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REVIEW ARTICLE

SYNTHETIC ŒSTROGENS

By E. C. Dodds,

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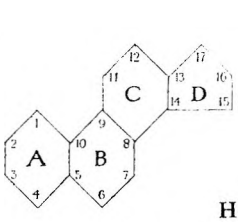
The field of chemotherapy in general has shown the large numbers of compounds which can be used for the same purposes. One only has to contemplate the development of the so-called "sulpha" drugs to provide an example of this. The first compound produced by Domagk[1] was the complex dye-stuff Prontosil. This substance obtained wide acceptance for the treatment of certain infections, but almost completely disappeared after the brilliant observations of Trefouel, Nitti and Bovet[2] that in the rabbit the compound was split at the azo linkage, liberating sulphonamide, and that this simple substance itself was active against certain infections. As we know, this was the start of a whole series of drugs of which there must be by now many hundreds on the market.

The same story can be told of the anti-syphilitic remedies, and the same is true of the anti-malarials. The physician wishing to treat either of these conditions has a wide range of compounds to choose from, many of them differing quite fundamentally in constitution, despite the fact that they all bring about the same therapeutic results.



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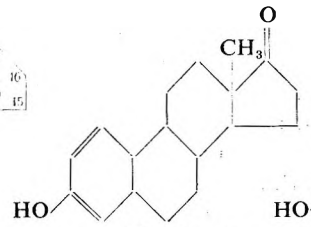
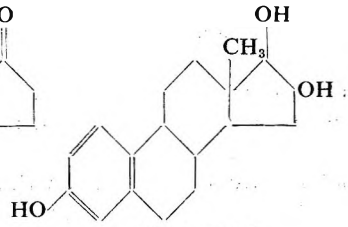
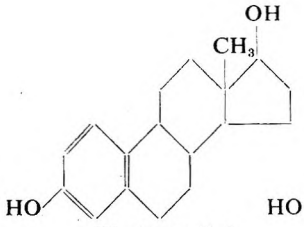


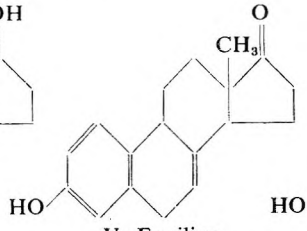
II. Œstrone



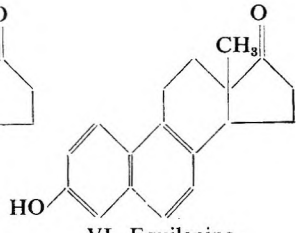
III. Œstriol



IV. Œstradiol



V. Equiline



VI. Equilenine

Up to the discovery of synthetic œstrogens, this phenomenon did not apply to the hormones. There has been only one adrenaline isolated from the suprarenal medulla, only one thyroxine from the thyroid gland and only one insulin from the pancreas.

The first indication that there might be a whole series of closely related substances secreted by one endocrine gland came with the isolation of five different œstrogenic substances from the urine of pregnancy, namely, œstrone, œstriol, œstradiol, equiline and equilenine (II to VI). These all have the same qualitative effect, though their quantitative effects are different.

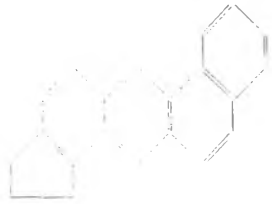
The same story was unfolded concerning the suprarenal cortex. It is now known that a very large number of steroid substances are produced by the cortex of the suprarenal gland, differing in constitution and differing considerably in their metabolic action.

Chemistry

Up to the present time the basic structure of the cyclopentenophenanthrene ring system (I) has been found indispensable for androgenic, progestational and adreno-cortical action. In the case of œstrogens it has been found possible to break away from this structure.

It is only necessary to review very briefly the work leading to the synthesis of stilbœstrol and its allied compounds. Experiments were begun in the Courtauld Institute about 1930 with the object of seeing how far it was possible to change the molecule of œstrogenic substances without destroying the biological activity. As all naturally-occurring œstrogens contain the phenanthrene system as part of the nucleus, a number of phenanthrene derivatives were prepared and tested by the vaginal smear method (Stockard and Papanicolaou[3]) on ovariectomised rats. In 1933, the substance 1-keto-1:2:3:4-tetrahydro-phenanthrene (Cook, Dodds and Hewett[4]) was found to be active in rats at a dose level of 100 mg./rat (VII). At the same time a certain similarity was noticed between the microscopic appearance of the cells of the vagina under the influence of œstrogens and the proliferation caused by the painting of carcinogenic hydrocarbons on the skin. Two of the most potent carcinogenic hydrocarbons, 5:6-cyclopenteno-1:2-benzanthracene (VIII) and 1:2-benzpyrene (IX) were tested and found to have definite,

VII. 1-keto-1:2:3:4-tetrahydro-phenanthrene



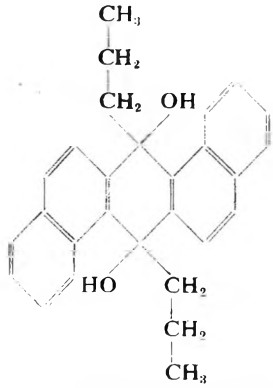
VIII. 5:6-cyclopenteno-1:2-benzanthracene

IX. 1:2-benzpyrene

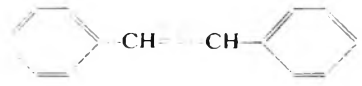
though slight œstrogenic activity (Cook and Dodds[5]). Moreover, it was found that by introducing groups in the 9:10-position of dibenzanthracene this could be converted into quite a powerful œstrogen. A series of 9:10-dihydroxy-9:10-dialkyl-1:2:5:6-dibenzanthracenes was specially

investigated, and the di-*n*-propyl substituent (X) was found to be active in a dose of 25 μg. in the rat (Cook, Dodds, Hewett and Lawson[6]).

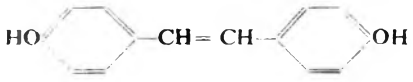
At this point it was decided to see whether the phenanthrene nucleus could be dispensed with and a series of compounds was made, the aim always being to find the simplest possible substance with the highest œstrogenic activity. Considerable activity was shown by certain compounds with only two benzene rings, particularly by stilbene (XI) and 4:4′-dihydroxystilbene (XII) (Dodds and Lawson[7]). An attempt was then made to "drop" one of the rings and the compound anol, *p*-hydroxypropenylbenzene (XIII), was tested. This appeared to be very highly active (Dodds and Lawson[8]), but when other workers attempted to repeat the observation, considerable variation was found in the different batches of anol, some having only very slight activity. The conclusion was that the activity in some batches of anol was due to



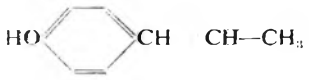
X. 9:10-Dihydroxy-9:10-di-*n*-propyl-1 : 2 : 5 : 6-dibenzanthracene



XI. Stilbene



XII. 4 : 4′-Dihydroxystilbene



XIII. Anol

a contaminant, probably a dimeride of anol. The unsymmetrical dimeride, di-anol (XIV), was tested, but though active, it was not sufficiently so to account for the high activity of some of the batches of anol (Campbell, Dodds and Lawson[9]). The other possibility was the symmetrical dimeride, 4:4′-dihydroxy-α:β-diethyl stilbene, later known as stilbœstrol (XV). This compound was synthesised by a combined team from Sir Robert Robinson's Department at Oxford and from the Courtauld Institute. When tested on rats by the vaginal smear method this was found to be the most powerful œstrogenic substance then known (Dodds, Golberg, Lawson and Robinson[10]). At the same time it was found possible to isolate another compound from the residue remaining from the anol crystallisation, and this compound was later known as hexœstrol (XVI) (Campbell, Dodds and Lawson[11]). A further compound, dienœstrol (XVII), was made a few months later (Dodds, Golberg, Lawson and Robinson[12]).

These compounds have now been used, particularly for the treatment of menopausal symptoms, for nearly ten years, and have been found to

replace the naturally-occurring œstrogens in every way, with the additional advantage that they are active by mouth. They have also been used since the publication by Huggins[13] of his observations on carcinoma of the prostate for the treatment of this condition, and have proved to be of great benefit in a large proportion of cases.

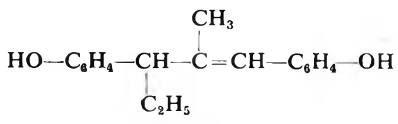
With the establishment of stilbœstrol, hexœstrol and dienœstrol as therapeutic agents, it looked as if the interest in this particular field had more or less come to an end. Recently, however, there have been a number of developments of entirely new synthetic œstrogens, and it is mainly with these that the present account is concerned.

In the first instance, we must abandon the use of the term " synthetic œstrogen," as pointed out recently by Horeau[14]. The synthesis of œstrone has now been effected, and therefore the naturally-occurring hormone could also be included under the heading of synthetic œstrogens. For the stilbœstrol type of substance it is better to employ the term " artificial œstrogens " in the future.

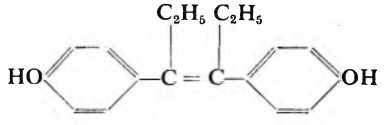
The total synthesis of œstrone was effected by Anner and Miescher[15]. Whilst this is of great theoretical importance, it would appear very unlikely that the synthetic product will ever compete with the production from natural sources. The natural œstrogens are prepared commercially either from the urine of certain pregnant animals, notably the mare, or from cholesterol by a degradation synthesis.

The fact that there were available three artificial œstrogens for use in therapeutics did not hinder the attempts to find others, since it was hoped by this means to find some clue as to the reason for the œstrogenic activity shown by substances with a constitution far different from that of the natural product.

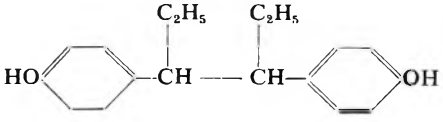
Recently, clinical interest has been shown in a substance produced by Inhoffen and Hohlweg[16] as long ago as 1938. These workers showed



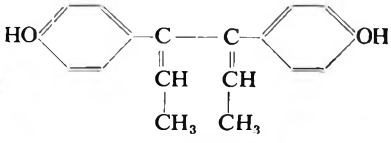
XIV. Di-anol



XV. Stilbœstrol



XVI. Hexœstrol

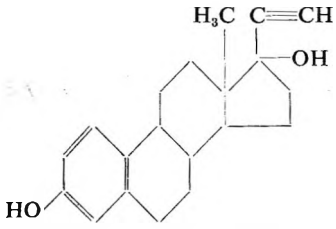


XVII. Dienœstrol

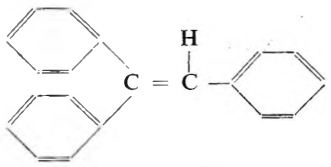
that it was possible to introduce an ethylenic linkage on to the 17-carbon atom in œstradiol. The resulting compound was called ethinyl œstradiol (XVIII). This derivative of the naturally-occurring substance was found to be active by mouth, but it was also stated to suffer from the same

disadvantages with regard to the production of side-reactions as stilbœstrol. There are no figures available to show the comparative potency of this substance as compared with the others, on laboratory animals, and therefore it will merely be referred to from the clinical point of view.

Attempts to produce substances of the same degree of activity as the stilbœstrol series have not been particularly successful. The activity of diphenylethylene was shown to be definite, but slight. Robson and his colleagues[17] studied the activity of triphenylethylene (XIX) and have shown that derivatives in this series have activity, but again on a much lower plane than the stilbœstrol series. Robson and his colleagues[18,19] also made some interesting observations on halogen substituted derivatives of triphenylethylene. These substances have not aroused the interest of clinicians.



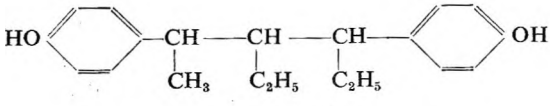
XVIII. Ethinyl œstradiol



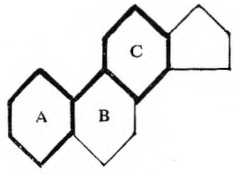
XIX. Triphenylethylene

Another modification of the stilbœstrol type of molecule was made by Blanchard and his colleagues[20,21] in the synthesis of octofollin, 2, 4-di(*p*-hydroxyphenyl)-3-ethyl hexane (XX). This is a derivative of hexane and the general resemblance to the stilbœstrol formula can be seen by comparing the formulæ. This substance is considerably less active than those of the stilbœstrol series, but it has been offered commercially and there are references to its activity in the human subject (Jaeger[22]).

In an attempt to explain the activity of synthetic œstrogens the author suggested (Dodds[23]) that some of the substances showing œstrogenic activity might be regarded as stages in the disintegration of the cyclopenteno-phenanthrene nucleus. For example, the activity of the diphenyl series might be explained by the opening of ring B in the manner shown in the diagram (XXI). With the discovery of stilbœstrol, however, this hypothesis was rather neglected, but recently it has been revived in a



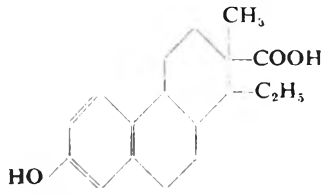
XX. Octofollin



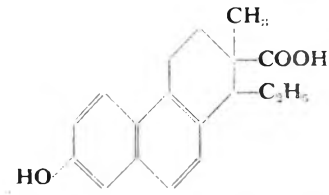
XXI.

very definite form, first by the striking work of Miescher and his colleagues, and later by Horeau and Jacques[24,25,26,27].

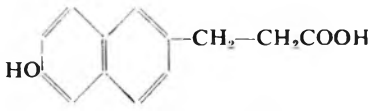
In 1933 Doisy and his colleagues[28,29] showed that it was possible to produce a very highly active substance from œstradiol by an oxidation process, but they failed to identify the compound so produced. The suggestion that highly active substances could be obtained in this manner suggested to Miescher and his colleagues that the disintegration of the œstrone molecule might produce substances of considerable activity. He therefore synthesised a number of compounds which corresponded to the œstrone molecule with the 5-membered ring opened. Two of these substances have shown great activity. These are referred to as doisynolic



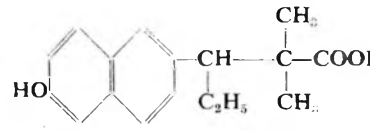
XXII. Doisynolic Acid



XXIII. *bis*Dehydrodoisynolic Acid



XXIV. Allenoic Acid



XXV. Dimethylethylallenolic Acid

acid and *bis*dehydrodoisynolic acid respectively (Miescher[24,25]) (XXII, XXIII). There have been extensive clinical trials with the 7-methyl derivative of *bis*dehydro-doisynolic acid. Miescher and his colleagues have published a number of papers on the synthesis of this compound and have also described[26] a shortening of the synthetic process, but even with this advantage the method of production is infinitely more costly than that of the simpler stilbene derivatives such as stilbœstrol.

Following up the disintegration idea still further, Horeau and Jacques[27] synthesised compounds which correspond to doisynolic acid with the 6-membered ring C opened. This yields a series of naphthalene derivatives, some of which have shown considerable activity. The parent substance has been called allenolic acid (XXIV) and the most active member of the series is dimethylethylallenolic acid (XXV), sometimes referred to as the Horeau acid.

Biological Activity

The biological standardisation of sex hormones and particularly of œstrogens has always presented very great difficulties. The œstrus reaction in the ovariectomised rat or mouse can only be treated quantitatively on a statistical basis, and the method proposed by Coward and Burn[30] is still the basis of all methods of standardisation. This in brief consists in an estimation of the amount of material required to produce full œstrus response in 50 per cent. of a group of ovariectomised animals. According to the size of the group, so vary the reproducibility and

accuracy of the result. To obtain a reproducibility of 20 per cent. some 20 animals must be used, and in order to get down to 10 per cent. 100 will be required. It can be seen that such a standardisation is very laborious, and in order to make the results of one laboratory comparable with those of another the League of Nations Committee on Biological Standards introduced the international standards of œstrone and œstradiol some years before the war. With the isolation and characterisation of the pure compounds, the difficulties of standardisation very largely disappeared and the dubious use of " rat units " fortunately disappeared from the literature.

The introduction of synthetic œstrogens raises a whole series of new difficulties, and the comparison of the potency of the various synthetic and artificial œstrogens becomes an impossibility. In the first instance, it will be remembered that the potency of an œstrogenic substance depends not only on the actual weight of material administered, but on the length of time over which the administration is spread. By and large one can say that the more one fractionates the dose, the greater will be the potency shown. One of the great difficulties with the synthetic œstrogens is their rate of absorption and destruction in the animal body. It is therefore very difficult to compare on any sound basis the activity of, say, stilbœstrol as against œstradiol. Again, the sensitivity of animals varies from laboratory to laboratory, and therefore it is impossible to compare potencies arrived at in one laboratory with those of another. In the Courtauld Institute a method of standardisation has been worked out, using ovariectomised rats and fractionated injections in sesame oil. By the use of this method it has been possible to arrive at the relative potency of the various synthetic substances which led to stilbœstrol. The results are only comparable in the one institution, and therefore it is not proposed here to make any suggestion that the potencies given are in any way absolute. By our method the following Table gives the potency of the synthetic œstrogens mentioned:

Substance	*Dose per Rat*
Stilbœstrol	0·3 to 0·4 μg.
Hexœstrol	0·2 μg.
Dienœstrol	0·4 μg.
*7-methyl-*bis*dehydro-doisynolic acid	0·5 μg.
Dimethylethylallenolic acid	3·0 to 4·0 μg.

* This is the figure obtained for the racemic compound. Miescher[24] has resolved this and has found that the dextro (+) compound is active in rats by single subcutaneous injection in oil in dose of 10·0 μg., whereas the lævo (−) compound is active in rats in a dose of 0·05 μg.

From this table it can be seen that by the methods employed, hexœstrol is the most potent substance of the series when administered by subcutaneous injection in oil. With regard to oral administration, it would

appear that in the rat the most potent substance is 7-methyl-*bis*dehydrodoisynolic acid, with stilbœstrol occupying second place.

Clinical Activity

The earliest tests of stilbœstrol were made under the ægis of the Medical Research Council in 1939 (Bishop, Boycott and Zuckerman[31], Winterton and MacGregor[32]). Since that time a vast literature has accumulated describing the testing and dosage of the various synthetic œstrogens. Out of this very extensive, and in many cases highly uncritical, literature a number of facts emerge:

1. That the synthetic œstrogens are active in the human subject by mouth, and that they are efficient in the treatment of the various gynæcological disorders.

2. That all products in a varying degree do cause side reactions, varying from slight nausea to, very rarely, severe symptoms such as vomiting, skin rashes, and so forth. By and large the reactions are never so severe as to necessitate the discontinuance of the treatment.

3. Astonishing diversity of opinion occurs on the relative potency and percentage of side reactions in these various compounds. In the first instance, the question of side reactions has been the subject of much speculation. Various groups of workers have claimed that one of the synthetic œstrogens is much less prone to produce side reactions than another, and from this it has been concluded that the toxicity is due to some peculiarity of the molecule. To the present writer this has always seemed an unlikely explanation, and in view of the fact that it is possible to get the same type of side reactions with compounds of such widely different structure as ethinyl œstradiol, doisynolic acid and allenolic acid, stilbœstrol, and so forth, it would appear much more reasonable to suppose that the toxicity is associated with the œstrogenic potency. It is known that the naturally-occurring œstrogens are rapidly destroyed in the body, whereas most of the synthetic œstrogens are excreted in the urine either unchanged in part or in conjugation with glucuronic acid.

It has been usual to assume that the sensitivity of all mammals is roughly the same for œstrogens, but there is now considerable evidence that such is not the case, and that it is most unwise to assume that the human female will react in the same way as the laboratory animals. The difficulty in the past has been the lack of any quantitative work on the subject. It is therefore with very great interest that the paper by Bishop, Kennedy and Wynn-Williams[33] has been received. These authors, recognising the lack of quantitative data, have attempted to standardise the œstrogens on the human subject by using œstrogen withdrawal bleeding as a criterion. If œstrogens are given to a menopausal woman with amenorrhœa, amelioration of the symptoms of the menopause occurs almost immediately. If, after a fortnight or so, treatment is suddenly stopped, a small vaginal hæmorrhage occurs. This has been termed œstrogen withdrawal bleeding. Bishop and his colleagues have used

this as an end-point in their standardisation, and by determining the minimum amount of orally active œstrogen necessary to induce this phenomenon, have been able to place the compounds tested in order of potency. The result was that, of the substances tested, stilbœstrol is the most potent. In view of the extremely important nature of their conclusions, the summary is quoted *in extenso:*

> " A method is described for comparing the potency of œstrogens in man. It consists in giving the œstrogen daily by mouth in 14-day courses to amenorrhœic women and recording whether œstrogen withdrawal bleeding takes place.
>
> " The results obtained indicate that dienœstrol is about a quarter, doisynolic acid about a fifth, and hexœstrol about an eighteenth as potent as stilbœstrol.
>
> " Investigation of the incidence of ' toxicity ' indicates that stilbœstrol is more likely to produce nausea in therapeutic doses than are dienœstrol, doisynolic acid and hexœstrol.
>
> " Reasons are given for choosing this end-point, and for the failure to devise any other suitable method of assessment at different levels of œstrogenic response, such as the relief of menopausal symptoms, the production of an œstrous vaginal smear, and the suppression of lactation."

This work is of very great interest in that it shows the folly of applying results obtained on animals to the human being. For example, there appears to be little doubt that 7-methyl-*bis*dehydro-doisynolic acid is highly potent in the rat and mouse by mouth, yet it appears from the results of Bishop and his colleagues to be relatively impotent in the human female.

Finally, ethinyl œstradiol has been the subject of a number of publications, and there is no doubt that it is able to replace the naturally-occurring œstrogens in the same way as stilbœstrol. A number of papers have appeared in America which show that menopausal symptoms can generally be controlled by daily doses of 0·05 to about 0·3 mg. (Wiesbader and Fillet[34], Groper and Biskind[35], Salmon *et al.*[36]). Birnberg *et al.*[37] have used ethinyl œstradiol with success for the treatment of the menopause and of amenorrhœa, for the suppression of lactation and for the induction or hastening of labour. Ethinyl œstradiol can also be used, like other œstrogens, for the treatment of carcinoma of the prostate (McCrea[38]). Some papers have appeared in which the potency of ethinyl œstradiol is compared with that of other œstrogens, natural and artificial. Harding[39] in a series of 47 cases used ethinyl œstradiol and other œstrogens to treat hypo-ovarian symptoms. Ethinyl œstradiol was shown to be the most active of the substances used, but like stilbœstrol was liable to cause " mild toxic reactions." Jeffcoate *et al.*[40] have compared ethinyl œstradiol with other œstrogens on its power to suppress lactation. By this criterion it is also shown to be the most

active. However, as pointed out by Bishop *et al.*[33], this is "an unsuitable method for the clinical assessment of œstrogens," and it is not one that lends itself to quantitative consideration. Finally, Soule[41] has compared ethinyl œstradiol, using œstrogen withdrawal bleeding as the endpoint, with stilbœstrol, α-œstradiol and "mixed œstrogens." The various œstrogens were only tested on 1 patient, so the results can hardly be considered as statistically significant, but it was shown in this case that ethinyl œstradiol was the most active, producing œstrogen withdrawal bleeding with a dose of 0·05 mg. per day for 21 days, as against a dose of 4 mg. of stilbœstrol for 13 days. Both these substances caused nausea when used in effective dosage.

Future Research

The success obtained in the field of synthetic œstrogens leads one to speculate as to future possibilities in the extension of research. Whilst it is always unwise to prophesy, there would appear to be two main lines of work.

Firstly, is it possible to synthesise compounds with a more selective action on the various tissues acted upon by œstrogens? As Parkes[42] has pointed out, the word œstrogen is rather an unfortunate one, since it focusses attention on only one aspect of these compounds' activities, namely, the production of œstrus changes in the vagina. He has suggested that the term "gynæcogenic" would be better, as this would include all the various activities associated with œstrogenic power, such as development of secondary sexual characteristics, action upon the uterus, breast and anterior lobe of the pituitary. Many have speculated as to whether it would be possible to synthesise a substance with selective action on the anterior lobe of the pituitary, whilst at the same time having little action on the breast, uterus and so on. The advantage of such a compound in the treatment of carcinoma of the prostate is obvious. It is the writer's opinion that there is no evidence that such compound could be found. Experience suggests that these compounds act in their entirety, and that it is not possible to segregate or separate the various actions. In other words, the results are due to œstrogenic activity *per se*.

The second line of speculation is whether it will be possible in the future to make synthetic analogues of the other steroid hormones. In other words, would it be possible to produce a compound with, let us say, androgenic activity, which bears no more resemblance to testosterone than does stilbœstrol to œstradiol. Whilst on general grounds it would seem possible that such compounds could be produced, until one has actually been synthesised and its action demonstrated it is obviously idle to speculate.

In conclusion it may be stated that the clinician has a wide selection of artificial œstrogens from which to choose to treat his patients by the oral route. Again, consideration of the evidence would seem to indicate that there is little to choose between any of these substances, and that they are likely to produce side reactions in direct proportion to their œstrogenic potency.

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RESEARCH PAPERS

SYNTHETIC COMPOUNDS RELATED TO THE CARDIAC GLYCOSIDES

PART 1—*p*-HYDROXYPHENYL-$\Delta\alpha$:β-BUTENOLIDE GLUCOSIDE

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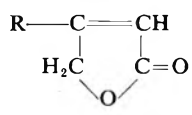
INVESTIGATIONS on the natural cardiac glycosides have revealed that the unsaturated lactone ring in the side chain of their aglucones is indispensable for the specific action on the heart. Thus Elderfield[1], Ruzicka[2], Reichsrein[3] and others have been engaged in the preparation of unsaturated lactones related to the natural aglucones, but the compounds they obtained did not show cardiotonic activity[4]. Comparing the potencies of some cardiac glycosides with those of their aglucones Chen[5] made it clear that glycosidal combination enhances the cardiotonic potency up to 12 times. So, whilst a synthetic aglucone of relatively small activity might show no activity whatsoever during pharmacological tests, it is much less likely that the potential cardiotonic activity of such a compound would be overlooked if it was examined in glycosidal form.

Some special monosaccharides have been found in nature only in combination with the cardiac aglucones, and might be supposed to have an optimum effect upon the activity of the aglucones with which they are combined. However, synthetic glucosides of strophanthidin, digitoxin and digoxin[6] possess a greater activity than the natural glycosides. Hence no specificity may be expected from the sugar fragment of the molecule. Certain comparatively simple molecules of the substituted butenolide structure have been prepared and examined for cardiotonic activity. These substances are shown in Table I.

TABLE I



R = CH_3[7]; -C_2H_5; -$CH_2.CH_2.CH_3$
= -C_6H_5; -C_6H_4(OH) [1 : 4]; -C_6H_4(OH) [1 : 3][8]; -C_6H_4(OCH_3) [1 : 3][9]
= *cyclo*hexyl; 2-chloro-*cyclo*hexyl; *cis* and *trans* 4-hydroxy *cyclo*hexyl[10]; 3 : 4-dihydroxy*cyclo*hexyl[11]
= Δ^3-*cyclo*hexenyl[11]
= *cyclo*pentanyl[12]
= α-naphthyl; β-naphthyl; 6-hydroxy-β-naphthyl, 6-methoxy-2-naphthyl; decahydro-β-naphthyl[1]
= 1-indanyl[3]

Although the methyl and the β-naphthyl derivatives showed a minute reaction in frogs (active at a dose of 2 mg./g.)[4]; all the other substances were inactive. This level of activity is hardly significant. However, none of the compounds has been converted into glycosides and tested in this form.

For these reasons it was decided to prepare some compounds which possess the characteristic unsaturated lactone ring in conjugation with simple hydroxylated carbon skeletons, and convert them into their respective glucosides before pharmacological testing. The first member of this series, *p*-hydroxyphenyl-Δ^{α}:β-butenolide glucoside, was obtained by the action of acetobromoglucose on *p*-hydroxyphenyl-Δ^{α}:β-butenolide[8] and subsequent deacetylation of the tetra-acetylglucoside thus obtained by means of barium methoxide. The glucoside was obtained as a white microcrystalline powder melting at 208° to 209°C.; it had a faint bitter taste and was very hygroscopic and freely soluble in alcohol and in water. It gave a positive Legal's test and was hydrolysed on boiling in water. The analytical figures were in accord with those required.

Pure acetobromoglucose necessary for the reaction was obtained in good yields by a modification of the process usually used for its preparation[13].

Neither the pure aglucone nor the glucoside showed any cardiotonic activity. The tetra-acetylglucoside was insoluble in ordinary solvents and thus could not be examined.

Experimental

Acetobromoglucose. The following method was found to be better than the normal method for the preparation of the compound.

Glucose penta-acetate (10 g.) was covered with commercial 50 per cent. solution of hydrogen bromide in glacial acetic acid (20 ml.) at 0°C. The mixture was left at room temperature overnight, then gradually poured with stirring into a large excess of ice-cold water. The acetobromoglucose, which separated as a white crystalline mass, was filtered, washed with ice-cold water and dissolved in warm methyl alcohol, and the solution kept in a refrigerator for 1 hour. The compound separated out in long colourless needles, which were filtered and recrystallised from *iso*propyl ether. Yield 95 to 98 per cent.; m.pt. 91°C.

p-O-Tetra-acetylglucosidoxyphenyl-Δ^{α}*:β-butenolide.* *p*-Hydroxyphenyl-Δ^{α}:β-butenolide[8] (1 g.) dissolved in 2 per cent. aqueous sodium hydroxide (10 ml.) was added to a solution of acetobromoglucose (2·5 g.) in acetone (10 ml.) and the mixture shaken for 5 hours. 2 per cent. sodium hydroxide solution (15 ml.) and acetobromoglucose (2 g.) were added and the mixture shaken for a further 12 hours. The precipitate that formed was filtered, washed with 10 per cent. sodium hydroxide solution and crystallised from alcohol. It formed colourless shining plates, m.pt. 195° to 195·5°C. Yield 36 per cent. Found; C, 56·72; H, 5·01; $C_{24}H_{26}O_{12}$ requires C, 56·89; H, 5·10 per cent.

p-Hydroxyphenyl-Δ$^{\alpha}$:β-butenolide glucoside. 2N Barium methoxide[14] (0·1 ml.) was added to a suspension of the glucoside acetate (0·4 g.) in methyl alcohol (40 ml.) and the mixture kept at ordinary temperature in a stoppered flask for 5 days. The solution was exactly neutralised by the addition of 0·5N sulphuric acid (1 ml.) and, after allowing to stand for half an hour, the precipitate was filtered off. The filtrate was evaporated to dryness under reduced pressure and the residue dissolved in methyl alcohol; on adding dry ether to the solution a white precipitate was thrown down. The precipitate was rapidly filtered, washed with ether followed by light petroleum and then kept in a vacuum desiccator until dry. The glucoside formed a white microcrystalline powder, m.pt. 208° to 209°C. Yield 90 per cent. Found: C, 54·5; H, 5·64 per cent.; $C_{16}H_{18}O_8$ requires: C, 56·5; H, 5·4 per cent.

The aglucone, glucoside acetate and glucoside gave a positive Legal's test.

Attempts to form the glucoside acetate by shaking in presence of active silver oxide in different solvents alone[15] and in presence of pyridine[16] gave amorphous brown products which could not be purified.

Thanks are due to Professor Buttle and Dr. Dyer, of the Pharmacological Department of this School, for the physiological testing of these compounds.

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SYNTHETIC COMPOUNDS RELATED TO THE CARDIAC GLYCOSIDES

PART II

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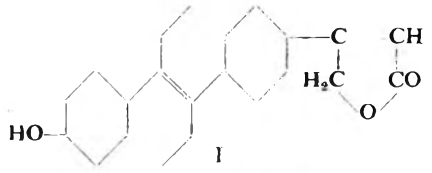
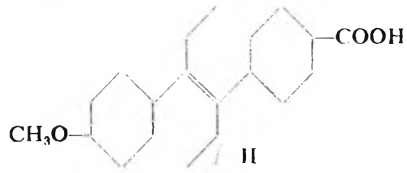
IN Part I of this series the *p*-hydroxyphenyl-Δ^{α}:β-butenolide and its β-glucoside were found to have no cardiac activity.[1] The success which has attended the use of the stilbene nucleus in the preparation of synthetic œstrogens naturally encourages the use of this nucleus in other fields of steroid derivatives. For this reason it was decided to attempt the preparation of a 4-hydroxy-4′-Δ^{α}:β-butenolido-α:β-diethylstilbene (I) in the hope that this molecule or the glucoside prepared from it would exhibit the desired activity.



I



II

Some little difficulty was encountered in the preparation of this compound, but at length its methyl ether was obtained. The starting point of the synthesis was, 4-methoxy-α:β-diethylstilbene-4′-carboxylic acid (II) which had been previously obtained in low yields by Jaeger and Robinson[2] and by Neher and Miescher[3], and therefore attempts were made to improve this preparation. The previous authors obtained (II) by the hydrolysis of the corresponding nitrile although difficulties had previously been encountered in the hydrolysis of such stilbene derivatives[4,5,6].

This difficulty was surmounted by the hydrolysis of 4-methoxy-4′-cyanodeoxybenzoin[2] with a mixture of acetic and sulphuric acids, when a good yield of 4-methoxy-4′-carboxy-deoxybenzoin was obtained in colourless needles melting at 223° to 224°C. It was slightly soluble in alcohol, ether and benzene. This acid was converted into its ethyl ester which, was obtained in fine white needles melting at 136° to 137°C. It gave the required analytical figures as did its 2:4-dinitrophenylhydrazone which crystallised from benzene-light petroleum mixture in red scales m.pt. 165° to 166°C.

The above ester was ethylated by means of sodium ethoxide in ethyl alcohol and the 4-methoxy-4′-carbethoxy-α-ethyl-deoxybenzoin obtained

was purified by distillation *in vacuo;* it formed a pale yellow oil boiling at 220° to 223°C./0·2 mm. Hg. pressure. The 2:4-dinitrophenylhydrazone separated as an oil and could not be induced to crystallise. The ester was easily hydrolysed to the free acid which, separated as an oil and solidified into a resinous mass which was subsequently crystallised from benzene-light petroleum mixture in thick needles melting at 125° to 126°C. Both the ester and the acid gave analytical figures according with those theoretically required.

The resulting 4-methoxy-4′-carbethoxy-α-ethyldeoxybenzoin was treated with ethyl magnesium iodide to yield the corresponding tertiary alcohol, but during the final distillation involved in the purification of the isolated product, water was eliminated from the molecule and ethyl 4-methoxy-α:β-diethylstilbene-4′-carboxylate was isolated in good yield and quantitatively hydrolysed to the desired stilbene acid (II). The conversion of this stilbene acid into the corresponding acid chloride was effected without difficulty with thionyl chloride and, without isolation, the product was treated with diazomethane to give the diazoketo derivative which, on warming with glacial acetic acid yielded 4-methoxy-4′-ω-acetoxyacetyl-α:β-diethylstilbene. This compound appeared as a thick oil boiling at 208° to 211°C. (bath temperature)/0·1 mm. Hg. pressure and formed a crystalline 2:4-dinitrophenylhydrazone melting at 232° to 233°C. Both the ketone and its dinitrophenylhydrazone gave the required analytical figures.

The above compound was treated with ethyl bromoacetate in presence of zinc to give the required lactone 4-methoxy-α:β-diethylstilbene-Δ^{α}:β-butenolide in the form of pale microcrystalline powder melting at 94° to 95°C. It gave a positive Legal's test, and the analytical figures accorded with the theoretical requirements.

Unexpected difficulty was encountered in the attempts made to demethylate this stilbene-butenolide and further work is in progress to achieve this end and then to convert the demethylated substance into its glucoside.

Preliminary pharmacological examination, for which we are indebted to Professor Buttle and Dr. Dyer of the Pharmacological Department of this School, indicated that the lethal dose for guinea-pigs was about 200 mg./kg.; the death was characteristic of the cardiac aglucones which suggests that the compound possessed approximately 1/1000 the potency of strophanthidin. This result is encouraging because it is more than probable that the demethylated compound would possess a much higher level of activity. Again the conversion of the compound into its glucoside would in all probability produce a further increase in activity. This work is therefore being continued in order to obtain the glucoside which might be expected to exhibit a reasonable level of activity.

Experimental

4′-Carboxy-4-methoxydeoxybenzoin. 4-Methoxy-4′-cyanodeoxybenzoin[2] (2·5 g.) was dissolved in glacial acetic acid (20 ml.), sulphuric acid (20 ml.) and water (20 ml.) were added and the mixture boiled under

reflux for 4 hours. The cooled mixture was diluted with water and filtered, and the precipitate recrystallised from 60 per cent. acetic acid. The acid was obtained in colourless needles. It is slightly soluble in alcohol, ether and benzene, m.pt. 223° to 224°C. Yield 82 to 85 per cent. Found C, 71·0; H, 5·13 per cent.; $C_{16}H_{14}O_4$ requires C, 71·11; H, 5·19 per cent.

Esterification of the acidic group. Hydrogen chloride was passed into a boiling alcoholic solution of the acid (2 g.) for 4 hours. The solution was concentrated under reduced pressure and the ester, which crystallised out, was filtered off and recrystallised from 90 per cent. alcohol. It formed fine white needles, m.pt. 136° to 137°C. Yield 85 per cent. Found C, 71·15; H, 6·14 per cent.; $C_{18}H_{18}O_4$ requires C, 72·50; H, 6·04 per cent. The 2:4-dinitrophenylhydrazone crystallised from benzene-light petroleum mixture as red scales, m.pt. 165° to 166°C. Found C, 60·34; H, 4·00; N, 11·1 per cent. $C_{24}H_{22}N_4O_7$ requires C, 60·00; H, 4·00; N. 10·2 per cent.

4'-Carbethoxy-4-methoxy-α-ethyldeoxybenzoin. 4'-Carbethoxy-4-methoxydeoxybenzoin (5·5 g.) was mixed with absolute alcohol (50 ml.) and the mixture raised to boiling. A solution of sodium ethoxide (0·5 g. of sodium in 10 ml. of alcohol) was added and after 10 minutes boiling, ethyl iodide (3 g.) was added and the mixture strongly boiled for 10 minutes more. Sodium ethoxide solution (0·25 g. of sodium in 6 ml. of alcohol) was added followed by ethyl iodide (1 g.). After 2 hours refluxing, another addition of sodium ethoxide (0·25 g. of sodium and 6 ml. of alcohol) and ethyl iodide (1 g.) was made and the whole refluxed for 6 hours. To isolate the desired compound, the neutral solution was diluted with water, acidified with dilute sulphuric acid and extracted with ether. The ethereal extract was washed with aqueous sodium carbonate, sodium thiosulphate solution and water respectively, then dried over anhydrous sodium sulphate. The residue remaining after the removal of ether was distilled under reduced pressure. 4'-Carbethoxy-4-methoxy-α-ethyldeoxybenzoin distilled as a pale yellow oil at 220° to 223°C./0·2 mm. Hg. pressure. Yield 90 per cent. Found C, 73·9; H, 6·91 per cent.; $C_{20}H_{22}O_4$ requires C, 73·6; H, 6·61 per cent.

The 2:4-dinitrophenylhydrazone separated as a semisolid mass which could not be crystallised.

Hydrolysis. The ester (1 g.) was boiled with 10 per cent. sodium hydroxide solution (20 ml.) for 1 hour, when it completely dissolved. Treatment of the clear solution with dilute sulphuric acid produced a milky precipitate, which solidified into a resinous mass. It was crystallised from a mixture of benzene and light petroleum in clusters of thick needles, m.pt. 125° to 126°C. Found C, 71·93; H, 5·77 per cent.; $C_{18}H_{18}O_4$ requires C, 72·50; H, 6·04 per cent.

4'-Carbethoxy-4-methoxy-α:β-diethylstilbene. An ethereal solution of ethyl magnesium iodide prepared from ethyl iodide (17·26 g.) and magnesium turnings (2·23 g.) in ether in the usual way, was added with stirring to a solution of 4'-carbethoxy-4-methoxy-α-ethyldeoxybenzoin

(10 g.) in dry ether (100 ml.), with continual stirring during the addition and for 1 hour more. The mixture, carefully protected from moisture, was left overnight and then refluxed for 2 hours. It was then cooled and decomposed by means of ice and hydrochloric acid; the oil that separated was extracted with ether, the ethereal extract dried over anhydrous magnesium sulphate, and the ether then removed. The residue was distilled *in vacuo* and the fraction boiling at 180° to 183°C/0·2 mm. Hg. pressure was collected. It formed a colourless thick oil and its solution in carbon tetrachloride decolorised bromine. Found C, 78·4; H, 7·77 per cent.; $C_{20}H_{22}O_3$ requires C, 78·1; H, 7·71 per cent.

4-Methoxy-4′-carboxy-α:β-diethylstilbene. The above ester (5 g.) was boiled with a mixture of alcohol (10 ml.) and 10 per cent. aqueous sodium hydroxide (10 ml.) for 1 hour. On cooling, the sodium salt of the acid crystallised out. It was filtered off and boiled with glacial acetic acid (20 ml.), when 4-methoxy-4′-carboxy-α:β-diethylstilbene separated in colourless needles on cooling, m.pt. 175° to 176°C. Yield 96 per cent.

4-Methoxy-4′-ω-acetoxyacetyl-α:β-diethylstilbene. 4-Methoxy-α:β-diethylstilbene-4′-carboxylic acid (5 g.) was dried by heating on a water-bath under reduced pressure for 2 hours. The dry acid was converted to its acid chloride by refluxing with thionyl chloride (20 g.) for 4 hours. On removal of the excess of thionyl chloride, the acid chloride remained as a semisolid mass, which was dissolved in ether and added at 0°C. to an ethereal solution of diazomethane[7] prepared from 60 g. of nitrosomethyl urea[8]. The mixture was kept at 0°C. for 1 hour, then at room temperature for 16 hours. Ether was removed under reduced pressure and the crude diazoketone thus obtained, mixed with glacial acetic acid (20 ml.) and heated on a water-bath for 2 hours after which no more nitrogen was evolved. The mixture was cooled, diluted with ether, shaken with water and then with sodium carbonate solution. The ethereal layer was separated and dried over calcium chloride; on removal of the ether, the ketol acetate was left as a thick oil, which was purified by distillation *in vacuo*. It distilled at 208° to 211°C. (bath temperature)/0·1 mm. Hg. pressure. Yield 70 per cent. Found C, 74·00; H, 6·8 per cent.; $C_{24}H_{28}O_4$ requires C, 75·4, H, 7·1 per cent.

The 2:4-dinitrophenylhydrazone, crystallised from alcohol in the form of an orange microcrystalline powder, m.pt. 233° to 234°C. Found C, 63·60; H, 5·37; N, 10·01 per cent.; $C_{30}H_{30}O_7N_4$ requires C, 63·73; H, 5·5; N, 10·25 per cent.

4-Methoxy-α:β-diethylstilbene-4′-Δ$^{α'}$:β′-butenolide. Zinc (2 g.) was added to a solution of 4-methoxy-4-ω-acetoxyacetyl-α:β-diethylstilbene (3 g.) in benzene (20 ml.) and the mixture raised to boiling on a water-bath. Ethyl bromoacetate (3 g.) in benzene (10 ml.) was then gradually added to the boiling mixture with stirring. After 2 hours refluxing and stirring, the reaction mixture was cooled and decomposed by means of ice and concentrated hydrochloric acid. The benzene layer was separated, washed with water and sodium carbonate solution respectively and dried over anhydrous sodium sulphate. After removing the benzene under

reduced pressure, the residue was heated on a water-bath under reduced pressure for half an hour, then mixed with a 50 per cent. solution of hydrobromic acid in glacial acetic acid and heated again for another half hour, and finally poured into a large volume of ice-cold water. The precipitate that formed was filtered and recrystallised from benzene. It formed pale yellow crystals, m.pt. 94° to 95° C. Yield 24 per cent. Found C, 79·8; H, 6·74 per cent.; $C_{23}H_{24}O_3$ requires C, 79·31; H, 6·92 per cent. The compound gave a positive Legal's test.

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THE HISTOLOGY OF BELLADONNA ROOT

PART IV

THE DIFFERENTIAL VALUE OF THE FIBRE/VESSEL RATIO

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BELLADONNA ROOT is defined in the British Pharmacopœia, 1948, as the root, or root and rootstock, of either *Atropa Belladonna* Linn., or *Atropa acuminata* Royle ex Lindley or a mixture of both species. In the earlier parts of this work, it was noted that the xylem of *A. Belladonna* root consists in the main of cellulosic parenchyma and scattered vessel strands, whereas in *A. acuminata* the cellulosic parenchyma is largely replaced by lignified fibrous tissue. Inspection of transverse sections of the roots of the two species did not indicate any differences in the numbers of vessels per unit area, hence it seemed likely that a differential character could be based on the ratio of the numbers of fibres to the numbers of vessel elements. It was therefore decided to investigate this character on both the whole drug and the powder and to adopt the term " fibre/vessel ratio " to indicate the number of fibres associated with one vessel element. In counting the fibres, it was anticipated that the somewhat similar fibre-tracheids might cause difficulty in their discrimination, and to avoid this they were included in the fibre count if they exhibited elongated simple pits. True tracheids, due to their characteristic elliptical pits, are readily distinguished.

In Part III of this work,[1] it was shown that the vessel index provided a means of differentiating the powdered drugs, but its application to the whole drug was not investigated in detail. It appeared desirable therefore to do this also in order to compare the two characters and to assess their value when combined in a discriminant function.

MATERIALS

Specimens of the whole drug were selected from a number of parcels of commercial material obtained from reputable wholesale druggists between 1940 and 1945, and supplied as either " English Belladonna root " or " Indian Belladonna root." Ten specimens of each variety were selected, ranging in diameter from the narrowest to the widest in the material and including examples at all the stages of xylem development present. The wider of these specimens consisted of both root and rootstock, and the narrower of root only. They were numbered 1 to 10 in decreasing order of diameter, which order corresponded approximately with their age, the widest specimens of either variety being about five years old.

The powdered material consisted of six samples of commercial powder, coded AB1 to AB6, supplied as *A. Belladonna* and five coded Aa1 to Aa5, supplied as " Indian Belladonna." This was the same material as that used for the determination of the vessel index and the cork cell number reported on in Part III of this work,[1] in Table I of which fuller details appeared.

A.—The Whole Drug

Number of observations. Since the fibres are more numerous than the vessel elements, it was found convenient in practice to count the numbers of the latter associated with groups of 10 fibres. To determine the minimum number on which the ratio should be based in order to obtain good agreement between successive results, one root of each species taken at random from the commercial drug was examined and the number of vessel elements associated with a total of 400 fibres determined in each case. The range of successive results based on count of 100 fibres and 200 fibres was respectively about 8 per cent. and 4 per cent. of the mean. Subsequent work was therefore based on a minimum count of 200 fibres.

Variation in the whole drug. The 10 roots of each species were softened by soaking in dilute alcohol and cut into 5 approximately equal lengths, which were numbered 1 to 5 from base to apex or crown. The entire portion, or a longitudinal sector according to the bulk, was disintegrated separately by means of Schultz's maceration fluid, following the method described below. The fibre/vessel ratio was calculated from observations of the number of vessel elements associated with 200 fibres ; at the same time, and using the same microscopical preparations, the vessel index 135μ[1] (i.e. the percentage of vessel elements wider than 135μ) was also determined.

Summary of Results

The values of the fibre/vessel ratio for the whole drug are given in Table I, and of the vessel index 135μ in Table II. The ranges of the positional values of the fibre vessel ratio are : *A. Belladonna*, 0·89 to **1·79** to **4·28** to 7.69, mean 3·04, standard deviation, 1·25 ; *A. acuminata*, 2·35 to **2·20** to **8·79** to 18·18, mean 5·49, standard deviation 3·29. For the vessel index 135μ, the corresponding ranges are : *A. Belladonna*, 0 to **1·42** to **7·14** to 10·07, mean 4·28, standard deviation 2·86 ; *A. acuminata*, 6·37 to **8·58** to **22·30** to 27·74, mean 15·44, standard deviation 6·86. The ranges of either character thus show a not inconsiderable overlap so that complete differentiation of the whole drug is not possible by these means. Fisher has shown that, in cases such as this, an im-

TABLE I

Fibre/vessel ratio—variation in the whole drug

Species	*A. Belladonna*						*A. acuminata*					
Root No.	Position					Root Mean	Position					Root Mean
	1	2	3	4	5		1	2	3	4	5	
1	1·39	1·62	1·75	2·27	2·63	1·93	5·26	6·06	6·25	6·90	7·69	6·43
2	2·99	2·90	3·70	3·23	4·76	3·52	8·00	10·53	10·53	12·50	12·50	10·81
3	2·25	3·77	3·70	3·70	4·08	3·50	2·86	3·85	3·85	5·88	5·56	4·40
4	3·77	3·70	4·55	4·35	7·69	4·81	6·45	10·00	11·11	14·29	18·18	12·01
5	0·89	1·35	1·32	1·74	1·90	1·44	2·60	2·35	2·86	4·44	4·08	3·27
6	2·25	2·27	2·70	3·13	2·94	2·66	2·99	2·86	3·12	3·12	3·08	3·03
7	1·55	1·92	2·25	2·47	3·23	2·28	2·74	3·57	2·94	3·57	2·53	3·07
8	3·28	3·51	4·55	4·26	4·55	4·03	3·08	4·00	4·65	5·00	5·71	4·49
9	1·87	2·25	2·99	3·13	3·28	2·70	2·60	3·51	3·57	5·26	5·56	4·10
10	2·60	2·94	3·08	3·03	5·88	3·51	3·03	3·45	3·64	2·86	3·70	3·34
Position Mean	2·28	2·62	3·06	3·13	4·09	3·038	3·96	5·02	5·25	6·38	6·86	5·494

provement may result from the use of a discriminant function X, which is a linear compound of the available measurements, i.e. $X=\lambda_1 x_1+\lambda_2 x_2$, the constants λ_1 and λ_2 being chosen so as to maximise the ratio of the difference between the specific means to the variance within the species.

TABLE II

135 μ VESSEL INDEX—VARIATION IN THE WHOLE DRUG

Species	*A. Belladonna*						*A. acuminata*					
Root No.	Position					Root Mean	Position					Root Mean
	1	2	3	4	5		1	2	3	4	5	
1	3·85	3·10	2·72	1·19	1·19	2·41	21·38	24·47	26·04	27·11	27·74	25·38
2	7·41	8·76	8·42	8·09	8·09	8·15	10·07	10·40	9·75	10·71	11·34	10·45
3	6·02	6·72	7·41	8·76	8·76	7·53	10·07	12·28	11·98	14·09	14·97	12·68
4	1·96	2·72	3·10	3·85	4·22	3·17	13·79	16·39	16·67	16·94	11·34	15·03
5	3·10	3·10	3·10	3·10	3·85	3·25	15·25	16·94	18·57	19·87	23·08	18·74
6	0·79	0·79	1·58	1·19	1·96	1·26	20·64	23·31	23·78	24·93	25·37	23·61
7	7·06	7·41	8·09	10·07	10·07	8·54	17·76	21·88	23·78	25·81	27·74	23·39
8	1·96	1·96	2·72	3·47	3·47	2·72	6·37	6·72	6·72	6·72	8·42	6·99
9	4·22	4·58	4·58	5·30	6·02	4·94	9·09	9·75	10·07	10·07	10·40	9·88
10	0·40	1·19	0·40	0·00	1·96	0·79	7·06	7·41	8·09	8·76	10·07	8·28
Position Mean	3·68	4·03	4·21	4·50	4·96	4·277	13·15	14·96	15·55	16·50	17·05	15·439

Accordingly, the discriminant function combining the positional fibre/vessel ratio and the vessel index values in Tables I and II was calculated. The calculation followed the method detailed by Mather[2], with the result $X=0{\cdot}004431x_1+0{\cdot}004760x_2$, where x_1 and x_2 are the vessel index and fibre/vessel ratio values respectively. For convenience in practice, a new function $X^1=225{\cdot}6832X$ was employed, i.e. X^1=vessel index+1·074 fibre/vessel ratio. The results are given in Table III and discussed later.

B.—THE POWDERED DRUG

Preliminary. Before the fibre/vessel ratio can be determined on the powdered drug, the method as applied to whole roots needs modifying to allow for the presence of broken elements. Accordingly, portions of two samples of commercial powdered Indian belladonna root, Aa1 and Aa2, were disintegrated and used for this preliminary work.

TABLE III

VALUES OF X^1 = VESSEL INDEX + 1·074 FIBRE/VESSEL RATIO FOR THE WHOLE DRUG

Species	*A. Belladonna*						*A. acuminata*					
Root No.	Position					Root Mean	Position					Root Mean
	1	2	3	4	5		1	2	3	4	5	
1	5·34	4·84	4·60	3·55	4·02	4·47	27·03	30·98	32·75	34·52	36·00	32·26
2	10·62	11·88	12·39	11·56	13·20	11·93	18·66	21·71	21·06	24·14	24·77	22·07
3	8·44	10·77	11·38	12·73	13·14	11·29	13·14	16·41	16·11	20·41	20·94	17·40
4	6·01	6·69	7·99	8·52	12·48	8·34	20·72	27·13	28·60	32·29	30·87	27·92
5	4·06	4·55	4·52	4·97	5·89	4·80	18·04	19·46	21·64	24·64	27·46	22·25
6	3·21	3·23	4·48	4·55	5·12	4·12	23·85	26·38	27·13	28·28	28·68	26·86
7	8·73	9·47	10·51	12·72	13·54	10·99	20·70	25·71	26·94	29·64	30·46	26·69
8	5·48	5·73	7·61	8·05	8·36	7·04	9·68	11·02	11·71	12·09	14·55	11·81
9	6·23	7·00	7·79	8·66	9·54	7·84	11·88	13·52	13·90	15·72	16·37	14·28
10	3·19	4·35	3·71	3·25	8·28	4·56	10·31	11·12	12·00	11·83	14·04	11·86
Position Mean	6·13	6·85	7·50	7·86	9·36	7·538	17·40	20·34	21·18	23·36	24·41	21·34

Three methods of assessing the numbers of whole elements equivalent to the fragmentary ones appeared worthy of trial. These were based on :—

(1) measurement of the total length of the fragments and division by the mean whole element length of the sample ;

(2) counting the numbers of fragmentary elements and multiplying by a factor equivalent to the ratio of the mean fragment length to the mean whole element length ;

(3) counting the fragmentary elements as whole ones according to a predetermined convention.

Method (1). The mean lengths of the broken elements based on 50 measurements were found to be :—fibre fragments—227·5μ ; vessel element fragments—184·5μ. Three slides were examined for each sample, the numbers of whole elements being counted, and the lengths of the broken elements in the same fields determined with the aid of a camera lucida. Results are incorporated in Table IV.

TABLE IV

Sample	*A. acuminata.* Aa1			*A. acuminata.* Aa2		
	Fibres	Vessels	Ratio	Fibres	Vessels	Ratio
Length of fragments μ	2158	579		7020	754	
Equivalent number whole ...	9·5	3·1	3·1	30·9	4·9	6·3
Counted number whole	142	23	6·2	189	30	6·3
Total as whole	151·5	26·1	5·8	219·9	34·9	6·3

Method (2). The three slides of each sample were re-examined and the numbers of whole and broken elements counted. The mean length of the vessel elements has already been recorded for the whole drug[3], viz. 252μ, hence the factor by which the number of fragments should be multiplied to estimate the equivalent number of whole elements is 184·5/252=0·732 which can be approximated to $\frac{3}{4}$. The mean length of the fibres (including fibre-tracheids as described above) was estimated as 450μ, hence the corresponding factor is 227·5/450=$\frac{1}{2}$, approximately. Results are incorporated in Table V.

Method (3). For this method, the following convention was adopted. Broken fibres were counted as whole if they tapered towards both ends, otherwise they were ignored. Broken vessel elements were counted as

TABLE V

Sample	*A. acuminata.* Aa1			*A. acuminata.* Aa2		
	Fibres	Vessels	Ratio	Fibres	Vessels	Ratio
Number of fragments	394	105		587	125	
Equivalent number whole ...	197	79	2·5	294	94	3·1
Counted number whole	240	39	6·1	399	68	5·9
Total as whole	437	118	3·7	693	162	4·3

whole if they exhibited portions of the perforation rim at both ends ; if visible only at one end, they were counted as half ; all other fragments were ignored. The number of such vessel elements associated with 300 such fibres was determined on the three slides of each sample examined previously. The fibre/vessel ratios were :—Sample Aa1, 6·25 ; Sample Aa2, 6·67.

The relative merits of the three methods were now considered before proceeding with the examination of the powdered drug. The standard by which they may be compared is the value of the fibre/vessel ratio obtained from counts of the whole elements only. This value is reasonably consistent for the first two methods, having regard to the relatively small numbers of elements on which it is based. The results for sample Aa2 by method (1) show that this method is capable of giving consistent results, but the process of measuring the broken elements is tedious and does not recommend itself in practice owing to the time involved. Method (2) does not suffer from the latter disadvantage, but the results do not suggest that an accurate estimate of the fibre/vessel ratio could be obtained in this way. The results obtained by method (3) are consistent with those obtained by counting whole elements only and the process is simple to apply in practice. Moreover, the number of elements that can be observed in a given time is much larger than that possible by method (1), thus reducing variation due to random effects. Having regard to the above considerations, it was decided to adopt method (3) for the subsequent work.

Variation with the fineness of the portion examined. Powdering causes some destruction of individual cells and it is necessary to determine whether the action is selective on either the fibres or the vessel elements. Powder samples Aa1 and AB3 were therefore passed through a series of standard sieves and the fibre/vessel ratio determined on the portions retained by each sieve.

TABLE VI

VARIATION OF THE FIBRE/VESSEL RATIO IN PORTIONS OF THE POWDER OF VARYING FINENESS

Sieve Number		10	22	25	30	36	44	60	85
Sample	AB.3	—	5·5	5·3	4·0	3·9	4·0	4·2	3·9
Number	Aa.1	8·5	7·3	7·1	7·1	6·4	5·5	5·3	4·8

From these results it is seen that the fibre/vessel ratio decreases with increasing fineness of powder and that the decrease is not large over the 25/44 range. Portions No. 60 and 85 contained a high proportion of broken elements and counting was correspondingly difficult. In view of this and also of that fact that the No. 22/44 portion had been employed previously[1] for vessel index determination, it was decided to employ the latter portion for subsequent work on powders.

Details of the method. About 1·0 g. of the 22/44 portion of the commercial powder was boiled gently with 30 ml. of Schultz's maceration fluid for about 10 minutes or until particles ceased to float on the surface, sufficient potassium chlorate being added meanwhile to maintain a steady evolution of chlorine. The macerated material was then collected on a

sintered glass filter, washed with water, transferred to a small test-tube with about 2ml. of water and disintegrated by vigorous shaking. The resulting suspension was then diluted with 3 or 4 volumes of suspending fluid containing a trace of thymol as preservative. Slides were prepared by further dilution such that each scan contained about 20 fibres and the fibre/vessel ratio calculated from observation of the number of vessel elements associated with 300 fibres. In counting, the convention for including broken elements in the count as described above (method 3) was adopted. Counting was done using a 1/6 in. objective and a ×6 eyepiece, and the whole slide covered systematically with the aid of a mechanical stage. Elements intersected by the field of view, but lying more than half within it, were included in the count, otherwise they were ignored.

Summary of Results

The values of the fibre/vessel ratio for commercial powders are given in Table VII. The ranges are :—

A. Belladonna, 2.30 to 4.55, mean 3.91.
A. acuminata, 6.54 to 12.05, mean 8.66.

These are distinct and the fibre/vessel ratio is thus a good differential character for the powdered drugs. The values for samples Aa1 and Aa2 are slightly higher than those obtained in the preliminary work, which may be accounted for by the fact that they relate to the 22/44 portions, whereas the preliminary work was done on the unsieved powders.

TABLE VII
Fibre/vessel ratio for commercial powders—22/44 portion

A. Belladonna		*A. acuminata*	
Code Number	Fibre/vessel ratio	Code Number	Fibre/vessel ratio
AB.1	4·07	Aa.1	6·54
AB.2	4·29	Aa.2	7·0
AB.3	3·91	Aa.3	8·85
AB.4	4·55	*Aa.4	12·05
AB.5	2·35	*Aa.5	8·85
AB.6	2·30		
Mean	3·91	Mean	8·66

* 44/60 portion.

Discussion of Results

The analysis of variance, Table VIII, for the fibre/vessel ratio determinations on the whole drug shows that the difference between the species is not very significant and hence the differential value of the character when applied to the whole drug is limited. The variance within the species *A. Belladonna* is contributed to almost equally by positional variation within the roots and by differences between them, while in *A. acuminata* root differences are more important. The increase in positional values from the base towards the apex of the roots is fairly uniform and the values at the mid-points approximate to the root means. The critical value for classifying whole roots is 4·27, i.e. half the sum of the specific means, and the expected proportion misclassified on the

basis of a value determined at one position taken at random, as calculated from knowledge of the normal deviate, is 32 per cent. The ranges for the root means are *A. Belladonna* 1·44 to 4·8 ; *A. acuminata*, 3·03 to 12·01, but little improvement in classification results from their use. However, it may be considered that values less than about 2·0 indicate

TABLE VIII

ANALYSIS OF VARIANCE IN TABLES I AND II

Character	Species	Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	Variance Ratio P=0·01
Fibre/vessel ratio Table I	*A. Belladonna*	Roots	9	46·97	5·22	17·7	3·1
		Positions	4	18·65	4·66	15·9	3·9
		Error	36	10·59	0·29		
	A. acuminata	Roots	9	486·34	54·04	31·4	3·1
		Positions	4	52·87	13·22	7·7	3·9
		Error	36	61·91	1·72		
		Species	1	150·82	150·82	21·8	7·0
		Error	98	677·33	6·91		
Vessel Index Table II	*A. Belladonna*	Roots	9	368·51	40·95	63·8	3·1
		Positions	4	9·38	2·35	3·7	3·9
		Error	36	23·10	0·64		
	A. acuminata	Roots	9	2126·68	236·30	99·5	3·1
		Positions	4	92·08	23·02	9·7	3·9
		Error	36	85·52	2·38		
		Species	1	3115·10	3115·10	117·1	7·0
		Error	98	2705·27	27·60		

A. Belladonna and greater than about 8·0 *A. acuminata*, intermediate values being inconclusive. In this connection, however, the fact must also be taken into account that the specimens of the whole drug were selected on a basis of diameter and development of xylem and were not taken at random. Reference to the results for commercial powders shows that the mean value of 8·66 for the *A. acuminata* material is considerably higher than that for the whole drug 5·49, indicating that specimens with high fibre/vessel ratios form the greater part of the drug from this species. Thus the misclassification rate of 32 per cent. may be unduly high when applied to specimens taken at random from the commercial drug.

The values of the vessel index for the whole drug also increase from the base towards the apex of the root, but the analysis of variance, Table VIII, shows this effect to be unimportant compared with that due to differences between the specimens. The difference between the species is highly significant, so that the vessel index is valuable for differentiating the whole drug. On the basis of a single determination, the expected misclassification rate is about 15 per cent., the critical value being 9·86 and if the root means are used this proportion is reduced to about 10 per cent. Inspection of the results in Table II does not suggest any close connection between the vessel index and the age of the specimen in *A. Belladonna* although in *A. acuminata* the values for the younger roots numbers 8, 9 and 10 are considerably lower than the mean of the other seven. The ranges for the root means, *A. Belladonna* 0·79 to 8·54, *A. acuminata* 6·99 to 25·38, are not inconsistent with those for another

selection of specimens reported on in Part III of this work, namely, *A. Belladonna* 0·3 to 16·02, mean 6·9 : *A. acuminata* 5·0 to 43·58, mean 19·87. Thus values of less than about 5 may be taken as indicating *A. Belladonna* and greater than 16, *A. acuminata.*

Comparison of the mean values of the vessel index and the fibre/vessel ratio for specimens of the whole drug suggests there is little correlation between them. This is borne out by the low correlation coefficients calculated from the data in Tables I and II, which are :—*A. Belladonna* 0·6, *A. acuminata* −0·15. The two characters may thus be considered as varying independently of one another. Their combination in a linear function yields a discriminant with a critical value for classifying purposes of 14·44 and an expected misclassification rate of 12 per cent.. This is a considerable improvement on the figure of 32 per cent. for the fibre/vessel ratio, but is very little superior to that of 15 per cent. when the vessel index is used alone. For this reason, use of the discriminant for other than borderline cases would not be justified.

The variation of the fibre/vessel ratio in the whole drug is not reproduced in the commercial powder owing to the thorough mixing of the elements on which it is based. Thus the values for commercial powders vary within narrow limits and, since the ranges for the two species are distinct, the fibre/vessel ratio provides a valuable character for their differentiation.

Summary and Conclusions

1. The term " fibre/vessel ratio " is adopted to signify the number of fibres (including fibre-tracheids exhibiting elongated simple pits) associated with one vessel element.

2. Consistent results are obtained when the fibre/vessel ratio is calculated from the number of vessel elements associated with not less than 200 fibres.

3. The variation in the whole drug of the fibre/vessel ratio and of the vessel index 135μ is investigated and their value as differential characters assessed.

4. Neither character is completely successful in differentiating the whole drug, but some improvement results by combining them in a linear function.

5. A method of determining the fibre/vessel ratio on the powdered drug is described.

6. The fibre/vessel ratio of commercial belladonna root powders decreases with the fineness of the portion examined, but is reasonably constant for the portion retained between a No. 22 and No. 44 sieve.

7. Fibre/vessel ratio values for commercial powders provide a means of differentiating the species from which they were prepared.

8. The ranges for the commercial powders examined are :—*A. Belladonna* 2·30 to 4·55 ; *A. acuminata* 6·54 to 12·05.

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THE PHARMACOLOGICAL ACTIONS OF THE CRYSTALLINE PRINCIPLES OF *AMMI VISNAGA* LINN.

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Attempts to isolate the crystalline principles of the fruit of *Ammi Visnaga* (in Arabic "Khella") date since 1897, when Mustapha[1] obtained by extraction with alcohol and ether, white silky needles, bitter in taste and sparingly soluble in water. Mustapha gave this substance the name of khellin. The work was continued by Malosse[2], Fantl and Salem[3], Fahmy and El-Keiy[4] and Samaan[5]. The crystalline substances obtained by these authors were, in many instances, not subjected to a satisfactory chemical analysis, the proof of their purity resting mainly on a rough examination of their physical properties. The small amount of consideration which most of these authors had paid to the results obtained by the preceding workers led to a considerable confusion of nomenclature; different names were frequently given to apparently the same substance. A similar confusion exists also in the pharmacological literature concerning *Ammi Visnaga,* different properties being sometimes attributed to the same crystalline substance.

The recent work of Späth and Gruber[6,7,8] placed the problem on a sound chemical basis. These authors isolated from the fruit of the plant three crystalline substances which on analysis proved to belong to the group of chromones. In a private communication to one of us (G.V.A.) Prof. Gruber states that, in addition to the three chromones, he had also detected the presence of a small amount of a coumarin, the analysis of which he has not, however, yet completed. Therefore, until further proof is available, only the three chromones, so far isolated and analysed, are to be considered.

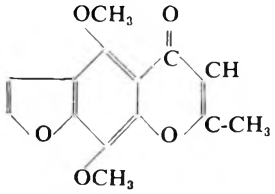
According to Späth and Gruber, these substances are:—

(1) *Khellin,* isolated by Mustapha and by Fantl and Salem and shown by Späth and Gruber[6] to be a dimethoxymethylfuranochromone (I) m.pt. 154°C.

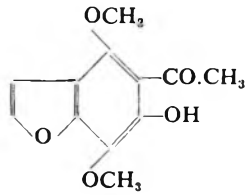
(2) *Khellol glycoside,* isolated by Fantl and Salem and shown by Späth and Gruber[8] to be a monomethoxymethylfuranochromone, oxyglycoside (II) m.pt. 175°C.

(3) *Visnagin,* isolated by Späth and Gruber[7] and shown by them to be a monomethoxymethylfuranochromone (III) m.pt. 144°C.

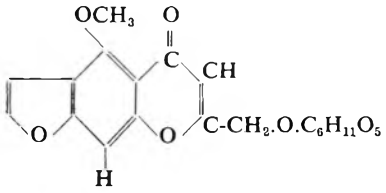
It can be seen from the structural formulæ given that the simplest compound is visnagin, that khellin is a methoxyvisnagin and that the khellol glycoside is an oxyglycoside of visnagin. Taking the visnagin radical as R, khellin is R-OCH_3 and khellol glycoside R-$OC_6H_{11}O_5$.



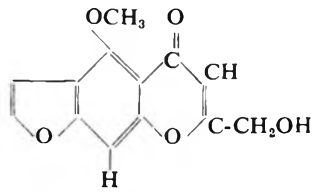
I. Khellin



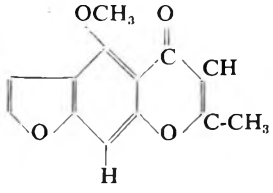
IV. Khellinon



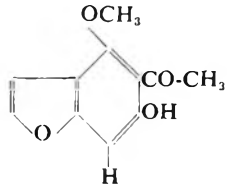
II. Khellol glycoside



V. Khellol



III. Visnagin



VI. Visnaginon

Späth and Gruber have also analysed a large number of derivatives of these compounds. The most interesting were obtained by the action of alkali and of acids. By alkali, khellin is split to a dimethoxyacetylhydroxy coumarone (m.pt. 99° to 101°C.) and visnagin to a monomethoxyacetylhydroxy coumarone (m.pt. 109° to 111°C.). The authors gave the name of khellinon to the first compound (IV) and visnaginon to the second (VI). The aglycone obtained by the hydrolysis of the khellol glycoside in acid was first isolated by Fantl and Salem[3], who gave it the name of khellol (m.pt. 179°C.). Späth and Gruber showed that the khellol is a hydroxyvisnagin (V). It was further shown that the glycoside, as well as the khellol, when treated with alkali give a product which is in all respects identical with visnaginon. In a simplified form the relations between these compounds are as follows:

Khellin is converted by alkali into khellinon
Khellon glycoside is converted by acid into khellol

Khellol glycoside, Khellol, Visnagin } are all converted by alkali into visnaginon

The decoction of the dried fruit of *Ammi Visnaga* has been used since ancient times by the population in the Middle East, and is frequently prescribed by the local physicians as a diuretic and as an anti-spasmodic in cases of ureteral stones. Samaan[9] investigated the pharmacological

action of khellin and of the glycoside (which he respectively called visammin and khellinin) and found that khellin increases the flow of urine and causes a relaxation of the visceral plain muscles. The interest in the crystalline constituents of *Ammi Visnaga* has been recently revived as the result of the demonstration by Anrep, Barsoum, Kenawy and Misrahy[10] that khellin causes a conspicuous dilatation of the coronary blood vessels without much affecting the systemic circulation. The interest in the substance was further stimulated by the promising results of the clinical observations upon the effect of khellin in the anginal syndrome (Kenawy and Barsoum[11], Anrep and *et al.*[12] Ayyad[13]).

Salama[14] showed in animals and man that none of the crystalline principles of *Ammi Visnaga* exerts a diuretic action, the increased formation of urine being entirely due to the fluid taken in the form of the decoction.

Samaan[15,16] ascribes the coronary vasodilator action of *Ammi Visnaga* not to khellin, but to the glycoside, which, according to him, causes in concentrations of 1 in 25,000 and even 300,000 a conspicuous increase of the coronary outflow of the isolated perfused rabbit's heart. In experiments in which an artificial spasm of the coronary blood vessels was induced by barium chloride, administration of the glycoside was stated to cause sometimes as much as a twelve-fold increase of the coronary outflow. On the other hand, according to Bagouri[17], the glycoside, even when used in high concentrations, exerts no action on the coronary vessels of the perfused heart, the coronary vasodilatation being entirely due to khellin.

It follows from the above that further research is required before a proper assessment of the pharmacological potency of the different crystalline principles of *Ammi Visnaga* can be made. We, therefore, undertook to investigate the comparative action of khellin, of visnagin, of the glycoside and of their derivatives by quantitative methods.

Methods of Preparation

The three natural crystalline substances of *Ammi Visnaga*, khellin, khellol glycoside and visnagin were prepared according to the method devised by Späth and Gruber. The substances were repeatedly crystallised from methyl alcohol and other solvents until their respective melting-points reached the maximum values given by the Austrian observers and did not change by further recrystallisation. The purity of the final products was controlled in the Pharmacognosy Laboratory and in the Faculty of Science of this University. The fission products, khellinon, visnaginon and khellol were obtained by the action of acid and alkali as recommended by Späth and Gruber. The purification of these substances presents no difficulty since they easily crystallise from methyl alcohol and give sharp melting-points, 100°C. for khellinon, 110°C. for visnaginon and 179°C. for khellol.

The Colorimetric and the Biological Assay of the Crystalline Principles of *Ammi Visnaga*

Colorimetric assay:—The moderately stable pink coloration which khellin gives, as discovered by Fahmy and El-Keiy[4], in contact with solid sodium hydroxide, served as the basis for the colorimetric assay of khellin and of visnagin. For quantitative work we used a saturated solution of potassium hydroxide and a 0·5 millimolar standard solution of khellin in water (0·13 mg. of khellin per ml.). Addition of 0·1 ml. of the khellin solution to 1·0 ml. of saturated potassium hydroxide solution gives an intensity of colour suitable for work with a sensitive colorimeter of the ordinary type or with a photoelectric colorimeter. As has already been shown by Fahmy, this method is less suitable for the estimation of the glycoside, which, in presence of strong alkali, gives a very unstable cherry-red colour. The products of acid and alkaline hydrolysis of the three natural substances give no pink coloration in presence of strong alkali.

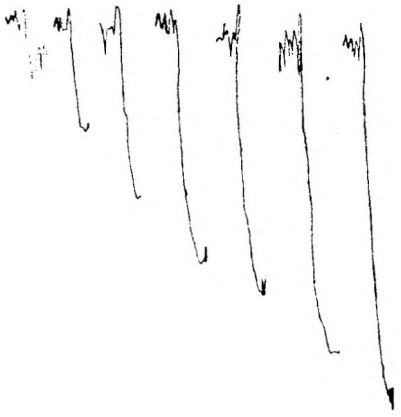


FIG. 1. Response of the rectal cæcum of the fowl to gradually increasing doses of khellin. The amounts administered are shown in μg.

Biological assay:—The biological method of assay used in this work was originally devised by Barsoum and Gaddum[18] for the estimation of adenosine. As a test object they used the rectal cæcum of the fowl suspended in Tyrode's solution in a bath 5 ml. in capacity. We find that the rectal cæcum can be satisfactorily used also for the comparative assay of the active principles of *Ammi Visnaga*. With sensitive preparations it is possible to make the assay with an accuracy of about 10 per cent. Figure 1 shows the reaction of the rectal cæcum to gradually increasing doses of khellin which, for this purpose, was dissolved in Tyrode's solution. An average preparation of the rectal cæcum is sensitive to about 2 μg. of khellin, i.e., a concentration of khellin in the bath of $2·5 \times 10^{-7}$. Figure 2 shows that repeated administration of the same dose of khellin gives the same degree of relaxation of the rectal cæcum, and Figure 3 serves as an example of a comparative assay of visnagin and of khellin showing that visnagin is about 30 per cent. less active than khellin.

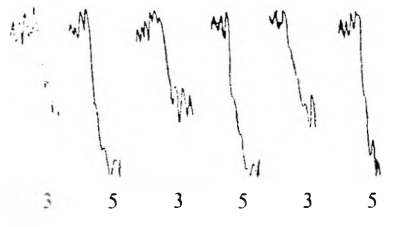
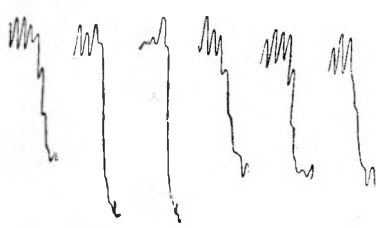


FIG. 2. Regular response of the rectal cæcum to alternate doses of 3·0 and 5·0 μg. of khellin.

Table I gives the results obtained with the three natural substances and with the products of their hydrolysis. The results of the colorimetric and of the biological assays given in Table I are means of not less than 50 separate estimations of the 3 natural substances and of their derivatives. Usually only 2 or 3 substances were assayed on one rectal cæcum. Before administration, the solutions were warmed to the temperature of the bath containing the cæcum. The doses of the substances were such as to cause 30 to 50 per cent. of the maximal relaxation of the intestinal muscle, which is the range of maximal discrimination of the preparation.



10 μg. Visnagin 10 μg. Khellin 15 μg. Visnagin 10 μg. Khellin 7 μg. Visnagin 10 μg.

FIG. 3. Comparative assay of khellin and of visnagin on the rectal cæcum of the fowl. The amounts of the two substances administered are given in μg.

TABLE I

COLORIMETRIC AND BIOLOGICAL ASSAYS OF KHELLIN, OF VISNAGIN, OF KHELLOL-GLYCOSIDE AND OF THE PRODUCTS OF THEIR HYDROLYSIS. ALL THE SUBSTANCES WERE PREPARED IN 0·5 MILLIMOLAR SOLUTIONS AND KHELLIN WAS TAKEN AS THE STANDARD FOR THE COMPARISON

EQUIVALENT DOSES IN μg.

	Khellin m.pt. 154°C	Visnagin m.pt. 144°C	Glycoside m.pt. 175°C	Khellinon m.pt. 100°C	Visnaginon m.pt. 110°C	Khellol m.pt. 179°C
Colorimetric assay	10	14·5	not tested	gives no colour reaction	gives no colour reaction	not tested
Standard deviation	—	0·5	—	—	—	—
Biological assay	10	15	over 300	30	50	40
Standard deviation	—	0·6	—	1·1	2·2	—
Percentage of activity in relation to that of Khellin	100	66	traces	33	20	25

Table I reveals the following points of interest:

(1) Colorimetric and biological assays show that the monomethoxy derivative, visnagin, is about 30 per cent. less active than the dimethoxy derivative, khellin. The agreement between the colorimetric and the biological assays is extremely satisfactory.

(2) Khellinon, the alkali-split product of khellin, loses its colour reaction, but partially retains its power to relax the smooth muscle. Biologically, khellinon is about one third as active as khellin.

(3) The biological action of the glycoside, as tested on the rectal cæcum, is not less than 30 times weaker than that of khellin. In fact it is questionable whether it has any action at all.

(4) Khellol, the product of acid hydrolysis of the glycoside, shows a considerably greater activity than the mother substance. Apparently the substitution of the glycoside radical by the hydroxyl group partially unmasks some of the latent activity of the rest of the molecule.

(5) Khellol is a monomethoxy derivative which differs from visnagin by containing a hydroxyl group. This difference is sufficient to cause a conspicuous diminution of the activity of khellol as compared with that of visnagin.

(6) Visnaginon, similarly to khellinon, gives no colour reaction. Its biological action is about 30 per cent. weaker than that of khellinon.

The action of khellin on the rectal cæcum is considerably weaker than that of adrenaline, 4 μg. of khellin being approximately equivalent in action to 0·01 μg. of adrenaline chloride. Khellin is, however, about 12 times more effective than aminophylline. The rabbit's uterus is also relaxed by khellin, especially when it has previously been contracted by administration of adrenaline, showing that khellin acts directly on the plain muscle and not on the sympathetic nerve endings.

The Comparative Action of Khellin, of Visnagin and of the Khellol Glycoside on the Coronary Circulation

At the time when Anrep and *et al*[10] made their observations upon the coronary vasodilator action of khellin in the heart lung preparation, the existence of the related monomethoxy compound, visnagin, was not yet known. So far, visnagin does not present much interest from the practical point of view, since it occurs in the fruit of *Ammi Visnaga* in very small amounts. However, in the future it might possibly be prepared synthetically. From the theoretical point of view it presents a greater interest because a comparison between visnagin, khellin and the glycoside might throw a light on the relation between the action of these substances and their molecular structure. The comparison of the action of khellin and of the glycoside on the one hand and of khellin and of visnagin on the other, was made on the standard heart-lung preparation on dogs by collecting the blood through a coronary sinus cannula. A few typical experiments, selected from amongst many others, are sufficient to illustrate the action of these substances.

Experiment 1. Heart-lung preparation; systemic output 650 ml./minute; aortic blood pressure 120 mm. Hg. For about 20 minutes the coronary outflow remained constant at 42 to 44 ml./minute. After a gradual administration of 40 mg. of the glycoside the coronary blood flow remained unchanged. Administration of 5 mg. of khellin increased it to 59 ml./minute; after another dose of 5 mg. of khellin the flow increased to 90 to 95 ml./minute. The total amount of blood in circulation was about 800 ml.

Experiment 2. Heart-lung preparation; output 500 ml./minute; aortic blood pressure 120 mm. Hg. The outflow of blood from the coronary sinus remained constant for over 15 minutes at 58 to 61 ml./minute. 4 doses of the glycoside, 20 mg. each, were administered at intervals of a few minutes; 80 mg. in all. The coronary blood flow remained unchanged although the drug was allowed to circulate for several minutes. Administration of 10 mg. of khellin rapidly increased the coronary blood flow to 120 ml./minute. The total amount of blood in circulation was about 700 ml.

Experiment 3. Demonstrated to the Cairo Clinical Society. Heart-lung preparation output 450 ml./minute, arterial blood pressure 130 mm. Hg. The outflow from the coronary sinus was 51 ml./minute. On administration of 45 mg. of the glycoside the coronary outflow remained the same. After administration of 15 mg. of khellin it increased to 250 ml./minute. The amount of blood in circulation was about 500 ml.

The action of khellin was extremely prolonged, the coronary blood flow remaining increased to the end of an experiment. In this, the effect of khellin greatly differs from that of amyl nitrite.

In some of the experiments the action of khellin on the coronary blood flow was more and in others less conspicuous than in the above examples. As regards the glycoside, no coronary vasodilator action could be demonstrated in the heart-lung preparation, even though its concentration was increased to 100 μg./ml.

We were also able to confirm the observation of Bagouri[17], who found that systemic blood vessels are much less sensitive to the vasodilator action of khellin than coronary blood vessels, and that the glycoside caused no coronary dilatation in the perfused rabbit's heart.

The glycoside, since it has no action on the coronary blood vessels, could be administered together with khellin in the same heart-lung preparation. This is not possible when comparing the action of khellin with that of visnagin. Both are coronary vasodilators, the action of which persists for a very long time. The action of the two drugs was, therefore, studied on two separate preparations which were made to work in, as nearly as possible, the same experimental conditions. The type and the weight of the dogs used for the two preparations were the same; the arterial blood pressure, temperature and the output of the heart were respectively maintained at the same levels, and the two hearts usually did not differ in weight by more than 5 g. In spite of these precautions, the individual variations of the coronary sinus outflow were too large to permit of a definite conclusion as regards the relative action of the two drugs. The observations made on the rectal cæcum would suggest that visnagin might possibly be a somewhat weaker coronary vasodilator than khellin. Observations made on two separate heart-lung preparations are not, however, sufficiently accurate to justify this conclusion. As compared with aminophylline the action of khellin on the coronary circulation in the heart-lung preparation is 4 to 6 times stronger.

Observations on the Alimentary Tract *in situ*

The experiments were made on dogs of 7 to 9 kg. weight, anæsthetised with chloralose, sodium luminal or nembutal. The abdomen was opened by a median incision and a loop of the jejunum, about 30 to 40 cm. in length, was tied off between two ligatures. A wide cannula was inserted into each end of the loop. The two cannulæ were then connected to a separating funnel the top of which was joined to a volume recorder. The funnel, filled with saline solution, was kept at

a suitable height, sufficient to fill but not to distend the intestinal loop. The contractions of the loop were recorded on a drum. Intravenous injection of khellin in doses of 5 to 10 mg. caused a rapid and conspicuous relaxation of the intestinal loop, the rhythmic movements being

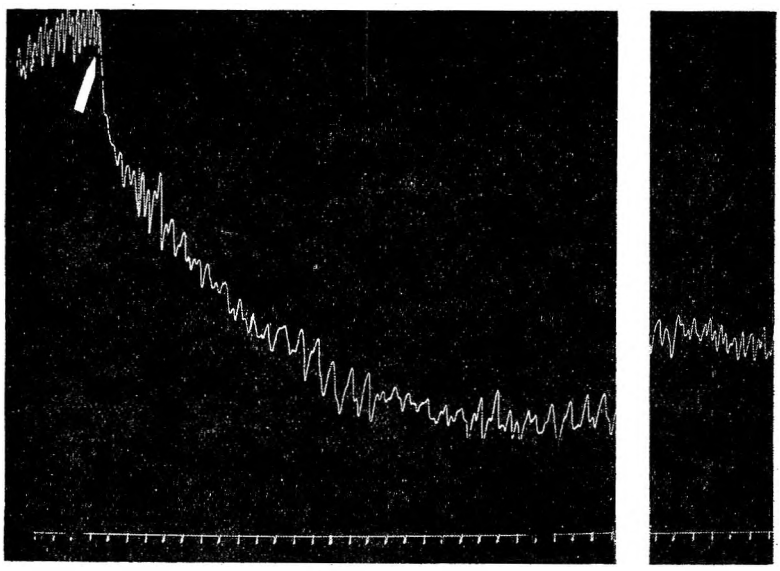


FIG. 4. Dog 10 kg. Left, effect of intravenous injection of 10 mg. of khellin on the intestinal movements recorded as described in the test. Right, 20 minutes later. Time in 10-second intervals.

reduced in rate and in strength. The relaxation of the intestine following administration of khellin was extremely prolonged, the recovery being slow and usually incomplete even after 1 to 2 hours. Figure 4 shows the effect of administration of khellin on the intestinal movements in the whole animal. Khellol glycoside even in greater doses caused no relaxation of the intestinal loop and no diminution of the rhythmic contractions (Fig. 5). In some experiments injections of the glycoside were followed by an increase of the intestinal tone.

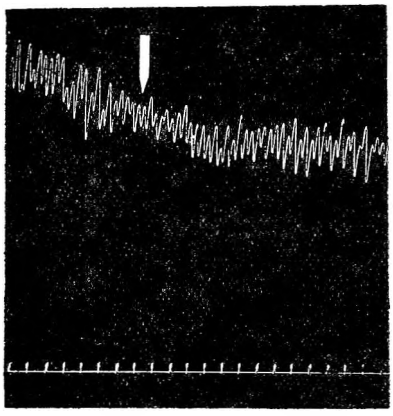


FIG. 5. Dog 9·5 kg. Effect of injection of 20 mg. of khellol glycoside on the intestinal movements. Times in 10-second intervals. The intestine was slowly relaxing before the injection.

OBSERVATIONS ON THE BRONCHIAL MUSCLES

Samaan[8] showed that khellin causes a relaxation of bronchial muscle, but did not study the effect by quantitative methods. Our ex-

periments were performed on perfused lungs of the guinea-pig, using the method of Sollmann and Oettingen[19]. The lungs were suspended in an air thermostat at a temperature of 38° to 40° C., the air being kept saturated with water. A cannula was introduced into the trachea, and the lungs were perfused with warm oxygenated Ringer-Locke solution in the same manner as the isolated heart. In order to allow an outlet, the surface of the lung was scarified in several places, the fluid being collected through a wide funnel in a graduated cylinder. All the measurements were made without opening the thermostat so as to avoid cooling the lungs. The results of some of the experiments are given in Table II.

TABLE II

ACTION OF KHELLIN ON THE BRONCHI OF THE PERFUSED LUNGS OF THE GUINEA-PIG

Initial flow in ml./minute	Concentration of khellin in mg./l.	Maximum flow in ml./minute	Percentage increase
5·0 to 5·4	10	42·0 to 43·0	700
4·4 to 4·8	10	39·0 to 39·6	750
2·5 to 2·6	6	11·6 to 12·0	350
3·3 to 3·5	4	9·8 to 10·2	195
3·5 to 3·7	2	5·2 to 5·4	47

Other similar experiments gave approximately the same results. The minimum effective concentration of khellin causing a 50 per cent. increase in the outflow was about 2 to 3 μg./ml. After replacing the khellin solution with Ringer-Locke solution the outflow of the fluid from the bronchial tree usually returned to its original volume in the course of 4 to 5 minutes. The effect of khellin is strong enough to antagonise considerable concentrations of histamine. When the lungs are perfused with 10 mg./l. of khellin, injections of 5 to 10 μg. of histamine diphosphate have only a negligible effect. In absence of khellin these doses cause a conspicuous broncho-constriction. The glycoside even in high concentrations causes no relaxation of the bronchi. As a broncho-dilator khellin is 4 to 6 times more effective than aminophylline.

As a result of the above observations administration of khellin was tried in a large number of patients suffering from bronchial asthma. With a few exceptions, presented by subjects with advanced emphysema or fibrosis of the lungs, khellin was extremely beneficial. The asthmatic attacks were cut short, the vital capacity of the subjects increased and the feeling of respiratory distress disappeared. On continuous administration of khellin the attacks either disappeared or became less frequent and less severe. The treatment of bronchial asthma with khellin will form the subject of another communication.

ESTIMATION AND DISTRIBUTION OF KHELLIN IN BLOOD AND TISSUES

The estimation of khellin in blood and tissues can be made by the colorimetric method or by the biological method on the rectal cæcum. The following procedure was adopted for the preparation of the final extracts suitable for quantitative estimations. Ten ml. of blood was

added to 100 ml. of alcohol; after filtration the precipitate was washed with three quantities of alcohol, each of 10 ml. The washings and the filtrate were mixed and evaporated on a water-bath, under reduced pressure, to a volume of about 5 ml.; 50 to 100 ml. of water was then added and the solution was treated in a separating funnel with 3 quantities of chloroform, each of 10 ml., to extract the khellin. The chloroform solution was evaporated to complete dryness and the residue was dissolved in exactly 1 or 2 ml. of distilled water, for the colorimetric test, or, of Tyrode's fluid, for the biological assay. This method presents the advantage that the khellin content of any reasonable quantity of blood or of other biological fluids can be concentrated in the 1 or 2 ml. of the final extract. The colorimetric or the biological assay was made against a standard 0·5 millimolar solution of khellin. The recovery of khellin by the above method is 95 to 100 per cent. When the method is used for tissues, a piece of an organ is weighed, ground with silver sand, treated with alcohol and extracted with chloroform as described for blood. Control observations showed that the extraction of khellin from tissues is somewhat less complete, ranging between 85 and 95 per cent. Extraction of khellin from fat is less satisfactory, khellin being highly soluble in lipoids. The accuracy of the colorimetric and of the biological method is the same.

Distribution of khellin in blood.—Khellin added to defibrinated blood or to blood rendered incoagulable by addition of heparin is taken up by the serum or plasma and by the red blood corpuscles. With concentrations varying between 1 and 200 μg./ml. the plasma or serum contained about 10 to 20 per cent. more khellin than the red blood corpuscles. This proportion is not changed by allowing the blood to stand for several hours before it is centrifuged. It is well known that the red blood cells are able to take up a large number of organic substances, some of which are easily given off, while others become fixed and, therefore, probably biologically inactive. Glucose, for example, belongs to the first group of substances and histamine to the second. We find that khellin is rapidly given off by the corpuscles when these are exposed to serum or Tyrode's solution containing no khellin. It is, therefore, obvious that the khellin of the red blood corpuscles is not pharmacologically wasted. It should be looked upon as a store which is readily released to the surrounding plasma.

After intravenous or intramuscular administration of khellin the drug at first appears in the blood in a high concentration. Within a few minutes the khellin concentration begins to diminish, and in about 20 to 30 minutes it reaches a steady level which is maintained for several hours. The rapid diminution of the khellin concentration in the circulating blood is not due to excretion by the kidneys or to destruction by the tissues, but to a gradual and more or less uniform distribution of the drug amongst all the organs of the body.

The drug remains in the circulation for an extremely long time. In dogs after injections of 10 to 20 mg./kg. khellin could be detected in

the blood as long as 36 hours later; 24 hour-samples of urine collected after the injection contain only traces of khellin. The conclusion must be, therefore, made that the khellin is not eliminated by the kidneys in an unchanged form.

Repeated administrations of khellin lead to its accumulation in the blood and tissues. Animals injected with doses of 10 mg./kg. had a concentration of 4 μg./ml. of blood, 24 hours after the first injection and 12 μg./ml. 24 hours after the ninth injection. A similar accumulation of the drug can be also demonstrated in man. For example, in subjects who received one injection of 200 mg. of khellin per day its concentration in the blood, 30 minutes after the first injection, was 4 to 5 μg./ml. and after the 5th injection, 12 to 17 μg./ml.

In order to study the distribution of khellin in the tissues the drug was injected intramuscularly in doses of 20 to 40 mg./kg., the administration of such large doses being necessary since only small samples of tissues could be used to obtain a perfect extraction. The dogs were killed at different intervals of time after the injections and their tissues analysed. In one set of experiments the first animal was killed 1 hour after the injection, the second 24 hours, and the third 36 hours later. The concentration of khellin in the blood of these animals was 30, 16 and 5 μg./ml. respectively. The concentration in the liver, muscle, brain, kidney and the mesentery varied between 20 and 40 μg./g. 1 hour after the injections, between 7 and 15 μg./g. 24 hours and between 2 and 5 μg./g. 36 hours later. It follows that khellin is rapidly distributed over the whole body. The concentration in the liver was always somewhat higher than in the other tissues. The figures obtained for the brain were the lowest, which is probably due to the difficulty of extraction of khellin from lipoid-containing tissues. The disappearance of khellin from the tissues is extremely slow and is not related to any particular organ.

Absorption of khellin and of the khellol glycoside from the Alimentary Tract

Khellin is absorbed from the stomach, from the small intestine and from the large intestine. The absorption from the stomach and from the small intestine was studied in anæsthetised dogs after complete separation of the pylorus from the duodenum. Khellin solutions were injected directly into the stomach or into the duodenum. In some of the experiments khellin was injected into a separated loop of the small intestine. Absorption from the large intestine was studied only in man. The blood samples were collected after the respective injections and assayed for khellin in the usual manner. The absorption from the alimentary tract is not followed by a temporary large increase of the khellin concentration in the blood as is the case with intramuscular absorption. Oral administration is, therefore, suitable for the maintenance of a high concentration of khellin in the blood, while intramuscular

administration is more suitable when it is desired to raise its concentration in a short time.

In man, after oral administration of 300 mg. the maximum concentration of 5 to 6 μg./ml. of blood was reached in 40 to 60 minutes. After rectal administration of 500 mg. dissolved in 50 ml. of alcohol (20 per cent.), the maximum concentration of 6 to 8 μg./ml. was reached in about 2 hours. It can be seen that the rate of absorption of khellin from the large intestine is not inferior to that from the rest of the digestive tract.

No evidence could be found to show that the khellol glycoside is converted in the body to active khellin. After oral or intramuscular administration of 4 to 5 g. of the glycoside, in non-anæsthetised dogs, no khellin could be detected in their circulating blood. Neither is there any evidence showing that the glycoside is absorbed from the intestinal tract. In dogs, anæsthetised with chloralose, a loop of the small intestine, about 50 cm. long, was tied off and its two ends were provided with cannulæ. After thoroughly washing the inside of the loop, 70 ml. of a solution containing 1 mg./ml. of khellin or of the glycoside in alcohol (20 per cent.) was injected into the loop, which was then closed and returned to the abdominal cavity. After 30 to 90 minutes the loop was emptied into a measuring cylinder and washed 2 or 3 times with alcohol (20 per cent.) to remove all traces of the injected substance. The amount of khellin or of the glycoside which escaped absorption was then determined. The khellin was determined as described before for blood and the glycoside, by measuring the reducing power of the intestinal content before and after hydrolysis in acid following a preliminary precipitation of the protein matter with alcohol (95 per cent.). The results of 4 experiments are given in Table III.

TABLE III

ABSORPTION OF KHELLIN AND OF KHELLOL GLYCOSIDE FROM THE SMALL INTESTINE AMOUNT OF FLUID, ALCOHOL (20 PER CENT.), INJECTED INTO THE LOOP WAS IN EACH CASE 70 ML. CONTAINING 70 MG. OF KHELLIN OR OF THE GLYCOSIDE

Substance injected	Duration of absorption in minutes	Amount of fluid not absorbed ml.	Amount of substance not absorbed mg.
Khellin	30	43	14
Khellin	30	41	17
Glycoside	30	48	68
Glycoside	90	26	72

CONCLUSIONS

1. A biological method of assay and a modification of the colorimetric method of assay of the active crystalline principles of *Ammi Visnaga* are described.

2. A comparative colorimetric and biological assay of khellin, of the khellol glycoside and of visnagin showed that the glycoside is biologically almost inactive and that the activity of visnagin is about one-third less than that of khellin.

3. The glycoside has no detectable action on the coronary circulation.

4. No difference could be detected between the coronary vasodilator action of khellin and of visnagin.

5. Kellinon and visnaginon, the products of alkali hydrolysis of khellin and visnagin respectively, give no colour reaction, but still exert some biological activity. The action of khellinon is about one-third that of khellin, and the action of visnaginon about one-third that of visnagin.

6. Khellol, the product of acid hydrolysis of the glycoside, has a definite biological action which is much stronger than that of the glycoside and is about 25 per cent. of that of khellin.

7. Khellin causes a conspicuous relaxation of the bronchi in the isolated lungs and a diminution of the intestinal tone in the whole animal. The khellol glycoside is in these respect inactive.

8. Khellin is rapidly absorbed from the small intestine, from the stomach and from the large intestine.

9. Intramuscular injections of khellin are followed by a rapid and conspicuous increase of its concentration in the circulating blood, which after some time gradually diminishes and finally becomes stabilised at an approximately uniform level. Oral administration is not followed by such a temporary large increase; the concentration of khellin increases gradually and reaches a stable level in about 30 minutes.

10. Khellin is not excreted in the urine in an unchanged form and it disappears from the blood and tissues at a very slow rate.

11. In man, oral or intramuscular administration of a single dose of 100 to 200 mg. of khellin raises its concentration, in the blood to above the minimal effective concentration, which has been shown to cause a coronary vasodilatation and relaxation of the bronchi. Due to the slow destruction of the drug, repeated administration leads to its accumulation in the body.

12. No evidence could be found that the khellol glycoside is converted in the body to khellin or that it can be absorbed from the intestine in an unchanged form.

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THE CORONARY VASODILATOR ACTION OF THE CRYSTALLINE PRINCIPLES OF *AMMI VISNAGA* LINN.

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The two main crystalline principles of the fruit of *Ammi Visnaga*, khellin and khellol glycoside, have been extracted by Mustafa,[1] Malosse,[2] Fantl and Salem,[3] Fahmy and El-Keiy,[4] and Samaan.[5] The chemical structure of the two compounds was determined by Späth and Gruber.[6,7] Samaan claims that khellin (named by him visammin) causes a relaxation of visceral plain muscle, while the glycoside (named by him khellinin) conspicuously dilates the coronary blood vessels of the perfused rabbit's heart.[8,9] The latter observation stands in direct contradiction with that of Anrep, Barsoum, Kenawy and Riad,[10] who find, in the heart-lung preparation on dogs, that the glycoside has no effect on the coronary circulation and that the relaxation of plain muscle, as well as the coronary vasodilation are both due to khellin. The question arises whether possibly both substances, khellin as well as the glycoside, have a coronary vasodilator action, the difference in the results being due to the difference in the species of animals used or to the fact that Samaan worked on isolated hearts perfused with Ringer-Locke's solution, while the experiments of Anrep *et al.* were performed on hearts supplied with blood.

The object of the experiments described in this communication was to compare the action of khellin and of the glycoside on the coronary circulation in the isolated perfused rabbit's heart. I should like to thank Prof. Fahmy and Dr. Haddad, of the Pharmacognosy Department, for the supply of the two substances. Some of the experiments were made with the khellin and the glycoside prepared in the Physiological Laboratory or obtained from pharmaceutical firms. These samples will not be described separately, since no difference in their action could be detected.

Comparative Action of khellin and of khellol glycoside on the Coronary Circulation of the Isolated Perfused Rabbit's Heart

The administration of the different substances was made by changing the fluid perfusing the isolated heart from oxygenated Ringer-Locke's solution to the same solution in which the one or the other of the two substances had been dissolved. A modified form of Langendorff's method was used for the perfusion. The coronary outflow was recorded by collecting the fluid in a measuring cylinder at intervals of 30 seconds. The perfusion pressure was kept at 100 to 120 mm. Hg. A few examples representing the average results obtained are given in Table I.

Khellin in such a large concentration as used in the above experiment, caused a diminution in the amplitude of the heart beat, which was in

EXPERIMENTS WITH KHELLIN

TABLE I

PERFUSION OF THE ISOLATED RABBIT'S HEART. KHELLIN CONCENTRATION 40 mg./l.

Perfusion Fluid	Coronary outflow in ml./minute measured at intervals of 30 seconds
1—Ringer Locke's solution	9, 9, 8·6, 9, 9, 9·2, 9, 9
2— ,, ,, with Khellin ...	12, 19, 24, 28, 35, 36, 38, 37·5, 38, 39
3— ,, ,, solution	35, 33, 28, 24, 22, 17, 16·5, 11, 11, 9, 9·5, 9, 9, 9·5, 9
4— ,, ,, with Khellin ...	12, 16, 19, 22, 28, 36, 38, 40, 39, 40, 39
5—7 minutes later	35, 36, 38, 35, 37, etc.
6—Ringer Locke's solution	33, 30, 26, 22, 20, 18, 14. 13, 10, 8·5, 8, 8

most cases of a temporary nature. It is unnecessary to give a detailed description of each experiment, instead, a summary, showing the initial coronary outflow and the maximum increase obtained with different concentrations of khellin, is given in Table II.

TABLE II

INITIAL CORONARY OUTFLOW AND THE MAXIMUM INCREASE OBTAINED WITH DIFFERENT CONCENTRATIONS OF KHELLIN

Average initial coronary outflow in ml./minute	Concentration of khellin mg./l.	Maximal coronary outflow during perfusion with khellin ml./minute	Increase per cent.
9·0	40·0	39·0	333
9·0	40·0	40·0	334
4·5	10·0	12·0	167
4·2	10·0	10·8	157
10·2	4·0	15·5	52
9·2	4·0	14·5	58

Concentration of 2 to 10 mg./l. caused no detectable effect on the heart beat. As regards the coronary circulation, the minimum effective concentration of khellin, for the rabbit's heart perfused with Ringer's solution, is somewhat below 2 mg/l., i.e., about double that given by Anrep, Barsoum, Kenawy and Misrahy[11] for the heart lung preparation.

In several experiments, solutions of 1 : 40,000 or 1 : 50,000 of barium chloride were used to induce an artificial spasm of the coronary blood vessels before perfusion with khellin. No special advantage was, however, gained by this procedure. In the presence of barium chloride, larger concentrations of khellin had to be used to cause an appreciable increase of the ordinary outflow. The coronary vasodilator action of khellin can be demonstrated on the normally beating heart as well as on the fibrillating heart.

EXPERIMENTS WITH KHELLOL GLYCOSIDE

The observations with the glycoside were carried out with the same technique as those with khellin. The results obtained in some of the typical experiments are summarised in Table III.

Every observation was made on a different heart. In all the above experiments, administration of khellin caused the usual coronary dilata-

TABLE III

EFFECT OF KHELLOL GLYCOSIDE ON CORONARY OUTFLOW

Average initial coronary outflow ml./minute	Concentration of the glycoside mg./l.	Average coronary outflow during perfusion with the glycoside ml./minute
11·0	4·0	10·5
6·5	4·0	6·8
10·8	20·0	11·2
8·8	20·0	8·8
12·0	20·0	11·5
7·4	40·0	5·2
5·8	40·0	4·4
4·7	100·0	4·5
5·9	100·0	5·2

tion. Attempts to demonstrate the coronary vasodilator action of the glycoside after inducing a coronary spasm by means of barium chloride were unsuccessful. In fact, in many experiments, administration of large doses of the glycoside caused some diminution of the coronary outflow, but never an increase. The diminished outflow does not necessarily indicate a vaso-constriction, since it may be accounted for by the mechanical effects accompanying the slight increase in the strength of the heart beat, which is usually observed on administration of large doses of the glycoside.

COMPARATIVE ACTION OF KHELLIN AND OF THE KHELLOL GLYCOSIDE ON THE ISOLATED CORONARY AND SYSTEMIC ARTERIES

The study of the action of drugs on isolated arterial rings was first made by Langendorff. A detailed description of the results obtained by this method was given by Cruickshank and Subba Raw.[12] These authors observed some fundamental difference in the reaction of coronary and systemic arterial rings to changes in temperature and to different drugs. Kountz,[13] working in this laboratory, confirmed the results of the previous workers and applied the method to human arterial rings. The same method was used for the study of the action of khellin and of the glycoside.

Intramuscular branches of the coronary arteries of the water buffalo were dissected and several rings, about 2 mm. thick, were joined together by means of silver wire, 3-4 rings in a chain. The rings were mounted in a 50-ml. bath containing oxygenated Ringer-Locke's solution at 37° C. The contractions of the rings were recorded by a light lever allowing a magnification of about 30 to 40 times. The drugs were administered directly into the bath.

Khellin, in doses of 0·5 mg., caused a definite relaxation of the rings. After replacing the khellin-containing solution with fresh Ringer-Locke's fluid the rings showed a partial recovery, never, however, completely regaining their original tone. Both the relaxation and the recovery were very slow. The difference between the action of khellin and of the glycoside was quite obvious. The latter caused no relaxation of the coronary rings, even though the doses were increased to about 10 times above those of khellin.

As regards the action of khellin upon rings of systemic arteries, I found that doses up to 2 mg. caused no relaxation. Evidently the systemic arteries are much less sensitive to the drug than the coronary arteries.

In order to gain further knowledge about the action of khellin and of the glycoside on systemic blood vessels, I made use of Pissemsky's method[14] of the perfused rabbit's ear. This method presents the advantage that the perfusing fluid need not be oxygenated or warmed.

Samaan found that the flow of fluid through the perfused toad may be as much as doubled by khellin in a concentration of 1 : 5,000. Such high concentrations present no therapeutic interest. My own observations confirm the statement of Anrep, Barsoum, Kenawy and Misrahy,[11] that khellin in concentrations which cause a conspicuous dilatation of coronary blood vessels has no effect on the systemic blood vessels, the latter being less sensitive. Khellin as well as the khellol glycoside, in concentrations up to 40 mg./l., caused no increase in the flow of the Ringer-Locke's solution through the perfused rabbit's ear.

Conclusion

1. Khellin causes a conspicuous increase in the coronary outflow in the isolated perfused rabbit's heart, the minimum effective concentration being about 2 mg./l.
2. The khellol glycoside causes no increase in the coronary outflow, even when administered in concentrations as high as 100 mg./l.
3. Isolated coronary rings are relaxed by khellin and are not affected by the glycoside.
4. The systemic blood vessels are considerably less sensitive to khellin than the coronary blood vessels.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Fluosilicates of the Alkaloids. Janot and Chaigneau. (*C. R. Acad. Sci., Paris,* 1948, **227,** 982.) Knowledge of the fluosilicates of the alkaloids and related substances has been extended to the analysis and optical rotation of 13 alkaloidal fluosilicates. The fluosilicic acid was determined as the potassium salt in alcohol (50 per cent.); in the case of the morphine compound as the fluorochloride of lead. The alkaloid was liberated with ammonia and extracted with ether, chloroform or amyl alcohol. The fluosilicates crystallised easily in prismatic needles or plates; the quinine and quinidine compounds showed a blue, those of morphine and corynanthine a green, fluorescence. The general formula was SiF_5H_2 $(alkaloid)_2$ XH_2O, except the compounds of morphine and codeine, both of which were anhydrous. H. F.

ANALYTICAL

Colorimetric Determination of Copper with Carbon Disulphide and Diethanolamine. W. C. Woelfel. (*Anal. Chem.*, 1948, **20,** 722.) Use has been made of the reaction of the *bis*-(2-hydroxy-ethyl)-dithiocarbamate of diethanolamine with the cupric ion forming a brownish yellow salt soluble in water, as the basis of a colorimetric method for the determination of copper. The reagent is prepared by mixing solutions of carbon disulphide and diethanolamine, both in methyl alcohol. Several advantages are claimed over the ordinary diethyldithiocarbamate procedure in that the solubility of the coloured copper salt eliminates the need for a stabilising colloid or for extraction with an organic solvent. Of the metals whose compounds are soluble under the conditions used, bismuth, chromium, cobalt, iron, mercury, nickel, silver, and uranium interfere seriously. Procedures are described for eliminating the interference of appreciable amounts of bismuth, chromium, ferric iron, and uranium. Among the anions studied, only cyanide, dichromate, nitric, and sulphite interfered appreciably. R. E. S.

Ephedra and Ephedrine in Nasal Sprays, Assay of. Report No. 6 of the Poisons Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts. (*Analyst,* 1948, **73,** 312.) The method of the British Pharmaceutical Codex, 1934, for the determination of the total alkaloids in ephedra was regarded as satisfactory. For the determination of ephedrine in sprays an aliquot portion of the spray is steam distilled in the presence of sodium chloride and sodium hydroxide, the ephedrine being collected in a standard excess of 0·05N sulphuric acid which is titrated against 0·05N sodium hydroxide. The distillation is continued until no further alkaloid is removed and the result is calculated as anhydrous ephedrine. R. E. S.

Ferric Chloride, Reactions of, in presence of Alcohol. L. Rosenthaler. (*Pharm. Acta Helvet.*, 1948, **23,** 271.) It is well known that the blue colour given by phenol with aqueous solutions of ferric chloride does not appear in dilute alcohol. If to 1 vol. of a 1 per cent. solution of ferric chloride

9 vols. of alcohol are added the solution after a time no longer gives the usual reactions of ferric salts. Apparently the whole of the iron is present in the form of a complex, or alternatively in a colloidal form. G. M.

***o*-Hydroxyquinoline Sulphate, Alkalimetric Titration of.** F. Reimers. (*Dansk Tidsskr. Farm.*, 1948, **22**, 181.) The pk_2 value for hydroxyquinoline was found to be about 10·6 (in 50 per cent. alcohol) and 11·4 (in 75 per cent. alcohol.) Thus the difference between pk_1 and pk_2 increases with increasing alcohol concentration. In addition, the colour of the titrated solution is brighter in alcohol than in water. The titration may be carried out as follows: 0·1 g. of *o*-hydroxyquinoline sulphate is dissolved in 20 ml. of alcohol (86 per cent. by weight) and titrated with aqueous 0·1 N sodium hydroxide to the colour change of bromocresol purple, or to a green colour with bromothymol blue. This method often gives higher results than bromimetric titration, showing the presence of excess of sulphuric acid.

G. M.

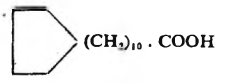
Methylene Blue Periodide as Volumetric Indicator. J. A. Gautier. (*Ann. pharm. Fr.* 1948, **6**, 171.) By the addition of iodine and hydriodic acid to methylene blue, a precipitate of the formula $B.HI.2I_2$ is obtained. This reaction may be used both in iodimetry and acidimetry. On the addition of a reducing agent, methylene blue is reformed, and gives a blue colour to the solution, while a larger quantity of a strong reducing agent decolorises the methylene blue forming the leuco base. A suitable indicator may be prepared by mixing a solution of methylene blue (0·0935 per cent.) with an equal volume of 0·01N iodine solution. This suspension should be used fresh. For iodimetry a drop or two is added to the solution being titrated just before the end-point, and titration is continued with thiosulphate until a blue colour appears in the solution. Alternatively, one drop of methylene blue solution may be added, though in this case there is a slight error equivalent to one drop of 0·01N iodine. This method is claimed to be superior to the use of starch. Since the compound is also decomposed by alkalis, it may also be used for acidimetric titrations but in this case it would not appear to offer any advantage over the usual indicators. G. M.

Starch in Plant Tissues, Determination of. G. D. Pucker, C. S. Leavensworth, and H. B. Vickery. (*Anal. Chem.*, 1948, **20**, 850.) The method consists of extraction of the starch from a 50 to 250 mg. sample of dried plant tissue with perchloric acid, precipitation with iodine under conditions that have been shown to be quantitative, decomposition of the starch-iodine complex and determination of the sugar produced by hydrolysis of the recovered starch. The results are independent of the composition of the starch of different species with respect to amylose and amylopectin content, in contrast to the colorimetric methods of starch estimation, but once the fundamental values for the starch from a given tissue have been determined in terms of sugar titrations and in comparison with a standard, e.g. a preparation of potato starch, the more rapid colorimetric method can be used in a series of determinations on the same tissue. For a variety of plant tissues results are accurate to within 2 per cent. E. N. I.

FIXED OILS, FATS AND WAXES

***dl*-Hydnocarpic Acid, Synthesis of.** D. G. M. Diaper and J. C. Smith. (*Biochem. J.*, 1948, **42**, 581.) The accepted structure assigned to

hydnocarpic acid has been confirmed by synthesis. The ester-acid chloride of sebacic acid reacts with ethyl sodioacetoacetate and the product yields a sodio derivative which reacts in the cold with *cyclo*-pent-2-enyl chloride to give a complex; this complex is hydrolysed mainly with the loss of the acetyl and carbethoxy groups to 10-ketohydnocarpic acid.



The keto acid was isolated as the semicarbazone which on heating with sodium ethoxide yielded *dl*-hydnocarpic acid. The synthetic acid did not depress the melting point of the natural *d*-acid and confirmation was obtained from the preparation and identity of the two dihydroderivatives in which there is no asymmetry. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Ascorbic Acid, Pure, Stability of Solutions of, and of Dehydroascorbic Acid. P. Guild, E. E. Lockhart and R. S. Harris. (*Science*, 1948, **107**, 226.) Because there appears to be lack of agreement between the content of ascorbic acid in foods as measured by the 2 : 6-dichlorophenolindophenol method and the 2 : 4-dinitrophenylhydrazine method, the suitability of these two methods for determining ascorbic or dehydroascorbic acid has been compared. The effects of oxalic acid and metaphosphoric acid on the stability of ascorbic and dehydroascorbic acids in solution have also been investigated. It was found that the 2 : 4-dinitrophenylhydrazine method gave higher results than the other since the reagent reacts with products related to ascorbic acid which are biologically inactive; this may prove helpful when assaying the original vitamin C content and not the actual content. The method using 2 : 6-dichlorophenolindophenol gave results which closely resembled the biologically active content of ascorbic acid and dehydroascorbic acid. Ascorbic acid was found to be stable for at least 12 days when kept at 4°C. in a solution containing 5 per cent. of oxalic acid and 10 per cent. of acetic acid; in a solution containing 5 per cent. of metaphosphoric acid and 10 per cent. of acetic acid it was stable for at least 8 days under similar conditions. Dehydroascorbic acid was unstable in neutral solution at room temperature, and neither oxalic nor metaphosphoric acids prevented the irreversible change into biologically inactive forms. For stabilising ascorbic acid, oxalic acid was more effective, more stable, more convenient and less expensive than metaphosphoric acid. L. H. P.

B_t, a New Vitamin of the B Group. G. Fraenkel, M. Blewett and M. Coles. (*Nature*, 1948, **161**, 981.) The common mealworm, *Tenebrio molitor*, has been shown to be a very suitable test subject for folic acid and certain other, still unidentified, B factors. When grown on an artificial diet consisting of casein, glucose, water-insoluble fraction from yeast, cholesterol, a salt mixture, and aneurine, riboflavine, nicotinic acid, pyridoxine, pantothenic acid, choline chloride and inositol in ample amounts, at least two more factors are essential for growth and survival, namely, folic acid and a factor contained in a norite filtrate from yeast or liver extract; in the absence of both these factors growth is very slow and mortality high. The authors have tentatively named the norite filtrate factor B_t (to indicate its activity on *Tenebrio*). The reported absence of significant amounts of folic acid or the conjugate, pteroylheptaglutamic acid, in well known commercial parenteral liver extracts was fully confirmed in tests with *Tenebrio*.

Further tests to ascertain the possible B_L effects of these extracts, showed no effect with one of the extracts in any concentration, and a positive effect with another extract in only a very large dose. It is obvious that the kind of activity for which the anti-anæmic concentrates have been developed must be entirely different from that of folic acid or B_L. S. L. W.

Schiff's Reagent, Use of Thionyl Chloride in the Preparation of. J. C. Barger and E. D. DeLamater. (*Science*, 1948, **108**, 121.) 1·0 g. of basic magenta is dissolved in 400 ml. of distilled water, 1· ml. of thionyl chloride is added and the mixture allowed to stand for 12 hours. The decolorised solution is cleared by shaking with 2 g. of charcoal and filtering immediately. Alternatively, the treatment with charcoal may precede the addition of the thionyl chloride. The *p*H of the resulting solution is 1·24, as opposed to 1·38 for the solution prepared in the usual manner. When used in the Feulgen reaction, Schiff's reagent prepared with thionyl chloride is a successful nuclear stain for fungi, *Blastomyces dermatitidis* and *Saccharomyces cerevisiae*, in addition to tissue sections of human thymus, kidney, liver and spleen. G. B.

Vitamin B_{12} and Thymidine, for growth of *Lactobacillus lactis*. W. Shive, J. M. Ravel and R. E. Eakin. (*J. Amer. chem. Soc.*, 1948, **70**, 2614.) A medium previously described is modified by adding an oleic acid source, tween 80, 10 mg./10 ml., enzymatic digest of casein, 10 mg./ml. or clarified tomato juice, 0·5 ml./10 ml., and Wilson's liver fraction LR, 10 μg/10 ml. and replacing a phosphate buffer by sodium acetate. For the growth of *Lactabacillus lactus* Dormer, thymidine can replace liver extracts containing the principles active against pernicious anæmia, half-maximum growth stimulation requiring 1 to 3μg./10 ml. Thymine (100 μg./10 ml.) is inactive. Probably vitamin B_{12} functions in the biosynthesis of thymidine. In the medium containing tomato juice, 1 ml. of aerated water in 10 ml. can replace liver extracts; this effect is enhanced by adding ascorbic acid. Aerated water is not effective in the medium made with enzymatic digest of casein, but 1 mg. of ascorbic acid in 1 ml. of aerated water/10 ml., can replace liver extracts. G. B.

Vitamin D. Potency of the U.S.P. Reference Cod-liver Oil. W. Dasler, C. D. Bauer, and M. van Nostrand. (*J. Lab. clin. Med.*, 1947, **32**, 1251.) Three fresh samples of calciferol from entirely different sources, were dissolved in corn oil and repeatedly assayed against the U.S.P. reference cod-liver oil No. 2. The results indicate potency-values of 50 ± 2 units/μg. for all three samples. Similar bioassays of samples of parallel physical and chemical purity made in 1937-1938 against an earlier U.S.P. reference oil gave values of 40 units/μg. This discrepancy in potency-value, confirming repeated observations of recent teams of workers, can only be explained upon the hypothesis that the reference standard cod-liver oil is deteriorating, and should therefore be replaced, as regards vitamin D, by a primary standard, viz., pure crystalline vitamin D_3. F. J. D.

BIOCHEMICAL ANALYSIS

***p*-Aminosalicylic Acid in Blood and Cerebrospinal Fluid, Determination of.** W. Klyne and J. P. Newhouse. (*Lancet*, 1948, **255**, 611.) A colorimetric method for the determination of *p*-aminosalicylic acid in blood and cerebrospinal fluid has been developed. The procedure is as follows:— Add 0·5 ml. of oxalated whole blood or 1 ml. of cerebrospinal fluid to

7 ml. of water, mix until caking occurs, add 3 ml. of 20 per cent. *p*-toluenesulphonic acid, allow to stand for 5 minutes and filter through a No. 40 or 42 Whatman paper. To 5 ml. of the clear filtrate add 1 ml. of citrate buffer solution, 0·75 M, and 2 ml. of 2 per cent. (Ehrlich's) *p*-dimethylaminobenzaldehyde reagent. Read the colour intensity with a photoelectric photometer using a blue filter, e.g., Ilford No. 602. Use a reagent blank consisting of 1·5 ml. of *p*-toluenesulphonic acid, 1 ml. of citrate buffer and 2 ml. of Ehrlich's reagent and made up to 8 ml. with water. A calibration curve is constructed from 3 standards of sodium *p*-aminobenzoate corresponding to 20, 10 and 4 mg. of *p*-aminosalicylic acid per 100 ml. Streptomycin (1000 μg/ml. of plasma) does not interfere with the estimation but the method cannot be used if other primary aromatic amines are present. E. N. I.

Salicylates in Blood, Determination of. M. Volterra and D. M. Jacobs. (*J. Lab. clin. Med.*, 1947, **32**, 1282.) Salicylates may be determined by a simple and rapid method based on the xanthoproteic reaction, in volumes of 1 ml. of blood serum or plasma, deproteinated by trichloracetic acid. The yellow colour subsequently developed by the reagents is directly proportional to the concentration of salicylates as observed in recovery-values ranging from 5 to 80 mg. per cent. determined either photoelectrically or by direct vision against a standard series of potassium dichromate units. Good agreement was obtained between the authors' method and that of Coburn for values ranging from 5 to 55 mg. per cent. F. J. D.

Streptomycin, Identification on Paper Strip Chromatograms. R. E. Horne, Jr. and A. L. Pollard. (*J. Bact.*, 1948, **55**, 231.) A paper strip chromatographic method is described for detecting the presence of streptomycin in culture filtrates, etc., using 3 per cent. ammonium chloride solution as the solvent, the mechanism involved being a salting-out process. The paper strips are spotted near one end with the solution under test and the "spot" dried. They are then suspended, spotted end downwards, so that the lower ends are immersed in the ammonium chloride solution, the whole being placed in a closed container so as to maintain a saturated atmosphere. The solvent moves the streptomycin in a sharp band near the advancing solvent front. After 4 to 12 hours, when the solvent front has reached the desired height, the strips are dried and developed by means of a modification of the Sakaguchi reaction. The dry strip is sprayed with N/2 sodium hydroxide and immediately with 0·25 per cent. α-naphthol. After 2 minutes it is sprayed with sodium hypochlorite prepared as described by Sakaguchi. Streptomycin is shown as a brilliant red band. H. T. B.

Vitamin A, Simultaneous Comparative Carr-Price Reactions for Determination of. W. Koch and D. Kaplan. (*J. biol. Chem.*, 1948, **172**, 363.) The difference in rates of fading of the Carr-Price colour of graded concentrations of standard vitamin A, provides the basis of a photoelectric method in which standard and "unknown" are matched simultaneously thereby cancelling errors. Initially, the currents generated by two photocells receiving the light from two reaction tubes containing two different concentrations of vitamin A in Carr-Price reagent, were balanced on a galvanometer sliding bridge scale. When the differences in fading-rates in seconds, for graded concentrations of vitamin A standard in U.S.P. units were plotted against bridge-readings, it was observed that with falling concentrations the curves

flatten, change their slope and (at 10 units) approach the 80 per cent. transmission line, used as a convenient arbitrary standard. Subsequently, a calibration diagram was constructed by joining each of the predetermined bridge readings equivalent to fading values at 15 to 180 sec. in 15 sec. intervals, for concentrations of 50, 40, 30 and 20 units respectively, to the point of intersection of the 10 unit abscissa with the arbitrary "80" bridge ordinate. Determination of "unknowns" were then made by entering each bridge-reading as a dot on the appropriate seconds line, tracing the best fitting line through the points back to the abscissa, and reading the vitamin A in units/ml. Evidence is offered that values obtained by this method are in fair agreement with figures measured by ultraviolet absorption. F. J. D.

PHARMACY

DISPENSING

Oils and Fats, Sterilisation of. H. Hurni. (*Pharm. Acta Helvet.*, 1948, **23**, 283.) A number of methods are given in various pharmacopœias for the sterilisation of oils and fats. The ordinary conditions of sterilisation in an autoclave do not apply since water is absent, and in fact bacteria are killed in the same time at any temperature, whether they are in fat or in dry air. It has been shown that fats and oils as used for pharmaceutical preparations are nearly always sterile. Suitable methods for sterilisation are, as follows: 4 hours at 140°C.; 3 hours at 145°C.; 2 hours at 150°C.; or filtration through a Berkefeld filter at 80° to 90°C. The Seitz filter is not effective for this purpose. G. M.

Vitamin A, Stability of, In Pills. P. Terp. (*Arch. Pharm. Chemi*, 1948, **55**, 513.) Pills were prepared according to the following two formulæ: I. lactose-starch granulate, 54 g.; vitamin concentrate, 8·4 g.; hydroquinone, 0·03 g.; aluminium oxide anhydrous, 9·6 g.; II. granulate, 41·4 g.; vitamin concentrate, 9 g.; hardened mustard oil, 12 g.; hydroquinone, 0·06 g.; aluminium oxide, anhydrous, 15·6 g. Both of these lost 40 per cent. in strength in 3 months. A further batch was made as above, with the addition of 0.25 per cent. of hydroquinone added to the granulate during granulation. These pills became black after a few days, and the vitamin was almost completely destroyed in a week. A number of pills were then made by the dropping method in which the molten mixture is solidified by dropping into cold alcohol (d. 0·883). Good results were obtained with the following formula: vitamin A concentrate (160,000 units/g.) 1 part; hardened arachis oil 8 parts; hydroquinone, 0·25 per cent. These pills showed a loss in strength of 12 per cent. after keeping for 12 months at ordinary temperature, and no loss over the same period in an ice chest. G. M.

GALENICAL PHARMACY

Penicillin Depot Preparations. J. Büchi and F. O. Gundersen. (*Pharm. Acta Helvet.*, 1948, **23**, 290.) Measures found effective for delaying the absorption of penicillin were: to avoid aqueous solutions, to surround the solid penicillin or sparingly soluble penicillin salt with an oil base, to choose the optimum degree of fineness, and to add substances (wax, adrenaline, aluminium stearate) which delay absorption. Certain proprietary preparations were effective, so that with these only one injection daily was necessary. Of the formulæ given, the following is the best; with an

injection of 1 ml., the effect lasts for more than 12 hours; and with 2 ml. for 24 hours; benzylpenicillin sodium cryst., 300,000 units; adrenaline, 0·3 mg.; sterile neutralised olive oil, to 1 ml. G. M.

Tincture of Iodine, Stability of. A. Tennøe. (*Dansk Tidsskr. Farm.*, 1948, **22**, 226.) An alcoholic solution of iodine, without potassium iodide, rapidly decomposes, and after 1 week's storage it already contains an amount of hydriodic acid greater than the limit allowed by the Danish Pharmacopœia (i.e. 0·13 per cent.). The reaction is apparently reversible, since if the preparation, after heating at 90°C, is then kept at ordinary temperature for some time, the acidity decreases and the free iodine increases. After 12 weeks' storage at 20°C., there was no appreciable difference between two preparations containing respectively 5·06 per cent. of iodine with 3·54 per cent. of potassium iodide, and 5·04 per cent. of iodine with 2·08 per cent. of potassium iodide. When kept at 90°C. for 4 weeks, the preparation with the smaller quantity of potassium iodide showed 50 per cent. more acidity than the other. G. M.

PHARMACOGNOSY

Hydrastis, Histological Peculiarities of its Adulterants. R. Lemesle. (*C. R. Acad. Sci., Paris*, 1948, **227**, 686.) Blaque and Maheu (Rev. gén. Bot. 1947, **54**, 138) have described the peculiarities of the pith and pericycle of the rhizomes of *Xanthorrhiza apiifolia* L'Hér. and of *Coptis Teeta* Wall, which are adulterants of *Hydrastis canadensis* rhizome. In this paper the xylem elements of these three rhizomes are described. Hydrastis rhizome has vessels with bordered pits, containing pectosic mucilage and of diameter 36 to 44μ. Wood parenchyma and fibres (without starch) are also present. In *Xanthorrhiza* the vessels are up to 55μ in diameter and have no bordered pits; parenchyma is absent. The fibres contain numerous spherical or polyhedral starch granules (4 to 6μ). In *Coptis*, narrow tracheidal vessels, such as occur in ipecacuanha, are present, maximum diameter 18μ. Parenchyma is absent and the fibres contain starch granules. J. W. F.

PHARMACOLOGY AND THERAPEUTICS

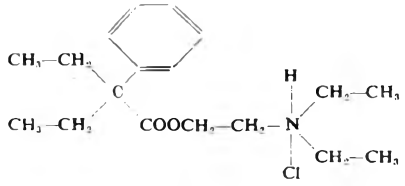
Caronamide, Effect on Penicillin Plasma Concentration in Children. M. Rapoport, F. B. Corneal, K. H. Beyer and W. F Verwey. (*Amer. J. med. Sci.*, 1948, **215**, 514.) Oral administration of caronamide to children, in a dosage of 0·2 g./kg. of body weight per day, together with penicillin increased the concentration of penicillin in the plasma to from 1·8 to 2·8 times the control values obtained with penicillin alone. With a caronamide dosage of 0·4 g./kg. of body weight per day the penicillin concentration was increased by from 2·8 to 14·5 times the control concentration. Toxic symptoms during 1 to 2 weeks administration were not sufficient to warrant discontinuance of caronamide treatment. Renal function tests before and after treatment showed that the drug does not irreversibly affect kidney function. H. T. B.

Cuprelone, Trigeminal Neuralgia Treated With. A. M. G. Campbell. (*Lancet*, 1948, **255**, 690.) Cuprelone, cupro-allyl-thiourea-sodium benzoate, contains 19 per cent. of copper. It was introduced as a non-toxic substitute for gold compounds. It is supplied in dry ampoules, containing 10 to 100 mg. and is dissolved in sterile distilled water for intravenous

injection; 100 mg. produced a rise in blood-copper level for 2 hours after injection. All the extra copper is probably excreted during the next 2 or 3 weeks. It was administered in 13 cases of trigeminal neuralgia and in some the pain was relieved; its toxic effects were negligible. H. F.

Dipole Moment and Physiological Activity. B. Melander. (*Farm. Revy*, 1948, **47**, 503.) In the hexachloro*cyclo*hexane series, maximum insecticidal activity is shown by the isomer with a dipole moment in the region of 4D; the two isomers with no dipole moment are inactive. A similar effect is observed with the same compound in the production of total colchicine mitosis in *Allium Cepa*. Local anæsthetic bases (procaine and xylocaine) have a moment about 4D, whereas the group of aliphatic alcohols and benzyl alcohol have moments 1·7 to 2 D. The latter group, unlike the former, do not produce convulsions when injected intravenously. Ether and chloroform have dipole moment 1·15 D. G. M.

Parpanit, Treatment of Parkinsonism with. W. F. Dunham and C. H. Edwards. (*Lancet*, 1948, **255**, 724.) The effects of parpanit, a synthetic compound, closely related to trasentin and pethidine (dolantin), and having the structural formula



were investigated in 25 cases of Parkinsonism, in all of which treatment with solanaceous drugs had been given for more than a year. The results showed no striking difference from those obtained with solanaceous drugs. The optimum individual dose varied from 0·025 to 0·1 g., usually 3 to 4 times a day. The side effects of parpanit, although similar to those of the solanaceous drugs, differed sufficiently in intensity and frequency to make it the drug of choice for some patients. E. N. I.

Penicillin Aluminium Salt in Mouse Protection Tests. R. D. Reid. (*Proc. Soc. exp. Biol. N.Y.*, 1947, **66**, 605.) Aluminium penicillin is a water-insoluble salt which has been administered in arachis oil suspension. A dose of 300,000 I.U. given intramuscularly to human subjects has been found to give blood levels of 0·03 I.U./ml. for 12 to 24 hours. The author compared the dosage required to give a survival rate of 50 per cent. in mice infected intraperitoneally with 1000 M.L.D. of a diluted culture of *Diplococcus pneumoniæ* Type 1, with the dosage of sodium penicillin in oil and wax and calcium penicillin in oil, giving injections for 14 days. The figures obtained were respectively 34 units, 40 units and 95 units.

H. T. B.

Penicillins F, G, K and X; Relative Antisyphilitic Activities. H. Eagle and R. Fleischman. (*J. Bact.*, 1948, **55**, 341.) The antisyphilitic activities of penicillins F, G, K and X, and of bacitracin were evaluated by a method based on the fact that an extremely small amount of treatment is sufficient to terminate syphilitic infection in rabbits, provided it is administered soon after inoculation and before the appearance of the primary lesion. Rabbits were inoculated intradermally with 2,000 *Treponema*

pallidum, and treated 4 days later with intramuscular injections of the various penicillins or bacitracin, repeated once daily for 4 days. It was found that penicillins F, K and X were approximately 8, 12 and 14 per cent. respectively as active as penicillin G, and a crude preparation of bacitracin assaying at 30 units/mg. was 10 per cent. as active. Comparison of the results obtained with those of other workers shows that the absolute and relative activities of the various penicillins and of bacitracin vary widely according to the method of assay. H. T. B.

Penicillin Suspension with Adrenaline for Gonorrhœa. A. Cohn and B. A. Kornblith. (*Amer. J. med. Sci.*, 1948, **215**, 506.) A total of 300 male ambulatory patients with acute gonococcal infections were treated intramuscularly or subcutaneously with a single dose of suspension of potassium penicillin in oil containing adrenaline. The suspension contained 300,000 units of potassium penicillin and 0·3 mg. of adrenaline in 1 ml. of vegetable oil, and dosage varied between 0·25 and 1 ml. Only exceptionally were 2 ml. doses used. Single injections of 150,000 units cured 97 of 100 patients treated, the criteria of cure including a bacteriological and clinical check for 2 or 3 weeks after treatment. The other 3 cases were promptly cured by a second injection of the same amount, indicating that the relapse strain had not become resistant to penicillin. All of 19 patients treated with 200,000 units in 1 ml. intramuscularly were cured. A table gives the results obtained on 154 of the patients treated, the remainder of the 300 not attending for final determination of cure. No untoward local or systemic reactions were observed. H. T. B.

Salicylazosulphapyridine, Therapeutic Action of. N. Svartz. (*Bull. schweiz. Akad. med. Wiss.*, 1948, **3**, 311.) It has been shown that all acid azo compounds have a marked affinity for connective tissue, particularly tissue rich in elastin; and their localisation in such tissues may be detected microscopically by the fluorescence. Such compounds should therefore be effective in ulcerative colitis and rheumatic polyarthritis. Cases of colitis were treated usually with 1 g. of salicyazosulphapyridine 6 times a day, the dose being decreased as the symptoms improved. It was found necessary to continue the treatment over a long period with 0·5 g. 2 to 3 times a day. Results of 119 cases show a considerable improvement or cure in 108 of them. For acute polyarthritis, 100 patients were treated (some with salicylazosulphathiazole) in the period 1941 to 1945. In 1947, 92 were reported free from symptoms. The results for chronic polyarthritis were less satisfactory, only about 40 per cent. showing any considerable improvement. Although the administration was generally *per os*, periarticular injection was sometimes useful. G. M.

Streptomycin, Effects on *Mycobacterium tuberculosis* Infection by Inhalation. C. Levaditi, A. Vaisman and P. Levy. (*C. R. Acad. Sci., Paris*, 1948, **227**, 987.) Mice were infected by injection with *Mycobacterium tuberculosis*, human strain H 512, and 30 were kept in an atmosphere containing streptomycin introduced under a pressure of 0·5 kg. for 6 hours a day for 6 weeks. The total amount of drug evaporated during this period amounted to 22 mega-units. Another batch of 20 mice were infected and kept as controls without treatment. Of these all died within 29 days. Of the mice treated with streptomycin, one died on the 46th day, the remainder were then destroyed and examined. From the results it appeared that streptomycin undoubtedly exerted therapeutic activity,

although it was lower than that showed by the subcutaneous injection of 1,000 to 2,000 units daily. H. F.

Tetra-ethyl pyrophosphate in Myasthenia Gravis. A. S. V. Burgen, C. A. Keele and D. McAlpine. (*Lancet*, 1948, **254**, 519.) T.E.P.P. was given to 3 patients either as a 0·5 per cent. solution in propylene glycol, intramuscularly, or as a 2 or 5 per cent. solution in propylene glycol, orally. By injection, it was found to be from a third to half as potent as prostigmine but its action lasted about twice as long. Side effects, similar to those produced by prostigmine, were experienced, but the action on the gut producing colic and diarrhœa was prevented by atropine. G. R. B.

BACTERIOLOGY AND CLINICAL TESTS

Aerosol OT, Synergistic Effect of, on Certain Germicides. G. V. James. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 336.) Rideal-Walker coefficients for dispersion of certain germicides in a 20 per cent. solution of a neutral castor oil soap show the following increases on the addition of 0·1 per cent. w/v of aerosol OT (di-octyl-sodiumsulphosuccinate): —phenol (1 per cent.), 0·6; cresol (1 per cent.), 0·9; *p*-chloro-*m*-xylenol (2 per cent.), 1·1; butylphenol (2 per cent.), 1·1; benzylcresol (2 per cent.), 1·6; cresantol-15 (3 per cent.), 2·2. Similar results are obtained with 0·4 per cent. of a commercial product described as the *bis*-ester of sodium sulphonated dicarboxylic acid. The increased germicidal activity appears to be stable over at least 4 months at room temperature. The use of sulphonated castor oil instead of castor oil soap results in a smaller enhancement of R.W. co-efficient. *Bacillus typhosus* was used as the test organism and 0·02 ml. pipettes replaced platinum loops to prevent errors due to differences in surface tension. G. B.

Penicillin: Induced Resistance and Oxygen Utilisation. W. D. Bellamy and J. W. Klimek. (*J. Bact.*, 1948, **55**, 147.) The observation that penicillin-resistant staphylococci grow more slowly than the parent strain, and almost exclusively at the surface of broth cultures, was confirmed on a penicillin-sensitive strain of *Staphylococcus aureus*, and a penicillin-resistant variant of this strain. Growth curves under aerobic and anaerobic conditions showed that the penicillin-resistant variant grew more slowly than the parent-sensitive culture, and had lost the ability to grow anaerobically. Strains of *Streptococcus fæcalis*, *Strep. mastitidis* and *Clostridium welchii* when treated in a similar manner failed to develop resistance to penicillin. It is suggested that the development of resistance is dependent on the power to grow under aerobic conditions. H. T. B.

Penicillin-Resistant Staphylococci. W. D. Bellamy and J. W. Klimek. (*J. Bact.*, 1948, **55**, 153.) The properties of penicillin-resistant variants of *Staphylococcus aureus* have been compared with those of the parent sensitive culture. The variant was 60,000 times more resistant to penicillin than the original culture, was Gram-negative, and had lost the ability to grow anaerobically. Increase in resistance is accompanied by a progressive loss of fermentative activity, but the resistant variant can synthesise nicotinic acid in quantities sufficient for growth. It produces an extracellular penicillinase when grown in the presence of penicillin. Serial transfers through a deficient medium will cause a reversion from Gram-negative rods to the original Gram-positive staphylococcus forms which have lost most of their resistance. H. T. B.

BOOK REVIEWS

THE UFAW HANDBOOK ON THE CARE AND MANAGEMENT OF LABORATORY ANIMALS, with an Appendix on Statistical Analysis. Edited by A. M. Worden. Pp. 368, 70 Figs. Bailliere, Tindall and Cox, London, 1947, 31s. 6d.

This handbook has been produced by the Universities Federation for Animal Welfare. It is intended as a practical introduction to the husbandry of laboratory animals for use by research workers and technicians. Both novices and experienced workers in fields involving the keeping of health stocks of animals for experimental purposes will find much of value to them in this book. The novice may be bewildered by the numerous variations in details which he will find reported from different laboratories. The experienced worker will be interested to learn what variations of detail have been found valuable by other workers. Scarcely any detail can be considered very much better than the rest and much depends on the availability of varieties of such things as food pots and water bottles, material for constructing cages and stands for cages, etc. One general principle, however, stands out pre-eminently, the simpler the arrangement the better. Simplicity in racks for holding cages, simplicity in the construction of the cage itself, simplicity in the food pots and water bottles all help towards cleanliness. Ledges in racks harbour dust and food, and getting it out with a brush spreads most of it into the air again to settle elsewhere. Labour and time are wasted. The best arrangement seen by the reviewer is that in her own laboratory, viz., racks made of gas piping, which, being cylindrical, affords the smallest possible area for collecting dust, and cages (not in her own laboratory) made of sheets of wire mesh with strong borders (for floor, walls and ceilings), held together with movable clips. These cages are hooked on the bars which hold the trays of the cages above, themselves loose and easily pulled out for cleaning. Each cage can be taken to pieces in nearly no time and many more cages can be sterilised at one time when in pieces than when whole. However, "chacun à son goût" (and his pocket). The animals considered in detail in this book are the rabbit, guinea-pig, Norway rat, black rat, mouse, wild house-mouse, wood mouse, deer mice, cotton rat, common or field vole, Orkney vole, golden hamster, ferret, hedgehog, pigeon, canary, amphibia, *Xenopus laevis* Daudin, and fresh water fish. Others dealt with more lightly are anthropoids, dogs and cats, horses, other ungulates, shrews, vampire bats, poultry, reptiles, marine forms and other vertebrates. Housing, nutrition, breeding, etc., and common diseases of each species are dealt with. In addition there are useful chapters on Law and Practice: The Rights of Laboratory animals, (much of which will be news to most animal workers), Pests of the Animal House and their Control, and an Appendix of 70 pages, a conspectus of the Elements of Statistical Analysis which should be read and digested if possible even by those readers who are already familiar with the recommended ways of planning and assessing the results of experiments. Another man's way of putting it is always worth examining.

K. H. COWARD.

LETTERS TO THE EDITOR

The Estimation of *d*-Tubocurarine Chloride

SIR,—A method for the estimation of *d*-tubocurarine chloride, suitable for standardisation of injections, depending on the development of a blue colour with Folin-Ciocalteu phenol reagent, has been described by Foster[1], and its application in the assay of the total quaternary alkaloids in crude curare has been described by Foster and Turner[2]. In our hands this method has not been found to give results more accurate than the ±5 per cent. claimed, and, as stated, it is certainly necessary in all cases to prepare a standard at the time the assay is made. The issue of the Therapeutic Substances Amendment Regulations, 1948, has emphasised the necessity for accurate control of this preparation. We have found the qualitative colour test given in these regulations—the development of a cherry-red colour when a few crystals of the substance are added to 0·5 ml. of Millon's reagent—to form the basis of a suitable colorimetric assay, accurate to ±2 per cent. and requiring less manipulation than with the Folin-Ciocalteu reagent.

To prepare Millon's reagent pure mercury is dissolved in twice its weight of nitric acid B.P., heating if necessary to complete solution. The solution is diluted with twice its volume of water, allowed to stand overnight and filtered if necessary. Standard colour solutions are prepared as follows. A standard 0·1 per cent. solution of *d*-tubocurarine chloride in water is prepared. 1, 2, 3, 4 and 5 ml. portions of this solution are diluted to 10 ml. with water and 5 ml. of Millon's reagent is added to each. A blank is prepared from 10 ml. of water and 5 ml. of reagent. The contents of each tube are thoroughly mixed and allowed to stand at room temperature for 2 hours. (Unless a standard solution is set up side by side on each occasion a test is made, it is desirable to standardise a temperature, e.g., 20 ± 1° C., at which colour development is allowed to proceed.) Maximum colour development is attained at this time, the variation in colour for a few minutes on either side being negigible. Shortly after 2 hours precipitation commences in the more concentrated solutions. Light transmission is measured on a suitable photoelectric absorptiometer, using a cell having a light path of 2 cm. and a filter with a maximum transmission at about 430 mμ. A straight line graph is obtained by plotting the logarithmic (density) readings against concentration. For the assay of a sample 2 mg. is a convenient quantity to use and a volume containing this amount is diluted to 10 ml. with water and 5 ml. of reagent added. After standing at room temperature for 2 hours, the colour is compared against a blank, which should contain in similar proportions any additional solvent known to be present in the test sample.

Ethyl alcohol, benzyl alcohol, glycerol and sodium metabisulphite, any of which may be encountered in injections, do not interfere with the development of the colour, and it is unlikely that any inorganic salts which may be used as buffers, or to prepare isotonic solutions, will affect the results. Phenol and chlorocresol interfere, producing a pink colour similar to that given by the alkaloid, and may be removed prior to colour development by extraction with chloroform, as described by Foster.[1]

Duncan, Flockhart and Co., Ltd., Edinburgh.
January 19, 1949.

A. M. PRYDE,
F. R. SMITH.

REFERENCES

1. Foster, *Analyst*, 1947, **72**, 62.
2. Foster and Turner, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 228.

NEW REMEDIES

The asterisk (*) after the name of an article indicates that the information given is derived from the makers' publications. Further information regarding these articles may be obtained by application to the Editor.

Cremosuxidine* is a suspension of succinylsulphathiazole 10 per cent., with pectin 1 per cent. and kaolin 10 per cent. It is indicated in the treatment of specific and non-specific diarrhœas, including bacillary dysentery, paradysentery, salmonellosis, diarrhœa of the new-born and summer diarrhœa; it is also of value in other conditions where a liquid form of succinylsulphathiazole is preferred to powder or tablets. The dose for adults is 2 to 3 tablespoonfuls 4 times daily, for children 1 to 2 tablespoonfuls 4 times daily, and for infants 2 to 3 teaspoonfuls 4 times daily, these doses being equivalent to a total daily dose of succinylsulphathiazole of 12 to 18 g. for an adult, 6 to 12 g. for a child, and 3 to 5 g. for an infant. For infants, the suspension may be incorporated with milk, or the infant's formula, up to 50 per cent. of the quantity of the feed, and will still pass through an ordinary teat. Cremosuxidine is supplied in bottles containing 4 or 16 fl. oz. S. L. W.

Etamon* is a proprietary form of tetraethylammonium chloride, a quaternary ammonium compound. It is indicated in the treatment of thromboangiitis obliterans, peripheral arteriosclerosis obliterans, thrombophlebitis, causalgia, and functional vascular disorders such as Raynaud's syndrome. It may also be employed diagnostically in many acrovascular conditions, and in neurogenic hypertension, to determine the contribution of sympathetic stimuli in the maintenance of vasospasm. The injections may be given either intravenously, in a dose of 1 to 5 ml., but not exceeding 7 mg./kg. of bodyweight, or intramuscularly, in a dose of 10 to 12 ml., but not exceeding 20 mg./kg. of bodyweight, the frequency of injections depending on the duration or relief of symptoms. It should be used with caution in patients with severe hypertension, especially in the presence of poor renal function or high diastolic pressure. It should not be used in cases with a recent coronary thrombosis, and only with caution in elderly and arteriosclerotic patients. It is issued in rubber-capped bottles containing 20 ml. of a sterile 10 per cent. aqueous solution. S. L. W.

Ferrivenin* is a preparation of saccharated iron oxide for intravenous injection, 5 ml. of a 2 per cent. solution containing 100 mg. of Fe. It is especially indicated in the treatment of refractory hypochromic anæmias. The total dose of ferrivenin required for the individual patient may be calculated by relating the hæmoglobin deficit of the patient to the fact that 25 mg. of iron are required to produce a 1 per cent. rise in hæmoglobin. A test dose of 1·5 ml. is usually given intravenously on the first day, followed by an injection of 3 ml. on the second day, and subsequent injections of 5 ml. daily or on alternate days until the course is completed. The injections should be given slowly and care taken to avoid perivenous leakage. Alternatively, the total required dose may be given by a single intravenous infusion. It is claimed that the injections are painless and non-toxic and do not give rise to thrombosis or embolism, and that the usual symptoms of nausea, indigestion, anorexia and diarrhœa, or constipation, associated with the oral administration of iron, do not occur. Ferrivenin is issued in ampoules containing 5 ml. of a 2 per cent. solution. S. L. W.

NEW APPARATUS

A LOW PRESSURE HYDROGENATOR OF WIDE APPLICATION

By A. L. Glenn

From the Pharmaceutical Chemistry Research Laboratories, the School of Pharmacy, University of London

Received January 28, 1949

A number of low-pressure catalytic hydrogenators have been described in the literature, most of them being intended for use in the determination of structure, in which the hydrogen absorbed by small quantities of material must be measured with great accuracy[1,2]. However, such apparatus is quite unsuitable for normal laboratory-scale hydrogenations. The small number of hydrogenators intended for synthetic work vary from simple devices[3,4,5,6], to those which are exceedingly complex[7]. The simpler designs are divisible into two groups; one kind will handle large volumes of hydrogen with ease and fair accuracy of measurement, but is somewhat inaccurate for quantities of 1 litre or less[5,8]. The other type is capable of dealing with small quantities with good accuracy, but requires too much refilling when large volumes are required[3,4,6].

In the present design an attempt has been made to produce a robust apparatus having as many advantages as possible compatible with simplicity of construction and safe operation; its main features are shown in the theoretical diagram (Fig. 1). The apparatus is easily moved from bench to

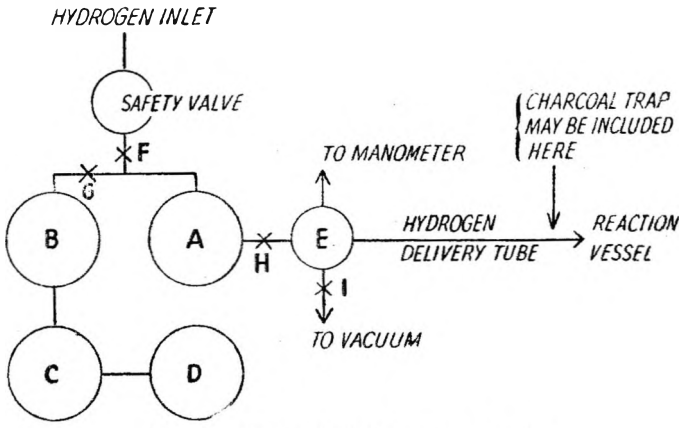


Fig. 1.—Theoretical diagram × = tap.

bench, since it has been built up on a laboratory trolley. Filling is almost instantaneous, for there is no large volume of liquid to displace, a decided advantage during large-scale hydrogenations. The hydrogen uptake is measured by means of a manometer in conjunction with a reservoir of known volume; this reservoir may have a capacity of either 1 or 4 litres, according to the position of a tap at the back of the apparatus. When the reservoir capacity is 1 litre, the delivery of a few hundred ml. of hydrogen results in a considerable fall in the manometer reading, which makes accurate measurement an easy matter. The 4-litre capacity is more convenient for larger volumes. During a series of small-scale hydrogenations, using the

1-litre capacity, the rest of the reservoir may be used for hydrogen storage, thus making the apparatus fairly independent of a cylinder.

The manometer has been placed in circuit at such a point that on closing the reservoir outlet tap the absorption rate, as measured by the manometer, is magnified. This interferes in no way with the initial and final measurements of the reservoir pressure, and is most useful for observing the rate of hydrogen absorption. The maximum pressure obtainable is about 2 atmospheres, but the apparatus will also operate at pressures below 1 atmosphere, a fact which may prove useful in the controlled hydrogenation of substances which absorb hydrogen with great vigour. In order to safeguard the operator, a safety valve has been incorporated, which cannot fail to leak above a predetermined pressure. An efficient charcoal trap has been included for use on those occasions when it is thought that catalyst poisons are being introduced either from the hydrogen used or from the apparatus itself.

The Reservoir.—This comprises four 1-litre bolt-head flasks, A, B, C and D, each flask being closed by a well-fitting rubber bung, through which the necessary connecting tubes pass. After wiring in the bungs, each flask is wrapped in cotton material and secured to a cork ring by means of insulation tape, so that the flasks are protected during movement of the apparatus. The four flasks are mounted on the lower tier of the trolley and are kept in fairly rigid and symmetrical arrangement by means of a length of brass strip, which is looped around each flask neck, and serves to keep the flasks about three inches apart. The reservoir compartment is covered in with thick plywood in order to protect the operator, whilst tap G, which controls the reservoir capacity, is mounted so that it projects through a hole in the board at the back of the reservoir compartment. The reservoir inlet and outlet taps, F and H, are mounted onto a plywood frame, screwed to the back of the manometer upright (Fig. 2). This reservoir system may be replaced with steel flasks if required.

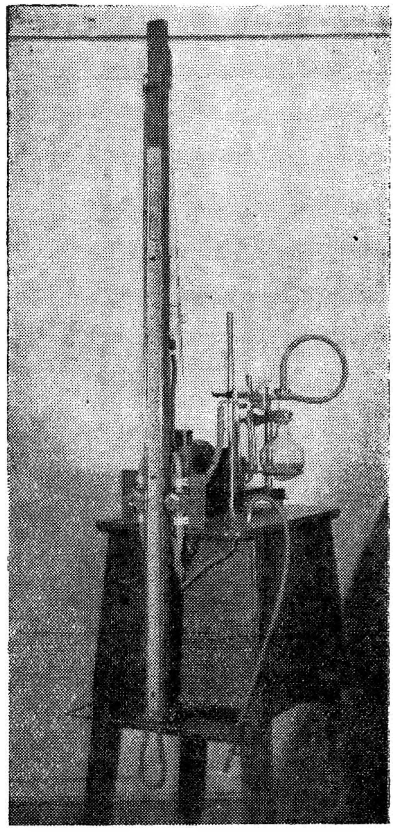


FIG. 2.—General view of apparatus

The Junction Tube.—The reservoir outlet tap, H, leads to a junction tube, E, in order to effect the connections referred to in the theoretical diagram. This consists of the open end of a thick-walled Pyrex boiling tube joined on to a length of 12 mm. tubing. Three side arms of the same tubing are then blown into the body of the boiling tube. The junction tube is situated in the reservoir

compartment, whilst the exhaust tap, I, is mounted in a rubber bung, which is fitted into a hole on the top of the trolley.

The Manometer.—An upright of oak ($\frac{7}{8}$" × $1\frac{1}{2}$") screwed to the edges of the upper and lower tiers supports a metre scale, which slides up and down the upright between two accurately fitted wooden rails. The manometer itself consists of two lengths of thick-walled capillary tubing (internal diameter = 1·5 mm.; external diameter = 6·5 mm.) joined together at the bottom of the " U " by means of a short length of pressure tubing. The use of pressure tubing in this case not only simplifies construction of the manometer and renders it less fragile than an all-glass U-tube, but enables one to vibrate the mercury column before reading to ensure that tailing has not distorted the levels. The two limbs of the manometer are fixed to the rails on the upright by loops of copper wire at intervals; the wire passes around the back of the upright, but not across the metre scale. In filling the manometer air bubbles should be removed by forcing the mercury into the open limb and pushing a long length of copper wire down the tube.

The Safety Valve.—The principle of operation is as follows (see Fig. 3). As the pressure in the flask, L, increases, mercury is forced into the vertical capillary tube OM; the quantity of mercury initially in L is adjusted so that, when the pressure has risen to about 2 atmospheres, the whole of the mercury is in the vertical tube. A further small increase in pressure then forces the mercury into the baffle chamber, J. Hydrogen escapes through the outlet tube K, which contains a small quantity of mercury to act as a seal, during evacuation of the apparatus; the outlet of K is fitted with a miniature mercury baffle. During this process the pressure in the apparatus falls rapidly, and the hydrogen supply must be cut off completely before the mercury in J will again return to the flask, L. Both L and J are constructed from 50-ml. Pyrex flasks, J being the most simple and efficient mercury baffle of several types tried. The tube ON has the same dimensions as that used for the manometer and passes to the bottom of the vessel, L, which is mounted on the bottom of the reservoir compartment as near to the manometer upright as possible. Connection between ON and J is made with a length of capillary pressure tubing, MN; this should be kept reasonably straight. The use of narrow-bore tubing in this part of the valve reduces the volume of mercury required, and hence lessens the problem of baffling; the baffle, J, is mounted on a bracket, screwed to the back of the manometer upright. After J, ordinary pressure tubing is used in order to reduce the resistance to hydrogen

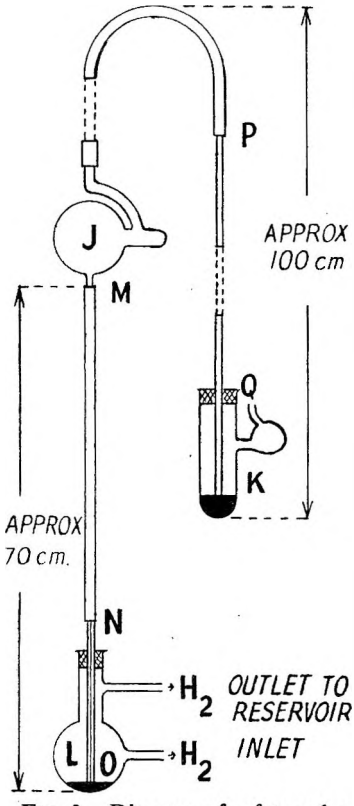


FIG. 3.—Diagram of safety valve

flow; PQ is a length of 6 mm. tube, which passes to the bottom of tube K; the latter is mounted on the top of the trolley immediately behind the manometer upright. The introduction of the correct volume of mercury is an

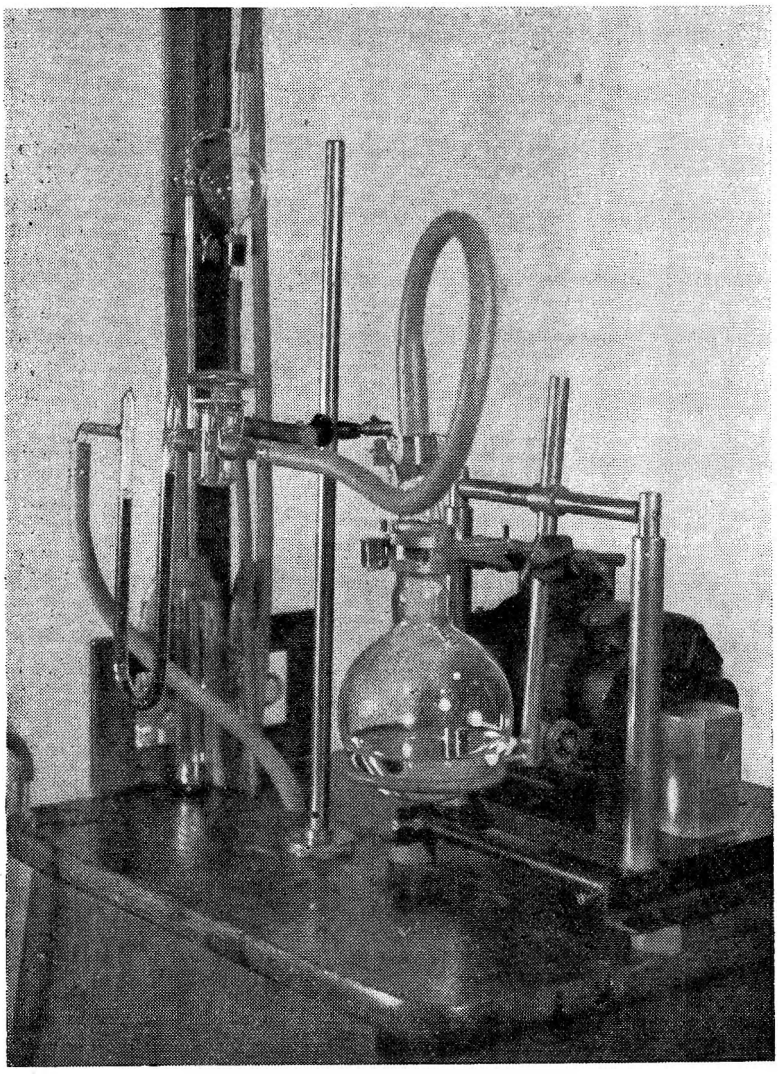


FIG. 4.—Close-up view of apparatus

easy matter; after removing the pressure tube from the top of J, small quantities of mercury are poured in until by trial and error the valve is found to operate at the desired pressure.

The Charcoal Trap.—When immersed in an acetone/solid carbon dioxide bath, this trap is capable of adsorbing 1 litre of hydrogen sulphide; it is therefore a useful adjunct where it is desired to rigidly exclude any catalyst poisons arising from the apparatus or the hydrogen supply. The construc-

tion should be apparent from Figure 4; the U-tube is filled with granular charcoal to give a total path of about 30 cm. length, and a glass wool plug is present at each end to prevent movement during the flow of gas. After use, immerse the trap in boiling water and pass a slow stream of hydrogen through for about an hour. At the end of this operation the trap should be quite free from moisture, otherwise it may block during the next hydrogenation.

The Shaker.—This was specially designed for the apparatus by Dr. W. J. Arrol. The 1/25th h.p. motor is suitably geared down to drive a rocking retort-stand by means of a shaft, the reaction flask being attached to the stand. The principle should be apparent by reference to Figure 4. The dimensions have been carefully worked out, so that the catalyst is very well shaken without undue splashing of the reaction mixture into the neck of the flask. This shaker appears to be much more satisfactory for this purpose than any previously encountered.

General Notes on Construction.—Most of the connections have been made with Portex Plastic Commercial Tubing No. 6c, and the associated glass work has been made from Pyrex tubing of 12 mm. external diameter, with which the plastic tubing forms a very tight-fitting joint. All glass junctions are narrowed at the tips and well-rounded off to facilitate the fitting of the plastic tube. In addition, each connection is doubly wired with No. 18 S.W.G. copper wire. When operating at 60 cm. of mercury above atmospheric pressure, the leakage rate of the whole apparatus is of the order of about 2 mm. an hour; for the 1 litre reservoir capacity this is equivalent to a leakage of approximately 3 ml. of hydrogen an hour, which may be ignored during any but the slowest hydrogenations. When desired, the leakage rate can be reduced further by working at a few cm. of mercury above atmospheric pressure.

The Taps.—Figure 5 shows the way in which high vacuum taps have been adapted to withstand pressure. The taps are well greased with Apiezon "L," and a slotted brass disc seated upon a rubber washer is wired into place, so that the tap is held firmly into its socket. This method has given very little trouble.

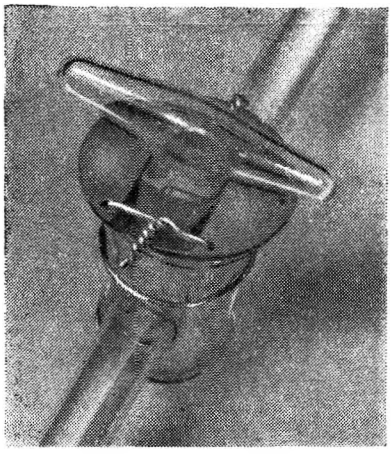


FIG. 5.—Close-up view of a tap

Calibration.—It is necessary to know the precise volumes of the reservoir when set at nominal capacities of 1 and 4 litres respectively. The apparatus should be filled with hydrogen and the drop in manometer pressure noted during the delivery of 500 ml.; a volumetric flask and a trough of water at room temperature is useful here. The volumes are then readily obtainable after correcting for aqueous vapour pressure. It is useful for practical purposes to calculate approximately the fall in manometer pressure corresponding with the delivery of 100 ml. of hydrogen in the case of both reservoir capacities. As will be seen below, in calculating the manometer drop for a given hydrogenation it is necessary to add the volume of

the dead space in the reaction vessel to the reservoir volume before arriving at the final volume for calculation purposes.

Calculation of Reservoir Volume.—Suppose 500 ml. has been collected over water at room temperature.

Let v = volume of reservoir in ml.

m_1 = initial manometer reading in mm.

m_2 = final manometer reading in mm.

a = atmospheric pressure in mm. of mercury

w = aqueous vapour pressure of water at room temperature in mm. Hg

$$\text{Then } v = \frac{760 \times 500}{(m_1 - m_2)} \left(1 - \frac{w}{a} \right)$$

Mode of Operation

(a) *Filling With Hydrogen.*—Close the hydrogen delivery tube by means of a pinch clip or, if the charcoal trap is being used, close the tap thereof. Connect the exhaust outlet to vacuum and close tap F; open taps G, H and I, and evacuate the apparatus. Then attach the hydrogen inlet tube to a cylinder and sweep out the air in the safety valve and associated tubing by applying just enough pressure to blow the valve, and then allowing hydrogen to bubble through for a few seconds. If the inlet tube is detached from the cylinder without previously closing by means of a pinch clip, air will diffuse into the valve, and must be swept out again before refilling. The apparatus is then filled by opening tap F and allowing hydrogen to flow in from the cylinder until the manometer registers about 60 cm., when tap F is closed. Tap H must be open during this operation. This sequence of operations is sufficient when the apparatus is in regular use, but when first used or after being unused for some time it is advisable to sweep out the apparatus thoroughly by repeating the above sequence two or three times, depending on the vacuum available.

(b) *Hydrogenation.*—A standard joint round-bottomed flask of suitable capacity is used as reaction vessel, and an adapter is needed to connect to the hydrogen delivery tube. The adapter is made by pulling out a standard cone and joining on to a short length of 12 mm. Pyrex tubing. The joint is greased with Apiezon "L" and held together by copper wire in conjunction with two bands of brass strip, as shown in Figure 4. Estimate, or measure when maximum accuracy is required, the dead space which will exist in the flask and adapter after adding the solution to be hydrogenated. This volume is added to the appropriate reservoir volume before calculating the manometer fall, which corresponds with the theoretical volume of hydrogen to be absorbed (see below). Then check that tap G is set to the desired reservoir capacity and close tap H. Mount the reaction vessel on the shaker and connect the adapter to the hydrogen delivery tube, open the tap on the charcoal trap, if this is in circuit.

Evacuate the reaction vessel by opening tap I, and after closing it admit hydrogen from the reservoir. Whether or not this operation is repeated depends upon the vacuum available and the volatility of the solvent, although where possible it should be repeated. At this point tap H should be open: if the manometer reading is too low, let in more hydrogen from the cylinder or, when the 1 litre capacity is being used, it is only necessary to open and close tap G. Note the manometer reading, making sure that tap F is closed and tap H open; start the shaker. In order to check that hydrogen is being absorbed, it is useful to close tap H at this point in

order to obtain the magnified pressure fall referred to above. During very slow hydrogenations it is advisable to correct for any change in atmospheric pressure and room temperature that has occurred during the process and to adjust the final manometer reading accordingly.

Calculation of the Required Manometer Drop

Let t_1 = temperature (in °C.)
s = volume of the dead space (in ml.)
h = volume of hydrogen in ml. theoretically required at N.T.P.
(The other symbols are as above)

$$\text{Then } m_1 - m_2 = 760 \times \left(\frac{\frac{h\,(273 + t_1)}{273}}{(v + s)} \right)$$

Correction for Temperature and Pressure Variations During Hydrogenation

Let m_2 = the originally calculated final manometer reading (in mm.)
m_3 = the corrected final manometer reading (in mm.)
t_1 = initial temperature (°C.)
t_2 = final temperature (°C.)
a_1 = initial atmospheric pressure (in mm. mercury)
a_2 = final atmospheric pressure (in mm. mercury)

$$\text{Then } m_3 = \left(\frac{(273 + t_2)\,(m_2 + a_1)}{(273 + t_1)} \right) - a_2$$

The author wishes to express thanks to Professor W. H. Linnell for encouragement and advice during this work. Also to Dr. W. J. Arrol for suggesting the use of the charcoal trap, to Mr. P. C. Barden for constructing the shaker and to Mr. V. Askam for checking the equations.

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