table 4. Time-course studies in the silkworm both with [1-¹⁴C] acetate and [1-¹⁴C] palmitic and stearic acids showed that the distribution of radioactivity in the fatty acids follow a pattern which was consistent with the present concepts of the pathways of fatty acid synthesis in higher animals^{137,149}. Lambremont¹⁵¹ showed that the pattern of incorporation of radioactivity in bollweevils was the same when either [1-¹⁴C] acetate or [2-¹⁴C] acetate was used. He also proved that the precaution of rearing insects aseptically for studying the *in vivo* synthesis of fatty acids was unnecessary.

The fat body of the silkworm is the major site of synthesis has been demonstrated as has been done in *in vitro* in other insects, but a study of the distribution of radioactivity in other organs suggested that lipid synthesis may take place in other organs also. That

diglycerides may be the intermediates in triglyceride synthesis was indicated by the fact that the considerable radioactivity acquired by the diglyceride fraction, after the administration of labelled fatty acids, decreased with time, while that of the triglyceride fraction went up. Since the radioactivity of stearic acid and lauric acid appeared in palmitic acid, it was argued that the ¹⁴C had entered the C₂ pool and was used again for *de novo* synthesis of palmitic acid. The silkworm handled the preformed and derived fatty acids almost in a similar manner with some minor differences¹³⁷.

A very rapid incorporation of radioactive glucose into lipid, at a rate much faster than that into glycogen, was found in *Prodenia*, while in *Locusta*, the amount was not as large as that when labelled acetate was used¹⁴². Vanhandel and Lum¹⁵² and Strong¹⁵³ found the pattern of distribution of radioactivity in the fatty acids to be same whether they used acetate or glucose. In *Cecropia* silkmoths, and larvae and pupae of *B. mori* only very low incorporation of radioactivity occurred from glucose-U-¹⁴C into the lipid^{72,138}. Vanhandel and Lum¹⁵² made the interesting observation that while female mosquitoes could synthesize large amount of triglycerides when fed on glucose, male mosquitoes and both sexes of houseflies were unable to do so. A similar difference was observed in *C. tarsalis*¹⁵⁴. Although the existing data do not allow one to explain these fundamental differences, the results do imply that there should be some control mechanism that regulates the entry of acetyl CoA originating from glucose either to the TCA cycle to get oxidized or to the fatty acid synthesizing system. Such differences between males and females of the same species to synthesize lipid components are not uncommon^{144,145}.

There is the problem of how the vast deposits of fat in the fat body is made available to other tissues for utilization. The data from studies on the metabolism of FFA *in vivo* in higher animals have been extensively reviewed and is consistent with the view that plasma FFA is the transport form of lipids which is available for most tissues as a substrate for oxidation^{155,156}. The presence of FFA in insects is accepted with scepticism. A good stride was taken in this direction when Teitz¹⁵⁷ and later Chino and Gilbert^{158,159} showed that, unlike in higher animals, diglycerides are released from the insects' fat body as an endergonic process in the presence of a specific factor in the haemolymph and that this along with FFA is carried in the haemolymph coupled with a

TABLE 4—PATTERN OF INCORPORATION OF RADIOACTIVITY INTO FATTY ACIDS BY VARIOUS INSECTS

	Percentage of radioactivity in the saponifiable fraction						
	<i>Bombyx</i> ⁶⁶	<i>Myzus</i> ¹⁵³	<i>Antho-</i> <i>nomus</i> ¹⁵¹	<i>Rhizo-</i> <i>stoma</i> ¹⁵⁰	<i>Peri-</i> <i>planeta</i> ¹⁴⁵	<i>Prodenia</i> ¹⁴²	<i>Locusta</i> ¹⁴³
Myristic acid	—	4.8	2.5	—	0.4	3	5
Palmitic acid	39	5.4	29.1	18.3	30.7	78	60
Stearic acid	32	24.0	14.2	—	9.9	9	22
Palmitoleic acid	—	20.0	8.5	39.8	—	—	—
Oleic acid	25	29.0	24.7	34.5	44.1	8	11
Linoleic acid	—	3.3	0.9	—	9.9	—	—
Linolenic acid	—	4.0	5.4	—	4.3	—	—

protein. Though the mechanism by which the diglycerides are utilized further is not known, it is certain that their release from the fat body is controlled by a factor in the haemolymph which may be a hormone. Then it will be of interest to know whether this factor is present in the haemolymph at all stages of growth or only at particular stages when the insect needs fat as an energy source, viz. adult life, since this will help to explain much of the debated results. This also means that fat body carries out the synthesis of triglycerides via the diglycerides, as well as releases fat via diglycerides, as endergonic processes. It will have to be decided whether both the processes follow the same pathways or not.

Based on these data some speculations may be made on the inability of the silkworm to utilize fat at the stages studied. It was shown that the administered fatty acids are built up into triglycerides and phospholipids and stored very rapidly¹³⁷. If the silkworm does not have the necessary factor in the haemolymph to release the neutral fat from the fat body, then it can be assumed that the inability to utilize fat is just due to the non-availability of the substrate for other tissues for utilization. In fact, Chino and Gilbert¹³⁸ also found that fatty acids were esterified at a fast rate and stored in the fat body. Incidentally, no sudanophilic staining protein could be found in the silkworm larval haemolymph¹⁶⁰ while this was observed in all the three insects studied by Chino and Gilbert¹⁵⁹.

Teitz¹⁴³ found up to 10 per cent of the radioactivity incorporated from [1-¹⁴C] acetate in the phospholipid fraction. Sridhara and Bhat¹⁴⁹ found a higher percentage of radioactivity in the phospholipid; also the percentage differed in different organs studied. The amount of radioactivity that was found in the phospholipid fraction after administering radioactive fatty acids was slightly less than that observed with labelled acetate. Taylor and Hodgson¹⁶¹ studied the biosynthesis of nitrogenous components of phospholipids and showed that the β -carbon of serine of the phospholipids of *P. regina* originated from glycine and that decarboxylation of serine yielded ethanolamine thus suggesting that the origin of these was similar to that in higher animals. However, that the next step in this pathway, viz. the conversion of ethanolamine to choline, is similar to that in rat liver was shown to be absent in the housefly¹⁶² whose phospholipid profile is very similar to that of *P. regina*.

Sridhara and Bhat⁶⁶ observed single peaks in the fractions obtained with 5 and 10 per cent ether in petrol during fractionation of unsaponifiables on alumina and these fractions could be ubiquinone and ubiquinone. However, these observations along with similar observations with [2-¹⁴C] mevalonate, can be taken as preliminary evidence for the biosynthesis of these components. The inability of the various insects to synthesize sterols has been repeatedly demonstrated. This inability is not due to a block between acetate and mevalonate has been proved in *M. domestica* and *B. mori*^{163,164}.

Summary

Investigations on the nutrition and metabolism of lipids in the animal systems are much more complex

than those involving the study of carbohydrates and proteins, and therefore progress in the former area has lagged behind. The considerable advances made during the past 15 years in the nutrition and metabolism of lipids has closely paralleled the growth of modern biochemistry and has led to the important conclusion that fat molecules deposited or synthesized in the cells of the body serve a variety of sophisticated functions. This development has also led to the introduction of many new and elegant quantitative methods.

The chemistry and biochemistry of insect lipids have not even acquired the status animal lipids had 25-30 years ago. Some studies have been made on lipids of whole insects and although the range of species covered is narrow, it is apparent that variations exist in their chemical composition. This is to be expected in view of the range of feeding habits and environments to which insects have become adapted. In contrast to the accumulation of analytical data on the composition of lipids, very little is known about their metabolism. A rough estimates of the number of reports published in this field until about 1960 is about 2 per cent or less of those published in other fields. The reasons for this situation are: (i) complications in working with these 'messy' substances; (ii) limitations because of the quantity of material available; (iii) the lack of standard techniques which have been developed only during the past two decades; and (iv) the baffling variations according to sex, age and species. However, a number of schools are now attacking these problems and the main questions posed are: (i) whether or not the lipid participates in the fast growth and the profound morphological changes that occur in insects; (ii) what purpose does the large amount of lipid stored by insects serve; (iii) whether deficiency and toxicity symptoms are similar to those observed in higher animals; (iv) how are the lipids absorbed from the gut in the absence of compounds like bile acids to facilitate micelle formation; (v) what are the metabolic functions of the high proportion of phospholipids found in some insects; (vi) how are the lipid components synthesized and deposited; and (vii) how the large deposits of lipid in the fat body are transported and delivered to various tissues. Added to these is the baffling relationship existing between farnesol, sterol, phagostimulants, food attractants, etc. These are a few questions which have been considered and discussed, and an attempt has been made to put forward hypotheses based on the available data.

Acknowledgement

The author records his deep sense of gratitude to Dr J. V. Bhat with whom all the author's work was carried out. His thanks are due to Dr M. B. Shyamala for her interest and encouragement. The author's work was supported by the grants from the Indian Institute of Science, the University Grants Commission, New Delhi, and the PL 480 programme.

References

1. CLAYTON, R. B., *J. Lipid Res.*, **5** (1964), 3.
2. HILDITCH, T. P., *The chemical composition of natural fats* (Chapman & Hall Ltd, London), 1956, 74.
3. GILMOUR, D., *Biochemistry of insects* (Academic Press Inc., New York), 1961, 203.

4. GILBY, A. R., *Annu. Rev. Entomol.*, **10** (1965), 141.
5. SHORLAND, F. B., cited in *Comparative biochemistry, a comprehensive treatise*, Vol. III, edited by M. Florkin & H. S. Mason (Academic Press Inc., New York), 1962, 1.
6. STRONG, F., *Hilgardia*, **34** (1963), 43.
7. SRIDHARA, S., *Some nutritional and metabolic aspects of the silkworm, Bombyx mori L.*, Ph.D. thesis, Indian Institute of Science, Bangalore, 1963.
8. SRIDHARA, S. & BHAT, J. V., *J. Insect Physiol.*, **11** (1965), 493.
9. DOMROSE, K. A. & GILBERT, L. I., *J. exp. Biol.*, **41** (1964), 573.
10. VILLENRUEVE, J. L. & LEMONDE, A., *Arch. int. Physiol. Biochim.*, **71** (1963), 143.
11. BARLOW, J. S., *Canad. J. Zool.*, **43** (1965), 291.
12. FAWZI, M., OSMAN, H. & SCHMIDT, G. H., *Biochem. Z.*, **334** (1961), 441.
13. PEPPER, J. H. & HASTINGS, E., *Bull. Mont. St. coll. Agric. exp. Statist.*, **413** (1943), 1.
14. SCOGGIN, J. K. & TAUBER, O. E., *Iowa St. Coll. J. Sci.*, **25** (1950), 99.
15. GILBERT, L. I. & SCHNEIDERMAN, H. A., *Gen. Comp. Endocrinol.*, **1** (1961), 453.
16. LAMBREMONT, E. N. & BLUM, M. S., *Ann. ent. Soc. Amer.*, **56** (1963), 612.
17. LUDWIG, D. & ROTHSTEIN, F., *Physiol. Zool.*, **22** (1948), 308.
18. PEARINCOTT, J. V., *J. cell. comp. Physiol.*, **55** (1960), 167.
19. MORAN, M. R., *J. N.Y. ent. Soc.*, **67** (1959), 213.
20. DITMAN, L. P., *Bull. Maryland Univ. agric. exp. Statist.*, **414** (1938), 183.
21. LEVINSON, Z. H. & SILVERMAN, P. H., *Biochem. J.*, **58** (1954), 294.
22. HERODEK, S. & FARKAS, T., *Magy. biol. Kutatoint. intesz Munkai*, **27** (1960), 9.
23. NIEMIEMKO, S., *Acta Biol. exp. vars.*, **14** (1947), 137.
24. KILBY, B. A., cited in *Advances in insect physiology*, Vol. I, edited by J. W. L. Beament, J. E. Treherne & V. W. Wigglesworth (Academic Press Inc., New York), 1964, 111.
25. DOLE, V. P., cited in *Chemistry of lipids as related to atherosclerosis*, edited by I. H. Page (C.C. Thomas, Springfield, Illinois), 1958, 189.
26. GIRAL, F., *J. biol. Chem.*, **162** (1946), 61.
27. NIEMIEMKO, W., cited in *The biochemistry of insects*, edited by L. Levenbook (Pergamon Press, New York), 185.
28. ALBRECHT, G., *Z. Vergl. physiol.*, **44** (1961), 487.
29. BERGMANN, W., *J. biol. Chem.*, **114** (1936), 27.
30. BACHSETZ, M. & ARAGON, A., *J. Amer. Pharm. Assoc.*, **31** (1942), 145.
31. BARLOW, J. S., *Nature, Lond.*, **197** (1963), 311.
32. BARLOW, J. S., *Canad. J. Zool.*, **43** (1965), 337.
33. DAWSON, B. M. C., *Biol. Rev.*, **32** (1957), 188.
34. ROSSITTER, R. J., *Clin. Chem.*, **11** (1965), 171.
35. STAKOTOS, A. N., *U.S. Army chem. Res. Develop. labs. Tech. Mem.*, No. 20-21 (1960), 8.
36. BEIBER, L. L., HODGSON, E., CHELDELIN, V. H., BROOKES, V. J. & NEWBURGH, R. W., *J. biol. Chem.*, **236** (1961), 2590.
37. FAST, P. G. & BROWN, A. W. A., *Ann. ent. Soc. Amer.*, **55** (1962), 663.
38. SRIDHARA, S. & BHAT, J. V., *Curr. Sci.*, **31** (1962), 240.
39. SCHMIDT, G. H. & OSMAN, H., *J. Insect Physiol.*, **8** (1962), 233.
40. FAIRBAIRN, D., *Canad. J. Biochem. Physiol.*, **34** (1956), 39.
41. BERGMANN, W. & LANDOWNE, R. A., *J. org. Chem.*, **23** (1958), 1241.
42. CHOJNACKI, T. & KORZYBSKI, J., *Acta biochim. polon.*, **9** (1962), 95.
43. CRONE, H. D. & BRIDGES, R. G., *Biochem. J.*, **89** (1963), 11.
44. CHAUDHARY, K. D. & LEMONDE, A., *Comp. Biochem. Physiol.*, **9** (1933), 343.
45. TAYLOR, J. F. & HODGSON, E., *Ann. ent. Soc. Amer.*, **57** (1964), 795.
46. HODGSON, E., CHELDELIN, V. H. & NEWBURGH, R. W., *Arch. Biochem. Biophys.*, **87** (1960), 48.
47. BEIBER, L. L. & NEWBURGH, R. W., *J. Lipid Res.*, **4** (1963), 397.
48. BEIBER, L. L., CHELDELIN, V. H. & NEWBURGH, R. W., *J. biol. Chem.*, **238** (1965), 1.
49. BRIDGES, R. G., RICKETTS, S. J. & COX, J. T., *J. Insect Physiol.*, **11** (1965), 225.
50. GUPTA, B. L. & KAMBOJ, V. P., *Experientia*, **18** (1962), 24.
51. LEVINSON, Z. H., *Proceedings, Eleventh international congress of entomology*, Vol. 3, 1960, 154.
52. LEVINSON, Z. H., *J. Insect Physiol.*, **8** (1962), 191.
53. BERGMANN, W., cited in *Comparative biochemistry, a comprehensive treatise*, Vol. III, edited by M. Florkin & H. S. Mason (Academic Press Inc., New York), 1962, 103.
54. LESTER, R. L. & CRANE, F. L., *J. biol. Chem.*, **234** (1959), 2169.
55. HELLER, J., SZARKOWSKA, L. & MICHALEK, H., *Nature, Lond.*, **188** (1961), 491.
56. LAIDMAN, D. L. & MORTON, R. A., *Biochem. J.*, **34** (1962), 386.
57. COHEN, C. F. & BARKAR, R. J., *J. cell. comp. Physiol.*, **62** (1963), 43.
58. GOLDSMITH, T. H. & WARNER, L. T., *J. gen. Physiol.*, **47** (1964), 433.
59. FRAENKEL, G. & BLEWETT, M., *J. exp. Biol.*, **22** (1946), 172.
60. FRAENKEL, G. & BLEWETT, M., *Biochem. J.*, **41** (1947), 475.
61. VANDERZANT, E. S., KERUR, D. & REISER, R., *J. econ. Ent.*, **50** (1957), 606.
62. GORDON, H. J., *Ann. N.Y. Acad. Sci.*, **77** (1959), 290.
63. DADD, R. H., *J. Insect Physiol.*, **4** (1960), 319.
64. DADD, R. H., *J. Insect Physiol.*, **10** (1964), 161.
65. CHIPPENDALE, G. M., BECK, S. D. & STRONG, F. M., *J. Insect Physiol.*, **11** (1965), 211.
66. SRIDHARA, S. & BHAT, J. V., *Biochem. J.*, **91** (1964), 120.
67. GRINDLEY, D. N., *J. exp. Biol.*, **29** (1952), 440.
68. LAMBREMONT, E. N., BLUM, M. S. & SCHRADER, R., *Ann. ent. Soc. Amer.*, **57** (1964), 526.
69. SHYAMALA, M. B. & BHAT, J. V., *J. sci. industr. Res.*, **21A** (1962), 26.
70. COLLIN, G., *Biochem. J.*, **27** (1933), 1373.
71. HOUSE, H. L. & BARLOW, J. S., *J. Nutr.*, **72** (1960), 409.
72. SRIDHARA, S. & BHAT, J. V., *Life Sci.*, **4** (1965), 979.
73. HSUEN, T. Y. & TANG, P. S., *Physiol. Zool.*, **17** (1944), 71.
74. CLEMENTS, A. N., *J. exp. Biol.*, **36** (1959), 665.
75. DADD, R. H., cited in *Advances in insect physiology*, Vol. I, edited by J. W. L. Beament, J. E. Treherne & V. W. Wigglesworth (Academic Press Inc., New York), 1963, 47.
76. AHRENS, E. H., HIRSH, J., INSULL, W., TSALTAS, T. T., BLOMSTRAND, R. & PETERSON, M. L., *Lancet*, **272** (1957), 943.
77. GRANDE, F., ANDERSON, J. T. & KEYES, A., *J. Nutr.*, **74** (1959), 120.
78. PAGE, I. H., cited in *Chemistry of lipids as related to atherosclerosis*, edited by I. H. Page (C. C. Thomas, Springfield, Illinois), 1958, 3.
79. SRIDHARA, S. & BHAT, J. V., *Proc. nat. Inst. Sci., India*, **29** (1963), 602.
80. LIPKE, H. & FRAENKEL, G., *Annu. Rev. Ent.*, **1** (1956), 17.
81. BROOKES, V. J. & FRAENKEL, G., *Physiol. Zool.*, **31** (1958), 208.
82. MONROE, R. E., *Ann. ent. Soc. Amer.*, **53** (1960), 821.
83. ITO, T., *Nature, Lond.*, **191** (1961), 882.
84. CLARK, A. J. & BLOCH, K., *J. biol. Chem.*, **234** (1959), 2578, 2589.
85. KAPLANIS, J. N. & ROBBINS, W. E., MONROE, R. E., SHORTINO, T. J. & THOMPSON, M. J., *J. Insect Physiol.*, **11** (1965), 251.
86. BECK, S. D. & KAPADIA, G. C., *Science*, **126** (1957), 258.
87. ROBBINS, W. E., KAPLANIS, J. N., LOULOUDES, S. J. & MONROE, R. E., *Ann. ent. Soc. Amer.*, **53** (1960), 128.
88. ROBBINS, W. E., *Steroid*, **4** (1964), 635.
89. SRIDHARA, S. & BHAT, J. V., *Arch. int. Physiol. Biochim.*, **72** (1964), 743.
90. CASIDA, J. E., BECK, S. D. & COLE, M. J., *J. biol. Chem.*, **224** (1957), 365.
91. RILEY, C. & NUNN, R. F., *Biochem. J.*, **74** (1960), 56.

92. WEST, C. E. & ANNISON, E. F., *Biochem. J.*, **92** (1964), 576.
93. NOLAND, J. L., *Arch. Biochem. Biophys.*, **52** (1954), 323.
94. ISHII, S., KAPPLANIS, J. N. & ROBBINS, W. E., *Ann. ent. Soc. Amer.*, **56** (1963), 115.
95. CLAYTON, R. B. & EDWARDS, A. M., *Biochem. biophys. Res. Commun.*, **6** (1961), 281.
96. THOMPSON, M. J., LOULOUDIS, S. J., ROBBINS, W. E., WATERS, J. A., STEELE, J. A. & MOSETTIG, E., *Biochem. biophys. Res. Commun.*, **9** (1962), 113.
97. THOMPSON, M. J., LOULOUDIS, S. J., ROBBINS, W. E., WATERS, J. A., STEELE, J. A. & MOSETTIG, E., *J. Insect Physiol.*, **9** (1963), 615.
98. ROBBINS, W. E., *Int. atom. Energy Ag. Bull.*, **5** (1963), 269.
99. SISSMAN, E. E., JENNY, N. A. & BECK, S. D., *J. pharm. Sci.*, **53** (1964), 1519.
100. SCHMALEK, P., *Z. Naturf.*, **16B** (1961), 461.
101. YAMAMOTO, R. T. & JACOBSON, M., *Nature, Lond.*, **196** (1962), 908.
102. KIRIMURA, J., SAITO, M. & KOBAYASHI, M., *Nature, Lond.*, **195** (1962), 729.
103. KARLSON, P., *Angew. Chem.*, **2** (1963), 175.
104. SCHMALEK, P., *Z. Naturf.*, **18** (1963), 516.
105. SCHNEIDERMAN, H. A. & GILBERT, L. I., *Science*, **143** (1964), 325.
106. WILLIAMS, C. M. & LAW, T. H., *J. Insect Physiol.*, **11** (1965), 569.
107. STEIN, G., *Biol. Zh.*, **82** (1963), 345.
108. ITO, T., KAWASHIMA, K., NAKAHARA, M., NAKANISHI, K. N. & TERAHARA, A., *J. Insect Physiol.*, **10** (1964), 225.
109. LEVINSON, Z. H. & BERGMAN, E. D., *Biochem. J.*, **65** (1957), 254.
110. VANDERZANT, E. S., *J. econ. Ent.*, **52** (1959), 1018.
111. VANDERZANT, E. S., *J. econ. Ent.*, **56** (1963), 357.
112. FORGASH, A. J., *Ann. ent. Soc. Amer.*, **55** (1962), 703.
113. THORSTEINSON, A. J. & NAYAR, J. K., *Canad. J. Zool.*, **41** (1963), 931.
114. JOHNSON, D. F., BENNETT, R. D. & HEFTMAN, E., *Science*, **140** (1963), 180.
115. NAITO, K. & HAMAMURA, Y., *J. agric. Chem. Soc., Japan*, **35** (1961), 848.
116. HAMAMURA, Y., HAYASHIYA, K., NAITO, K., MATSURA, K. & NISHIDA, J., *Bull. Fac. text. Fib. Kyoto Univ.*, **3** (1962), 567.
117. MURTHY, M. R. V., *Studies on the nutrition of the silkworm, Bombyx mori L.*, Ph.D. thesis, University of Bombay, 1954.
118. FISHER, L. R. & GOLDIE, E. H., *Proceedings, Third international congress on photobiology, Copenhagen, 1960*, 153.
119. GLOOR, H. & WISS, O., *Arch. Biochem. Biophys.*, **83** (1959), 216.
120. GLOOR, H. & WISS, O., *Biochem. biophys. Res. Commun.*, **1** (1959), 182.
121. PHILLIPS, W. E. J., *Canad. J. Biochem. Physiol.*, **39** (1961), 855.
122. JOHNSON, B. C. & WOLF, G., *Vitam. & Horm.*, **18** (1960), 457.
123. DIPLOCK, A. T., GREEN, J. & BUNYAN, J., *Biochem. J.*, **95** (1965), 138.
124. WILLIAMS, C. M., BARNES, L. A. & SAWYER, W. H., *Biol. Bull., mar. biol. Lab.*, **84** (1943), 263.
125. BARRON, E. S. G. & TAHMISIAN, T. N., *J. cell. comp. Physiol.*, **32** (1948), 57.
126. WIGGLESWORTH, V. B., *J. exp. Biol.*, **26** (1949), 150.
127. WEIS-FOGH, T., *Phil. Trans. R. Soc.*, **B237** (1952), 1.
128. ZEBE, E. C., cited in *The biochemistry of insects*, edited by L. Levenbook (Pergamon Press, New York), 197.
129. REES, K. R., *Biochem. J.*, **58** (1964), 196.
130. SACKTOR, B., *J. Biophys. biochem. Cytol.*, **1** (1955), 29.
131. GONDA, O., TRAUB, A. & AVIDOR, Y., *Biochem. J.*, **67** (1957), 487.
132. MEYER, H., PREISS, B. & BAUER, S. H., *Biochem. J.*, **76** (1960), 27.
133. SIAKOTOS, A. N., *Fed. Proc.*, **18** (1959), 144.
134. NELSON, W. L., *Proc. 10th Congr. Entomol.*, **2** (1958), 359.
135. ZEBE, E. C., *Biochem. Z.*, **332** (1960), 328.
136. SRIDHARA, S., *J. Insect Physiol.*, **11** (1965), 33.
137. SRIDHARA, S., RAVI RAU, U. & BHAT, J. V., *Biochem. J.*, (1965), (in press).
138. CHINO, H. & GILBERT, L. I., *J. Insect Physiol.*, **11** (1965), 287.
139. GILBERT, L. I. & SCHNEIDERMAN, H. A., *Amer. Zool.*, **1** (1) (1961), 11.
140. SACKTOR, B., cited in *Physiology of Insecta*, II, edited by M. Rockstein (Academic Press Inc., New York), 1965, 483.
141. KREBS, H. A., *Endeavour*, **16** (1957), 125.
142. ZEBE, E. C. & MCSHAN, W. H., *Biochim. biophys. Acta*, **31** (1959), 513.
143. TEITZ, A., *J. Lipid Res.*, **2** (1961), 182.
144. ROBBINS, W. E., KAPPLANIS, J. N., LOULOUDIS, S. J. & MONROE, R. E., *Ann. ent. Soc. Amer.*, **53** (1960), 128.
145. LOULOUDIS, S. J., KAPPLANIS, J. N., ROBBINS, W. E. & MONROE, R. E., *Ann. ent. Soc. Amer.*, **54** (1961), 99.
146. SEDEE, P. D. J. W., *Arch. int. Physiol. Biochim.*, **69** (1960), 295.
147. CLAYTON, R. B., *J. biol. Chem.*, **235** (1960), 3421.
148. CLAYTON, R. B., EDWARDS, A. M. & BLOCH, K., *Nature, Lond.*, **195** (1962), 1125.
149. SRIDHARA, S. & BHAT, J. V., *Biochem. J.*, **94** (1965), 700.
150. MIURA, K., VONK, H. J., ZANDEE, D. I. & HOUX, N. W. H., *Arch. int. Physiol. Biochim.*, **73** (1965), 65.
151. LAMBREMONT, E. N., *Comp. Biochem. Physiol.*, **14** (1965), 419.
152. VANHANDEL, E. & LUM, T. M., *Science*, **134** (1961), 1979.
153. STRONG, F., *Science*, **140** (1963), 933.
154. TAKATA, N. & HARWOOD, R. F., *Ann. ent. Soc. Amer.*, **57** (1964), 749.
155. FREDRICKSON, D. S. & GORDON (JR), R. S., *Physiol. Rev.*, **38** (1958), 585.
156. HAVEL, R. J., *Metabolism*, **10** (1961), 1031.
157. TEITZ, A., *J. Lipid Res.*, **3** (1962), 421.
158. CHINO, H. & GILBERT, L. I., *Science*, **143** (1964), 359.
159. CHINO, H. & GILBERT, L. I., *Biochim. biophys. Acta*, **98** (1965), 94.
160. SRIDHARA, S. & ANANTASAMY, T. S., *Proc. Indian Acad. Sci.*, **38** (1963), 19.
161. TAYLOR, J. F. & HODGSON, E., *J. Insect Physiol.*, **11** (1965), 281.
162. BRIDGES, R. G. & RICKETS, J., *Biochem. J.*, **95** (1965), 41.
163. KAPPLANIS, J. N., DUTKY, R. C. & ROBBINS, W. E., *Ann. ent. Soc. Amer.*, **54** (1961), 114.
164. SRIDHARA, S. & BHAT, J. V., *Life Sci.*, **4** (1965), 161.

REVIEWS

NONLINEAR PARTIAL DIFFERENTIAL EQUATIONS IN ENGINEERING by William F. Ames (Academic Press Inc., New York), 1965. Pp. xii+510. Price \$ 16.00

With the development of high speed computation the problem of obtaining solutions of nonlinear partial differential equations in engineering has attracted considerable attention. The book provides a comprehensive survey of methods which are used in solving nonlinear partial differential equations. This book contains 8 chapters: (1) The origin of nonlinear differential equations; (2) Transformation and general solutions; (3) Exact methods of solution; (4) Further analytic methods; (5) Approximate methods; (6) Further approximate methods; (7) Numerical methods; and (8) Some theoretical considerations. An appendix is devoted to elements of group theory.

There is an extensive bibliography of about 600 references. The wealth of information contained in this book makes it a 'must' on the reading list of everybody connected with the study of nonlinear partial differential equations of practical interest.

M. K. JAIN

ELEMENTARY PARTICLE AND HIGH ENERGY PHYSICS edited by M. Lévy & Ph. Meyer (Gordon & Breach Science Publishers, New York), 1965. Pp. 362. Price \$ 7.95 (paper); \$ 14.50 (cloth)

The present volume comprises the lectures given at the Cargèse Summer School of Theoretical Physics in 1963 and deals with selected topics in the theory of elementary particles.

In the first lecture of the series, Van Hove gives a rapid but clear account of the theoretical and experimental situation regarding cross-sections for interactions involving strongly interacting particles at high energies. He deals with the various Pomernanchuk theorems and their generalizations (to include statements on differential cross-sections), the Regge pole approach to high energy scattering and its limitations, calculations using unitarity and the essentially imaginary nature of the high energy scattering amplitudes. He ends up with reviewing three models for inelastic collisions including the multiperipheral model.

Charap in his article on complex angular momentum theory of particles with spin starts with a historical account of the S-matrix approach to strong interaction physics, its difficulties and the main results achieved and then goes on to discuss the 'inessential complications due to spin' when considering potential scattering in the complex angular momentum plane. Extension to the relativistic case is briefly discussed. Jacob in his second talk dealing with spin effects in the Regge pole approach to high energy physics continues this theme and considers the polarization effects arising from the interference between several Regge poles in the case of p-p scattering and the inadequacy of this procedure even when spin effects are taken into

account in explaining the absence of 'shrinkage' in pion-proton scattering properly. His first $t_{p,p}^{(1)}$ about the newly discovered baryon and meson resonances, their quantum numbers and their phenomenological analysis, including brief mention of the Ball and Frazer and Peierls mechanisms and the Chew-Low extrapolation procedure.

Gourdin gives an account of unitary symmetry and the eightfold way (though, as the author himself confesses, the rapid progress in the subject has made it somewhat outmoded). Methods of extracting experimental consequences of the theory in the form of relations between the reaction amplitudes for various processes are given and violation of the symmetry and the mass formulae for baryons and mesons are discussed.

In the (logically) most satisfying article of the book, Schwinger treats us to the topic of the relativistic theory of fields and some problems of quantization of nonabelian gauge fields. After introducing the action principle for quantum mechanical systems he goes on to consider the question of when a quantum field theory can be considered relativistic and derives an important commutation relation between energy densities at two points in various ways (in one method introducing the gravitational field). (Incidentally, the relation was independently given by Dirac.) Extension of these ideas to particles with spin one necessitates consideration of gauge invariance. The electromagnetic field is considered at length as an introduction to the more general case of nonabelian gauge fields corresponding to charged vector particles (which might be required to explain the existence of various internal symmetries. The success of SU_3 symmetry makes the necessity for such particles doubtful.)

In the final article Meyer summarizes the phenomenological study of the weak interactions of strange particles.

The volume shows evidence of the haste with which it has been prepared to make it available to the scientific community quickly. A whole page of references, which should follow after page 362, seems to be missing. There are a number of mistakes and the English of some of the articles has a 'continental' flavour. These, however, do not detract from the usefulness of the volume.

K. VENKATESAN

ANALYSIS OF ESSENTIAL NUCLEAR REACTOR MATERIAL edited by C. J. Rodden (Division of Technical Information, US Atomic Energy Commission, Washington DC), 1964. Pp. 1280

In late forties, barring a few nations who had made spectacular advances the rest had barely started work in the field of atomic energy. Even in USA, amongst the very many hurdles in the development of this new field, one was the development of suitable analytical methods. Therefore, the publication in 1950 of the book *Analytical Chemistry of Manhattan Project* edited by Dr Rodden was a real

boon to the laboratories which were newly coming in the field since it presented in one volume the gist of the vast work in analytical chemistry in USA.

Since then, enormous developments have taken place in the concepts of reactor designs and materials, bringing new analytical problems to the fore. This book projects all these developments from a practical viewpoint of an analytical chemist, saving him once again considerable efforts on preliminaries to new work.

As the title implies this is essentially a book for analytical chemists engaged in atomic energy projects. Considering, however, that besides atomic energy there are, of late, major developments in many other fields such as transistor technology and space research, analytical chemists engaged on testing materials on such projects would also find enough material in this book to tackle corresponding problems.

It is as difficult for a reviewer to review in detail this volume of 1200 pages dealing with various materials, methods, concentrations and techniques as was probably also the case with the editors before writing this book. It, therefore, stands to reason that the editor defined the scope of this book in the preface thus: "In the writing up of this book an attempt has been made to provide the analytical chemist with the information needed to carry out many analyses involved in nuclear technology without the necessity of further literature searching. The effort to make this book a laboratory reference book is not without disadvantage, the foremost of which is that much of the information included had to be strictly practical in nature with the physical and inorganic chemistry aspects of the subject held to a minimum."

This aspect of practical utility has been adhered to in the whole presentation. The book would thus serve a nice reference book to analytical chemists engaged on developing newer methods and a practical guide for the analysts and technicians.

The book deals with the analysis of nuclear fuels and fertile materials like uranium, thorium, plutonium, moderators like beryllium, graphite and heavy water, neutron absorbers like boron and coolants like sodium, Na-K and polyphenyls. In addition methods for determination of trace elements in these materials, the analysis of expended fuels and various alloys of uranium, plutonium and thorium are also presented. Separate chapters are devoted to the instrumental techniques of mass spectrometry, X-ray spectroscopy, electrochemical methods and radiochemical methods so essential for the analysis of nuclear materials. Exhaustive bibliographies have been given at the end of each chapter though it appears that the literature survey after 1960 may not be very exhaustive. This probably was inevitable.

The editors have really done a service to the analytical chemists in the field in bringing out this very useful volume.

V. T. ATHAVALA

ORIENTED NUCLEI: POLARIZED TARGETS AND BEAMS
by J. M. Daniels (Academic Press Inc., New York), 1965. Pp. xii+278. Price \$ 9.00

The purpose of this book, according to the author, is to present in one volume a concise account of

nuclear orientation. Since the parity violation in beta decay was experimentally verified, physicists have been keenly interested in the methods of orienting nuclei and their use in designing new experiments. Till now, for information on this rapidly developing subject, one had to turn to research papers and review articles spread over many journals, over the past few years. This book, therefore, fulfils the need felt by students and research workers in physics.

The book, which runs into some 250 pages, is divided into seven chapters. After the introductory chapter, there is a chapter each devoted respectively to thermal equilibrium methods and non-thermal equilibrium methods of orienting nuclei. The former describes the so-called 'Brute Force' method and Bleaney-Gorter-Rose method of nuclear orientation, while the latter describes transient nuclear orientation, including microwave and optical pumping.

One chapter gives experimental and technical details associated with both these groups of methods, such as production of low temperatures and adiabatic demagnetization, problems of heat transfer and apparatus for microwave pumping.

The next two chapters are concerned with beams of polarized particles and polarized ion sources and give valuable information to nuclear physicists, who wish to study nuclear reactions with polarized particles. The final chapter describes some experiments which can be carried out with oriented nuclei, in particular, angular distribution of radiations and nuclear reactions and scattering. These are the better known examples of what can be done with oriented nuclei. In fact, they open up a rich new area of investigation for nuclear and solid state problems.

The book is easy to read and gives a very useful list of references for those who may wish to go deeper into a particular aspect of the subject matter. It will serve as a good survey of a new subject for postgraduate students and as an adequate starting point for research workers in this branch of physics.

B. V. THOSAR

ADVANCES IN HETEROCYCLIC CHEMISTRY: Vol. 4, edited by A. R. Katritzky (Academic Press Inc., New York), 1965. Pp. xiii+462. Price \$ 15.00

The contents include the following chapters: Covalent hydration in nitrogen-containing heteroaromatic compounds: I—Qualitative aspects (A. Albert & W. L. F. Armarego); Covalent hydration in nitrogen heteroaromatic compounds: II—Quantitative aspects (D. D. Perrin); Recent advances in oxazoline chemistry (R. Filler); Isothiazoles (R. Slack & K. R. H. Wooldridge); Hetarynes (H. J. Den Hertog & H. C. van der Plas); and Reactivity of azine, benzoazine and azinoazine derivatives with simple nucleophiles (R. G. Shepherd & J. L. Fedrick). Isothiazoles, covalent hydration in nitrogen heteroaromatic compounds and hetarynes are topics which have been reviewed for the first time. Like the previous volumes, Vol. 4 is a very valuable addition to the literature of heterocyclic chemistry, and the authors have themselves made substantial contributions to the areas covered by them. The chapter on reactivity of azine derivatives with

simple nucleophiles is the longest, comprising nearly 300 pages; together with Illuminati's chapter on nucleophilic heteroaromatic substitution in Vol. 3, a separate volume could well have been devoted to this important subject. The chapter on isothiazoles, very well written as it is, occupies only 14 pages, because the parent compound was only described in 1956 by Adams and Slack and less than 250 derivatives have been reported so far. In view, for instance, of the activity of simple derivatives of isoxazole and imidazole as oral hypoglycaemic agents, this novel and simple heterocyclic system is of great interest in the search for new chemotherapeutic agents. Benzynes chemistry has developed rapidly during the last ten years, and arynes derived from heterocyclic ring systems, which may be expected to differ in several properties from carbocyclic arynes, are discussed fully in the chapter on hetarynes. The phenomenon of $C=N$ hydration in pteridines was first observed by Albert in 1951, and Albert and his collaborators have now made an extensive study of such covalent hydration. Lastly, Filler has given an excellent account of the new developments in oxazolone chemistry which have wide synthetic, structural and mechanistic interest; earlier work, reviewed in 1957, was based on azlactones as intermediates for amino acids and peptides and on the suspected presence of an oxazolone ring in penicillin before the β -lactam structure was established.

K.V.

OPTICAL ROTATORY DISPERSION AND CIRCULAR DICHOISM IN ORGANIC CHEMISTRY by Pierre Crabbe (Holden-Day Inc., San Francisco), 1965. Pp. xv + 378. Price \$ 12.95

Of the most important tools, currently available, for the determination of absolute stereochemistry of optically active natural organic products, are the Optical Rotatory Dispersion (ORD) and the closely related Circular Dichroism (CD). The first book on ORD, of interest to organic chemists, was published by Prof. Djerassi in 1960, but the number of applications appearing in recent years has been so extensive that a need for a more up-to-date work was evident. Dr Crabbe's book fulfils this gap eminently. Moreover, it is the first work dealing with the phenomenologically related method of CD.

The book is divided into twelve chapters and is equally useful to both the beginner and the practising research chemist, as the treatment is quite lucid and broad with extensive bibliography. The first five chapters are introductory in nature while the remaining chapters deal with applications of the techniques to saturated ketones, compounds with inherently dissymmetric chromophores, unsaturated ketones, optically active dienes, polypeptides, proteins, etc.

The book is very well produced.

SUKH DEV

ORGANOMETALLIC SYNTHESIS: Vol. I — TRANSITION METAL COMPOUNDS by R. Bruce King (Academic Press Inc., New York), 1965. Pp. xii + 186. Price \$ 6.50

The chemistry of organometallic compounds has been the subject of intense research in recent years,

and procedures for the preparation of most of these compounds are widely scattered in the voluminous literature. In this book the author has compiled for the first time the preparative methods pertaining to the transition metal compounds. It begins with a foreword stressing the objective followed by a preface on the scope of the volume.

The book is broadly divided into two parts. Part I which consists of four chapters deals with the different techniques employed in organometallic chemistry. The first three chapters describe in some detail the preparation of the compounds, their isolation and purification, and identification in that order. Under identification the area of usefulness as well as the limitations of the physico-chemical instruments commonly employed such as UV, visible and IR spectrophotometers and NMR have been clearly brought out. Mention is also made of the potential use of mass spectrometry in the determination of molecular weights as also of structure through fragmentation patterns. The chapter ends with a brief note on the application of the Mössbauer resonance spectroscopy to organo-iron compounds. The fourth and final chapter in Part I is devoted to precautions against oxidation, toxic materials, and fire and explosion. For the isolation of the organometallic cations, the author recommends the use of the equally well crystallizable tetrafluoroborates and hexafluorophosphates in place of the rather explosive perchlorates.

Part II consists of precise and specific directions for the preparation of over 60 organometallic compounds of transition metals. Every procedure is claimed to have been either tested in the author's laboratory or confirmed independently by two or more groups of workers. References to original articles are given wherever necessary. Special mention should also be made of the excellent illustrations of the experimental set-ups which will help in particular the beginner who is keen to get started.

Perhaps a short chapter touching on the modern trends and concepts of organometallic chemistry in relation to bonding and structure would not have been out of place in this volume. On the whole the book is very readable and well printed and should indeed be welcome to every synthetic chemist.

P. R. SUBBARAMAN

GENERAL CHEMISTRY by N. Glinka; translated from the Russian by David Sobolev (Gordon & Breach Science Publishers, New York), 1965. Pp. 694. Price \$ 17.50

This is the first American edition of the book originally printed in English in Russia several years ago. It has no preface or foreword. The introductory chapter deals with matter and motion, substances and their changes, subject and method of chemistry, and birth and initial stages of chemistry.

Chapters II-VIII, which may be regarded as physical chemistry, are concerned with atoms and molecules, periodic law of Mendeleev, atomic structure, structure of molecules and of solid substances, development of the periodic law, chemical kinetics

and chemical equilibrium. Chapters IX-XXV deal with nonmetallic and metallic elements and their compounds, properties, and uses by groups according to the periodic table.

The organic chemistry, needed in a general chemistry course, is included under carbon group. A brief account is also given of fuel gases, plastics, fibres and rubbers, proteins, sugars and starches, oils and fats. Under silicon, mention is made of glass, cement, ceramics and silicones of technical importance. Colloids too are included in this chapter, with particular emphasis on technically and industrially important colloids.

There is a very commendable bias towards practical applications of chemical principles, useful applications of chemical substances in daily life, agriculture and in industry. It is also of interest to be told of the achievements of Russian scientists but not many non-Russians would appreciate the omission of well-known non-Russian scientists or the insistence on 'materialistic dialectics' (p. 19) or 'the eternity of matter and its motion' (p. 18).

The presentation remains definitely old-fashioned. Mention is made of quantum mechanical theory of chemical bond, electron orbitals, shape and properties of molecules, energy and so on, but they are rarely applied to actual chemical substances in the course of the book. The absence of sections 4 and 5 of Chapter I found in the Russian edition of 1958 in the contents (p. 9) is conspicuous.

L. M. YEDANAPALLI

SILLIMANITE by E. R. Varley (Her Majesty's Stationery Office, London), 1965. Pp. v+165. Price 15s.

In industry, the term sillimanite covers andalusite kyanite, sillimanite and mullite. Different aspects of this mineral group, viz. mineralogy, origin and mode of occurrence, grade and reserves, mining and beneficiation, calcination, uses, trade requirements, specifications and testing, production, marketing and prices, and substitutes and synthetic materials are treated briefly.

A brief account of sillimanite deposits of various countries is given in two sections, namely 'Sillimanite in Commonwealth Countries' and 'Sillimanite in Foreign Countries'. Tables comprising up-to-date data on production and exports are given for leading producers like India and the USA.

The author has taken pains to make an exhaustive literature survey and has brought between the covers of a slim book all the important information available on sillimanite group of minerals. The bibliography provided at the end of the volume is comprehensive and should prove very helpful to workers in the field. The volume should serve as a ready reference book on the subject.

D. R. ANANDA KUMAR

THE ANATOMY OF THE LABORATORY MOUSE by Margaret J. Cook (Academic Press Inc., New York), 1965. Pp. 143. Price 35s.

A comprehensive anatomy of the mouse has not been made so far, although certain parts of it, such as vertebral column, have been investigated in detail. The present anatomical atlas remedies this deficiency to a certain extent. The various illustrations arranged under the heads externals, skeleton, viscera and the circulatory system are followed by an index. All the illustrations, mostly line drawings, are clear and attractive. The illustrations of circulatory system drawn in red and blue greatly facilitate the distinguishing of arteries from veins.

The author has undertaken a commendable effort in bringing out this edition. This book will be a valuable addition to the college libraries.

KULDIP CHAND

PUBLICATIONS RECEIVED

MOLECULAR BIOPHYSICS edited by Bernard Pullman & M. Weissbluth (Academic Press Inc., New York), 1965. Pp. x+452. Price \$ 19.50

DYNAMICAL THEORY OF GROUPS AND FIELDS by Bryce S. Dewitt (Gordon & Breach Science Publishers, New York), 1965. Pp. xv+248. Price \$ 2.95 (paper); \$ 5.95 (cloth)

CALCULUS OF VARIATIONS AND PARTIAL DIFFERENTIAL EQUATIONS OF THE FIRST ORDER: Vol. 1, by C. Carathéodory (Holden-Day Inc., San Francisco), 1965. Pp. xvi+171

STARCH: CHEMISTRY AND TECHNOLOGY: Vol. 1 — FUNDAMENTAL ASPECTS edited by R. L. Whistler & Eugene F. Paschall (Academic Press Inc., New York), 1965. Pp. xviii+579. Price \$ 22.00

PRINCIPLES OF SENSORY EVALUATION OF FOOD by M. A. Amerine, R. M. Pangborn & E. B. Roessler (Academic Press Inc., New York), 1965. Pp. x+602. Price \$ 19.50

THEORY OF OPTIMUM AERODYNAMIC SHAPES edited by Angelo Miele (Academic Press Inc., New York), 1965. Pp. xix+454. Price \$ 16.50

SULFONATION AND RELATED REACTIONS by E. E. Gilbert (John Wiley & Sons Inc., New York), 1965. Pp. xi+529. Price \$ 16.50

HIGH PRESSURE MERCURY VAPOUR LAMPS AND THEIR APPLICATIONS edited by W. Elenbaas (Centrex Publishing Co., Eindhoven), 1965. Pp. xi+303. Price Rs 44

THERMAL NEUTRON SCATTERING by P. A. Egelstaff (Academic Press Inc., New York), 1965. Pp. xv+523. Price 115s.

ALLOYING BEHAVIOUR AND EFFECTS IN CONCENTRATED SOLID SOLUTIONS: Vol. 29, edited by T. B. Massalski (Gordon & Breach Science Publishers, New York), 1965. Pp. ix+445. Price \$ 9.50 (paper); \$ 22.50 (cloth)

NOTES & NEWS

New absolute temperature scale for the range 4-14°K.

The Institute for Basic Standards of the National Bureau of Standards (NBS) has established a new absolute scale of temperature for the region between 4° and 14°K., based on the 'acoustical thermometer'. The new scale bridges the gap between the lower limit (10°K.) of the NBS 1955 provisional scale and the temperatures (2-5°K.) defined by the T_{68} helium vapour pressure scale. Thus, the NBS is now in a position to calibrate thermometers for industry at one degree intervals over the range 2-20°K.

Accurate measurement of temperatures below 20°K. plays an important role in the aerospace and other industries, e.g. in the development of cryogenic fuels for rockets and missiles, work on solid state devices for spaceborne computers, design of high field magnets and studies on the phenomenon of superconductivity.

The acoustical thermometer works on the principle that the absolute temperature is proportional to the square of the speed in an ideal gas (i.e. at zero pressure). It consists of a resonant (variable path, fixed frequency sound wave) tube which is used to determine the wavelength and hence the speed of the sound wave. In practice, a real gas is to be used and the effect of pressure has to be considered. The speed of the sound wave is determined at sufficiently low pressures such that a plot of speed versus pressure is linear and it is extrapolated to zero pressure, thus eliminating the need for pressure corrections. For measuring temperatures below 20°K. germanium resistance thermometers are usually employed. Those submitted for the NBS calibration service are compared with standard germanium resistance thermometers of high sensitivity that have been calibrated with reference to the acoustical thermometer [*Tech. News, Bull. U.S. nat. Bur. Stand.*, 49 (No. 7) (1965), 102].

First observation of Hall effect in superconductors

Hall effect has been observed for the first time in superconductors (niobium and indium) by scientists at the Bell Telephone Laboratories, Murray Hill, New Jersey. Several earlier attempts to detect the Hall effect in tin and lead in superconducting state were not successful and this even led to theoretical speculations that Hall effect would be unmeasurable in a superconductor. Even though, theoretical investigations of the Magnus force on the quantized flux lines moving in a type II superconductor indicated that the Magnus force on the flux line will lead to a large Hall angle, experimental results on type II superconductors of short mean free path did not bear out this anticipation. Subsequent studies pointed out the flaws in earlier theoretical investigations. In view of this historical background the present observation of the Hall effect is significant.

The Hall effect was detected in a niobium plate (15.2 mm. long, 3.2 mm. wide and 0.43 mm. thick) cut from a single crystal (residual resistance ratio = 1550), when the magnetic field applied across the specimen is gradually increased. Current leads were soldered across the ends of the sample to ensure uniform current density and a pair of transverse (Hall) leads were soldered to the narrow edges at the middle. The potentials were recorded continuously on the y-axis of an x-y recorder whose axis is driven by a flux meter linear in H . Recordings of the transverse voltages were made for the four combinations of forward and reverse current and forward and reverse field and the Hall voltage was determined from these observations. The Hall voltage is found to appear at the same magnetic field (H_0) where the resistive voltage of the sample also begins to appear. The tangent of the Hall angle is found to be proportional to the magnetic

field. Studies made with a type I superconductor in the intermediate state (where the resistance of the specimen is non-zero at high current densities) show that in this case also the Hall voltage and resistance voltage vanish at the same field, though the field dependence of the Hall voltage in the intermediate state appears to be quite different from that in the mixed state [*Phys. Rev. Lett.*, 14 (1965), 790].

Raman laser excited by an ordinary laser

Observations made using an ordinary (non-giant) ruby laser at the National Bureau of Standards, Boulder, Colorado, have indicated that it is possible to observe stimulated Raman effect in benzene not only at the hitherto observed ν_2 Stokes line but also at the $2\nu_2$, $3\nu_2$ and $4\nu_2$ and the ν_1 Stokes line of benzene. Though earlier investigations have shown that Raman active materials in an optical cavity builds up optical radiation at one or more Raman-shifted frequencies, these were observed only when the excitation was due to strong radiations from a giant-pulse laser.

The observations were made on a ruby rod (75 mm. length and 6 mm. diam. polished to $\lambda/10$ and parallel to 2 sec. of arc) with a dielectric anti-reflection coating on one end and a multilayer dielectric coating (99.6 per cent reflectivity) on the other. The concentration of Cr^{3+} ion was 0.04 per cent and the rod axis was perpendicular to the optical axis of the ruby crystal. A plane mirror of 95 per cent reflectivity and the end of the ruby rod with high reflectivity formed an optical resonator for both the ruby and the Raman laser. A Raman cell which has anti-reflection coated optical flats as windows and forming an effective optical length of 95 mm. was inserted as the optical resonator and benzene was used as the Raman active liquid. Observations made on the stimulated Raman radiations showed that the beam contained ν_2 , $2\nu_2$, $3\nu_2$ and $4\nu_2$ Stokes lines as well as the first ν_1 Stokes line. The threshold exciting power for laser action in the ν_2 Stokes line has been measured to be 9.5 kW. From

the analysis of the rate equation for the Raman Stokes line, the scattering cross-section for the ν_2 Raman line of benzene has been determined as $\sigma = 0.46 \times 10^{-28} \text{ cm}^2$. It is also shown from qualitative analysis of the rate equation that the efficiency of Raman laser action can be improved to more than 30 times the value actually observed in the present investigation, when a continuous operating Ar gas laser under optimum choice of operating is used as the stimulator instead of the ruby laser [*Proc. Inst. elect. electron. Engrs.*, **53** (1965), 146].

Synthesis of cubane, a new box-like compound

Synthesis of a new molecule, called cubane, because of its box-like chemical structure, has been achieved at the University of Chicago, USA. The cubane molecule (X) differs from other hydrocarbons in two respects: (i) the carbon atoms form a cube producing a box-like or cage structure with a cavity in the centre, and (ii) each carbon in this molecule is bonded to three carbon atoms and one hydrogen atom. Cubane derivatives have potential practical applications in combating virus infections and as pesticides, fungicides and herbicides.

For obtaining cubane, the bromocyclopentadienone dimer (I) is first converted to the bisethylene ketal by refluxing with ethylene glycol and excess *p*-toluene sulphonic acid in benzene solution. Selective regeneration of the 1-carbonyl group with conc. aq. hydrochloric acid gives the 1-keto-8-ethylene ketal (II) in 85 per cent yield which under ultraviolet radiation in benzene solution leads quickly to the cage compound (III) in nearly quantitative yields.

Hot aq. 10 per cent potassium hydroxide readily converts (III) to the acid (IV). The *t*-butyl ester (V), prepared by the reaction of the acid chloride of (IV) with *t*-butyl hydroperoxide and pyridine in anhydrous ether, undergoes ready radical fragmentation in boiling cumene. Some of the alkyl radicals thus formed (40 per cent) combine with the connate *t*-butoxy radicals, but the major fraction (55 per cent) escapes this and extracts hydrogen from the solvent to give (VI). Hydrolysis of the ketal (VI) in 75 per cent aq. sulphuric acid gives the hydrate of ketone (VII) from which the anhydrous ketone is generated by desiccation in boiling benzene and crystallization from hexane. Cubane carboxylic acid (VIII) forms slowly from (VII) on refluxing with 25 per cent aq. potassium hydroxide. Cubane (X; m.p. $130-13^\circ$) is produced by thermal decomposition at 150° of the *t*-butyl perester (IX) of cubane carboxylic acid in diisopropylbenzene [*J. Amer. chem. Soc.*, **86** (1964), 3157].

Disulphide interchange and the three-dimensional structure of proteins

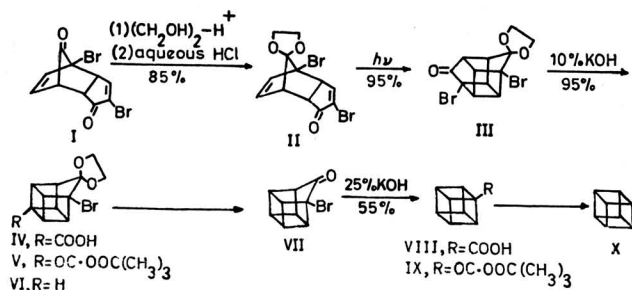
According to Anfinsen the information necessary for the correct folding of a protein resides in the linear sequence of the amino acids of the polypeptide chain. Thus the tertiary structure of a protein is completely defined by its own primary structure. To obtain experimental evidence for the above concept, D. Givol, F. De Lorenzo, R. F. Goldberger and C. B. Anfinsen [*Proc. nat. Acad. Sci., Wash.*, **53** (1965), 676] have studied the action of a microsomal sulphhydryl disulphide-interchange enzyme on proteins having single

and multichains. They have also attempted to find out whether the information contained in the sequential arrangement of the amino acids in proteins having only single peptide chains is affected when they are converted to multichain proteins by chemical modification or by proteolytic cleavage. In this study RNase and chymotrypsinogen were chosen to represent single-chain proteins. The 'C-protein' derivative of RNase prepared by cleavage of peptide bonds adjacent to methionine residues in the protein, using cyanogen bromide and α -chymotrypsin, represent multichain proteins. A study has also been made of the effect of disulphide-interchange enzyme on insulin.

Oxidation of reduced RNase either spontaneously in the presence of 8M urea or by incubation with dehydroascorbic acid produces an enzymatically inactive product. This inactive product is rapidly activated by the disulphide-interchange enzyme in the presence of β -mercaptoethanol. However, incubation of the interchange enzyme under similar conditions with the 'C-protein' derivative of RNase resulted in aggregation and precipitation, indicating formation of polymeric products due to incorrect pairing of half-cystine residues.

Chymotrypsin, reversibly denatured by 8M urea, regained 85 per cent of the activity on a 100-fold dilution with 0.1M Tris buffer (*pH* 7.5) with or without $10^{-3}M$ β -mercaptoethanol. The presence of the interchange enzyme and β -mercaptoethanol ($10^{-3}M$) in the diluting solution caused rapid inactivation of chymotrypsin. However, when chymotrypsinogen was subjected to similar treatment, the protein was found to be not susceptible to inactivation by the disulphide-interchange enzyme. It was concluded that the inactivation of α -chymotrypsin by the interchange enzyme was due to random rearrangement of the disulphide bonds.

The authors have provided evidence in support of the hypothesis that the information for the correct three-dimensional structure of a single-chain protein is contained in its amino acid sequence and that this information is seriously affected when the



original single-chain polypeptide is fragmented.

Incubation of insulin with the disulphide-interchange enzyme in the presence of β -mercaptoethanol led to precipitation of the hormone. Analysis of the precipitated protein after performic acid oxidation showed that it contained an increased concentration of the B-chain. It was concluded from this that during the process of interchange new intrachain disulphide bonds were formed leading to the separation of the A- and B-chains.

Further evidence for the change in the three-dimensional structure of insulin on treatment with the disulphide-interchange enzyme was obtained from the immunoassay of insulin carried out essentially by the procedure of Berson *et al.* The chromatographic pattern of a solution of ^{131}I labelled insulin incubated with bovine serum albumin (BSA), the interchange enzyme and β -mercaptoethanol in 0.1M Tris buffer (pH 7.2) revealed a loss in the capacity of the hormone to complex with the antibody, indicating a change in the antigenically specific structure of the hormone.

The results with insulin support the view that the hormone is derived, by a zymogen-like conversion, from a protein originally synthesized as a single chain, containing the information for the correct pairing of the disulphide bonds.—B. N. MANJULA

Role of labile sulphide in metalloflavoprotein catalysis

Absorption spectra of metalloflavoproteins differ from those of other flavoproteins in showing absorption at wavelengths longer than 500 μ , enhanced absorption in the 450 μ region and less distinct or even totally absent absorption maximum in the 370 μ region. The deviations from the absorption spectra of free flavins are correlated to some extent with the metal content of the enzyme. The direct correlation between metal content and spectral characteristics, together with the results of studies with model systems, have led to the suggestion that some form of interaction between the metal and prosthetic group might be responsible for

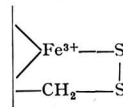
the observed deviations. The presence of labile sulphide in a flavoprotein, succinic dehydrogenase, together with the effect of urea, and mercurials on the spectrum and rate of reaction with iron chelators suggested that a more complex arrangement might be involved.

A recent study on xanthine oxidase and other metal flavoproteins [Brumby, P. E., Miller, R. W. & Massey, V., *J. biol. Chem.*, **240** (1965), 2222] has provided evidence regarding the involvement of labile sulphide groups in flavoprotein catalysis. The reduction of liver xanthine oxidase under anaerobic conditions in the presence of mercurials (e.g. *p*-chloromercuribenzoate) resulted in extensive degradation, but inhibition during turnover was not accompanied by such an effect, suggesting that for this to take place the enzyme must be in a more reduced state than was attained during catalysis. Such an explanation would be compatible with the observed correlation between the rates of inactivation and of secondary spectral changes. In the biphasic pattern of absorbance, the rapid primary phase may be indicative of the formation of an intermediate in which the flavin was at least reduced partially and which was probably insensitive to inactivation by mercurials. The slow secondary phase might then represent transfer of electrons through this intermediate to some other acceptor leading to the formation of a structure very sensitive to inactivation and degradation by mercurials. This enhanced sensitivity implies that mercurials react with some sulphhydryl groups available under reducing conditions.

The increased sensitivity to mercurials under reducing conditions was not caused by substrate inducing a conformational change in protein which 'uncovers' the sulphhydryl groups, since the same increase in sulphhydryls titrating in the presence of hypoxanthine was obtained under a variety of conditions.

The peculiar lability of this structure (low pH, boiling urea, high concentration of mercurials) clearly implies a different disulphide bond from that normally found

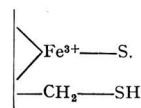
in proteins. The correlation between the number of these labile sulphide groups and the number of iron atoms suggests that one linkage of the disulphide ~~in the~~ protein may be through an iron atom, the other presumably being contributed by a cysteine residue of the protein (I)



(I)

As all the examined metalloflavoproteins contain sulphide residues, it was reasonable to conclude that these have some common function. The ability of the substrate to reduce the labile sulphide of xanthine oxidase suggests that such reduction may be concerned in catalysis.

In the case of dihydroorotic dehydrogenase, evidence has been presented [Miller, R. W. & Massey, V., *J. biol. Chem.*, **240** (1965), 1453] that in this catalysis the iron sulphide functions as an iron sulphur radical (II)



(II)

— P. A. KUMAR

Estrogen action and ribonucleic acid

The mode of action of estrogen has been the subject of several recent investigations. According to a current concept, the estrogen acts by inducing DNA-primed RNA biosynthesis. The newly synthesized RNA pervades the cells of the target tissue—the uterus—and produces structural and enzymatic alterations. Thus estradiol induced vaginal cornification in ovariectomized rats could be prevented by an intravaginal application of actinomycin D, which is known to inhibit the synthesis of DNA dependent RNA synthesis. It has been shown that estradiol, through a specific receptor system in the uterus, stimulates the synthesis of certain proteins necessary for the induced synthesis of new RNA which in

turn supplies the information essential for the synthesis of new proteins and manifestation of hormone action.

Biochemical studies have shown that uterine alkaline phosphatase increases after estrogen administration. Androgen and progesterone do not affect the enzyme activity. Ovariectomy results in the regression of the uterus and decrease in the enzyme activity. These effects of ovariectomy could be reversed by the administration of estrogen. RNA isolated from the uteri of intact female rats applied to the uterus causes stimulatory changes similar to those induced by estrogen [Segal, S. J. & Davidson, D. W., *Abstracts of the sixth international congress of biochemistry*, New York, 1965, 733].

In a recent investigation the role of RNA in affecting the alkaline phosphatase activity of the mouse uterus has been studied [Maher, M. & Niu, M. C., *Proc. nat. Acad. Sci., Wash.*, **53** (1965), 764]. As in the rat, ovariectomy in the mouse results in a reduction in the uterine alkaline phosphatase activity. Intrauterine administration of RNA isolated from pooled intact mature mice results in 50 per cent increase in the enzyme activity over the control ovariectomized saline treated uteri in three days. This effect of increasing the enzyme activity in ovariectomized mice is specific to RNA isolated from pooled uteri of intact mature mice. In contrast, RNA isolated from other sources like liver had no effect on the enzyme activity. Ribonuclease treatment or boiling of the uterine RNA destroyed its effect in restoring the enzyme activity in ovariectomized mice. Boar seminal vesicle RNA (an organ rich in alkaline phosphatase) is effective in increasing the enzyme activity.

Histological studies reveal a striking effect of RNA treatment. The endometrium of sexually mature mouse is characterized by columnar epithelium, glandular proliferation and relatively high content of alkaline phosphatase. Ovariectomy produces morphological changes in addition to a change in the enzyme activity. Histological examination shows the stimulatory effect of RNA treatment as seen in the increase in

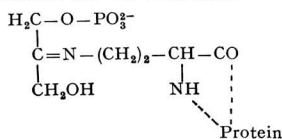
the number of endometrial glands over the control uteri. Uterine RNA from pooled uteri of mature intact mice is able to reverse the effect of ovariectomy, demonstrating that the effect of estrogen on the uterus is mediated by RNA. This effect of uterine RNA sustains for 3 days after administration.

It is unlikely that the observed increase in enzyme activity is due to contamination of the uterine RNA by the enzyme activity itself as the procedure of RNA preparation is not favourable for such a contamination.—M. R. SAIRAM

Mechanism of action of aldolases

Deoxyribose-5-phosphate aldolase which catalyses the reversible reaction, acetaldehyde + D-glyceraldehyde-3-phosphate \rightleftharpoons 2-deoxy-D-ribose-5-phosphate was discovered in mammalian liver and as a constitutive enzyme in *Escherichia coli*.

Rabbit muscle aldolase has been found to interact with dihydroxyacetone phosphate to yield a Schiff's base intermediate in which ϵ -amino groups of specific lysine residues of the enzyme are involved. It may be concluded that the active intermediate formed with aldolase has the structure:

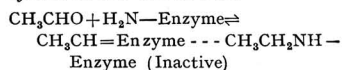


Reduction of the Schiff's base intermediate with borohydride followed by acid hydrolysis permitted the isolation and identification of the reduced lysine substrate derivative as N $^{\epsilon}$ - β -glyceryl lysine.

Propionaldehyde has been found to serve as a substrate for deoxyribose-5-phosphate aldolase. A new series of 2-methyl-2-deoxy-sugars was formed when the enzyme was incubated with propionaldehyde and suitable acceptor aldehydes.

Reduction of the enzyme with sodium borohydride at pH 6 in the presence of acetaldehyde resulted in loss of enzyme activity. This has been shown to result by

blocking of the ϵ -amino group of lysine at the active site.



It has been proposed by O. M. Rosen, P. Hoffee and B. L. Horecker [*J. biol. Chem.*, **240** (1965), 1517] that activation of substrates in enzyme catalysed aldol condensation reaction involves the formation of a carbanion which condenses with the acceptor carboxyl group to form the product. When acetaldehyde was incubated in tritiated water with deoxyribose-5-phosphate aldolase there was a rapid uptake of tritium which approached 3 equivalents per mole, while in the case of propionaldehyde only one equivalent per mole was incorporated. The presence of a carboxylic acid was essential for tritium exchange into the aldehyde and presumably for the formation of the active carbanion.

On incubating ^{14}C -propionaldehyde with DL-glyceraldehyde-3-phosphate and enzyme in potassium maleate buffer a 2-methyl-2-deoxy sugar was obtained as evidenced by sequential oxidation with periodate and bromine. Methylmalonic acid was the single radioactive product formed.

Unlike the reaction with acetaldehyde, product formation with propionaldehyde occurred to a greater extent with L-glyceraldehyde-3-phosphate than with the D-isomer. The K_{eq} measured in the direction of product formation was $31.0M^{-1}$ for the former and $1.24M^{-1}$ for the latter. The evidence suggests that D-glyceraldehyde-3-phosphate may function as an acceptor aldehyde, but steric hindrance of furanose ring formation shifts the equilibrium towards dealdolization.—K. V. JOHN

Order of induction and deinduction of the enzymes of the lactose operon in *Escherichia coli*

The lactose operon in *Escherichia coli* consists of a group of linked genes controlling the synthesis of β -galactosidase, galactoside permease and thiogalactoside transacetylase. The polycistronic concept of messenger RNA controlling the synthesis of the enzymes of a

single operon is gaining experimental support. It is of interest to know the sequence of translation of the messenger RNA as, for example, in the case of the lactose operon, with the basic assumption that a single messenger RNA molecule covers the entire lac operon.

Studies on the early kinetics of β -galactosidase induction have shown that after the addition of the inducer, the rate of enzyme synthesis becomes constant after a lag period of 2-3 min. In *Escherichia coli* Hfrc (+z+y+) the lag period for the appearance of transacetylase after the addition of the inducer (IPTG) is 5 min. [Alpers, D. H. & Tomkins, G. M., *Proc. nat. Acad. Sci., Wash.*, **53** (1965), 797]. These authors have not been able to detect any transacetylase activity during the lag period even with the very sensitive method of assay employed. The presence of an inhibitor during the lag period has been ruled out. It has also been shown that the interval between galactosidase and transacetylase appearance is independent of permease activity. The possibility of the formation of an inactive enzyme precursor which may slowly assemble into the active form of transacetylase is also not considered feasible on the basis of experimental facts. An explanation that the transacetylase can be released from the ribosomes only as a complex with galactosidase cannot be substantiated since the lag period is very much the same in a strain which makes twice as much galactosidase as normal but only the wild type level of acetylase. Thus the information for β -galactosidase synthesis is translated earlier to that of transacetylase and the translation has to begin at the operator end of the messenger and proceed away from it. Since the biosynthesis of a protein chain proceeds from the N- to the C-terminals, it can be held that the N-terminal amino acids of the β -galactosidase and transacetylase are coded for by the proteins of DNA (and messenger RNA) closest to the operator.

The kinetics of deinduction of β -galactosidase and transacetylase, where the inducer concentration is suddenly reduced by dilution, indicate that the synthesis of

transacetylase proceeds for 1-2 min. after the synthesis of galactosidase has stopped. Deinduction can prevent the attachment of new ribosomes to the operator end of the messenger in which case the ribosomes that are already attached to the messenger can complete their journey and fall off after the translation of the acetylase gene. It has also been indicated that deinduction brings about degradation of messenger RNA. The experimental results indicate that such a degradation should proceed sequentially from the operator end. Since the enzymes implicated in messenger RNA breakdown in *Escherichia coli* (K^+ activated phosphodiesterase and polynucleotide phosphorylase) degrade nucleotides from their free 3'-hydroxyl end, it is obvious from the experimental data on the order of induction and deinduction of the enzymes of the lac operon, that translation of the messenger should also begin at the free 3'-hydroxyl end of polynucleotide.—G. PADMANABAN

Progress Reports

Weizmann Institute of Science, Israel

The annual report of the Weizmann Institute of Science, Israel, for the year 1962-63 makes a broad survey of its achievements in different natural sciences.

The determination of the f -values for some of the transition states in helium, the development of effective methods for treating the seismic problem, the discovery of the existence of the diffracted pulses and the design and construction of a new computer called the GOLEM are some of the important achievements of the Applied Mathematics Section. A complete solution has been obtained for the propagation of a compressional pulse in a liquid sphere. The diffracted pulses have been found to appear in regions where they are disallowed by ray theory. A method has been developed for solving exactly a system of linear equations with rational coefficients by converting it to a number of systems of congruences, modulo certain prime numbers and assimilating the solutions of the

congruences using Chinese Remainder Theorem. The new GOLEM computer has inherent speed, high precision and some special features, e.g., automatic control, flowgating and interplay.

In the Biochemistry Section, studies on photophosphorylation catalysed by chloroplast, ribosomes and isolation, purification, properties and biosynthesis of ribonucleic acids have yielded important results. While studying the mode of formation of ATP, a protein has been extracted from chloroplasts which may play a part in the transfer of energy to ATP. Chloroplasts lacking this protein have been found to be unable to convert light energy to ATP. The study of the complex series of photoreductions catalysed by indophenol dyes has shown that these dyes, representing an unusual group of acceptors that do not promote the formation of ATP during their reduction, behave in this manner due to a delicate balance of various properties of the dyes under certain experimental conditions and when the conditions are changed in some way or other, the dyes have been found to either inhibit or promote the formation of ATP by chloroplasts. Several potent new compounds inhibiting the formation of ATP and the transport of oxidizing and reducing power in chloroplasts have been discovered.

In *Esch. coli*, RNase has been found to be attached exclusively to the 30S ribosome and this has led to the suggestion that the enzyme has a role in protein biosynthesis, and that the protein synthesizing apparatus may be divided between the 30S and the 50S ribosomes. About 10 per cent of the DNase activity of *Esch. coli* has been found to be attached to the 70S ribosomes. Most of the enzyme has been found to be detached when the ribosomes are dissociated into 30S and 50S particles and becomes firmly reattached when the particles are caused to reassociate and again form 70S ribosomes. Ribosomal β -galactosidase has been found to behave quite differently from the ribosomal DNase. A general method for the isolation of *t*-RNA specific for a single amino acid has been developed.

Pseudouridine has been isolated from commercial uridine by boiling in hydrazine hydrate. A new method has been developed for purifying the polynucleotide phosphorylase of *Esch. coli*.

In the Biodynamics Section, nidation, ovulation, mannoheptulose and the biological aspects of the breeding habits of desert rodents were under investigation.

Immunological studies on gonadotropins have led to the improvement and purification of the material, and to clear understanding of the various components. Enough technical and theoretical information leading to the betterment of the techniques for measurement of gonadotropins in urine and blood and for the detection and quantitation of biological and immunological activity, as well as of impurities in concentrated and relatively purified extracts has been obtained. In the Biophysics Section, some of the physico-chemical properties of polyamino acids in solution have been explored theoretically as well as experimentally. New techniques have been worked out for the selective chemical cleavage of peptides and proteins. A method for the synthesis of L-cysteine peptides has been devised. A new procedure for the synthesis of poly-L-lysine, via ϵ , N-trifluoro-acetyl- α , N-carboxyl-lysine anhydride has been evolved. A new technique has been worked out for the preparation of poly-L-arginine from poly-L-ornithine using 1-guanyl-3,5-dimethyl pyrazole nitrate as a guanidization reagent. A method of selective NBS-cleavage of histidyl bonds has been developed. Optical rotatory dispersion and infrared studies made on solutions of poly-1-benzyl-L-histidine (PBLH) in the solvent system chloroform-dichloroacetic acid (CHCl_3 -DCA) have indicated that this polymer exists in three different forms depending on the solvent composition. The investigation of the protein complexes occurring in soybean has shown that soybean meal contains, in addition to free oligo- and polysaccharides, neutral carbohydrates linked to proteins. The presence in soybean of a material associating reversibly with the triglycoside 1,3,5-tri-(β -glycosyloxyphenylazo)-2,4,6-tri-

hydroxy-benzene to form specific precipitates has been detected. A new method for the isolation of large quantities of cell walls and for fractionation by column chromatography of the lysozyme digests of these walls has been devised.

In the Cell Biology Section, studies have been made on the molecular determinants of cell differentiation. It has been found that the thymus induces the differentiation of cells originating elsewhere in the organism. A theory suggesting a selective action of chemical carcinogens in tumour induction has been developed. In the Chemical Immunology Section, the synthesis of highly radioactive antigenic and non-antigenic multichain polypeptides, making use of tritiated alanine, has been made. A method for the immuno-specific antibodies to small antigens, natural or synthetic, has been developed. A substance has been found in a multichain copolypeptide that induces in guinea-pigs a prolonged state of delayed hypersensitivity not associated with detectable circulating antibodies. Poly-DL-alanyl trypsin and poly-DL-alanyl chymotrypsin have been synthesized and characterized.

In the Electronics Section, several new instruments have been designed. A detailed study of the symmetry of the linear optical properties of magnetic materials has led to the prediction of a new optical effect, namely nonreciprocal birefringence. An automatic recording, constant velocity Mössbauer spectrometer for ^{57}Fe has been designed and constructed. A set of five instruments has been designed and constructed for the measurement of minute vibrations (with amplitude of the order of a few Angstroms) and for providing suitable permanent facilities for future research in magnetism. The suspension of a body without any corpuscular contact, with no surrounding fluid and without a servomechanism has been made possible. A new magnetic device consisting of a very small magnet in a plastic sheet, that can be introduced into such tubes in the human body as blood vessels or the oesophagus, has been developed. To enable simultaneous EEG recording from a few brain loca-

tions an arrangement for multiplexing in transistorized networks has been developed.

In the Experimental Biology Section, studies on N-hydroxyurethane metabolism have led to the development of a chemical method for its estimation in pure solutions and biological fluids. Work on the exploration of the possibility of obtaining a cell-free protective factor from heterologous sources (sheep spleen) has shown promising results. Study on the role of progesterone in mammary carcinogenesis has led to the unexpected finding that this hormone apparently enhances metastasis development among the mice bearing mammatrophic tumours. Several instruments have been developed for implantation of electrodes into the brain.

In the Genetics Section, the study of chemical carcinogenesis in tissue culture has shown that a hereditary cell transformation, resulting in a change of cellular organization can be induced by a short-term treatment of hamster and mouse cells with 3,4-benzopyrene and 3-methyl cholanthrene. Study on differentiation of lymphocytes and antibody formation has shown that after seeding on a feeder layer of mouse embryo cells, lymphocytes from the thymus of adult mice can differentiate to mast cells that produce histamine.

In the Infrared Spectroscopy Section, studies have been made on the properties of small molecules. While carrying out experiments on pressure-induced shifts two new effects have been observed. When DCl gas is perturbed by HCl, lines associated with ground states having odd J numbers have been found to shift towards higher frequencies, and those with even J towards the lower frequencies. When hydrogen fluoride is perturbed by xenon the pressure-induced shift of lines has been observed. A theory has been developed of vibration rotation interactions of the general linear molecule.

In the Isotope Research Section, the development of micromethods for the analysis of stable isotopes in materials of biochemical interest, the low level counting of the annihilation radiation of positron emitting isotopes and a double tracer technique for the

determination of the rate of iodination of thyroxine in humans are the important achievements. An improved large liquid scintillation counting system for the detection of naturally occurring tritium and ^{14}C and a pulse handling unit for low level radioactivity counting systems have been designed.

In the Nuclear Physics Section, studies have been made on nuclear structure, nuclear reactions, many-body theory and the Mössbauer effect. Mössbauer effect has been found to depend primarily on the source atom rather than on the host lattice. The measurement of the magnetic moments of the first excited states in even tungsten isotopes and in one isotope of osmium has shown the strong dependence of the magnetic moments on the atomic number. A new experimental technique for measuring mixing ratios between electric and magnetic transition probabilities by angular correlation methods in some particular low energy electromagnetic transitions and a new method for high resolution fast neutron spectroscopy have been developed. A heavy particle spectrometer consisting of specially designed electrostatic lenses and an electronic circuit capable of automatic and precise subtraction of random coincidences from recorded coincidence counts have also been developed.

Among the new compounds prepared in the Organic Chemistry Section are two isomeric bisdehydro-[12] annulenes and a number of A-homo-steroids with a heterocyclic ring attached to ring A and 3-methylcyclobutene. Electrophilic substitution reactions, such as nitration, sulphonation, etc., have been carried out with macrocyclic compounds for the first time. The structures of elateric acid, cannabidiol, a major constituent of hashish, antibiotic cannabidiolic acid, oleuropeic acid and pentaphenylphosphorane have been determined. Five nitrogen free compounds of a terpenoid nature have been isolated from a plant of the *Solanaceae* for the first time.

In the Photochemistry Section, investigation of the applicational aspects of the 'photochemical memory' system has been carried on. A 'flash photolysis' apparatus capable of operating in a

wide temperature range has been built.

In the Plant Genetics Section, much attention has been paid to the morphogenesis of reproductive plant. A proper radiocarbon tracing technique for both IAA and its precursor tryptophane and a technique for X-ray induced growth stimulation of corn seedling irradiated 24 hr after the beginning of germination (hydration) and growth under controlled light and temperature conditions have been found out.

Dr L. B. Singh

Dr Lal Behari Singh has been appointed Director, National Botanic Gardens, Lucknow.

Dr Singh (b. 1 June 1921) had his early education in Sultanpur district, UP. After graduating in agriculture from the Agra University in 1943, he proceeded to England where he obtained the Ph.D. degree in Pomology from the University of London in 1948. The same year he was appointed Assistant Professor of Horticulture, Government Agricultural College, Kanpur. In 1949, he joined the Fruit Research Station, Saharanpur, as Horticulturist and in 1957 he became the Director of the Horticultural Research Institute, Saharanpur, where he worked for five years and raised the Institute to the status of a first rate research institution.

Dr Singh's research work on papain during 1956-57 at Saharanpur earned for him the distinction of being the first to receive the Rafi Ahmad Memorial Award. He also won the Watumull Award, the first international award in the field of agriculture conferred by the Watumull Foundation, Hawaii, in 1961, for his notable work in the field of horticulture.

Dr Singh proceeded abroad in 1958 for special training at the leading horticulture research institutes in USA, UK and at other institutions in Europe for a short period under the Rockefeller Foundation Grant.

Dr Singh has published 59 research papers and is the author of three books on horticulture. His book on mango has been considered as an outstanding publication. He has also been the Editor of *Horticultural Advances*

(1957-62). Dr Singh is a Fellow of the Royal Horticultural Society, London.

New Periodicals

Brain Research

This new monthly journal (two volumes per year), to be published by Elsevier Publishing Co., will start appearing early in 1966. The journal is intended to serve as a medium for the publication of original research reports, short communications, review articles and book reviews dealing with the central or peripheral nervous system and related subjects. Its scope will cover the following disciplines: neuroanatomy, neurochemistry, neurophysiology, neuroendocrinology, neuropharmacology, neurocommunications, behavioural sciences, molecular neurology and biocybernetics. Results of clinical studies of fundamental importance having a direct bearing on the knowledge of the structure and function of the brain, spinal cord and the peripheral nerves will also be published. Subscription rate for the journal is \$ 16.00 or £ 5.12.6 per volume.

Announcement

■ *Atomic and Molecular Data Information Centre* — A specialized information centre called the 'Atomic and Molecular Processes Information Centre', which will collect, store, evaluate and disseminate information relating to atomic and molecular interactions from all over the world, has been established at the Oak Ridge National Laboratory, USA. At present, the activities of the centre will be limited to atomic and molecular cross-section data, and other particle collision process information in three areas: the interaction of heavy particles; particle penetration through matter; and excitation, dissociation, ionization and detachment by external electric and magnetic fields. Inquiries have to be addressed to C. F. Barnett, Director, Atomic and Molecular Processes Information Centre, Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, Tennessee, USA [*Sci. Inform. Notes* 7 (No. 3) (1965), 9].

New Publications

THE WEALTH OF INDIA

Industrial Products: Part VI (M-Pi)

Contains comprehensive account of development and present position of 28 Indian industries, important among them being Machinery, Motor Vehicles, Petroleum Refining, Pharmaceuticals, Paints & Varnishes, Paper & Paperboards, Metal Containers & Closures, Perfumery, Opium Alkaloids, Mica & Mica Products, Oxygen, Pin & Clips, Needles, Match and Papier Mache.

Pages xiv+315+xii

Demy 4to, 6 plates and 89 illustrations

Price Rs 28.00 (\$ 8.00, sh. 56)

DRUG ADDICTION

With Special Reference to India

by

R. N. CHOPRA & I. C. CHOPRA

Contains two parts consisting of 12 chapters dealing with: Drug addiction as a world problem, Addiction producing drugs, Psychiatric and clinical aspects of drug addiction, Treatment of drug addiction, Minor drug habits, Tobacco habit, Alcohol, Barbiturates, Cannabis, Opium, Cocaine, Control of drug addiction in India.

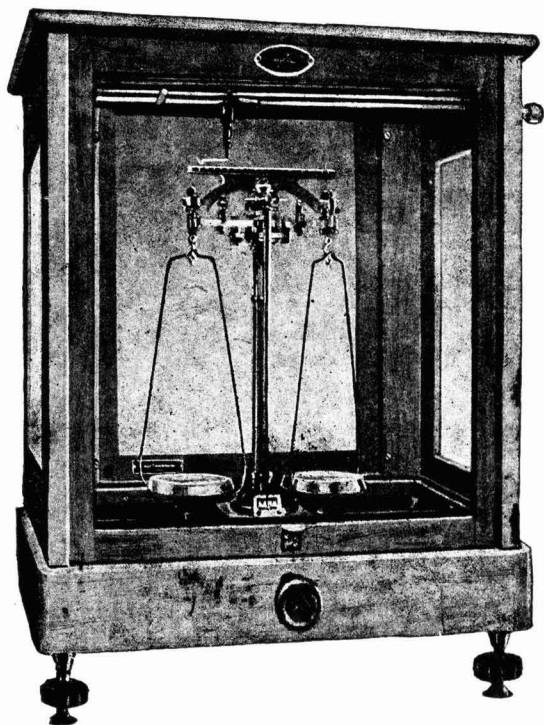
Pages xiii+264

Royal 8vo

Price Rs 12.00 (\$ 3.50, sh. 24)

Copies available from:

**Publications & Information Directorate, CSIR
Hillside Road, New Delhi 12**



'LAB-CHEM'

**ANALYTICAL BALANCES &
WEIGHTS**

for

**INDUSTRIAL, RESEARCH & COLLEGE
LABORATORIES**

Manufactured by

**LAB-CHEM BALANCE WORKS
BOMBAY II**



Contact Sole Selling Agents:

INDIA SCIENTIFIC TRADERS

DEALERS IN LABORATORY EQUIPMENT
OF EVERY DESCRIPTION

PEERBHOY MANSION

**460 SARDAR VALLABHBHAI PATEL ROAD
BOMBAY 4 (BR)**

Phone: 76336

Gram: 'Esvijack'

SETT & DE

16 Ganesh Chandra Avenue

Calcutta 13

Phone : 23-9588

manufacturers of

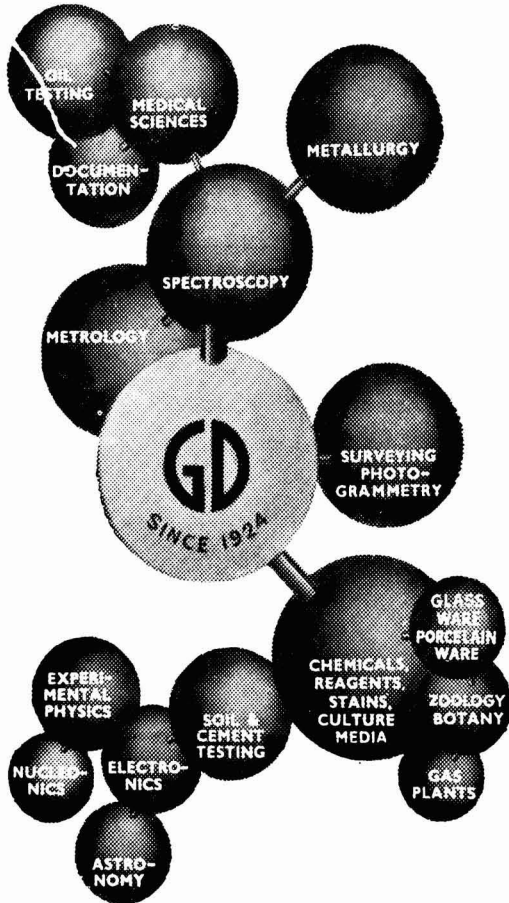
**MOVING COIL & MOVING IRON TYPE VOLTMETERS, AMMETERS (PORTABLE &
SWITCHBOARD TYPES), RESISTANCE BOXES, P.O. BOXES (PLUG &
DIAL), G.P.O. DETECTORS, EXTERNAL SHUNTS, RHEOSTATS
A.C./D.C. CONVERSION EQUIPMENT, ETC. ETC.**

for

LABORATORY AND INDUSTRY

OUR SERVICE DEPARTMENT IS ALSO AT YOUR DISPOSAL

A RANGE FROM THE HOUSE OF
SCIENTIFIC INSTRUMENTS



**GD GORDHANDAS
DESAI PVT. LTD.**

*Equipment and instruments for all branches of science,
technology and industry*

KERMANI BLDG. SIR PHEROZSHAH MEHTA RD., BOMBAY-1.
P.B. 1252, MADRAS-1. P.B. 328, CALCUTTA-1. P.B. 192, NEW DELHI-1.

OUR OWN LINE OF PRODUCTS

"GD" - Pressovac

Combined Pressure and Vacuum Pumps

"GD" - Optika Microscopes

Student and Medical Models

"GD" - Centrofix

Laboratory Centrifuges, 2, 4 and 8 tube models

"GD" - Stirrers

Laboratory and Industrial Electrical Stirrers, with or without hotplates

"GD" - Balances

Student and Precision, Chemical Models

"GD" - Laboratory Glassware

"GD" - Hardware

Gas and Water Taps, Burners, Clamp, etc.

"GD" - Orsat Gas Analysis Apparatus

"GD" - Biological Supplies

Demonstration and Museum Specimens, Charts, Agricultural, Medical and Veterinary Models, Micro prepared Slides, Stuffed Animals, Embalmed Specimens, Skeletal Preparations, Plastic Embedded Specimens, Slide Cabinets, Insect Boxes, Insect Cabinets, Card Index Cabinets, Vasculums, Herbarium Presses, Biokits, Specichromograms, Stains Alizarine Preparations, Aquaria, Micro Cover Glasses, Microtomes and Accessories, Trays, Inspissator

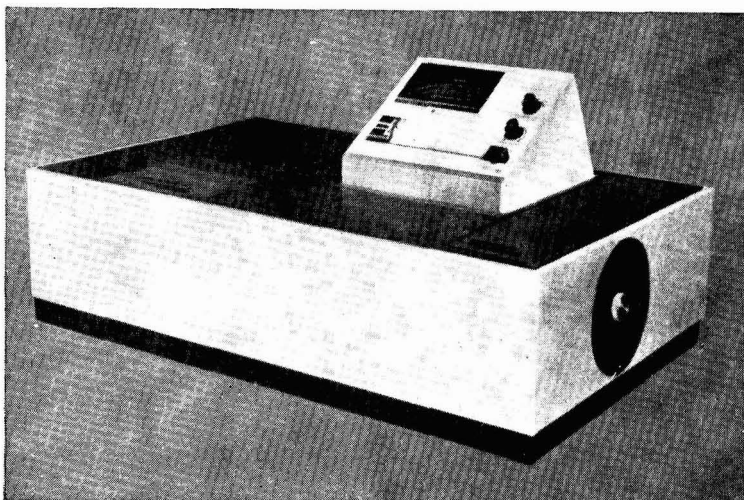
Bausch & Lomb

Spectronic 600E Spectrophotometer

Today there are enough good double beam UV Visible Spectrophotometers to make a choice difficult until you see the **Spectronic 600E**.

With two 1200 groove/mm. Gratings in series, you can take performance for granted. Wavelength accuracy and bandpass are 0.5 nm. over the entire range. . . stray light is less than 0.5 per cent.

And when you discover the low price, the choice becomes obvious.



Accessories Available

- V.O.M. RECORDERS
- DT-20 DIGITAL READOUT
- REFLECTANCE ATTACHMENT
- END-ON PHOTOMULTIPLIERS
- CONSTANT TEMPERATURE CELL
- MICRO CELLS
- FLAME ATTACHMENT
- U-V REFLECTANCE ATTACHMENT
- FLUORESCENCE ACCESSORY

For full information, please write for illustrated catalogue to
SOLE AGENTS

MARTIN & HARRIS (PRIVATE) LTD.

SCIENTIFIC DEPARTMENT

SAVOY CHAMBERS, WALLACE STREFT, BOMBAY 1 BR