REVIEW ARTICLE

INSTRUMENTS EMPLOYED IN POTENTIOMETRIC DETERMINATIONS

By C. Morton, B.Sc., Ph.C.
Head of the Chelsea School of Pharmacy

Introduction

The theory, technique, and applications of potentiometric analysis are fully described in well-known textbooks, e.g., those of Britton[1] and Clark.[2] Britton[3] has contributed a review of the electrode reactions upon which potentiometric determinations are based and, in a valuable series of articles, Furman[4,5,6] has summarised recent advances in this field of work, including brief references to progress in instrumentation: as far as the author is aware, however, no comprehensive review of the history and development of instruments for use in potentiometric analysis has appeared in recent years.

The potentiometric determination of an ion consists essentially in the measurement of the voltage of a cell, the E.M.F. of which is a function of the concentration of the ion. Prior to the investigation by Haber and Klemensiewicz[7] of the electromotive properties of the glass electrode, the cells used in potentiometry were of comparatively low electrical resistance and, in measuring the E.M.F. by means of the Poggendorf potentiometer, a moving coil galvanometer served as a satisfactory null point detector. In their fundamental studies of the behaviour of the glass electrode Hughes,[8] Kerridge,[9] MacInnes and Dole,[10] Dole,[11] and MacInnes and Belcher[12] used the quadrant electrometer as a null point indicator. Ballistic methods in which the cell is allowed to charge a condenser, the capacitor being subsequently discharged through a ballistic galvanometer, have been used by Brown,[13] Morton,[14] and Britton.[15] Neither the quadrant electrometer nor the ballistic galvanometer attained general popularity in potentiometric estimations, and it was not until the invention of the thermionic valve potentiometer that the problem of measuring the E.M.F. of cells of high electrical resistance was satisfactorily solved. Due to their high sensitivity, robust construction, portability, high input resistance, and simplicity of adjustment, electronic instruments have now superseded other devices for the measurement of E.M.F.

Goode's Valve Voltmeter

Thermionic valves were first employed in the measurement of electrode potentials by Goode,[16] in 1922. The principle upon which the design of Goode's valve voltmeter is based may be described with the aid of the simplified circuit diagram of Figure 1. The filament or cathode F of the triode valve T, when heated by a current supplied by the battery B_1, emits electrons. The anode A, which takes the form of a metallic sheath surrounding the cathode, is charged positively with respect to the cathode by means of the anode battery B_2, and thus attracts the electrons

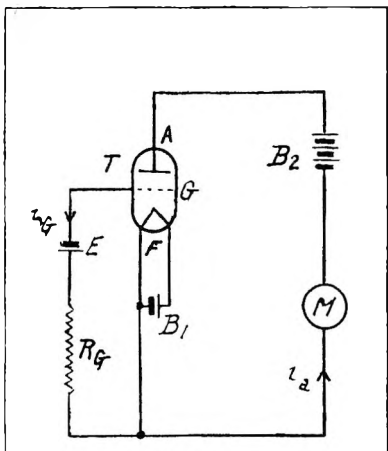


FIG. 1.

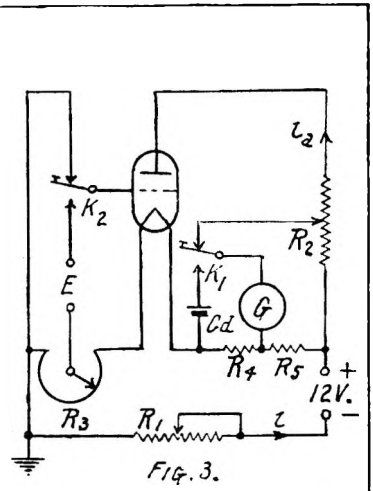


FIG. 3.

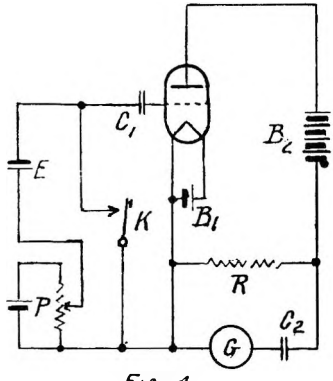


FIG. 4.

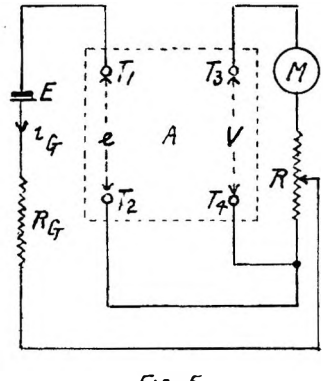


FIG. 5.

emitted by the hot cathode: the stream of electrons collected by the anode, in returning to the filament through the external circuit, sets up a unidirectional anode current i_a which actuates the microammeter or galvanometer M. In traversing the evacuated space between the cathode and anode, the electrons pass through the meshes of a perforated structure G known as the control grid. As this grid is usually charged negatively with respect to the cathode, comparatively few electrons are collected by it, and the grid current i_g flowing through the external grid circuit is extremely small by comparison with the anode current i_a: nevertheless, due to its proximity to the cathode, the grid exercises a powerful electrostatic control over the anode current which may, in fact, be reduced to zero when the grid is sufficiently negative with respect

to the cathode. This effect is illustrated by the characteristic curves 1 and 2 of Figure 2, in which the anode current i_a of the ME 1400 valve is plotted for anode voltages of 30 and 45 respectively, as a function of the grid voltage V_g. It will be seen that, except at highly negative grid voltages, the relationship between anode current and grid voltage is approximately linear: the microammeter M (Fig. 1) may accordingly be calibrated in such a manner that its readings indicate directly the value of a voltage E applied to the grid circuit.

Goode's valve voltmeter, subsequently modified by Williams and

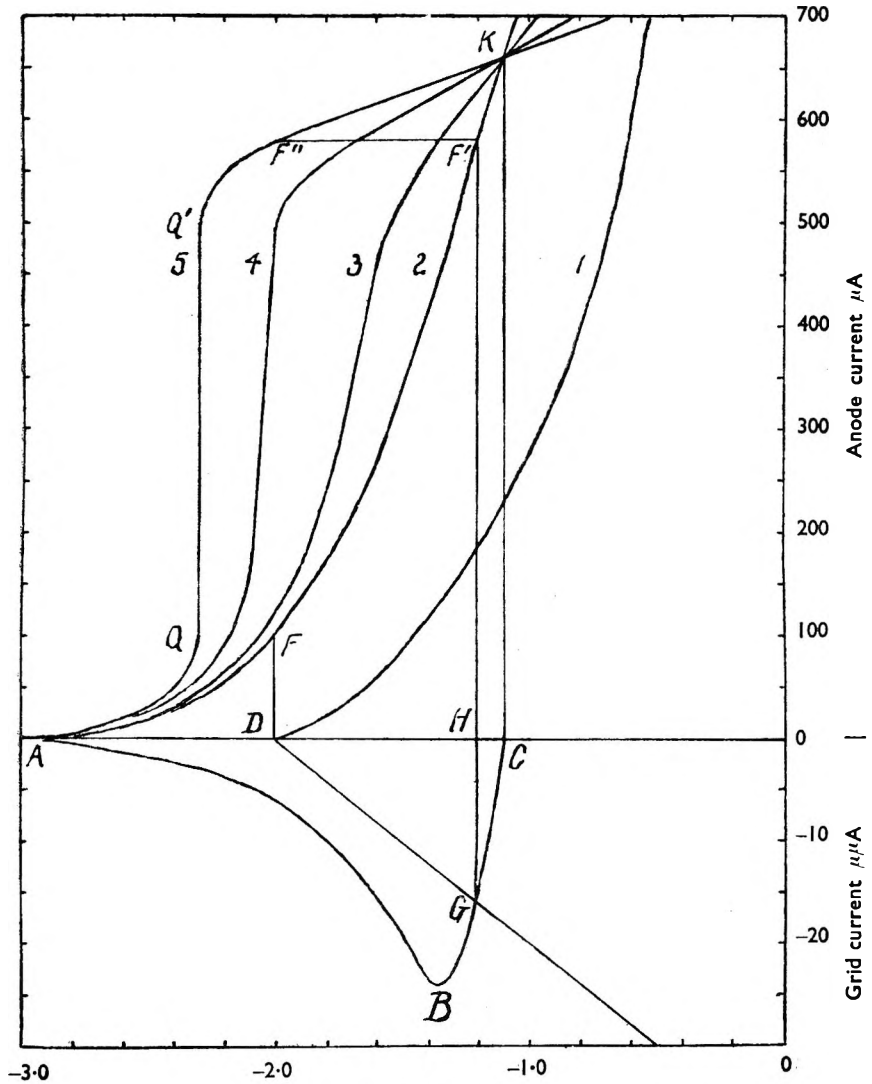


FIG. 2. Grid Voltage.

Whitenach,[17] Bienfait[18] and other workers, has been found to yield satisfactory results in the location of the end-point of potentiometric titrations. Unfortunately, the accuracy is seriously impaired by changes in the voltages of the batteries and by the gradual decline in the emission of electrons from the cathode as the valve ages.

The Valve Potentiometer

The valve potentiometer[19] differs essentially from the valve voltmeter in that the readings are given, not by a calibrated microammeter in the anode circuit, but by a standard potentiometer in the grid circuit of the valve: the potentiometer may be calibrated with any desired degree of accuracy, and retains its calibration unimpaired by changes in valve characteristics or battery voltages. The principle of the valve potentiometer may be applied in various ways, one of which is illustrated by the simplified circuit diagram[20,21] of Figure 3.

The adjustable rheostat R_1, the calibrated potentiometer R_3, the filament of the valve, the standardising resitance R_4 and the resistor R_5 are connected in series, this series circuit being supplied with a current i of 100 mA. from a 12 volt source, such as an accumulator or one of the commercial 12 volt mains units now available. The voltage drop across the potentiometer due to this current provides the normal operating grid potential or "grid bias" of the valve; similarly, the voltage dropped across the series-connected resistors R_4 and R_5 supplies the anode voltage of the valve. On depressing the key K_1, the Weston or cadmium cell Cd, the E.M.F. of which is 1·0183 volt, is connected, in series with the galvanometer G, across the standardising resistance R_4 (the ohmic value of which is 10·183 ohms), and the variable rheostat R_1 is adjusted until the galvanometer is undeflected: by this means the potentiometer current and the grid, filament, and anode voltages of the valve are accurately standardised in a single operation. On releasing K_1 and adjusting the movable contact on the anode resistance R_2 until the galvanometer is again undeflected, the voltage drop across this resistance due to the anode current i_a flowing through it is counterbalanced by the equal and opposite voltage across the resistance R_5 due to the current i: during this operation, the grid is earthed via the key K_2. Finally the latter key is depressed, thereby connecting the cell E, in series with an opposing voltage derived from the potentiometer R_3, to the grid of the valve, and the potentiometer control is adjusted until the galvanometer is once more undeflected: it is clear that on the completion of this adjustment the grid is again at earth potential. It follows that the voltage impressed on the grid by the potentiometer is now equal and opposite to that of the applied voltage E: this E.M.F. may be read on the calibrated scale of the potentiometer.

With few exceptions, the instruments used in the measurement of electrode potentials since 1928 have been of the potentiometric type: amongst the many workers who have applied this principle in various ways are Stadie,[22] Dubois,[23] Partridge,[24] Elder,[25] Fosbinder,[26] Muller,[27] Harrison,[28] Greville and Maclagan,[29] and Voegtlin, de Eds and Kahler,[30]

Recent developments in electronics have made it possible to stabilise the calibration of Goode's valve voltmeter, and there is at the present time a tendency to revert to this type of instrument in routine analytical work, the valve potentiometer being retained for applications in which measurements of high precision are essential. Before discussing the developments which have led to the reinstatement of the valve voltmeter, however, it is advisable to consider the modifications which are necessary in order to adapt electronic instruments for use with electrodes or non-aqueous solutions of high electrical resistance.

Grid Current

It has been pointed out that the grid current of a triode valve is extremely small by comparison with the anode current. For valves of the type used in radio receivers, the maximum grid current under normal operating conditions varies from 10^{-8} to about 5×10^{-6} amperes and, provided that the resistance of the cell does not exceed a few thousand ohms, no difficulty arises. The average resistance of a glass electrode, however, is about 10^8 ohms, and that of an electrochemical cell containing a non-aqueous solution may greatly exceed this value. Reverting to Figure 1, it is evident that if the true E.M.F. of the cell is E and its resistance R_g, the apparent E.M.F., i.e., the reading given by the instrument, is $E - i_g R_g$, where i_g is the grid current: for example, if $i_g = 10^{-8}$ amperes and $R_g = 10^8$ ohms, the error in the reading is 1 volt. Hence, unless precautions are taken to exclude grid current, or to apply corrections for its effects, the readings of the instrument are of no value.

In Figure 2, the grid current of the ME 1400 valve, when operated at an anode potential of 45 volts, is plotted as a function of the grid voltage. It will be seen that both the grid and anode currents are vanishingly small when the grid is highly negative, e.g., at the point A. As the negative potential applied to the grid is decreased, electrons are able to pass through the meshes of the grid in increasing numbers, and the anode current increases (curve 2): this increase in anode current is accompanied by a proportionate increase in grid current, which attains a maximum value at the point B. The grid current throughout this region of the characteristic curve, i.e., between the points A and B, is due mainly to the fact that the highly negative grid attracts and collects positive ions produced by the bombardment of the residual gas molecules in the incompletely evacuated glass envelope by the electron stream. With further reduction in the negative voltage applied to the grid, the latter loses its capacity for attracting positive ions and the grid current declines until, at the point C (usually known as the "contact potential point" or "free grid potential") it again becomes zero. Reduction of the negative potential of the grid beyond this point causes the latter to attract negative electrons instead of positive ions: in consequence the grid current, after passing through zero at the point C, changes sign and rapidly increases in the reverse direction. As the valve is not designed for operation under conditions of positive grid current, this portion of the characteristic curve is of no practical value, and is not shown in the diagram.

The effect on the performance of a valve voltmeter of including an electrochemical cell of high resistance in the grid circuit is most readily demonstrated by a graphic method. The characteristic of the ME 1400 valve, when operated at an anode potential of 45 volts with zero grid resistance, is represented by curve 2 of Figure 2. Normally the grid is maintained at an average potential of -2 volts with respect to the cathode by means of an accumulator or other source of E.M.F.; this negative "grid bias" is represented by O D, and the corresponding anode current is given by D F, viz., 100 μA. The effect on the anode current of the inclusion of a high resistance in the grid circuit may be investigated by drawing, from the point D, a load line of slope equal to $-1/R_g$, where R_g is the value of the grid resistance: for example, the load line D G, which has a slope of 20 $\mu\mu$A./V., corresponds to a value of $R_g = 5 \times 10^{10}$ ohms. At the point G the load line intersects the grid current characteristic, and the grid current at this point is given by G H, viz., 16 $\mu\mu$A. The applied E.M.F. of -2 volts represented by O D is now partially offset by the opposing voltage drop due to the flow of grid current through the grid resistance. This voltage drop is given by D H, viz., 0·8 volt: the resultant or true E.M.F. between the grid and cathode is thus only $-1{\cdot}2$ volt, and is represented by O H. It will be seen that the effect of introducing a resistance of 5×10^{10} ohms into the grid circuit is to increase the anode current from a value given by F D, viz., 100 μA., to the much higher value represented by F'H, i.e., 580 μA. It is also clear that, with an applied E.M.F. of -2 volts, the error introduced into the reading of the thermionic voltmeter by the inclusion of a resistance of 5×10^{10} ohms in the input circuit is that represented by D H, viz., 0·8 volt, corresponding to a percentage error of 40 per cent. in the reading.

By drawing, from various points along the grid voltage axis, a number of load lines parallel to D G, and noting their points of intersection with the grid current characteristics, curve 5 (in which the anode current is plotted as a function of the applied voltage in the presence of a grid resistance of 5×10^{10} ohms) has been constructed: for example, the point F″ on this curve was obtained by drawing a line F'F″ through F' equal in length and parallel to D H. Anode characteristic curves corresponding to values of $R_g = 2 \times 10^{10}$ (curve 4) and $R_g = 10^{10}$ (curve 3) are also included in the figures. If the grid current were constant for all values of grid voltage, these curves would be parallel to the normal characteristic (curve 2), and the simple addition of a constant correction would suffice to compensate for the effect of the grid resistance on the calibration of the instrument: however, due to the curvature of the grid characteristic, the anode characteristics 3, 4 and 5 are convex with respect to the normal characteristic, and no such simple correction can be applied. The convexity of the characteristics increases rapidly with the value of the grid resistance and, if the latter exceeds 5×10^{10} ohms, the grid loses control over the anode current. It is, of course, true, that resistances of this magnitude are only occasionally encountered in potentiometric determinations. The ME 1400 valve, however, is one of a type known as "electrometer valves" in which, by methods described

later in this review, grid current is very greatly reduced: during the early development of the valve potentiometer, such valves were unknown, and effects similar to those described above were obtained with glass electrodes having resistances as low as 10 megohms.

Early Attempts to Minimise Errors Due to Grid Current

The effects of grid current on the performance of thermionic voltmeters and potentiometers have been discussed in general terms by Metcalf and Thompson,[31] Morton,[32] Nottingham[33] and others. One of the earliest attempts to adapt the valve potentiometer for use with the glass electrode was that of Stadie,[22] who pointed out that, when this method is used, the grid voltage is restored to a predetermined value on the completion of each adjustment: on the assumption that the grid current is constant at constant grid voltage, a simple correction can be applied to compensate for the voltage drop across the glass electrode due to the flow of grid current across the glass membrane. Unfortunately, slight changes in cathode temperature or anode current due to decline in the battery voltages or other causes may produce considerable changes in grid current, even when the grid is maintained at constant potential. Moreover, as Morton[34] has pointed out, the glass electrode has an exceptionally high temperature coefficient of resistance, and the changes in its resistance (and therefore in the voltage dropped across it due to grid current) resulting from fluctuations in ambient temperature may vitiate the measurements: for these and other reasons, Stadie's method has not been generally adopted.

Morton[32] proposed a circuit arrangement in which the effects of grid current were eliminated by a "sum and difference" method: the device was in some respects inconvenient, and failed to gain general acceptance.

A method which has been more widely adopted is that in which, when using the valve potentiometer, the operating grid potential is adjusted to the "contact potential point," e.g., to the point C in Figure 2. At this point the grid current is zero, and the inclusion of a high resistance in the grid circuit has no effect on the anode current, the value of which is given by C K: it is for this reason that the anode characteristics 2, 3, 4 and 5 intersect at the point K. The method, which has been used by a number of workers, notably Garman and Droz,[35] is inconvenient in that, in order to provide for adjustment of the mean grid voltage to the contact potential point, an additional control (which usually takes the form of an auxiliary potentiometer) is required, and frequent readjustment of this control is necessary: further, as the contact potential point lies in the steepest part of the grid current characteristic, slight errors in adjustment lead to errors in measurement of considerable magnitude.

The Impulse-type Valve Electrometer

The circuit diagram of Figure 4 will serve to illustrate the basic principles of this device, which was described by Morton[36] in 1931. A blocking condenser C_1 is connected in series with the grid, which is thus effectively isolated, in so far as direct current is concerned, from the external grid

circuit: the latter includes the glass electrode cell E, which is connected in series with an adjustable opposing voltage derived from the calibrated potentiometer P, and the short-circuiting key K. A second blocking condenser C_2, of much greater capacitance, is connected, in series with the galvanometer G, across a high resistance R included in the anode circuit of the valve: since the galvanometer is isolated, by the capacitor C_2, from the anode circuit, it is unaffected by the slow changes in anode current (due mainly to the gradual decline in the voltages of the batteries B_1 and B_2) which would otherwise cause drift of the electrical zero of the instrument. Electrons accumulate on the isolated grid until the latter attains the contact potential indicated by O C in Figure 2, the potential thereafter being automatically maintained at this value. It should be pointed out that the condenser C_1 is virtually in series with the grid-cathode capacitance of the valve, and the combined capacitance is exceedingly small. On depressing the key K, no effect will be observed when the E.M.F. of the cell E is exactly counterbalanced by the opposing voltage derived from the potentiometer P: if this condition does not obtain, the condenser C_1 and the grid acquire a transitory charge due to the unbalanced E.M.F., and the resultant sudden increase in anode current produces a ballistic throw of the galvanometer pointer. The process of measurement thus consists in adjusting the potentiometer P until there is no transient deflection of the pointer on depressing the key.

A discussion of the design of multi-stage amplifiers suitable for use in conjunction with impulse-type electrometers is beyond the scope of the present review: details are given by Fox and Groves,[37] Morton,[38] Chun-Yu Lin,[39] Dole,[40] Ellis and Kiehl,[41] and Goodhue.[42] Using this method, Chun-Yu Lin claims recently to have obtained a sensitivity of 100,000 μA/V., as compared with an average of 100 μA/V. for commercial *p*H meters.

The Electrometer Valve

The electrometer valves manufactured in this country were originally intended for use in the measurement of ionisation and photoelectric currents, and credit for their application in 1930 to the determination of glass electrode potentials is due to Harrison.[28] In designing these valves, various expedients have been adopted with a view to minimising grid current, which may by these means be reduced to less than 10^{-14} ampere. For example, the cathode of the E T 1 valve is placed centrally, with the grid and anode on either side: by this means the grid is removed from the electron stream, and the internal resistance of the valve is reduced to such an extent that an adequate performance is obtained with an anode potential of 4 to 6 volts. As this is below the ionisation potential of the residual gas molecules within the valve, the most prolific source of grid current, viz., the production of positive ions as a result of the bombardment of the molecules by the electron stream, is thereby removed. Other manufacturers achieve similar results by producing "electrometer tetrodes" in which a positively charged screen grid is interposed between the cathode and the control grid: this positively-charged grid serves the

additional purpose of repelling the positive ions which are emitted by the hot cathode and which would otherwise be attracted to the control grid. In yet another type, developed in America, the anode takes the form of a perforated structure situated between the cathode and the control grid: "inverted triodes" of this type have been used in glass electrode measurements by Cherry.[43] Double electrometer valves specially designed for high zero stability have been described recently by Derbyshire[44] and Little.[45]

By comparison with the valves used in radio reception, electrometer triodes and tetrodes are both costly and inefficient, and it is not surprising that many attempts have been made to adapt normal broadcast receiving valves for use as substitutes. Adaptations of this kind have been described by Johnson,[46] Johnson and Neitzert,[47] Gabus and Poole[48] and others, but the circuits are intended for use with American valves which are not readily obtainable in this country.

Any of the circuit arrangements previously described may be adapted for use with electrometer triodes merely by readjustment of the grid, cathode, and anode voltages to the values recommended by the valve manufacturers: in the case of the impulse-type electrometer (Fig. 4), the condenser C_1 may be omitted. If it is desired to use an electrometer tetrode, somewhat more elaborate modification of the circuit arrangement is necessary: for this type of valve, the circuits described by Du Bridge and Brown,[49] Barth,[50] and Penick,[51] which possess exceptionally high zero stability, are of especial value.

Negative Feedback

During the period 1934–1945, thermionic electrometers and other *p*H-measuring devices were described by Mouquin and Garman,[52] Goyan, Barnes and Hind,[53] Penther, Rolfson and Lykken,[54] Penther and Rolfson,[55] Buras Jun. and Reid[56] and other workers. Throughout this period, important advances were made in the general theory and practice of electronics, and the outstanding feature of the post-war period has been the application of new principles established by electronic engineers to the instruments used in potentiometry. Of these, perhaps the most important is the development of negative feedback, which has rendered possible the reinstatement of Goode's valve voltmeter in a greatly improved form.

Both negative and positive feedback have been used, for various purposes, over a period of many years: in 1932, for example, the writer[38] applied positive feedback to a potentiometer recorder in order to enhance the sensitivity. It is only within recent years, however, that the value of negative feedback (or degeneration, to use an alternative term) as a means of stabilising the calibration of a thermionic voltmeter has been fully appreciated. The underlying principles have been discussed by Tellegen and Henriquez,[57] Lewis,[58] Farren[59] and other authors, and may be explained with the aid of Figure 5, in which A represents an amplifier, such as a valve voltmeter, to the output terminals T_3 and T_4 of which a microammeter M, in series with a load resistance R, is connected. The

combination of the microammeter M and its series resistance R constitutes a voltmeter, the readings of which indicate the output voltage V. The glass electrode cell (represented in the diagram as a source of voltage E in series with a high resistance R_g) is connected, in opposition to a voltage αV obtained by feeding back a fraction α of the output voltage V, to the input terminals T_1 and T_2. Using the notation of the diagram, and ignoring for the present the influence of the grid current i_g and resistance R_g in the input circuit, we have

$$V = \mu e \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where μ is the voltage amplification provided by the amplifier. But

$$e = E - \alpha V \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

hence

$$V = \mu(E - \alpha V) \text{ or } V = \mu E/(1 + \mu\alpha) = E/(\tfrac{1}{\mu} + \alpha) \quad .. \quad (3)$$

Evidently when μ is sufficiently great, equation (3) reduces to

$$V = E/\alpha \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

and if, in addition, the whole of the output voltage V is fed back to the input circuit (an arrangement which is known as a "cathode follower" circuit)—we have

$$\alpha = 1 \text{ and } V = E \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

As the term μ has disappeared from equations (4) and (5) it follows that under these conditions the output voltage V and the calibration of the microammeter M are independent of changes in the characteristics of the valves and therefore also of changes in the voltage of the power supply to the amplifier.

Deflection *p*H meters in which the principle of negative feedback has been applied are now obtainable commercially, and various circuits have been described; one of the most recent is that of Thorp.[60] As Sowerby[61] and other authors have shown, negative feedback is also valuable as a means of increasing the input resistance of a valve voltmeter or *p*H meter.

The Feedback Potentiometer

The simple theory of the feedback potentiometer[62] may be explained with the aid of the circuit diagram of Figure 5. It has been shown above that, on the application of a voltage E to the input terminals T_1 and T_2, the output voltage V indicated by the microammeter M is given by

$$V = E/(\tfrac{1}{\mu} + \alpha) \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

If now the voltage E increases by an amount δE, the output voltage will also increase, but may be restored to its former value by increasing the feedback factor α (by means of a sliding contact on the potentiometer R) by an amount $\delta\alpha$ such that the relationship

$$V = (E + \delta E)/(\tfrac{1}{\mu} + \alpha + \delta\alpha) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

is satisfied. From (6) and (7) we have

$$\delta\alpha V = \delta E \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

that is, the increment in feedback voltage is equal to the increment in applied voltage. Assuming that the potentiometer R has been suitably calibrated with any desired degree of accuracy, its readings give directly the E.M.F. of the cell or *p*H value of the solution: as the term μ does not appear in equation (8), it follows that, even if the amplifier consists of a single stage of low voltage amplification, the readings are independent of the valve characteristics. In order to permit of accurate adjustment, the microammeter is replaced in practice by a standardising resistance, across which a galvanometer is connected in series with a Weston cell: the process of measurement consists in adjusting the potentiometer R until the galvanometer is undeflected.

Zero Stability

It has been pointed out that a prolific source of inconvenience and error in the use of electronic *p*H meters is drift of the electrical zero of the indicating instrument as a result of slow changes in valve characteristics or supply voltage. Using two independent lines of approach, electronic engineers have in recent years made striking progress in the design of D.C. amplifiers and thermionic electrometers of high zero stability.

(1) Reverting to the impulse-type valve electrometer (Fig. 4), it is clear that if the key K is replaced by a mechanical interruptor or vibrator operating at a suitable frequency, the unbalanced E.M.F. due to the glass electrode cell E and potentiometer P will be applied intermittently to the grid circuit, giving rise to an alternating current in the anode circuit which may be amplified by a conventional A.C. amplifier and, after rectification, used to operate a D.C. indicating or recording instrument. Successive stages in the amplifier may be isolated, in so far as direct current is concerned, by means of blocking condensers or transformers, thereby eliminating drift due to slow changes in the anode currents of the intermediate stages. The design of a high-insulation vibrator suitable for use with glass electrodes is by no means a simple problem, but *p*H meters operating on this principle have been produced. As an alternative to the use of a vibrator, the capacitance of the condenser C_1 may be varied at a convenient frequency, e.g., by using as one of its plates a vibrating diaphragm or reed. The latter method had been developed, under the name of the dynamic condenser electrometer, by Palevsky,[63] Thomas and Finch[64] and other workers, and has been applied to the measurement of glass electrode potentials by Kraus.[65]

(2) Methods of reducing zero drift by using two triodes of similar type in a balanced bridge circuit have been used for many years, the earliest being those of Brentano,[66] Wynn-Williams,[67] and Brentano and Ingleby.[68] A more recent circuit of this type, developed by Schmitt[69] and Richter,[70] has been modified for use in the measurement of glass electrode potentials by Buras and Reid.[56] The exacting requirements, as regard zero stability, of the amplifiers used in encephalography and electrocardiography have led to the extension of the balanced bridge method to multi-stage amplifiers by Offner,[71] Matthews,[72] Miller,[73] Parr and Walter,[74] Johnston[75] and many other workers.

Prinz[76] has recently described an ingenious method, based on negative feedback principles, of automatically compensating a D.C. amplifier for zero drift and input current, and has suggested the application of the device to *p*H-measuring instruments.

The Trend of Future Development

Reference has been made to a number of recent advances in electronics which may have an important bearing on future progress in potentiometric instrumentation. These include (1) the principle of negative feedback, (2) the dynamic condenser electrometer, and (3) the development of biological amplifiers of extremely high zero stability and voltage amplification. The intelligent application of these new devices may lead to striking improvements in the performance of instruments used in potentiometry. In order to meet the need for portable radiation detectors suitable for use in Civil Defence, miniature electrometer valves with very modest power requirements are now in production: this has made possible the construction of miniature *p*H meters, the power consumption of which may be no greater than 50 mW, as compared with 1,200 mW for the instrument illustrated in Figure 3. It may be anticipated that the present trend towards the development of compact apparatus of economical power consumption will continue.

In conclusion the author regrets that, owing to the limited scope of this review, it has not been found possible to describe the work of numerous investigators, some of whom have made valuable contributions to the development of potentiometric instrumentation.

References

1. Britton, *Hydrogen Ions*, 1942, London: Chapman and Hall, Ltd.
2. Clark, *The Determination of Hydrogen Ions*, Baillière, Tindall and Cox.
3. Britton, *J. sci. Instrum.*, 1946, **23**, 89.
4. Furman, *Industr. Engng. Chem., Anal. Ed.*, 1930, **2**, 213.
5. Furman, *ibid.*, 1942, **14**, 367.
6. Furman, *Anal. Chem.*, 1950, **22**, 33.
7. Haber and Klemensiewiez, *Z. Phys. Chem.*, 1909, **67**, 385.
8. Hughes, *J. Amer. chem. Soc.*, 1922, **44**, 2860.
9. Kerridge, *Biochem. J.*, 1925, **19**, 611; *J. sci. Instrum.*, 1926, **3**, 404.
10. MacInnes and Dole, *J. Amer. chem. Soc.*, 1930, **52**, 29; *Industr. Engng. Chem., Anal. Ed.*, 1929, **1**, 57.
11. Dole, *J. Amer. chem. Soc.*, 1931, **53**, 4260; *J. Chem. Phys.*, 1934, **2**, 862.
12. MacInnes and Belcher, *J. Amer. chem. Soc.*, 1931, **53**, 3315.
13. Brown, *J. sci. Instrum.*, 1924, **2**, 12.
14. Morton, *ibid.*, 1930, **7**, 187.
15. Britton and Robinson, *Trans. Faraday Soc.*, 1932, **28**, 531.
16. Goode, *J. Amer. chem. Soc.*, 1922, **44**, 26; 1925, **47**, 2483.
17. Williams and Whitenach, *J. phys. Chem.*, 1927, **31**, 519.
18. Bienfait, *Rec. Trav. chim. Pays-Bas*, 1926, **45**, 166.
19. Morton, *Trans. Faraday Soc.*, 1928, **24**, 14.
20. Morton, *J. sci. Instrum.*, 1932, **9**, 289; British Patent No. 396,817.
21. Morton and Best, *J. Soc. chem. Ind.*, 1933, **52**, 6T.
22. Stadie, *J. biol. Chem.*, 1929, **83**, 477.
23. Dubois, *ibid.*, 1930, **88**, 729.
24. Partridge, *J. Amer. chem. Soc.*, 1929, **51**, 1.
25. Elder, *ibid.*, 1929, **51**, 3256.
26. Fosbinder, *J. phys. Chem.*, 1930, **34**, 1299.
27. Muller, *Z. Elektrochem.*, 1930, **34**, 1299.
28. Harrison, *J. chem. Soc.*, 1930, 1528.

29. Greville and Maclagan, *Trans. Faraday Soc.*, 1931, **27**, 211.
30. Voegtlin, de Eds and Kahler, *Amer. J. Physiol.*, 1929, **91**, 225.
31. Metcalf and Thompson, *Phys. Review*, 1930, **36**, 1489.
32. Morton, *J. chem. Soc.*, 1931, 2983.
33. Nottingham, *J. Franklin Inst.*, 1930, **209**, 287.
34. Morton, *J. chem. Soc.*, 1934, 256.
35. Garman and Droz, *Industr. Engng. Chem. Anal. Ed.*, 1939, **11**, 398.
36. Morton, *J. chem. Soc.*, 1931, 2977.
37. Fox and Groves, *J. Soc. chem. Ind.*, 1932, **51**, 7T.
38. Morton, *J. chem. Soc.*, 1932, 2469.
39. Chun-Yu-Lin, *J. sci. Instrum.*, 1944, **21**, 48.
40. Dole, *The Glass Electrode*, Chapman and Hall, Ltd., 1941, 60.
41. Ellis and Kiehl, *Rev. sci. Instrum.*, 1933, **4**, 131.
42. Goodhue, *Iowa State Coll. J. Sci.*, 1935, **10**, 7.
43. Cherry, *Trans. electrochem. Soc.*, 1937, **72**, 33.
44. Derbyshire, *Electronic Engn.*, 1946, **18**, 277.
45. Little, *ibid.*, 1947, Nov., 365.
46. Johnson, *Phys. Review*, 1936, **50**, 170.
47. Johnson and Neitzert, *Rev. sci. Instrum.*, 1934, **5**, 196.
48. Gabus and Poole, *ibid.*, 1937, **8**, 196.
49. Du Bridge and Brown, *ibid.*, 1933, **4**, 532.
50. Barth, *Z. Physik.*, 1934, **87**, 399.
51. Penick, *Rev. sci. Instrum.*, 1935, **6**, 115.
52. Mouquin and Garman, *Industr. Engng. Chem., Anal. Ed.*, 1937, **9**, 287.
53. Goyan, Barnes and Hind, *ibid.*, 1940, **12**, 485.
54. Penther, Rolfson and Lykken, *ibid.*, 1941, **13**, 831.
55. Penther and Rolfson, *ibid.*, 1943, **15**, 337.
56. Buras, Jun. and Reid, *ibid.*, 1945, **17**, 120.
57. Tellegen and Henriquez, *Wireless Engr.*, 1937, **14**, 409.
58. Lewis, *J. sci. Instrum.*, 1938, **15**, 353.
59. Farren, *Wireless Engr.*, 1938, **15**, 23.
60. Thorp, *Electronic Engn.*, 1945, Sept., 671; *Instrum. Practice*, 1947, **1**, 132.
61. McG. Sowerby, *J. sci. Instrum.*, 1944, **21**, 42.
62. Morton, *Trans. Faraday Soc.*, 1948, **44**, 588: British Patent No. 593,920.
63. Palevsky, Swenk and Grenchik, *Rev. sci. Instrum.*, 1947, 298.
64. Thomas and Finch, *Electronic Engn.*, 1950, **22**, 395.
65. Kraus, Holmberg and Borkowski, *Anal. Chem.*, 1940, **22**, 341.
66. Brentano, *Nature, Lond.* 1926, **108**, 532.
67. Wynn-Williams, *Trans. Camb. phil. Soc.*, 1927, **23**, 811.
68. Brentano and Ingleby, *J. sci. Instrum.*, 1939, **16**, 81.
69. Schmitt, *ibid.*, 1938, **15**, 136.
70. Richter, *Electronics*, 1943, Nov., 112.
71. Offner, *Rev. sci. Instrum.*, 1937, **8**, 20.
72. Matthews, *J. Physiol.*, 1934, **81**, 28 P.
73. Miller, *Electronics*, 1941, **14**, 27.
74. Parr and Walter, *J. Instn. elect. Engrs.*, 1943, **90**, 129.
75. Johnston, *Wireless Engr.*, 1947, 24, 231.
76. Prinz, *J. sci. Instrum.*, 1947, **24**, 328.

RESEARCH PAPERS

STUDIES IN THE EFFECT OF SUBSTITUTION ON THE LOCAL ANÆSTHETIC ACTIVITY OF PYRAZOLINE DERIVATIVES

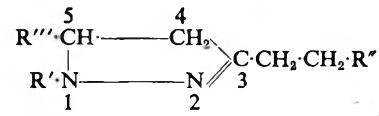
PART I. 1-PHENYL-5-(3′-METHOXY-4′-*n*-PROPOXYPHENYL)-3-β-DIALKYLAMINO (PIPERIDINO; MORPHOLINO)-PYRAZOLINES

BY T. B. B. CRAWFORD, H. B. NISBET and D. RITCHIE

From the Heriot-Watt College, Edinburgh and The Department of Pharmacology, University of Edinburgh

Received January 7, 1952

PYRAZOLINES of the type I are known to possess local anæsthetic activity (Nisbet et al.[1]) and it has been shown that when R‴ in position 5 is a phenyl group, alkoxy substitution on this group has a profound influence upon the physiological activity of the molecule (Levvy and Nisbet[2]). Similar effects have been observed by alkoxy substituents in the analogues of metycaine, benzoyl-γ-(2′-methyl-piperidino)-*n*-propanol hydrochloride (McElvain and Carney[3]).



(I)

In order to study the effect of alkoxy substitution and also the effect of varying the tertiary amino group — R″ in the pyrazolines of type I, a series of compounds have been prepared in which:

R′ = C_6H_5
R″ = $N{\cdot}(CH_3)_2$, $N{\cdot}(C_2H_5)_2$, $N{\cdot}(n\text{-}C_3H_7)_2$, $N{\cdot}(n\text{-}C_4H_9)_2$, morpholino and piperidino.
R‴ = 3-methoxy-4-*n*-propoxy-phenyl.

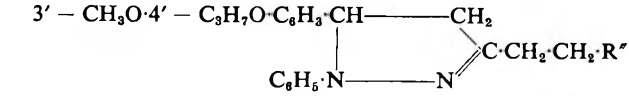
The local anæsthetic values of the compounds in the form of tartrates have been compared with procaine hydrochloride by intradermal injection in the back of the guinea-pig (Bülbring and Wajda[4]) and with cinchocaine hydrochloride on the cornea of the same animal. The relative toxicities of two of the most potent compounds and of procaine hydrochloride and cinchocaine hydrochloride were determined by intraperitoneal injection in mice.

The results of the pharmacological tests are summarised in Table I and consideration of the data indicates that variation of the tertiary amino group R″ substituted on the 3-ethyl group of the pyrazoline molecule causes a change in the local anæsthetic power of the compound. The order of descending potencies in the intradermal weal test is given in the series:—

$N{\cdot}(C_2H_5)_2 > N{\cdot}C_5H_{10} > N{\cdot}(CH_3)_2 > N{\cdot}(n\text{-}C_3H_7)_2 > N{\cdot}(n\text{-}C_4H_9)_2 > N{\cdot}C_4H_8O$

TABLE I

SUMMARY OF THE RESULTS OF THE PHARMACOLOGICAL TESTS ON CERTAIN PYRAZOLINE DERIVATIVES



R″	Guinea pig intradermal weal test		Corneal test on guinea-pig		Toxicity LD 50 (mice) mg./kg. i.p.
	Relative potency compared with procaine hydrochloride	Therapeutic value	Relative potency compared with cinchocaine hydrochloride	Therapeutic value	
Dimethylamino	4·0	—	0·15	—	—
Diethylamino	7·0	3·5	0·25	0·95	125
Di-*n*-propylamino	2·5	—	0·06	—	—
Di-*n*-butylamino	3·5	—	0·05	—	—
Piperidino	6·0	4·5	0·1	0·52	170
Morpholino	1·5	—	—	—	—
Cinchocaine hydrochloride	—	—	1·0	1·0	33
Procaine hydrochloride ..	1·0	1·0	—	—	225
Cocaine hydrochloride ..	—	—	—	—	110

While in the corneal test the order of potency is given by the series:—

$$N{\cdot}(C_2H_5)_2 > N{\cdot}(CH_3)_2 > N{\cdot}(C_5H_{10} > N{\cdot}(n\text{-}C_3H_7)_2 > N{\cdot}(n\text{-}C_4H_9)_2 > N{\cdot}C_4H_8O$$

This variation in potency with change in the R″ group will perhaps be more easily appreciated by reference to Figures 1 and 2. Figure 1 illustrates graphically the results obtained in the intradermal weal test. The anæsthetic effect, measured in arbitary units to be described later, has been plotted against the concentration of the solution employed, using a logarithmic scale. The observation first made by Sinha[5] that a linear relationship exists between the two functions has again been confirmed. Since the slope of the regression lines are approximately equal to that of the procain hydrochloride, it is possible to compare with some degree of accuracy the relative potencies of the drugs with procaine hydrochloride as standard (Bülbring and Wajda[4]).

The results obtained from the corneal test are shown graphically in Figure 2 which records the relationship between duration of anæsthesia and the concentration of the solution employed, using a logarithmic scale.

The therapeutic values of two of the most potent compounds have been determined, the therapeutic value being defined as the ratio of the relative potency to the relative toxicity. By intradermal injection 1-phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-piperidino-ethyl-pyrazoline tartrate has a therapeutic value of 4·5 and 1-phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-diethylamino-pyrazoline tartrate has a value of 3·5 with procaine hydrochloride as standard. The corresponding values for the corneal test in the guinea-pig being 0·52 for the piperidino derivative and 0·95 for the diethylamino derivative with cinchocaine hydrochloride as standard.

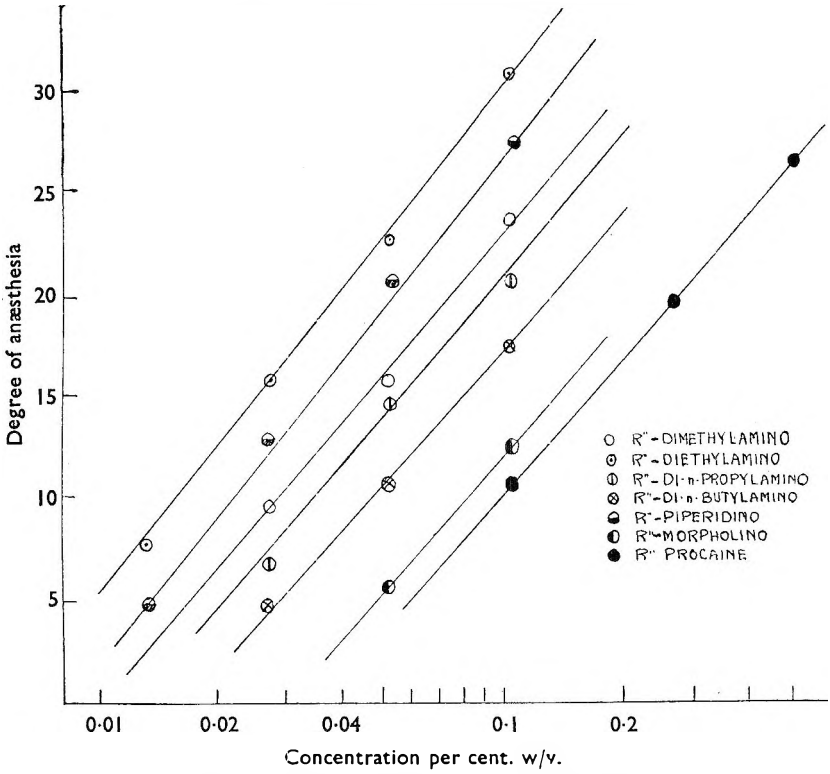
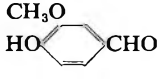


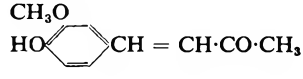
FIG. 1. Intradermal weal test in guinea-pigs.

SYNTHESIS OF THE PYRAZOLINE COMPOUNDS

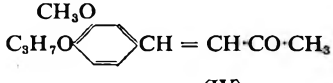
The compounds used in this investigation were prepared by general methods described by Nisbet *et al.*[1] Vanillin (II) is converted to vanillidene acetone (III) and the latter by treatment with *n*-propyl-*p*-toluenesulphonate and sodium hydroxide to 3-methoxy-4-*n*-propoxy-benzylidene acetone (IV). The alkylated benzylidene acetone (IV) on treatment with formaldehyde and the hydrochlorides of various secondary bases (Mannich reaction) yields the unsaturated ketones (V). The phenylhydrazones of the ketones (VI) on treatment with dilute hydrochloric acid are isomerised to the pyrazoline hydrochlorides (VII), the bases of which are converted to the tartrates.



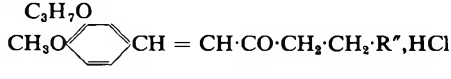
(II)



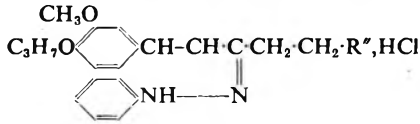
(III)



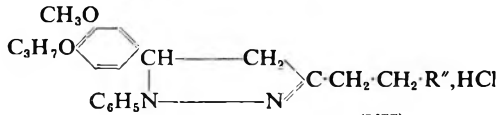
(IV)



(V)



(VI)



(VII)

3-*Methoxy*-4-n-*propoxy-benzylidene acetone.* IV. Vanillidene acetone (105 g.) and *n*-propyl-*p*-toluene sulphonate (107 g.) are dissolved in ethanol (205 ml.) by heating under a reflux condenser. Potassium hydroxide (35 g.) dissolved in the minimum of water is added and the refluxing continued for 1½ hours. The contents of the flask are poured into water (2·5 l.) and the oil which separates allowed to crystallise.

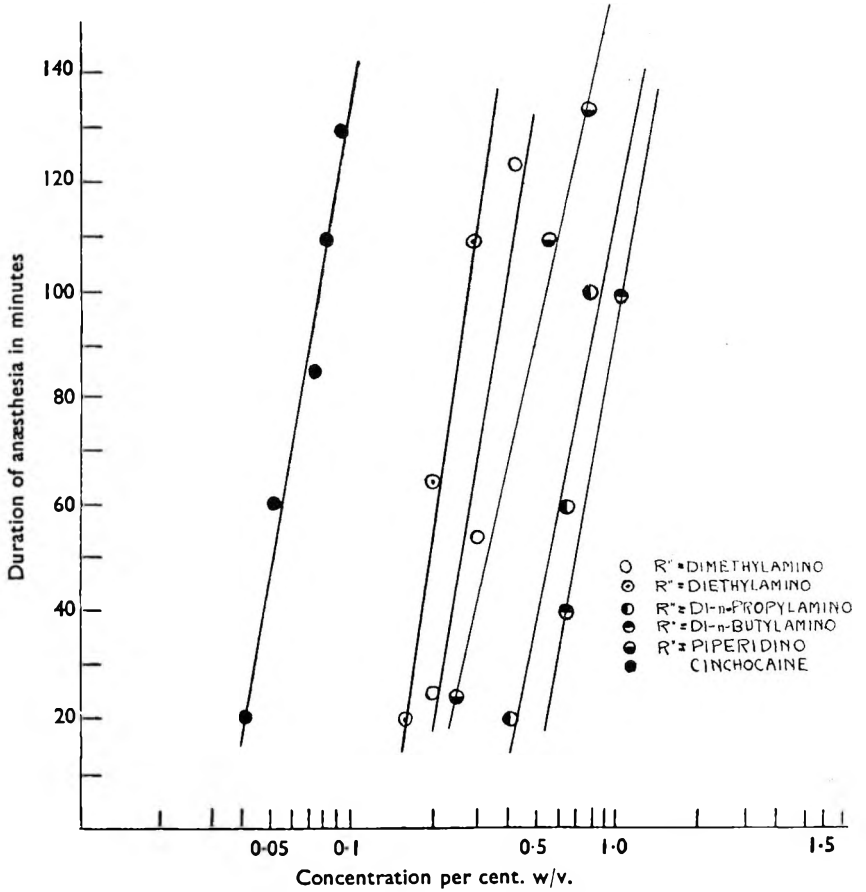


FIG. 2. Corneal test on guinea-pigs.

The solid is collected on a filter, washed, dried and recrystallised from acetone to give pale yellow crystals m.pt. 92° to 93° C. Yield 51 per cent. Found: C, 71·06; H, 7·59; $C_{14}H_{18}O_3$ requires C, 71·8; H, 7·69 per cent.

1-*Dimethylamino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride*, (V). 3-Methoxy-4-*n*-propoxy-benzylidene acetone (10 g.) and dimethylamine hydrochloride (3·25 g.) are dissolved in ethanol (15·0 ml.) by heating. Paraformaldehyde (2·2 g.) is added in small portions and the heating, under a reflux condenser, continued for 30 minutes. The crystalline solid which forms on cooling and scratching the internal surface of the flask is recrystallised from ethanol to give white needles m.pt. 133° to 134° C. Yield 51 per cent. Found: N, 4·1 per cent.; $C_{17}H_{25}O_3N$,HCl requires N, 4·27 per cent.

Phenylhydrazone of 1-*dimethylamino*-5-(3′*methoxy*-4′-n-*propoxy*-*phenyl*) Δ^4-*penten*-3-*one hydrochloride* (VI). The unsaturated ketone $C_{17}H_{25}O_3N$, HCl (4·0 g.) is dissolved in ethanol (20·0 ml.) and phenylhydrazine (1·25 g.) in acetic acid (1·25 g.) added. The phenylhydrazone crystallises on standing and is recrystallised from ethanol to give yellow needles m.pt. 163° to 164° C. Yield 71 per cent. Found: N, 10·1 per cent.; $C_{23}H_{31}O_2N_3$,HCl requires N, 10·06 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-3-*β*-*dimethylamino*-*ethyl*-*pyrazoline tartrate* (VII). The phenylhydrazone $C_{23}H_{31}N_3O_3$,HCl (2·5 g.) is heated under a reflux condenser on a steam-bath with 0·1 N hydrochloric acid (25·0 ml.) for 30 minutes. On cooling, the pyrazoline separates as a green oil and is isolated by making the solution slightly alkaline with sodium hydroxide and extracting with ether. The tartrate is formed by the addition of an ethanolic solution of tartaric acid to the base and recrystallising from ethanol-light petroleum (1-1) to give pale yellow crystals m.pt. 79° to 80° C. Yield 44 per cent. Found: N, 7·5 per cent.; $C_{23}H_{31}O_2N_3$,$C_4H_6O_6$ requires N, 7·9 per cent.

The other pyrazoline compounds of the series were prepared in a similar manner from 3-methoxy-4-*n*-propoxy-benzylidene acetone (IV).

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-3-*β*-*diethylamino*-*ethyl*-*pyrazoline tartrate* (VII). M.pt. 47° to 48° C. Yield 38 per cent. Found: N, 7·3 per cent.; $C_{25}H_{35}O_2N_3$,$C_4H_6O_6$ requires N, 7·5 per cent.

1-*Diethylamino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 103° to 104° C. Yield 43 per cent. Found: N, 3·5 per cent.; $C_{19}H_{29}O_3N$,HCl requires N, 3·9 per cent.

Phenylhydrazone of 1-*diethylamino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 143° to 144° C. Yield 72 per cent. Found: N, 9·42 per cent.; $C_{25}H_{35}O_2N_3$,HCl requires N, 9·43 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-3-*β*-*di*-n-*propylamino ethyl pyrazoline tartrate* (VII). M.pt. 54° to 55° C. Yield 28 per cent. Found: N, 7·3 per cent.; $C_{27}H_{39}O_2N_3$,$C_4H_6O_6$ requires N, 6·96 per cent.

1-*Di*-n-*propylamino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 124° to 125° C. Yield 46 per cent. Found: N, 3·48 per cent.; $C_{21}H_{33}O_3N$,HCl requires N, 3·65 per cent.

Phenylhydrazone of 1-*di*-n-*propylamino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 154° to 155° C. Yield 84 per cent. Found: N, 9·02 per cent.; $C_{27}H_{39}O_2N_3$,HCl requires N, 8·87 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-3-β-*di*-n-*butylamino-ethyl-pyrazoline tartrate* (VII). M.pt. 58° to 59° C. Yield 29 per cent. Found N, 7·04 per cent.; $C_{29}H_{43}O_2N_3$,$C_4H_6O_6$ requires N, 6·83 per cent.

1-*Di*-n-*butylamino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 93° to 94° C. Yield 43 per cent. Found: N, 3·42; $C_{23}H_{37}O_3N$,HCl requires N, 3·4 per cent.

Phenylhydrazone of 1-*di*-n-*butylamino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 143° to 144° C. Yield 74 per cent. Found: N, 8·81 per cent.; $C_{29}H_{43}O_2N_3$,HCl requires N, 8·4 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-3-β-*piperidino*-*ethyl pyrazoline tartrate* (VII). M.pt. 65° to 67° C. Yield 42 per cent. Found: N, 7·2 per cent.; $C_{26}H_{35}O_2N_3$,$C_4H_6O_6$ requires N, 7·35 per cent.

1-*Piperidino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 146° to 147° C. Yield 46 per cent. Found: N, 4·2 per cent.; $C_{20}H_{29}O_3N$,HCl requires N, 3·8 per cent.

Phenylhydrazone of 1-*piperidino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 168° to 169° C. Yield 64 per cent. Found: N, 9·2 per cent.; $C_{26}H_{35}O_2N_3$,HCl requires N, 9·18 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-3-β-*morpholino-ethyl-pyrazoline tartrate* (VII). M.pt. 64° to 65° C. Yield 32 per cent. Found: N, 8·52 per cent.; $C_{25}H_{33}O_3N_3$,$C_2H_3O_3$ requires N, 8·43 per cent.

1-*Morpholino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 164° to 165° C. Yield 55 per cent. Found: N, 3·8 per cent.; $C_{19}H_{27}O_3N$,HCl requires N, 4·0 per cent.

Phenylhydrazone of 1-*morpholino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 158° to 159° C. Yield 85 per cent. Found: N, 8·96 per cent.; $C_{25}H_{33}O_3N_3$,HCl requires N, 9·14 per cent.

Pharmacological Tests

The local anæsthetic potency of each compound was compared with procaine hydrochloride by intradermal injection on the back of the guinea-pig and with cinchocaine hydrochloride by local application to the cornea of the same animal.

Intradermal Weal Test. In the guinea-pig weal test, the method described by Bülbring and Wajda[4] with some slight modification was used. Procaine hydrochloride was taken as standard in preference to cocaine hydrochloride, as Bülbring and Wajda[4] have shown that the vaso-constrictor action of cocaine makes it unsuitable as a standard for the determination of the relative local anæsthetic potency of new compounds which possess no such vaso-constrictor action. 4 guinea-pigs were used for each test. The hair was removed by means of hand clippers 24 hours previous to the test, during which time any irritation

resulting from the clipping had disappeared. Solutions of the drug for test were prepared in 0·9 per cent. saline solution and a volume of 0·25 ml. of each of 4 concentrations of the compound under test and of procaine hydrochloride was injected intradermally along each side of, and at the same distance from the midline of the back of, each animal. Bülbring and Wajda[4] found that the sensitivity of the skin increased from the back to the front. In order to overcome the errors which would arise from this variation, it was the practice in these experiments to inject each concentration at a different relative site on the back of the animal in each of the 4 guinea-pigs as indicated in Table II. The weal formed from each injection was marked out in ink and the enclosed area tested for anæsthesia by pricking with a pin. After observing the animal's normal reaction to the prick, the marked area was tested 6 times at intervals of 3 to 5 seconds. This test was applied every 5 minutes for half an hour and the number of times the pricks failed to produce a response were added up for each concentration and out of a possible 36 gave an indication of the degree of anæsthesia. The results of the test are given in Table III and shown graphically in Figure 1.

TABLE II

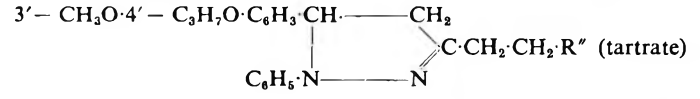
POSITION OF THE INTRADERMAL INJECTIONS ON THE BACK OF THE GUINEA-PIG. 0·25 ML. OF EACH SOLUTION INJECTED INTRADERMALLY

Animal 1	Animal 2	Animal 3	Animal 4
T_4 P_1	P_4 T_3	T_1 P_3	P_3 T_2 Head
P_3 T_2	T_1 P_2	P_4 T_3	T_4 P_1
T_1 P_2	P_2 T_2	T_4 P_1	P_4 T_3
P_4 T_3	T_4 P_1	P_2 T_2	T_1 P_2 Tail

T_1, T_2, T_3 and T_4, Test solutions 0·0125, 0·025, 0·05 and 0·1 per cent.
P_1, P_2, P_3 and P_4, Procaine solutions 0·125, 0·25, 0·5 and 1·0 per cent.

TABLE III

THE MEAN NUMBER OF PRICKS (WITH STANDARD ERROR) OUT OF A POSSIBLE 36, FAILING TO ELICIT A RESPONSE AFTER INTRADERMAL INJECTION OF THE DRUG IN GUINEA-PIGS



Concentration of the solution injected (per cent. w/v)	0·1	0·05	0·025	0·0125
R″				
Dimethylamino	24 ± 4·5	16 ± 3·5	10 ± 3·5	3 ± 1·3
Diethylamino	31 ± 4·5	23 ± 1·3	16 ± 1·8	8 ± 1·7
Di-*n*-propylamino	18 ± 3·4	11 ± 2·1	7 ± 1·6	0
Di-*n*-butylamino	21 ± 2·3	15 ± 3·4	7 ± 1·8	0
Piperidino	28 ± 2·2	21 ± 2·8	13 ± 2·1	5 ± 1·7
Morpholino	13 ± 4·7	6 ± 2·5	0	0
Concentration of solution injected (per cent. w/v)	1·0	0·5	0·25	0·125
Procaine hydrochloride	36 ± 0	27 ± 2·5	20 ± 2·3	11 ± 4·2

Corneal Tests. The compounds were also examined for their topical local anæsthetic effect by application to the cornea of an animal. The cornea of the rabbit was first used for this purpose but it was subsequently

found that the cornea of the guinea-pig gave more consistent results. Chance and Lobstein[6] have also found the guinea-pig to be the better subject for this test.

Cocaine hydrochloride was first used as the standard but this was found unsatisfactory. On plotting the logarithm of the concentration against duration of anæsthesia, it was found that over a limited range of concentrations, the regression lines for the pyrazolines were parallel, but these were not parallel to that for cocaine hydrochloride. In order to find a suitable reference standard, various local anæsthetics were tested and it was found that cinchocaine hydrochloride had a regression line parallel to that of the pyrazolines (see Figure 3) and it was, therefore, selected as standard.

The local anæsthetic, dissolved in normal saline was applied by means of a dropping tube to the eye of the guinea-pig which was held in such a position as to allow the cornea to be covered by a film of the solution

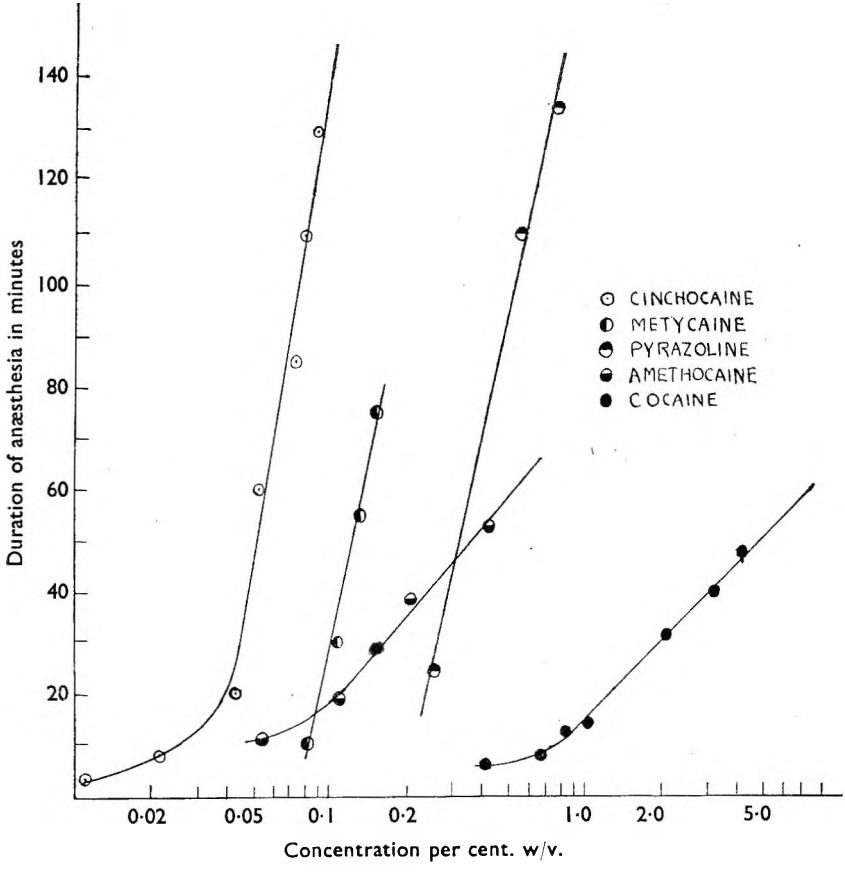


FIG. 3. Corneal test on guinea-pigs.

Pyrazoline = 1-phenyl-5-(3′-methoxy-4-*n*-propoxyphenyl)-3-β-piperidinoethylpyrazoline tartrate

for a period of 15 seconds. If the guinea-pig blinked during this period the film was renewed. The cornea was tested every 5 minutes by touching the centre 3 times with a hog's bristle attached to a glass rod. The duration for each concentration was obtained by noting the time when 2 or more positive responses were obtained from the 3 stimulations. The results are shown graphically in Figure 2 in which duration of anæsthesia has been plotted against the logarithm of the concentration of the solution. From these figures the approximate relative potency compared with cinchocaine hydrochloride as given in Table I was calculated.

Toxicity Tests. The approximate median lethal dose was calculated by intraperitoneal injection of mice weighing about 20 g. A suitable concentration of the drug was used and the dose, calculated in mg./20 g. mouse, was varied by varying the volume of the fluid injected. The volume injected did not at any time exceed 0·6 ml./20 g. of body weight. The results for these tests are summarised in Table IV and from these results the approximate values for the median lethal dose as given in the last column of the Table were found.

TABLE IV

RESULTS OF TOXICITY TESTS OBTAINED BY INTRAPERITONEAL INJECTION OF MICE

Drug	Dose mg. /20g.	Number of mice injected	Number of deaths	Mortality per cent.	LD 50 mg/20 g.
Cinchocaine HCl	0·4	6	0	0	
	0·5	7	0	0	
	0·6	6	1	18	0·66
	0·7	7	5	70	
	0·8	7	7	100	
Procaine HCl ..	1·0	10	0	0	
	2·0	20	0	0	
	4·0	20	8	40	4·5
	6·0	10	8	80	
	8·0	20	20	100	
R_2 = Diethylamino ..	2·0	10	0	0	
	3·0	10	9	90	
Compound A.	3·5	10	8	80	2·5
	4·0	20	16	80	
	4·5	8	8	100	
R_2 = Piperidino ..	3·0	10	3	30	
	4·0	10	8	80	
Compound B.	5·0	10	10	100	3·4
	6·0	10	10	100	

Compound A. 1-Phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-diethylamino-ethyl-pyrazoline tartrate.

Compound B. 1-Phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-piperidino-ethyl-pyrazoline tartrate.

SUMMARY

1. Several analogues of 1-phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-dialkylamino (piperidino, morpholino)-ethyl-pyrazoline tartrate have been prepared.

2. The pharmacological tests, which were of a screening nature to obtain approximate values, indicate that the compounds are potent local anæsthetics both by intradermal injection and by absorption through mucous membrane.

3. From the pharmacological examination it appears that the activity of the molecule attains a maximum when the size of the secondary amino group in the 3-ethyl position is diethylamino.

4. Of the series 1-phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-diethylamino-ethyl-pyrazoline tartrate was found to be the most potent having a therapeutic value of 3·5 with reference to procaine hydrochloride by intradermal injection on the guinea-pig and a value of 0·95 with reference to cinchocaine hydrochloride on the cornea of the guinea-pig, the relative toxicity having been determined by intraperitoneal injection in mice.

A grant from the Carnegie Trust for the Universities in Scotland towards the cost of the animals used in this research is gratefully acknowledged.

References

1. Nisbet, *J. chem. Soc.*, 1938, 1237.
2. Levvy and Nisbet, *ibid.*, 1938, 1572.
3. McElvain and Carney, *J. Amer. chem. Soc.*, 1946, **68**, 2592.
4. Bülbring and Wajda, *J. Pharmacol.*, 1945, **85**, 78.
5. Sinha, *ibid.*, 1936, **57**, 199.
6. Chance and Lobstein, *ibid.*, 1944, **82**, 203.

BRAZILIAN JALAP

Part III. An Examination of the Resin and Some Comparisons with the Resins from Vera Cruz jalap and Orizaba jalap

By E. J. Shellard

From the School of Pharmacy, College of Technology, Bristol

Received February 4, 1952

Introduction

In Parts I and II[1,2] some indication was given of the histological and pharmacognostical features of 4 samples of commercial Brazilian jalap, together with some comparisons with Vera Cruz jalap and Orizaba jalap. Since, however, it is the so called resins of these drugs that are more commonly used in medical and veterinary practice, it was considered desirable to make an examination of Brazilian jalap resin in order to provide a quick and reliable method of identifying it and distinguishing it from the resins of Vera Cruz jalap and Orizaba jalap.

The first reference to work on Brazilian jalap resin appears to be that given by Holmes[3] in 1915, who reported that experiments by Passmore on this particular sample of drug showed "over 20 per cent. of resin answering all the B.P., 1914, and U.S.P. VIII tests for the resin of true or Vera Cruz jalap but only 0·85 per cent. soluble in ether." In 1918 Scoville[4] and Ewing and Clevenger[5] made further examination of the resin obtained from Brazilian jalap, but since that date there is no evidence of further investigation. The resins of Vera Cruz jalap and Orizaba jalap have, on the other hand, both been extensively and critically examined. Much of this work has been concerned with the physical properties and chemical constants of the resins and it is by means of these characters that the resins are at present distinguished.

Materials

Samples of resin were prepared by the method given in the British Pharmaceutical Codex, 1949, from the samples of Brazilian jalap A, B, C and D, Vera Cruz jalap and Orizaba jalap referred to in Parts I and II.

Only small quantities of Brazilian jalap A and B were available so that it was not possible to obtain sufficient resin to enable all the tests to be carried out on these.

Experimental

Loss on Drying at 100° *C.* Determined by the method of the British Pharmacopœia the results obtained were—Brazilian jalap A 3·58, B 4·24, C 3·08, D 2·15; Vera Cruz jalap 3·66; Orizaba jalap 3·38 per cent. All percentages are calculated with reference to the resins dried at 100° C.

Solubility in Organic Solvents. Scoville[4] and Ewing and Clevenger[5] extracted Brazilian jalap resin with various solvents successively in a continuous extraction apparatus, a procedure which had previously been employed by Power and Rogerson[6,7] for the resins of Vera Cruz and

Orizaba jalap. For reference purposes the percentages extracted per solvent as reported by these workers are given in Table I.

TABLE I

EXTRACTION OF JALAP RESIN WITH VARIOUS SOLVENTS

Solvent (in order used)	Brazilian jalap resin (Scoville)	Brazilian jalap resin (Ewing and Clevenger)	Vera Cruz jalap resin (Power and Rogerson)	Orizaba jalap resin (Power and Rogerson)
	per cent.	per cent.	per cent.	per cent.
Light petroleum..	2·09	2·1	1·9	6·2
Ether	2·376	5·4	9·7	64·8
Chloroform ..	2·424	73·4	24·1	0·6
Ethyl acetate ..	(All remainder) 93·1	14·2	22·0	24·8
Ethanol		4·7	38·8	2·3

It will be noted that there is considerable discrepancy between the amounts of Brazilian jalap resin extracted with chloroform as given by Scoville and by Ewing and Clevenger, and it is difficult to attempt an explanation of this. In order to obtain some comparative figures for the resins available, Brazilian jalap resin C and D, Vera Cruz jalap resin and Orizaba jalap resin were successively extracted in a Soxhlet apparatus with the same solvents as those referred to in Table I. In view of previous criticisms of the method, care was taken to ensure that the solvents were dry and in the case of ether and chloroform, that they were free from ethanol.

The percentages of resin extracted by the various solvents are given in Table II.

TABLE II

EXTRACTION OF JALAP RESINS WITH VARIOUS SOLVENTS (AUTHOR'S RESULTS)

Solvent (in order used)	Brazilian jalap resin	Brazilian jalap resin	Vera Cruz jalap resin	Orizaba jalap resin
	Sample C	Sample D		
	per cent.	per cent.	per cent.	per cent.
Light petroleum..	2·86	1·94	2·66	2·36
Ether	2·94	3·61	4·01	73·26
Chloroform ..	48·12	50·11	22·25	1·12
Ethyl acetate ..	42·33	43·06	22·08	19·84
Ethanol		all remainder		

Scoville[4] further determined the solubility of Brazilian jalap resin in various solvents directly and it was decided to make similar determinations of all the resins available. The percentage resin soluble in the solvents are given in Table III, together with Scoville's figures for comparison.

It must be noted that whereas Scoville obtained his figures by shaking 1 g. of resin with about 4 times its volume of solvent for several hours, filtering, evaporating to dryness and weighing the residue, the method used here was to use 100 ml. of solvent, shake occasionally during 24 hours, decant or filter, recover most of the solvent, evaporate to dryness and weigh.

TABLE III

SOLUBILITY OF JALAP RESIN WITH VARIOUS SOLVENTS

Solvent	Brazilian jalap resin					Vera Cruz jalap resin	Orizaba jalap resin
	Scoville	A	B	C	D		
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Light petroleum	3·7	—	—	2·84	1·96	2·58	2·62
Ether	5·9	6·26	3·83	3·72	3·94	5·25	74·72
Chloroform	19·55	52·31	43·44	53·38	56·55	20·15	71·41
Benzene	5·3	—	—	4·25	3·87	5·23	90·64
Acetone	99·4	—	—	98·42	97·92	99·04	98·86

The figures obtained for the percentage soluble in chloroform shows some degree of uniformity for all 4 samples of Brazilian jalap resin and, although the number of samples examined are too few to suggest limits for the solubility of this resin in this solvent, it appears to be between 40 and 60 per cent.

Solubility in Water. Scoville[4] also reported on the solubility of Brazilian jalap resin in water, stating that on shaking 1 g. of resin mixed with washed sand with 100 ml. of distilled water for several hours at room temperature 0·535 g. was found to be dissolved and he commented that 53·5 per cent. was an abnormally high figure for the solubility of a resinous body in water.

It is possible that this figure does not represent the true figure for the amount of resin soluble in water but rather that for the maximum solubility of the resin substance in water at room temperature. In order to ascertain how much of the resin was actually water-soluble 3 quantities each of 1 g. (Sample D) were shaken continuously for 3 hours with 50 ml., 100 ml. and 500 ml. of distilled water respectively. The aqueous extractives were then poured into tared dishes, evaporated to dryness and weighed. Further similar quantities of distilled water were then added and the process repeated until the 3 quantities of resin had been extracted with 200 ml., 500 ml. and 2000 ml. of distilled water respectively. The results obtained are given in Table IV.

TABLE IV

RESIN EXTRACTED BY WATER FROM 1 G. OF BRAZILIAN JALAP RESIN FROM SAMPLE D

	Volume of water used for each extraction	Weight of resin soluble in each volume of water					Total weight of resin extracted
		1	2	3	4	5	
		g.	g.	g.	g.	g.	g.
1	50 ml.	0·1668	0·1640	0·1188	0·0502	—	0·4998
2	100 ml.	0·2340	0·2240	0·0706	0·0208	0·0072	0·5606
3	500 ml.	0·3842	0·3464	0·0702	0·0056	—	0·8064

It will be seen from these results that the amount of resin extracted by water varies with the volume of water used. On all occasions when the resin was shaken with water there was considerable frothing and it was considered that the anomalous results might be due to some hydrolysis of the glycoside constituent of the resins. However, when the aqueous extractive was heated with Fehling's solution no reduction occurred. No further attempt was made to explain the results. The figures do,

however, confirm the work of Scoville that the water-soluble portion of Brazilian jalap resin is abnormally high for a convolvulaceous resin. They also suggest that in order to obtain reproducible results a standardised method must be adopted.

Determination of the water-soluble matter according to the method A of the British Pharmacopœia (using distilled water) gave the following results:—Brazilian jalap resin—A. 20·66; 20·62. B. 32·71; 32·73. C. 22·06; 22·11. D. 21·75; 21·70 per cent.; Vera Cruz jalap resin—0·56; 0·54 per cent.; Orizaba jalap resin—0·32; 0·35 per cent.

There was considerable frothing with the Brazilian jalap resins which persisted for some time, that for Sample B persisting for several hours. The *p*H of the distilled water used was 5·4. The *p*H of the aqueous extractives were:—Brazilian jalap resin—A. 4·35; B. 4·30; C. 4·40; D. 4·35; Vera Cruz jalap resin—4·85; Orizaba jalap resin—5·30.

Specific Rotation.—Although these convolvulaceous resins are not constant in composition, previous workers have given some consideration to the specific rotation of resins which have been decolorised by refluxing ethanolic solutions with animal charcoal. Guigues considered that the optical activity had a better diagnostic value than ether-solubility. Cowie[9] and Power and Rogerson[6,7] have also examined various convolvulaceous resins for optical activity and Ewing and Clevenger[5] have given the only reported specific rotation for Brazilian jalap resin.

As a further comparison between the resins, the specific rotation was determined on samples of resins after purifying as far as possible by refluxing ethanolic solutions with animal charcoal for four hours. The resulting colour in each case was pale straw yellow. The results are given in Table V, together with figures obtained by the previous workers.

TABLE V

SPECIFIC ROTATION

		Guigues[8]	Cowie[8]	Power and Rogerson[6,7]	Ewing and Clevenger[5]
Brazilian jalap	—	—	—	—	−48·5°
Sample A	−19·67°	—	—	—	—
„ B	−20·48°	—	—	—	—
„ C	−20·14°	—	—	—	—
„ D	−20·00°	—	—	—	—
Vera Cruz jalap	−37·6°	−36·0°	−37·3°	−37·0°	—
Orizaba jalap	−25·4°	−24·45°	−27·0°	−23·0°	—

It will be observed that in spite of probable variation in the composition of the resins of each type of jalap, the specific rotation obtained by various workers is fairly constant, except in the case of Brazilian jalap resin, where the value obtained by Ewing and Clevenger is more than double that obtained for each of the 4 samples examined.

Acid Values, Saponification Value, Melting-point. In order to complete the comparative examination of the physical properties and chemical constants of the 3 varieties of resin, acid values, saponification values and melting points were determined on the purified resins. The results

obtained for the acid values and saponification values are given in Table VI, together with values given by previous workers for comparative purposes.

TABLE VI

ACID VALUES AND SAPONIFICATION VALUES

	Acid value		Saponification value	
Brazilian jalap resin—	—	Scoville 23·1	—	Scoville 141·6
Sample C	24·58	—	164·1	—
„ D	23·73	—	158·8	—
Vera Cruz jalap resin ..	17·15	Power and Rogerson 15	142·3	Power and Rogerson 140
Orizaba jalap resin ..	21·65	Power and Rogerson 20	182·4	Power and Rogerson 180

The melting-points of the purified resins obtained by the method described in the B.P. Appendix IV, AI were:—

Brazilian jalap resin *ali samples*	94° to 100° C.
Vera Cruz jalap resin	138° to 144° C.
Orizaba jalap resin	121° to 127° C.

Examination under Screened Ultra-violet Light. When the resins were powdered, spread in thin layers and examined in screened ultra-violet light, it was quite easy to distinguish between them by their appearance, which was as follows:—Brazilian jalap resin (all samples), yellowish brown; Vera Cruz jalap resin, pale pinkish violet; Orizaba jalap resin, deep bluish purple.

Elementary Chromatograms. Adsorption chromatograms were prepared from the varieties of resins with heavy magnesium oxide as the adsorbent. Both tube and disc chromatograms were prepared using:—(i) ethanolic solutions of the resins; (ii) ether solutions of ether-soluble portions; (iii) ethanolic solutions of ether-insoluble portions; (iv) chloroform solutions of the chloroform-soluble portions; (v) ethanolic solutions of the chloroform-insoluble portions.

The chromatograms were developed with the same solvent as that used to prepare the solutions. When observed in daylight it was possible to distinguish pale coloured zones in most of the chromatograms but they were not so distinctive as to enable the various resins to be characterised. It was possible to note a difference between the chromatogram of the

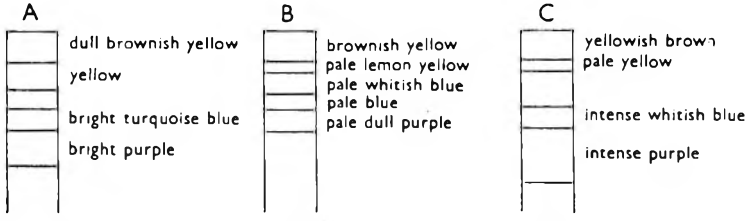


FIG. 1. Appearance of the chromatograms of the resins in screened ultra-violet light. A. Vera Cruz jalap. B. Brazilian jalap, Samples A, C and D. C. Orizaba jalap.

resin of Brazilian jalap B and the other Brazilian jalap resins. The appearance of the chromatograms in screened ultra-violet light was, however, most striking. The results obtained with the tube chromatograms when examined in screened ultra-violet light are given diagrammatically in Figures 1 and 2. It is, however, impossible to describe

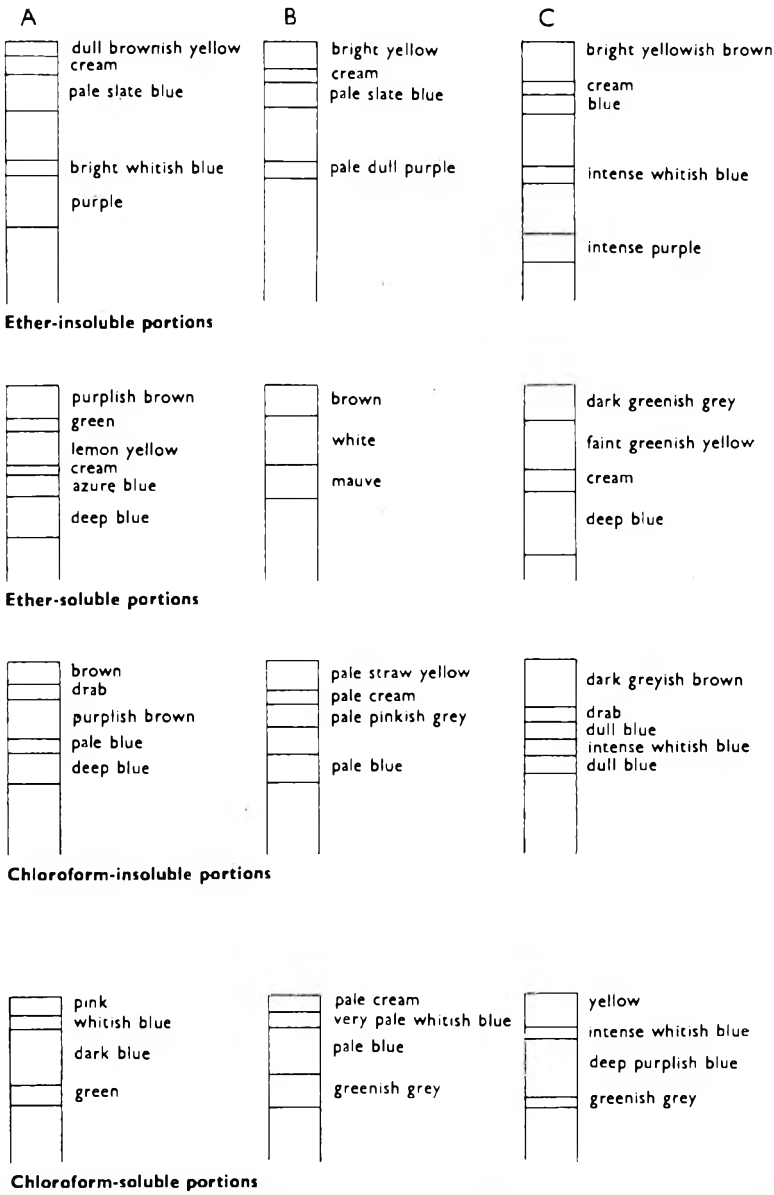


FIG. 2. Appearance of chromatograms in screened ultra-violet light. A. Vera Cruz jalap resin. B. Brazilian jalap resin. C. Orizaba jalap resin.

adequately the actual colours observed, and it is necessary therefore to make or emphasise the following points:—(1) the chromatogram of the resin of Brazilian jalap B differed from the resins of the other samples of Brazilian jalap by having a rather yellower first zone and a second narrow zone which was cream in colour rather than pale lemon yellow. Further, the following whitish blue zone was far less intense; (2) in all the chromatograms prepared from Brazilian jalap resin the blue and purple zones were pale and dull, while in the resins from Vera Cruz and Orizaba jalap the blue and purple zones were bright and often intense; (3) when adsorbed on magnesium oxide, β-methylæsculetin gives a bright pale yellow fluorescence.

Summary and Conclusions

The resins obtained from the 4 samples of Brazilian jalap have been examined and compared with the resins of Vera Cruz jalap and Orizaba jalap. The appearance of the chromatograms under ultra-violet light indicated that the resin of Brazilian sample B differed slightly from those of the other three samples which appeared to be identical.

Brazilian jalap resin can be characterised by:—

(i) Slight solubility in ether.
(ii) Considerable solubility in water, the aqueous solutions giving copious persistent frothing when agitated.
(iii) A yellowish brown colour when observed in screened ultra-violet light.
(iv) The almost complete absence of blue and purple zones from the chromatograms when viewed in screened ultra-violet light.
(v) Specific rotation (of the purified resin) about $-20°$.
(vi) Melting-point (of the purified resin) of 94 to 100° C.

Brazilian jalap resin may be distinguished from Vera Cruz jalap resin by the following tests:—

(i) Appearance in screened ultra-violet light.
(ii) Appearance of chromatograms in screened ultra-violet light.
(iii) Solubility in water.
(iv) Specific rotation (of purified resins).
(v) Melting-point (of purified resin).

It may be distinguished from Orizaba jalap resin by the above tests and, in addition, by—

(vi) Solubility in ether.

References

1. Shellard, *J. Pharm. Pharmacol.*, 1951, **3**, 233.
2. Shellard, *ibid.*, 1951, **3**, 285.
3. Holmes, *Pharm. J.*, 1915, 571.
4. Scoville, *J. Amer. pharm. Ass.*, 1918, **7**, 785.
5. Ewing and Clevenger, *ibid.*, 1918, **7**, 855.
6. Power and Rogerson, *J. Amer. chem. Soc.*, 1910, **32**, 80.
7. Power and Rogerson, *J. chem. Soc.* (*Trans.*), 1912, **101**, 1.
8. Guigues, *Bull. Soc. Chim.*, 1908 (4). **3**, 872.
9. Cowie, *Pharm. J.*, 1909, 89.

A COLOUR REACTION FOR CERTAIN MERCAPTOIMIDAZOLES USING IODOBISMUTHOUS ACID

By RONALD A. MCALLISTER

From the Biochemical Laboratory, Royal Samaritan Hospital, Glasgow

Received November 26, 1951

THE present writer[1,2] has already described the colorimetric determination of 1-methyl-2-mercaptoimidazole using 2 : 6-dichloroquinone chloroimide. The reaction has also been applied to the determination of propylthiouracil in urine (McAllister[3]), and to the determination of these compounds in tablet form (McAllister and Howells[4]). Further work aimed at investigating the binding of mercaptoimidazoles on plasma proteins prompted the need for a confirmatory colour reaction for 1-methyl-2-mercaptoimidazole, other than the chloroimide reaction mentioned above. For this purpose the reactions of this compound with metals were examined. As would be expected, mercuric salts precipitated the mercaptoimidazole from aqueous solution. In common with certain other organic thiocompounds, e.g., thiourea and dimercaptothiodiazole, the 1-methyl-2-mercaptoimidazole was found to react with bismuth salts with the formation of yellow complexes of the metal. Of the bismuth salts examined, bismuth sulphate (acid) was the only one found to give a stable colour, but this colour reaction, as well as being non-specific, was also found to be insufficiently sensitive to be used on an analytical basis. It was found, however, that when an excess of iodide ions was present in the reaction mixture, an intense red colour was produced and red needles crystallised out. This colour reaction would appear to be due to the formation of a complex iodide. Since slightly acid solutions of bismuth salts react with iodides, with the formation of yellow iodobismuthous acid, the latter would appear to be the active colour reagent. The reaction forms the basis of the colour test for certain mercaptoimidazoles reported here.

One interesting outcome of this work has been that no other metallic or acid radical in place of iodide has been found to simulate the colour reaction. This has resulted in the use of a bismuth mercaptoimidazole compound as a specific colour reagent for iodides, and this has been reported in detail elsewhere (McAllister[5]).

Reagents. (1) Freshly prepared 10 per cent. solution of potassium iodide, Analar. (2) Bismuth sulphate (acid).

To prepare the reagent, which must be made up fresh, add 50 mg. of bismuth sulphate to 5 ml. of the 10 per cent. aqueous solution of potassium iodide. Mix well, then add 1 ml. of N sulphuric acid. Set aside for a few minutes to allow the undissolved bismuth salt to settle. Use the clear supernatant liquid for the test.

Test. Place a small crystal of the drug, or if in tablet form use one tablet, in the depression of a spot test plate. Add 1 drop of the reagent; the crystal immediately turns a scarlet colour, which upon standing or

on agitation, forms a red microcrystalline precipitate, if the 1-methyl-2-mercaptoimidazole or other derivative shown in Table I is present. If the test is applied to the compound in tablet form, the addition of a further drop of the reagent results in the coloured complex diffusing out to form a red ring round the tablet.

The addition of an excess of iodide ions to an acid solution of a bismuth salt results in the formation of yellow iodobismuthous acid. When this solution is added to a mercaptoimidazole of the type shown, an insoluble red complex iodide is produced.

The coloured complex is practically insoluble in water, and dilute acids. Reducing agents, e.g., hydrazine sulphate, have no effect, but oxidising agents rapidly destroy it with the liberation of iodine. As would be expected, alkalis destroy it giving a colourless solution, probably due to the labile nature of the sulphydryl group under such conditions. When in suspension in aqueous solutions, the red complex exhibits a slight red fluorescence, which is quenched when the mixture is extracted with an organic solvent such as ethyl acetate, the resulting solution having an orange-yellow colour. The data presented in Table I show the results

TABLE I

THE IODOBISMUTHOUS ACID REACTION FOR MERCAPTOIMIDAZOLES

Compound	Colour reaction
1-Methyl-2-mercaptoimidazole ..	Scarlet: forms red needles
4-Methyl-2-mercaptoimidazole ..	Orange: not very sensitive
2-Mercaptoimidazole	Red: sensitivity about the same as for the 4-methyl derivative
4-Amino-methyl-2-mercaptoimidazole	No reaction

Sensitivity of the test for the 1-methyl derivative is in the region of 100μg.

obtained when the reaction was applied to various synthetic mercaptoimidazoles. The following compounds gave no colour reactions:— 2-thiouracil, 4-methyl-2-thiouracil, propylthiouracil, thiourea, l-cystine, *p*-ethylsulphonylbenzaldehyde thiosemicarbazone, *p*-acetylaminobenzaldehyde thiosemicarbazone, lactose, lactates, and sulphonamides. It will be seen that the colour test shows a high degree of specificity. In connection with the reaction with various mercaptoimidazoles, it is of interest to note that the colour response was best with the 1-methyl-2-mercaptoimidazole showing a varying response to the others, and none with the 4-amino-methyl derivative. Although insufficient data are presented, further work in this connection might give sufficient information for the test to be used for orientation purposes.

SUMMARY

A colour reaction for certain mercaptoimidazoles is described. This is based upon the red coloured complex iodide which is produced, when a slightly acid solution of iodobismuthous acid is added to their solutions.

References

1. McAllister, *Nature, Lond.*, 1951, **167**, 863.
2. McAllister, *J. Pharm. Pharmacol.*, 1951, **3**, 506.
3. McAllister, *J. Clin. Path.*, 1951, **4**, 432.
4. McAllister and Howells, *J. Pharm. Pharmacol.*, 1952, **4**, 259.
5. McAllister, *Nature, Lond.*, in the press.

THE BACTERIOSTATIC ACTION OF BASIC DI- AND TRIPHENYLMETHANE DERIVATIVES

PART II. THE RELATIONS BETWEEN CHEMICAL STRUCTURE AND BACTERIOSTATIC EFFECTS

BY E. FISCHER, M. GENSELOVICH and P. RONA

From The Research Department of 'Szabó Hnos., Kessler & Cía., Buenos Aires, Argentina

Received November 26, 1951

THE results of bacteriostatic assays conducted with certain basic diphenylmethane derivatives, have been published in a previous paper.[1] It has been found that among different methane carbon atom derivatives of bis(*p*-dimethylaminophenyl)methane, those with a quinoid structure had a bacteriostatic activity, whereas others, without such a structure were inactive. These results were in accord with those obtained in earlier work on methane carbon atom derivatives of basic triphenylmethane dyes.[2]

The bacteriostatic potency of the active diphenylmethane derivatives (auramine and Michler's hydrol) was substantially inferior to that of triphenylmethane dyes, but the stability of their methane carbon atom substituted leuco-derivatives greatly facilitated the study of the relations between chemical structure and bacteriostatic activity.

In view of the data published by Browning and Gilmour,[3] and by Kligler,[4] related to the antibacterial activities of different triphenylmethane dyes, it was to be expected that non-methylated diaminodiphenylmethane compounds would have a weaker bacteriostatic effect than the corresponding bis-dimethyl derivatives (bis(dimethylaminophenyl)methane compounds, like Michler's hydrol and auramine).

We compared, therefore, the bacteriostatic activity of three non-methylated diaminodiphenylmethane derivatives: bis(*p*-aminophenyl)-methane, *p*-diaminobenzohydrol and *p*-diaminobenzophenone, with that of auramine and Michler's hydrol. Bis(*p*-aminophenyl)methane, the reduced non-quinoid member of the series, is the proper homologue of the triphenylmethane leucobases; *p*-diaminobenzohydrol is a potentially quinoid compound, which shows a fuchsin red colour in moderately acid solutions, corresponding to the triphenylmethane carbinol bases and to Michler's hydrol, all of them being oxidised members of their series. *p*-Diaminobenzophenone, like its homologue (Michler's ketone), is a more highly oxidised form than the carbinols, without the possibility of a tautomeric quinoid structure.

The above mentioned diphenylmethane derivatives have been prepared in our laboratory according to the methods described by Rivier and Farine[5] (bis(*p*-aminophenyl)methane and *p*-diaminobenzophenone) and by Watson and Meek[6] (*p*-diaminobenzohydrol). Melting-points: *p*-diaminodiphenylmethane, 90° C. (Rep. 87 to 94°); *p*-diaminobenzohydrol, 98° (Rep. 98°); *p*-diaminobenzophenone, 241° (Rep. 244° to 245°).

2 g. quantities of the substances to be tested were dissolved in 100 ml. of

a mixture of 4 parts of ethanol and 1 part of 0·1 hydrochloric acid. These stock solutions were then diluted with different amounts of buffered nutrient broth (*p*H 7·0). The technique of the bacteriological test was the same as in our previous experiments. The results of the assays with *Staphylococcus aureus* are shown in Table I, and with *Streptococcus fæcalis* in Table II. The results were read by comparison of the inoculated tubes with a non-inoculated control tube containing the same dilution of the test substance. The assay was repeated several times, with the same results.

TABLE I

GROWTH OF *Staphylococcus aureus* IN 24 HOURS, IN BROTH

In the presence of:	1:5000	1:10,000	1:20,000	1:40,000	1:80,000
Bis(*p*-aminophenyl)methane	+	+	+	+	+
p-Diaminobenzohydrol	±	+	+	+	+
p-Diaminobenzophenone	+	+	+	+	+
Michler's hydrol	−	−	−	−	+
Auramine	−	−	−	−	+

+ Signifies normal growth ± Signifies weak growth − Signifies no growth

TABLE II

GROWTH OF *Streptococcus fæcalis* IN NUTRIENT BROTH, IN 24 HOURS

In the presence of:	1:5000	1:10,000	1:20,000	1:40,000	1:80,000
Bis(*p*-aminophenyl)methane	+	+	+	+	+
p-Diaminobenzohydrol	±	±	+	+	+
p-Diaminobenzophenone	−	−	−	−	+
Michler's hydrol	−	−	−	−	+
Auramine	−	−	−	−	+

Summarising the results of these experiments, we may say that bis(*p*-aminophenyl)methane and *p*-diaminobenzophenone showed no bacteriostatic activity against *Staphylococcus aureus* and *Streptococcus fæcalis* in the concentrations tested. *p*-Diaminobenzohydrol caused a very slight inhibition of growth. The dimethylated substances, Michler's hydrol and auramine, showed the same degree of efficacy as in our previous experiments.[1]

The results are in accordance with our expectations, among the non-methylated bis-(*p*-aminophenyl)methane derivatives only the quinoid *p*-diaminobenzohydrol is active and its activity is considerably weaker than that of the corresponding dimethylated compound (Michler's hydrol).

We made certain observations on the effect of adding broth of *p*H 7·0 to the stock solutions of the test substances (Table III). As Table III shows, only auramine preserves its colour and solubility under these conditions. Evidently, the salt of Michler's hydrol hydrolyses at *p*H 7·0, and changes into the non-quinoid hydrol form. In other experiments we have observed, systematically, the colour and solubility of auramine and Michler's hydrol at different *p*H values. Two drops of a 1 per cent. solution of these substances, in a mixture of 70 parts of ethanol and 30 parts of 0·1N hydrochloric acid, were added to 5 ml. of buffer mixtures. The *p*H was determined potentiometrically. The colour and transparency were read after 2 hours at room temperature (Table IV).

TABLE III

COLOUR AND TRANSPARENCE OF THE NON-INOCULATED DILUTIONS OF THE TESTED SUBSTANCES IN BROTH

		Dilution with broth of *p*H 7		
	Ethanolic acid stock solution (1 : 50)	1:100 (Final concentration 1 : 5000)	1:200 (Final concentration (1 : 10,000)	1:400 (Final concentration 1 : 20,000)
Bis(*p*-aminophenyl)methane	Slightly yellow, clear	Very slightly turbid	Clear	Clear
p-Diaminobenzohydrol ..	Red, clear	Turbid, greenish colour	Slight milky opalescence; greenish	Clear
p-Diaminobenzophenone ..	Slightly yellow, clear	Slightly turbid	Clear	Clear
Michler's hydrol	Blue, clear	White, turbid	Slight milky opalescence; white	Clear
Auramine	Yellow, clear	Yellow, clear	Yellow, clear	Yellow, clear

TABLE IV

COLOUR AND TRANSPARENCE AT DIFFERENT *p*H VALUES

*p*H	Michler's hydrol	*p*H	Auramine
4·8	Blue, clear	9·4	Yellow, clear
5·2	Blue, almost clear	9·8	Light yellow, fine cryst. precipitate
5·6	Light blue, milky	10·2	Almost colourless, fine cryst. precipitate
6·0	Light blue, milky	10·6	Almost colourless, fine cryst. precipitate
6·4	White, milky	11·0	Colourless, precipitated

The differences between the behaviour of Michler's hydrol and that of auramine at different *p*H is clearly shown in Table IV. Auramine is soluble and coloured up to *p*H 9·4 and begins to lose its colour and solubility at *p*H 9·8, precipitating in the form of crystals. Michler's hydrol is soluble with a blue colour, only below *p*H 5·2; above this *p*H it gradually loses its colour and takes on a milky appearance; at *p*H 6·4 the bluish tint is entirely absent.

The different behaviour of Michler's hydrol and of auramine in these experiments, is possibly the consequence of their differing basic strength or of a lactam formation between the hydroxyl group and one of the amino groups of Michler's hydrol. Their quantitatively equal bacteriostatic effect at *p*H 7 shows that, in this case, similarities in the molecular configuration have a more marked influence on antimicrobial potency, than solubility and actually quinoid or benzenoid state. It seems, therefore, that the hypothesis forwarded by Stearn and Stearn,[7] postulating a close correlation between basic strength and bacteriostatic effects of dyes, is not fully applicable to diphenylmethane compounds. This hypothesis, originally stated in connection with observations on triphenylmethane dyes, has been successfully applied to acridine derivatives by Albert, Rubbo, Goldacre, *et al.*[8] Its validity for triphenylmethane dyes has been, however, questioned by Ingraham[9] and ourselves.[10]

Fosse[11] showed that Michler's hydrol forms a methane carbon atom semicarbazide derivative, which has a bluish colour in acetic acid. We

prepared this substance according to the description of this author, to test it in bacteriostatic experiments. We also prepared, for the same purpose, the violet coloured salt of tetrakis (dimethylaminophenyl)ethene, a compound of which the carbinol base corresponds to two molecules of Michler's hydrol joined together at the methane carbon atom (Willstätter and Goldmann[12]) and finally, the orange coloured salt of *p*-dimethylamino-triphenylmethanol (Baeyer and Villiger[13]), which is a monoamino triphenylmethane derivative.

It is a well known fact that the bacteriostatic effect of triphenylmethane dyes is inhibited in the presence of proteins. We assayed, therefore, the three above mentioned compounds, and auramine and Michler's hydrol also in the presence of 20 per cent. horse serum. The results of the bacteriostatic assay are shown in Table V, which shows that the semicarbazide

TABLE V

GROWTH OF *Staphylococcus aureus* IN BROTH

In the presence of:	Dilutions of the tested substances			
	1:40,000	1:80,000	1:160,000	1:320,000
Auramine	−	+	+	+
Auramine + 20 per cent. of serum	±	+	+	+
Michler's hydrol	−	+	+	+
Michler's hydrol + 20 per cent. of serum	±	+	+	+
Semicarbazide of Michler's hydrol	−	−	+	+
Semicarbazide of Michler's hydrol + 20 per cent. of serum	±	+	+	+
Tetrakis(dimethylaminophenyl)ethene-hydrochloride	+	+	+	+
Tetrakis(dimethylaminophenyl)ethene-hydrochloride + 20 per cent. of serum	+	+	+	+
p-Dimethylaminotriphenylmethane-hydrochloride	−	−	−	±
p-Dimethylaminotriphenylmethane-hydrochloride + 20 per cent. of serum	−	−	+	+

of Michler's hydrol is somewhat more active than Michler's hydrol and auramine. Tetrakis(dimethylaminophenyl)ethene-hydrochloride is without activity in the same concentration, whereas the monoamino triphenylmethane derivative shows the highest activity among the tested substances. Horse serum inhibits the effects of every one of the active substances. The inactivity of tetrakis(dimethylaminophenyl)ethene derivative agrees with the observations of Albert, Rubbo, Goldacre, Davey and Stone,[8] according to which, *NN'-bis*(5-acridyl)ethylenediamine, obtained by joining together two molecules of 5-methylacridine, is much less active in bacteriostatic experiments than the parent substance (dystherapeutic effect of doubling the molecular size).

Comparing the bacteriostatic activity of dimethylaminotriphenylmethanol-hydrochloride with that of Michler's hydrol and of the corresponding diaminotriphenyl- and triaminotriphenylmethane derivatives, we concluded that: (1) Whereas triaminotriphenylmethane derivatives have practically the same degree of activity as diaminotriphenylmethane derivatives (Browning and Gilmour[3]; Kliger[4]), the elimination of a second amino group from the molecule diminishes very markedly the bacteriostatic effect. (2) Eliminating one phenyl group from the triphenylmethane molecule diminishes the bacteriostatic effect in a higher

degree than the elimination of two amino groups of the same, the monoaminotriphenylmethane derivative being more effective than the diaminodiphenylmethane derivative. The maximal efficacy is reached in the aminophenylmethane series in compounds with three phenyl groups and at least two alkylated amino groups.

Summary

1. *p*-Diaminobenzohydrol, a non-methylated homologue of Michler's hydrol, is less active bacteriostatically than the latter, in accordance with similar observations made on triphenylmethane dyes by Browning and Gilmour and by Kligler.

2. The potentially quinoid compounds auramine and Michler's hydrol are bacteriostatically active to an equal degree, in spite of Michler's hydrol being actually non-quinoid and auramine actually quinoid at the *p*H of the bacteriological medium.

3. The introduction of the semicarbazide radical into Michler's hydrol at the methane carbon atom augments, to a certain degree, the bacteriostatic activity.

4. The doubling of the size of Michler's hydrol to tetrakis(*p*-dimethylaminophenyl)ethanediol abolishes the bacteriostatic effect.

5. The dimethylated monoamino triphenylmethane derivative is more active than the corresponding dimethylated diaminodiphenyl derivatives, but is considerably less active than the homologous diaminotriphenylmethane and triaminotriphenylmethane compounds.

6. Serum inhibits the bacteriostatic action of all substances tested in our experiments.

References

1. Fischer, Garcés and López, *J. Bact.*, 1946, **51**, 1.
2. Fischer, Hoffmann, Prado and Boné, *J. Bact.*, 1944, **48**, 439.
3. Browning and Gilmour, *J. Path. Bact.*, 1913, **18**, 144.
4. Kligler, *J. exp. Med.*, 1918, **27**, 463.
5. Rivier and Farine, *Helv. chim. Acta.*, 1929, **12**, 865.
6. Watson and Meek, *J. chem. Soc.*, 1915, **107**, 1567.
7. Stearn and Stearn, *J. exp. Med.*, 1930, **51**, 341.
9. Ingraham, *J. Bact.*, 1933, **26**, 573.
8. Albert, Rubbo, Goldacre, Davey and Stone, *Brit. J. exp. Path.*, 1945, **26**, 160.
10. Fischer and Muñoz, *J. Bact.*, 1947, **53**, 381.
11. Fosse, *Ann. Chim.*, 1909, **18**, 400.
12. Willstätter and Goldmann, *Ber. dtsch. chem. Ges.*, 1906, **39**, 3765.
13. Baeyer and Villiger, *Ber. dtsch. chem. Ges.*, 1904, **37**, 2848.

ANALYTICAL NOTES

From the Analytical Control Division of May and Baker, Limited

(I) The Official Limit Test for Chloride in Bromides

By L. W. Cumming and Miss M. B. Rhodes

Received June 1, 1951

The official method for the determination of chloride in bromides of ammonium, calcium, potassium and sodium and in hydrobromic acid is based on that evolved by Caven[1] who although concerned mostly with relatively large amounts of chloride, reported results of 0·085 and 0·081 per cent. of KCl in a determination on potassium bromide. Under routine control conditions high results of poor reproducibility have been obtained from time to time.

The official instructions are brief, no indication of the method of titrating the chloride, for example, being given. Caven filtered off the silver chloride but mentions direct titration in acid solution using the "shaking method"; in these laboratories Caldwell and Moyer's[2] modified Volhard method is used. The British Pharmacopœia specifies a rapid current of air both during the one minute boiling and whilst cooling, whereas Caven passed a gentle stream of air when heating to boiling and during actual ebullition, but increased the aspiration rate at the commencement of the cooling stage. The method is apparently misquoted in a standard reference book[3] in which it is not specified that a stream of air be passed whilst boiling, nor is a thermometer, as used by Caven, shown in the diagram.

Effect of Omitting Aspiration of Air during the one minute Boiling Stage. If air is not aspirated through the solution during ebullition, higher results are obtained. Thus, when air was passed whilst boiling and cooling 10 determinations yielded figures between 0·07 and 0·17 per cent. of Cl (mean 0·11 per cent.), whereas when air was passed only while cooling, the corresponding range was 0·11 to 0·21 per cent. of Cl (mean 0·16 per cent.). Reproducibility was poor in both series; this was not due to errors in the titration stage since direct Volhard titrations of comparable amounts of chloride alone gave a recovery of 100 to 102 per cent. of Cl. The discrepancy between the two series of results could be due either to loss of chloride when air was passed whilst boiling and cooling, or to incomplete removal of bromide when air was passed only whilst cooling. That the lower results of the first series were not due to loss of chloride was shown by applying the official procedure to chloride alone, when recoveries of 98 to 102 per cent. of Cl were obtained; hence it was inferred that the higher results of the second series were due to incomplete removal of bromide.

Effect of Rate of Aspiration of Air and of Temperature. With high aspiration rates throughout (greater than 1 cu. ft./hr.) it was impossible to maintain boiling, even with a full Bunsen flame, during the one minute specified, and high results were obtained (Table I). With low aspiration

TABLE I

EFFECT OF ASPIRATION RATE AND OF TEMPERATURE ON RECOVERY OF CHLORIDE* ADDED TO POTASSIUM BROMIDE

Aspiration rate cu. ft./hr.		Temperature during 1 minute period ° C.	Recovery per cent.
1 minute boiling period	Cooling period		
1·2 to 3	1·2 to 3	104	101 101 102 102
1·2 to 3	1·2 to 3	103	135 129 125 127
1	2	105 to 106 (boiling)	99 99 99 101 100
Less than 1	Less than 1	106	120 220

* 3·55 mg. of Cl added to 1 g. of potassium bromide containing 1·07 mg. of Cl.

rates throughout high results were again obtained but with aspiration rates of about 1 cu. ft./hr. during the boiling period and changing to a brisk current (about 2 cu. ft./hr.) at the commencement of the cooling period, correct results were obtained. To obtain reproducible and correct results it is therefore necessary to include a thermometer in the apparatus and to regulate the rate of air stream during the 1-minute boiling period in order to maintain a temperature of 105° to 106° C.

PROPOSED METHOD

To 1 g. of bromide or 5 ml. of dilute hydrobromic acid, in a 500-ml. distillation flask, fitted with a rubber bung carrying a thermometer and tapered air inlet tube which will extend to the bottom of the flask, add 75 ml. of water (70 ml. of water in the case of dilute hydrobromic acid) and 25 ml. of nitric acid. Heat the solution and when it boils commence passing a gentle stream of air and continue boiling (105° to 106° C.) for 1 minute. Remove the source of heat and pass a brisk stream of air for 20 minutes. Add 5 ml. of 0·1N silver nitrate and 5 drops of nitrobenzene, shake and then titrate the excess of silver nitrate with 0·1N ammonium thiocyanate using ferric ammonium sulphate solution as indicator. 1 ml. of 0·1N silver nitrate is equivalent to 0·003546 g. Cl.

SUMMARY

1. The effect of variation of conditions in the official limit test for chlorides in bromides has been determined.

2. Temperature and aspiration rate are particularly important. A temperature of 105° to 106° C. must be maintained during the 1-minute boiling period and an aspiration rate of not less than about 2 cu. ft./hr. is necessary during the cooling period.

REFERENCES

1. Caven, *J. Soc. chem. Ind.*, 1909, **28**, 505.
2. Caldwell and Moyer, *Industr. Engng. Chem., Anal. Ed.*, 1935, **7**, 38.
3. Bennett and Cocking, *Science and Practice of Pharmacy*, Vol. 2, Churchill, London, 1933, 97.

(II) THE THIOCYANATE TITRATION OF MERCURY AND THE STANDARDISATION OF AMMONIUM THIOCYANATE SOLUTION

BY L. W. CUMMING and MISS S. SPICE

Received June 1, 1951

IN the British Pharmacopœia and British Pharmaceutical Codex mercury in certain inorganic and organic compounds is determined ultimately by thiocyanate titration for which, however, conditions are given in outline only. No definite temperature is specified, acidity ranges from 0·4N to 5N in different monographs, and final volumes and titres also vary.

Although standardisation of thiocyanate against mercury was recommended[1] when the cause of low assays on mercuric oxide was discussed at the British Pharmaceutical Conference in 1933 the importance of the exact conditions of the titration was not mentioned.

The effect of varying conditions in the thiocyanate titration of mercury has been studied by several workers. As regards temperature, Karaoglanov[2] pointed out that at higher temperatures the end-point appears too soon owing to the ionisation of mercury thiocyanate. Kolthoff,[3] confirming Rupp's[4] observation that at lower temperatures acid concentration was less important, recommended a temperature of 15° C. Results obtained in these laboratories show that the end-point is less definite and that lower results of poor reproducibility are obtained at temperatures higher than 15° C. In the official assays for mercury in mercury ointment and in mercurochrome there is no instruction to cool the warm solutions before titrating, hence a temperature greater than 15° C. might easily arise.

Only at low temperatures are the results independent of acid concentration, as shown by Rupp and Kolthoff; the latter recommends a concentration of 0·6N, the advantage of a relatively low acid concentration being a lower sensitivity to temperature differences. The acid concentration in official assays, which varies between 0·4N and 5N, may with advantage be brought within a narrower range, that for mercuric oxide being increased to about 0·7N by using the same acid/water ratio as used for mercury. On the other hand, the acid concentrations for dilute mercuric nitrate ointment, mersalyl and mercurochrome should be reduced somewhat; in our experience 10 ml. of nitric acid is sufficient to dissolve the amalgam in these cases, the acidity at the end-point being 2N to 2·5N.

The amount of indicator does not appear to be so critical but Karaoglanov obtained low results with less than 1 ml. of 10 per cent. ferric nitrate whilst Kolthoff recommends the use of 2·5 ml. of 10 per cent. ferric alum solution

Standardisation of Thiocyanate Solution. The British Pharmacopœia does not specify standardisation methods, but the United States Pharmacopœia directs that ammonium thiocyanate solution shall be standardised against silver nitrate solution, which may be made from reagent pure silver nitrate or standardised in turn gravimetrically *via* silver chloride. Pure mercury, and not silver, was recommended by Jones[1] for the standardisation of ammonium thiocyanate solutions intended for use in mercury assays; no figures were given in support. It is clear from the previous discussion that this procedure would be of value only if identical conditions were adhered to in both standardisation and subsequent determinations. Kolthoff and van Berk[5] obtained identical factors using mercury and silver nitrate and concluded that mercury was an excellent primary standard for the standardisation of thiocyanate provided correct conditions were used. Table I, giving results obtained by the present authors, shows that thiocyanate may be standardised against mercury, silver or silver nitrate solution provided that any end-point correction[3] in the standardisation of the latter is taken into consideration.

TABLE I

STANDARDISATION OF 0·1N THIOCYANATE

Method	Factor
Against silver nitrate standardised with sodium chloride by Mohr's method:—	
(*a*) With no end-point correction	1·001
(*b*) With end-point correction	1·003
Against silver nitrate standardised gravimetrically as AgCl	1·004
Against silver wire by Volhard method	1·004
Against mercury (redistilled in vacuo)	1·004

Hence if silver nitrate solution standardised against sodium chloride by Mohr's method,[6] and used for the assay of sodium and potassium chlorides, is used for the standardisation of thiocyanate solutions, the end-point correction in the standardisation of the silver nitrate must not be overlooked.

REFERENCES

1. Jones, *Quart. J. Pharm. Pharmacol.*, 1933, **6**, 554.
2. Karaoglanov, *Z. Anal. Chem.*, 1943, **125**, 406.
3. Kolthoff and Stenger, *Volumetric Analysis*, Vol. II, Interscience, New York, 1947, 255, 336.
4. Rupp, *Arch. Pharm.*, 1903, **241**, 444; *Chem.-Ztg.*, 1908, **32**, 1077.
5. Kolthoff and van Berk, *Z. Anal. Chem.*, 1927, **71**, 339.
6. *Methods of Analysis, A.O.A.C.*, Sixth Edition, Washington, 1945, 809.

(III) THE DETERMINATION OF IODINE IN ORGANIC COMPOUNDS BY ALKALINE REDUCTION

BY C. W. BALLARD and MISS S. SPICE

Received January 8, 1952

METHODS for the determination of iodine in organic compounds fall into three classes based on ignition,[1,2,3] oxidation[4,5,6,7] or reduction.[6,8,9] The ignition method is widely used in the British Pharmacopœia but has been

criticised[3,5,10] and the United States Pharmacopœia has adopted an alkaline oxidation method. Reduction with zinc and acetic acid is used in the British Pharmacopœia for iodoxyl and iodised oil; zinc and nitric acid have been used,[11] whilst a method using zinc and sulphuric acid was devised by Powell and Taylor[13] and applied to injection of diodone, injection of iodoxyl, pheniodol and chiniofon. This method lacks precision (4 results for chiniofon in their Table II have a total spread of 1·7 per cent.), the titre is small (7 ml. for chiniofon), and it is not specific for iodine. Reduction with zinc and alkali, used in an identity test for pheniodol,[14] was suggested *inter alia* by members of a Pharmacopœia Sub-Committee as the basis of an assay; optimum conditions have been determined and the following method developed.

Method

Weigh accurately an amount of material containing about 0·5 g. of iodine into a 250-ml. conical flask and dissolve in a mixture of 12 ml. of solution of sodium hydroxide and 20 ml. of water, warming if necessary. Add 1 g. of zinc powder, attach a condenser and reflux gently for 30 minutes. Cool, add through the condenser 20 ml. of water, filter through cotton-wool, wash the flask with 2 quantities of water, each of 15 ml., and pass the washings through the filter. To the filtrate add 25 ml. of hydrochloric acid, cool to room temperature, add 10 ml. of solution of potassium cyanide and titrate with 0·05M potassium iodate until the dark brown solution which is formed becomes light brown; add 5 ml. of mucilage of starch and continue the titration until the solution is colourless. Each ml. of 0·05M potassium iodate is equivalent to 0·01269 g. of iodine.

From Table I, which gives results obtained by this and other methods on a range of substances, it is seen that the method is precise and accurate.

TABLE I

Iodine content by different methods of various iodocompounds

Iodocompound	Proposed alkaline zinc reduction	Ignition with sodium carbonate (B.P.)	Reduction with zinc and acetic acid (B.P.)	Reduction with zinc and sulphuric acid (Powell)	Alkaline oxidation with permanganate (U.S.P.)
Pheniodol* (Theory 51·37 per cent. of iodine)	51·35 51·3 51·37	51·05[a] 51·1[a] 51·0[b] 51·1[b]		49·7 51·1 51·3 50·8	
Mean	51·34	51·06		50·7	
Iodophthalein	55·42 55·46	55·14 55·03			
Iodoxyl	51·10 51·08	51·10 51·10	51·07 51·05		
Chiniofon	30·52 30·49 30·49 30·54	29·61 29·65		29·47[c] 29·68[c] 29·41[c]	29·83 29·2 29·53

* Specially synthesised and purified by recrystallisation.
a Using Meker burner.
b Using electric furnace at 700° C.
c Yellow colour still present after 3 hours.

Ignition with sodium carbonate gave slightly lower results except with chiniofon where the discrepancy was appreciable; Powell and Taylor also obtained low results in this case.

Notes on the Method

1. About 0·4 g. of zinc is consumed; for quantitative reduction not less than 10 ml. of sodium hydroxide solution and a reduction time of 20 minutes are required.

2. The reduction products of pheniodol, iodophthalein, iodoxyl and chiniofon, identified respectively as α-phenyl-β-(4-hydroxyphenyl)-propionic acid, phenolphthalein, N-methyl-4-pyridone-2:6-dicarboxylic acid and 8-hydroxyquinoline-5-sulphonic acid were found not to interfere with the iodate titration.

3. In the B.P. the end-point acid concentration for Lang's iodate titration[12] varies from the equivalent of 0·64N in the case of iodophthalein to 1·42N in the case of iodoxyl. For accurate results not lower than 1N was found necessary.

Summary

A method for the determination of iodine in iodophthalein, chiniofon, iodoxyl and pheniodol is described. The method is simple and rapid and is more accurate than the ignition method of the British Pharmacopœia.

References

1. Schiff, *Liebig's Ann.*, 1879, **195**, 293.
2. Piria, *Z. anal. Chem.*, 1906, **45**, 572.
3. Block and Waters, *J. Amer. pharm. Ass., Sci. Ed.*, 1945, **34**, 192.
4. Baubigny and Chavanne, *C.R. Acad. Sci., Paris*, 1903, 1198.
5. Butler and Burdett, *Anal. Chem.*, 1939, **11**, 237.
6. Clark and Jones, *J. Ass. off. agric. Chem., Wash.*, 1942, **25**, 755.
7. Bergenfors and Schell, *Svensk. farm. Tidskr.*, 1949, **53**, 219.
8. Schwenck, Papa and Ginsberg, *Anal. Chem.*, 1943, **15**, 576.
9. Pesez and Poirier, *Bull. Soc. chim.*, 1948, 379.
10. Garratt, *Drugs and Galenicals*, Chapman & Hall, London, 1957, 161.
11. Monthulé, *Ann. Chim. analyt.*, 1912, 133.
12. Lang, *Z. anorg. Chem.*, 1922, **122**, 332.
13. Powell and Taylor, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 373.
14. Foster and Williams, *J. Pharm. Pharmacol.*, 1949, **1**, 60.

(IV) The Stability and Preservation of Litmus Solutions

By L. W. Cumming

Received January 8, 1952

Litmus solution prepared as specified in the British Pharmacopœia is distinctly alkaline (*p*H 8) and rapidly undergoes fermentation, becoming acid and less sensitive. Prideaux[1] suggests phenol as a preservative but gives no details as to its use.

A quantity of litmus solution prepared according to the British Pharmacopœia was divided into 3 equal portions which were stored (*a*) in an amber glass-stoppered bottle, (*b*) in a clear glass-stoppered bottle, (*c*) in a clear glass-stoppered bottle after the addition of 0·5 g. of phenol per 100 ml.

TABLE I

Mode of storage	Initial	4 weeks	8 weeks	12 weeks	12 months
	*p*H	*p*H	*p*H	*p*H	*p*H
(*a*)	8·18	7·02	6·83	6·69	—
(*b*)	8·17	6·78	6·68	6·56	—
(*c*)	8·04	8·03	8·01	8·02	7·98

of solution. Table I gives the *p*H of the stored solutions after different periods. The solution with added phenol had retained its original *p*H and colour whereas the unstabilised solutions became more acidic and reddish in colour; traces of sediment were observed in all 3 solutions. For some years a more sensitive litmus solution has been prepared in these laboratories from the official solution by diluting, and subsequently adjusting the *p*H. Litmus solution is prepared as directed in the British Pharmacopœia and 100 ml. of this solution then diluted with 150 ml. of water, just sufficient dilute acetic acid being added to give a very slight red colour. The solution is boiled for a few seconds then cooled and any slight acidity removed by adding dilute solution of ammonia until a purple colour is obtained, followed by boiling. The solution may then be stabilised by the addition of 0·5 g. of phenol per 100 ml. This procedure normally results in a solution having a *p*H of 7 to 7·5 but a more accurate adjustment may be made if the *p*H be determined during adjustment. Two drops of this stabilised solution in 10 ml. of distilled water gave a reddish-purple colour, changing on the addition of 0·03 ml. of either 0·01N acid or 0·01N alkali. A litmus solution prepared and stabilised as above (initial *p*H 7·29) was found to be stable during 3 months general laboratory use (final *p*H 7·26).

REFERENCE

1. Prideaux, *The Theory and Use of Indicators*, Constable, London, 1917, 354–5.

(V) The Determination of the Acid Value of Benzoin

By L. W. Cumming

Received January 8, 1952

In the official method, a hot ethanolic extract of the material is titrated with ethanolic potassium hydroxide solution, using phenolphthalein as internal indicator. Owing to the dark colouration produced during the titration the end-point is very indistinct and probably appreciably overrun. At the request of the British Pharmacopœia Commission the use of other internal and of external indicators was therefore investigated.

Internal Indicators. Using the official method, determinations by several operators on a sample of benzoin used throughout the work gave results from 117 to 131 (mean 124); no improvement resulted when two similar ethanolic extracts, one only of which contained phenolphthalein, were titrated side by side and compared at equal titres throughout the titration. The experiment was repeated using a photoelectric colorimeter to measure the colours, but in the region of the

expected equivalence-point a slight *decrease* in optical density was noted. α-Naphtholphthalein, suggested by King,[1] was found to be more suitable than phenolphthalein but the detection of the colour change required considerable practice. The results were somewhat lower than those obtained using the official method.

External Indicators. Phenolphthalein papers showed only a transitory pink colour at the end-point, whilst the use of phenolphthalein solution on a "spotting tile" was unsatisfactory since a brown precipitate formed which tended to mask the faint pink end-point colour. However, the addition of the hot extract to about 3 ml. of an aqueous phenolphthalein dilution resulted in a white suspension which aided the detection of the end-point and, moreover, this method gave lower results than the official method. To check accuracy the potentiometric method of Parks and Lykken[2] was applied and the potentiometric end-point was in accordance with that obtained using aqueous phenolphthalein solution as external indicator. Although in a cold *iso*propanol-benzene mixture as used by Parks and Lykken, hydrolysis of balsamic esters was slight, in hot ethanol it was appreciable and since the official method requires the titrated extract to be refluxed for a few minutes to break up any precipitate, it is clearly desirable to prescribe a definite period. Determinations by several operators using the proposed method gave results from 111 to 114 (mean 112·5) as compared with 117 to 131 (mean 124) by the official method.

Proposed Modified Method.

(*a*) *Aqueous Phenolphthalein Solution.* Dilute 1 volume of solution of phenolphthalein B.P. with 3 volumes of distilled water.

(*b*) *Apparatus.* A series of 3 in. × ½ in. ignition tubes each containing 3 ml. of aqueous phenolphthalein solution.

(*c*) *Procedure.* Extract 5 g. of powdered benzoin with 50 ml. of boiling 90 per cent. ethanol by continuous extraction for about 2 hours. Titrate the hot solution with N ethanolic potassium hydroxide until the addition of 0·1 ml. of the solution to the external indicator results, on mixing, in a permanent pink colour. Reheat the solution for a further minute and complete the titration (up to 0·1 ml. of titrant is required).

SUMMARY

1. The official method for the determination of the acid value of benzoin has been modified and more precise and accurate results obtained using phenolphthalein as an external indicator.

2. Hydrolysis occurs during the titration and a definite period of re-heating after the first end-point is suggested.

The authors wish to thank the Directors of Messrs. May and Baker Ltd. for permission to publish these notes.

REFERENCES

1. King, Private communication to B.P. Commission.
2. Parks and Lykken, *Anal. Chem.*, 1950, **22**, 1444.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Ambergris, Identification of. P. J. Hardwick and E. Q. Laws. (*Analyst*, 1951, **76**, 662.) The note records a simple chemical means for the identification of genuine ambergris which consists of ambreine, *epi*coprostanol, arachidic acid, ambroporphyrines, a ketonic fraction and a liquid paraffin; the method used entails the chromatographic separation of ambreine and *epi*coprostanol. The dried sample is extracted with ether, the fatty acids removed by shaking with aqueous alkali, the extract being evaporated to dryness and the residue dissolved in light petroleum. For the adsorption column alumina (Brockmann Grade III) is used and the light petroleum solution is adsorbed on it with the aid of a further quantity of light petroleum. A narrow yellow band, fluorescing green in ultra-violet light, moves rapidly down the column, the elution being continued until this band is washed into the receiver. The column is developed with a 1 + 1 mixture of ethyl ether and light petroleum when immediate separation of broad yellow bands at the top of the column begins and a series of bands that are fluorescent in ultra-violet light appears, a green and a yellow one travelling rapidly down the column. The green and yellow fluorescent fractions are collected separately, the yellow fraction containing the ambreine; a red fraction remaining in the column contains *epi*coprostanol which is eluted with benzene. The ambreine could be prepared in crystalline form by slow evaporation of the solvent; the crystals were colourless needles, m.pt. 83° C., showing a positive optical rotation and giving a rose colour with the Liebermann reagent. The ash of good quality ambergris ranged from 0·5 to 5·0 per cent., although a very inferior material from the outside of a large mass contained 57 per cent. of ash, consisting essentially of the phosphates of calcium and magnesium with a small proportion of sodium chloride and traces of copper, silicon, manganese and chromium. R. E. S.

Colchicine, Colorimetric Estimation of. J. S. King. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 424.) Pure colchicine alkaloid (2·5 mg.) is dissolved in 25 ml. of N hydrochloric acid in a 125-ml. Erlenmeyer flask. A funnel is inserted into the neck of the flask, which is then warmed on the steam-bath for 1 hour, removed, cooled to room temperature and the volume made up to 25 ml. with N hydrochloric acid. Graduated amounts containing up to 0·5 mg. of this solution are made to 5 ml. with N hydrochloric acid and 0·1 ml. of a 5 per cent. solution of ferric chloride is mixed with each. These solutions are then read in a colorimeter for standard transmittance at 470 mμ. This establishes the standard curve, which follows Beer's law up to 0·5 mg. Samples of unknown colchicine content are run in the same way, after separation of interfering materials. The jade green colour is formed immediately, and its intensity makes it suitable for identification of as little as 4 μg. of colchicine on a spot-plate. The procedure has been successfully applied to pharmaceutical mixtures, and a detailed example of such an application is given. S. L. W.

Dithizone as an Indicator in the Volumetric Determination of Zinc. J. P. Mehlig and A. P. Guill. (*Anal. Chem.*, 1951, **23**, 1876.) This work was

performed to establish the conditions for the use of dithizone as an indicator in the titrimetric determination of zinc with potassium ferrocyanide. In the titration, the zinc solution, buffered to *p*H 4·0 by potassium hydrogen phthalate, was run from a burette into the standard potassium ferrocyanide solution with constant shaking until 1 drop of dithizone in chloroform turned pink. It was not possible to titrate the zinc solution with the standard ferrocyanide solution since the ferrocyanide, unless in considerable excess, did not discharge the pink colour of the indicator in a reasonable time: the dithizone zinc complex was stable within a *p*H range of 3·5 to 5·0. All cations which form insoluble ferrocyanides in the presence of hydrochloric acid interfere with the titration and may be removed by the Waring procedure (*J. Amer. chem. Soc.*, 1904, **26**, 4) consisting of precipitation of copper with aluminium strips followed by precipitation of zinc sulphide in formic acid solution, the separated zinc sulphide being dissolved in hydrochloric acid. R. E. S.

Ethanol in Ether, Determination of, by Infra-red Absorption. A. A. Colon and H. A. Frediani. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 607.) A rapid and convenient method for the determination of small amounts of ethanol in ether depends upon the absorption band in the region of 2·83 μ. As this band is due to the –OH group allowance must be made for the water present. For the preparation of calibration curves a pure sample of ether may be prepared as follows. Ethanol and water are removed from absolute ether by treatment with anhydrous calcium chloride followed by sodium ribbon added until evolution of gas ceases, filtration and distillation. Known proportions of ethanol and water are added, the absorptions are determined and calibration curves prepared. When a sample of ether is to be examined, its water content is determined by the Karl Fischer method and its absorption is determined at 2·83 μ. Using the calibration curves the ethanol content is calculated. Samples whose absorption is too high for convenient measurement may be diluted with ethanol-free ether. The accuracy of the method is about ± 2 per cent. G. B.

Hydrastine in Hydrastis, Fluorimetric Determination of. E. Brochmann-Hanssen and J. A. Evers. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 620.) Hydrastine and canadine, but not berberine, are extracted from finely powdered hydrastis with ether in the presence of ammonia. The alkaloids are taken into aqueous solution and 5 ml. is oxidised by treatment with 10 ml. of nitric acid at 50° C. for 30 minutes, the solution is diluted and the blue-green fluorescence measured in a suitable fluorimeter. The fluorescence, which is due to hydrastinine and opianic acid, oxidation products of hydrastine, is dependent on temperature and fades on exposure to ultra-violet radiation. The hydrastine content is calculated from a standard curve prepared with pure hydrastine. The method is rapid and reasonable accuracy can be achieved. Impurities extracted from the hydrastis do not interfere. G. B.

Opium, Colorimetric Determination of Morphine in. A. Mariani, S. Guarino and O. Mariani Marelli (*Annali Chim.*, 1951, **51**, 661.) The official methods for the determination of morphine in opium are all long, troublesome and give doubtful results. The colour reactions devised by Guarino (*Arch. sci. biol.*, 1946, **31**, 115; *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 67) form the basis of a colorimetric method which is rapid and accurate. The reaction adopted is that of oxidation by excess of iodic acid in an acid medium and subsequent reaction with a trace of ferric chloride in a solution made alkaline with sodium bicarbonate or ammonium carbonate and then determining the absorption in

a spectrophotometer. The greatest absorption is at the wavelength of 510 to 520 μ and this should be chosen for the test, and as the colour is so deep that a high dilution is necessary for reading, the yellow colour of the solution of opium can be neglected except for deeply coloured samples, low in morphine. In the latter case a reading of the solution after adding the sodium bicarbonate should be taken and deducted from the final reading after adding the ferric chloride. The most delicate part of the reaction is the time allowed for oxidation with iodic acid before making alkaline. With pure morphine this should be 30 seconds, but with an opium solution made with lime it is slower and 2 to 4 minutes should be allowed. In a solution in the neighbourhood of 0·8 mg. in 50 ml. the curve follows Beer's law perfectly and $E_{1\text{ cm.}}^{1\text{ per cent.}} = 57{\cdot}14$. The authors compared the method of the Italian Pharmacopœia with their method using the lime solution obtained in the Italian Pharmacopœia method after the addition of hydrochloric acid, and also using the hydrochloric acid solution obtained by Eder and Wäckerlin's method (*Quart. J. Pharm. Pharmacol.*, 1937, **10**, 680). The last results were from 25 to 30 per cent. higher than those of the official method and 15 to 19 per cent. higher than the second method. This they attribute to the incomplete extraction of the morphine in the official concentration, and the loss of morphine in the precipitation. They recommend that 1 g. of opium be extracted with lime water sufficient to make a volume of 100 ml. and that 1 ml. of this solution be diluted with 9 ml. of 0·1 N hydrochloric acid. Good concordance was obtained on repeated determinations as well as with the addition of known quantities of morphine to extracts of opium. H. D.

Particle Size of Fine Powders, Determination of. C. Rossi and R. Baldacci. (*J. appl. Chem.*, 1951, **1**, 446.) The sedimentation curves for suspensions of kaolins from Central Europe, Italian bentonites and clays have been obtained by using a hydrostatic balance provided with a normal plunger. The concentration of the suspension should not exceed 2 per cent. by volume, so that for a powder of medium density (e.g., 2·5 g./ml.) an upper limit of 5 per cent. by weight is permissible; boiling or evacuation are necessary to remove all gas bubbles, and deflocculants are also added depending on the material under examination, although further detailed treatment may also be required to produce a final suspension which is satisfactory for measurements of specific gravity against time. Investigations undertaken to establish the accuracy of the method, and the theoretical calculation of the sedimentation curves are briefly described and the possibility of applying Stokes' law to the mass settling of kaolins is also discussed. The possibility (*a*) of determining the average diameter of powder particles by means of the tangent to the curve at zero time, and (*b*) of deducing from experimental sedimentation curves both the curves relating to size distribution (the number of particles as a function of equivalent diameter) are emphasised. Regular curves from sedimentation measurements, particularly with kaolins, were obtained only when conditions were such as to avoid flocculation, achieved by adding suitable electrolytes in quantities which determined the smallest sedimentation volume. The average equivalent diameters of the powders examined were deduced from sedimentation curves and were compared with statistical measurements of dimensions made by direct microscopic observations on two kaolins; Bohemian kaolin had an equivalent average diameter of 2·8 mμ and Zettlitz kaolin an equivalent average diameter of 2·11 mμ, these figures corresponding to results of 2·7 and 2·18 mμ respectively, calculated from equations using the sedimentation curves.

R. E. S.

Quercetin in Rutin, Chromatographic Estimation of. J. Naghski, C. S. Fenske Jr. and J. F. Couch. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 613.) Quercetin may be separated from commercial samples of rutin chromatographically and estimated spectrophotometrically. A solution of commercial rutin is dissolved in methanol or *iso*propanol, placed on the filter paper and developed with ethyl acetate saturated with water, using a descending chromatographic technique. Quercetin ($R_F = 0{\cdot}90$) is eluted while rutin ($R_F = 0{\cdot}05$) is retained on the paper. The quercetin solution is evaporated and the residue dissolved in absolute ethanol, aluminium chloride is added and the absorption determined at 440 mμ. The result is calculated from a standard curve. Recovery of quercetin is more complete from mixtures with rutin than when starting with quercetin alone. Certain flavonols which may be present in commercial rutin interfere, but this can be suppressed by using ethyl acetate, 35, benzene, 15, water, 50 as the developing solvent. G. B.

ORGANIC CHEMISTRY

***p*-Aminosalicylic Acid, Derivatives of.** W. Hückel and K. Janecka. (*Arch. Pharm., Berl.*, 1951, **284**, 341.) A description is given of the methods of preparation, and properties, of the following derivatives of *p*-aminosalicylic acid:—2-methoxy-4-aminobenzoic acid; methyl *p*-*N*-dimethylaminosalicylate; *p*-acetylaminosalicylic acid; methyl *p*-acetylaminosalicylate; 4-acetylamino-2-acetoxybenzoic acid; ethyl 4-acetylamino-2-acetoxybenzoate; *p*-acetylaminosalicylyl chloride; methyl *p*-acetylaminosalicylate, with the corresponding ethyl and *iso*propyl esters; methyl and ethyl *p*-aminosalicylates; ethyl *p*-acetylaminothiolsalicylate; and ethyl *p*-aminothiolsalicylate. G. M.

Iodinated Phenyl- and Pyridyl-alkanoic Acids as Contrast Agents. S. Archer, J. O. Hoppe, T. R. Lewis and M. N. Haskell. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 143.) Compounds of the following series were synthesised:—(1) α-(3:5-diiodo-4-aminobenzamido)alkanoic acids, (2) α-(4-amino-3:5-diiodobenzenesulphonamido) alkanoic acids, and (3) α-(3:5-diiodo-4-pyridone) alkanoic acids. Series (1) was prepared by condensing *p*-nitrobenzoyl chloride with the α-amino acid, reduction and iodination. Series (2) was prepared by condensing acetamidobenzenesulphonyl chloride with the amino-acid, removing the acetyl group and iodinating. Series (3) was prepared by condensation of 3:5-diiodo-4-pyridone with the α-bromo acid, and an analogous series was prepared from 3:5-diiodo-4-pyridinethiol. The compounds were tested as gall-bladder contrast agents in the cat and the following observations were made for series (1) and (2). The amino group (or other group such as hydroxyl) in the aromatic ring merely serves to facilitate formation of the iodinated compound and has little influence on cholecystographic effect. The alkyl group is of the greatest importance in obtaining a good contrast medium, although the bridge which links carboxyl to the iodinated nucleus has some effect. The most active compounds were derived from heptoic acid in series (1) and hexoic acid in series (2). Diiodopyridonealkanoic acids were not absorbed when administered orally, but the diiodopyridylthioalkanoic acids passed into the gall bladder and were effective agents. G. B.

Phenyl-alkyl-carbinols, Derivatives of Basically Substituted. A. C. Kjær and P. V. Petersen. (*Acta chem. scand.*, 1951, **5**, 1145.) Carbinols of general formula $C_6H_5{\cdot}C(R.)\ (OH){\cdot}CHR_2\ CH\ R_3R_4$ have been prepared in an investigation of substances with potential analgesic and spasmolytic action.

Those compounds in which R_1 was C_6H_5 or C_6H_{11}, R_2 either H or CH_3, R_3 was H and R_4 either $N(CH_3)_2$ or piperidino were prepared by the action of either phenylmagnesium bromide or *cyclo*hexylmagnesum bromide upon the corresponding aminoketones; the latter are readily accessible by typical Mannich reactions. Related alcohols of the type $C_6H_5C(R.)(OH)\cdot CHR_2 CHR_3R_4$ in which R_3 was methyl are prepared by a Grignard reaction with the corresponding amino and ethyl esters. None of these alcohols exhibited any significant spasmolytic or analgesic effect. Ester hydrochlorides of these carbinols with acetic, propionic, butyric and benzoic acids were unstable, being hydrolysed in water within a few hours. The free bases corresponding to the ester hydrochlorides were immediately hydrolysed in aqueous solution. Attempts to prepare ethers from the carbinols by reaction with alkyl halides in the presence of sodium, were unsuccessful. Treatment with thionyl chloride in an attempt to replace OH by Cl resulted in dehydration and the formation of unsaturated compounds, which in some cases showed considerable spasmolytic activity. One chloro compound only was isolated $(C_6H_5\cdot)_2\ C\cdot Cl\cdot CH\cdot CH_3\cdot CH_2\cdot N(CH_3)_2\cdot HCl$ and this was readily hydrolysed to the carbinol. No ether could be prepared from the latter compound by the action of sodium propylate in absolute propanol. The formation of the ether $(C_6H_5)_2\cdot C(OCH_2COCH_3)\cdot CH(CH_3)\cdot CH_2\cdot N(CH_3)_2$, HBr by the reaction of bromoacetone on 1:1-diphenyl- 2-methyl-3-dimethylamino propanol is recorded; this compound was unstable in aqueous solution. J. B. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Neovitamin A Esters and Neoretinene. P. D. Dalvi and R. A. Morton. (*Biochem. J.*, 1951, **50**, 43.) The fraction of vitamin A from fish liver oils reacting readily with maleic anhydride is the all-*trans* form, the remainder being neovitamin A, a *cis*-isomer in which only the double bond nearest to the $-CH_2OH$ or $-CH_2OCOR$ group possesses the *cis* configuration. The existence of neovitamin A makes it necessary to compare very closely the ultra-violet absorption shown by all-*trans* and neovitamin A, the precise form of the absorption curve being important in the analysis of liver oils when allowing for irrelevant absorption. Practical work on weakened alumina as adsorbent indicated that vitamin A ester concentrates could be enriched by chromatography until practically free from glycerides, sterol esters and other substances; repeated chromatographic adsorptions accumulated the neovitamin A esters in the strongly held fractions. The ultra-violet absorption spectra of the best neovitamin A ester fractions were measured in various solvents, the results being quoted on a basis of $E_{max.} = 1{\cdot}00$ and compared with the corresponding curves for all-*trans* vitamin A acetate. The natural neo-esters showed, relatively to the all-*trans* ester, higher absorption from 220 to 280 mμ and from 330 to 390 mμ; there was apparently a *cis*-peak near 250 mμ. In the region 280 to 330 mμ the differences between the neo-esters and the all-*trans* esters were small, but not negligible. Plotted on the basis of $E_{max.} = 1{\cdot}00$, the curves for neovitamin A alcohol showed higher intensity of absorption from 220 to 290 mμ and 330 to 390 mμ as compared with the all-*trans* free vitamin A. The differences, though small, were quite sufficient to influence corrections for irrelevant absorption if neovitamin A predominated over the all-*trans* form. Neovitamin A alcohol on oxidation over manganese dioxide (light petroleum solution) yielded neoretinene. The spectrum of neoretinene was slightly displaced in the direction of shorter wavelengths as compared with

retinene. It was concluded that the occurrence of neovitamin A in a fish liver oil could result in over-correction in spectroscopic assays if its presence were neglected. Many natural products contained about 25 per cent. of the total vitamin A in the neo form; if, however, this is confirmed it seems likely that for ester concentrates "over-correction" need not reach 5 per cent. The difficulty of saponification of neovitamin A esters was the only chemical difference observed in addition to the difference in speed of reaction with maleic anhydride. R. E. S.

Rutin and Quercetin, Relative Stability in Alkaline Solutions. E. B. Dechene. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 495.) Solutions of rutin and quercetin in aqueous ethanolic and ethanolic solutions of sodium hydroxide, ethylenediamine and hydrochloric acid in various concentrations were refluxed or allowed to stand at room temperature and the concentration of the flavonol in the solutions determined by the aluminium chloride colour reaction. Little or no decomposition of rutin occurred in aqueous-ethanolic solutions containing about 2 moles of sodium hydroxide or 30 moles of ethylenediamine per mole of rutin, and only slight decomposition occurred in solutions containing about 4 moles of sodium hydroxide per mole of rutin. Quercetin showed a rapid rate of decomposition in aqueous-ethanolic solutions containing about 2 moles of sodium hydroxide or 30 moles of ethylenediamine per mole of quercetin. Considerable decomposition of rutin occurred in solutions containing slightly less than 17 moles of sodium hydroxide or 60 moles of ethylenediamine per mole of rutin. Paper chromatograms of the alkaline solutions of rutin showed no detectable amounts of quercetin, indicating that the latter was decomposed as fast as it was produced by the hydrolysis of rutin. Considerable amounts of quercetin were detected on paper chromatograms of the acid solutions of rutin, indicating that rutin is readily hydrolysed in these conditions whereas quercetin is stable. G. R. K.

Starch and Ion Exchange Resin Chromatography for the Separation of ^{15}N-labelled Amino-acids. S. E. G. Aqvist. (*Acta. chem. scand.*, 1951, **5**, 1031.) Protein hydrolysates containing ^{15}N– labelled amino-acids from yeast and *E. coli*, obtained from 25 to 50 g. batches of protein, were separated on the cation exchange resin, Dowex 50, after being separated initially by electrodialysis into acidic, basic and neutral amino-acid fractions. Detailed information as to column size, packing and preliminary washing of the ion exchange resin are given. Each amino-acid mixture was applied to the column in 1·5 N hydrochloric acid and eluted in a large volume of the same solvent. Clear cut separation of all the common amino-acids was obtained by this method, with the exception that some overlays did occur with the three amino acids, methionine, *iso*leucine and leucine. Separation of protein hydrolysates corresponding to 250 to 300 mg. of protein and containing ^{15}N– labelled amino-acids was also effected on columns of potato starch following the general procedure described by Stein and Moore, though on a much larger scale. The starch column must be freed from organic impurities and metal ions before it is used in the separation of amino acids. A certain amount of fatty impurity, retained by the starch, may be eluted later with the amino-acid fractions, but this is thought to be preferable to the loss of resolving power which follows the more thorough preliminary extraction with solvents. Acid-propanol-butanol is recommended as the solvent system for these large-scale separations rather than benzyl alcohol as described by Stein and Moore (*J. biol. Chem.*, 1948, **176**, 337.) Identification of the different amino-acids separated was by paper chromatography. J. B. S.

Vitamin A, Spectroscopic Properties of All-*trans* Vitamin A and Vitamin A Acetate. H. R. Cama, F. D. Collins and R. A. Morton. (*Biochem. J.*, 1951, **50**, 48.) A detailed examination is made of the whole problem of the spectrophotometric estimation of vitamin A. The photoelectric spectrophotometer used was calibrated with potassium chromate and dichromate solutions; results are given which provide a basis for testing the performance of other instruments. Natural and synthetic vitamin A were found to be indistinguishable. The values of $E_{1\text{ cm.}}^{1\text{ per cent.}}$ and $\lambda_{max.}$ for vitamin A acetate and for vitamin A alcohol in *cyclo*hexane, light petroleum, ethanol and *iso*propanol are given. The values of $\epsilon_{max.}$ in *cyclo*hexane and light petroleum were the same for the free acetate and for the alcohol although values of $\epsilon_{max.}$ in ethanol and *iso*propanol for the alcohol were higher than for the ester. Detailed figures are given for the absorption intensities expressed as fractions of $E_{max.}$ at wavelength intervals of 1 mμ over the range 310 to 340 mμ together with the appropriate correction equations for eliminating irrelevant absorption in vitamin A acetate and alcohol solution in the solvents *cyclo*hexane, ethanol, *iso*propanol and light petroleum. All the absorption curves showed a second maximum at about 250 to 253 mμ, approximately 550. The value of the factor for converting $E_{1\text{ cm.}}^{1\text{ per cent.}}$ to I.U./g. was not strictly constant, but varied with the solvent and the state of combination of the vitamin A. A complete absorption curve for the reaction product of vitamin A and antimony trichloride has been obtained, the value of $\epsilon_{max.}$ being 144,900 $\pm$ 870 at 620 mμ. Detailed studies have been made of a sample of cod liver oil, two high potency oils and the International Standard Preparation of vitamin A acetate; it is concluded that the International Standard Preparation is perhaps not quite up to standard, results for $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 328 mμ as low as 5·09 instead of 5·21 being recorded. It is emphasised that although for a wide range of products the $E_{1\text{ cm.}}^{1\text{ per cent.}}$ value at 325 to 328 mμ on the oil or the "unsponifiable fraction" is a good guide to potency, it is necessary in aiming at an accurate assay to take full account of the presence of anhydrovitamins A, vitamin A_2, kitol, epoxides and other substances. It is generally, except with low potency oils, a better procedure to use the whole oil since the correction procedure when applied to unsaponifiable materials tends to give low results. It is also necessary to determine the neo/all-*trans* ratio, so that in addition to the main test based on ultraviolet absorption, a number of subsidiary tests are essential.

Appendix. Correction Procedure. A correction procedure can be developed which makes use of all the wavelengths at 2 mμ intervals from 310 to 340 mμ. As only 3 wavelengths are necessary in order to correct for linear irrelevant absorption, 13 degrees of freedom are available for the determination of the error. The following formulæ are derived by the method of least squares. In the case of vitamin A acetate in *cyclo*hexane,

$$\bar{E} = \frac{21760\ \Sigma\ \alpha\ E\ -\ 19{\cdot}824\ \Sigma\ \lambda\ E\ -\ 13488\ \Sigma\ E}{1297{\cdot}422}$$

In the case of vitamin A alcohol in *cyclo*hexane,

$$\bar{E} = \frac{21760\ \Sigma\ \alpha\ E\ +\ 2{\cdot}848\ \Sigma\ \lambda\ E\ -\ 20735{\cdot}36\ \Sigma\ E}{1461{\cdot}4985}$$

where E is the measured extinction at wavelength λ, α is the ratio of the absorption of pure all-*trans*-vitamin A to the absorption at wavelength $\lambda_{max.}$, $\bar{E}$ = extinction actually due to vitamin A at $\lambda_{max.}$. R. E. S.

BIOCHEMICAL ANALYSIS

Amines, Paper Chromatography of. J. M. Bremner and R. H. Kenton. (*Biochem. J.*, 1951, **49**, 651.) The R_F values of filter paper chromatograms of a large number of amines in various solvents have been determined and tabulated. The values were obtained with one batch of filter paper and represent the means of several determinations. In agreement with Bates-Smith (*Biochem. Soc. Symp.*, 1949, No. 3, 62) the values were found to vary from batch to batch of filter paper, but to be reasonably constant with individual batches. Ninhydrin is an effective reagent for the detection of primary aliphatic amines on paper chromatograms. Some compounds containing a secondary aliphatic amino group also give the ninhydrin colour reaction, but tertiary amines do not appear to give the reaction. The results obtained suggest that amines likely to be present in biological materials could be separated and identified by paper chromatography. Relationships between the molecular structure of the amines studied and their R_F values are also discussed. J. R. F.

Formaldehydogenic Corticosteroids in Urine, Estimation of. J. Rabinovitch, J. Decombe and A. Freedman. (*Lancet*, 1951, **261**, 1201.) The significant features of the method described are as follows. The first morning specimen of urine is collected in a vessel which contains 300 mg. of sodium sulphite, 3 ml. of glacial acetic acid is immediately added and the container tightly corked, a 50-ml. sample of the specimen being used for the assay. This procedure inhibits bacterial growth and the α-ketol group is protected against oxidation. Acid hydrolysis of the urine liberates corticosteroids from conjugation, and maximum hydrolysis with little destruction is achieved by heating the urine for 15 minutes in a closed flask at 60° C. with 0·12 ml. of concentrated sulphuric acid and 60 mg. of sodium sulphite, cooling, and adjusting to a *p*H of 6 to 7. With other methods of assay the formaldehyde formed by treatment with periodic acid is removed from the reaction mixture for estimation, but by treating the solution directly with calculated quantities of silver sulphite and filtering off the silver iodide formed, separation of the formaldehyde is obviated and the solution is ready for colorimetric estimation. From a calibration curve obtained from estimation of urinary extracts with added known quantities of cortisone the corticosteroid content of the specimen may be calculated. A single estimation need not take longer than 6 or 7 hours, and four estimations can be done in 8 or 9 hours. The range of excretion in the night urine of normal men, established by this method, is from 55 to 190 μg. S. L. W.

Gentisic Acid in Serum, Determination of. J. Lowenthal. (*J. Lab. clin. Med.*, 1951, **38**, 916.) A method of determination is described based on the fact that gentisic acid in a buffered solution (*p*H 3 to 4) is oxidised by ferric ions, ferrous ions being produced. Potassium fluoride is added to render the excess of ferric iron colourless through complex formation; the concentration of the ferrous iron is then determined photoelectrically by the formation of the coloured ferrous iron-ortho-phenanthroline complex. The accuracy of the method was tested by adding various quantities of gentisic acid to serum; satisfactory recoveries of gentisic acid were obtained. Analyses on single samples of serum stored in the refrigerator for several days gave reproducible results. Normal serum not containing gentisic acid, gave values equivalent to 0·5 to 1·0 mg. per cent. of gentisic acid; this figure can be neglected in comparison with gentisic acid levels experienced in practice. R. E. S.

Vitamin B_{12}, Plate Assay with *Escherichia coli*. E. Harrison, K. A. Lees and F. Wood. (*Analyst*, 1951, **76**, 696.) A microbiological method for the cup-plate assay of vitamin B_{12} with a mutant of *Escherichia coli* as test organism is presented. The assay is claimed as an advance over methods previously published of this series because (*a*) a simple chemically defined medium is used, (*b*) changes in the E_H of the test medium have little effect on the response, (*c*) the zones of exhibition are reproducible and well defined in character and (*d*) specificity and general freedom from interfering effects and inexplicable variations are shown to a marked degree. The inoculum consisted of a small volume of culture grown overnight in peptone water; the assay plates could be incubated at any temperature between 27° and 37° C. The sensitivity of the method was such that zones of exhibition could be obtained with solutions containing 1 μg. of vitamin B_{12} per ml. The dose response line was rectilinear over the range 0·005 to 5·0 μg per ml. and hence a (2 + 2) assay design was normally used; results are given relating to the effects of times of incubation, and of standing before incubation. A streptomycin-resistant strain of the test organism was developed for the direct assay of the vitamin B_{12} content of *Streptomyces griseus* fermentation samples. Thymidine did not interfere with the assay, but methionine gave zones of exhibition, a solution of 1 mg. per ml. giving a zone of approximately 35 mm.; dilutions of 100 and 10 μg. per ml. also stimulated growth, but the zones were indistinct and not measurable. Standard errors of 0·14 to 0·23 mm. per zone were obtained, 0·18 mm. being the normal error encountered in assays on Petri dishes or large glass plates. R. E. S.

CHEMOTHERAPY

Cinnoline Compounds in *T. Congolense* Infections. E. M. Lourie, J. S. Morley, J. C. E. Simpson and J. M. Walker. (*Brit. J. Pharmacol.*, 1951, **6**, 643.) A series of quaternary quinoline, cinnoline and quinazoline compounds were tested for activity against *Trypanosoma congolense*, using mice as the experimental animals. Evidence was obtained suggesting activity in a substance having two cinnolinium residues joined by a simple linkage and of a number of such compounds tested the most active was N^1:N^3-bis(4′-aminocinnolyl-6′)-guanidine methiodide, designated "528." The therapeutic index represented by LD10/CD90 is 11·6 compared with 12·5 for antrycide, the average LD10 being 1·03 mg./20 g. and the average CD90 being 0·089 mg./20 g. as compared with 0·49 mg. and 0·039 mg. for antrycide. H. T. B.

Barbiturates, Anæsthetic Properties of some new *N*-benzylated and *NN*′-dibenzylated Compounds. F. Sandberg. (*Svensk. farm. Tidskr.*, 1952, **52**, 31.) The following compounds were prepared and tested for anæsthetic properties:— 5:5-dialkylbarbituric acid, 5-allyl-5-*iso*propylbarbituric acid, 5-allyl-5-phenylbarbituric acid, 5-ethyl-5-*iso*-amylbarbituric acid and their 1-benzylsubstituted and 1:3-dibenzylsubstituted derivatives. The introduction of an *N*-benzyl group into the compounds was attended with a reduction in hypnotic efficiency and in toxicity, and a reduction in duration of action. No general rules were apparent for the influence of a single radical in the 5-position upon the anæsthetic properties. The *NN*′-dibenzylated compounds did not exhibit anæsthetic activity. A. H. B.

PHARMACY

DISPENSING

Sodium *p*-Aminosalicylate, Intravenous. R. G. Douris and J. Bory. (*Thèrapie*, 1951, **6**, 371.) A 3 per cent. aqueous solution of sodium *p*-aminosalicylate, as recommended by Paraf for intravenous use, is almost isotonic. It may be sterilised by heating, since the proportion decarboxylated is 1·3 per cent. (100° C. for 1 hour), 1·6 per cent. (115° for ½ hour), or 2 per cent. (120° for 20 minutes), and this proportion of *p*-aminophenol does not appear unduly toxic. Oxidation products which are formed slowly in the cold and more rapidly on heating, are pyrogenic in rabbits. Oxidation may be prevented by the use of sodium bisulphite, sulphite or hyposulphite, but the quantities necessary are too large to permit prolonged therapeutic use. Alternatively, the solution may be stabilised with sodium formaldehyde sulphoxylate (patented).

G. B.

Sodium Bisulphite, Sterilisation of Solutions of. S. A. Schou and J. M. Rhodes. (*Dansk Tidsskr. Farm.*, 1951, **25**, 365.) The effect of sterilisation on solutions of sodium bisulphite is important, since this compound is sometimes added as a preservative to other solutions. On sterilisation, oxidation to sulphuric acid occurs with a consequent decrease in *p*H, since the buffer action is very small. In a solution containing 0·1 per cent. of sodium bisulphite and 0·8 per cent. of sodium chloride, the *p*H changed on autoclaving from 3·44 to 0·96. Bubbling nitrogen through the solution causes a rise in *p*H, owing to loss of sulphur dioxide: such solutions also become more acid on autoclaving, though to a smaller extent than those sealed in air. G. M.

GALENICAL PHARMACY

Adrenaline and Analogues, Rate of Loss of Potency in Solution. J. C. Munch, A. B. Sloane and A. R. Latven. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 526.) Solutions of adrenaline and vaponefrin containing 0·5 per cent. of chlorbutol and adjusted to *p*H 4 were packed in ½-oz. amber glass bottles and stored under a variety of conditions. In a refrigerator at 5° C., adrenaline showed no loss of potency in 5 months, whereas vaponefrin showed no significant change in colour or potency up to 40 months. In the dark at room temperatures which ranged from 20° to 35° C., adrenaline began to change colour in 2 to 4 weeks but showed no significant change in potency; vaponefrin showed no change in potency over 3 months, and after 40 months one bottle showed a slight colour and precipitate but a loss of only 7 per cent. in potency, or substantially the same as that shown by a companion bottle kept at 5° C. When stored on a laboratory shelf in direct sunlight, adrenaline showed no significant loss over a month but did develop marked colour change and some precipitation; vaponefrin began to develop a pinkish-brown colour and a sediment after a year and loss in potency varied from 16 per cent. after 8 months to 12 per cent. after 40 months. Adrenaline solutions stored at room temperature in direct sunlight in frequently opened bottles showed marked losses in potency and definite discoloration within a week. Vaponefrin solutions stored similarly showed little loss in potency or discoloration in the first 3 months; after 29 months the decrease in potency was about 16 per cent., and after 40 months, about 24 per cent. When stored at 37° C., adrenaline

maintained its potency for a week whereas vaponefrin lost only 25 per cent. of its potency after 104 months. At 55° C., adrenaline again maintained its potency for 1 week but showed marked changes in colour and a loss of over half its potency in 1 month. Vaponefrin showed no change in colour over 15 months and a loss of potency of only 22 per cent. in 40 months, similar to that shown by solutions stored in sunlight in frequently opened bottles. The assays were performed on dogs by the U.S.P. method. G. R. K.

PHARMACOGNOSY

Caffeine in Cacao Beans. W. G. C. Forsyth. (*Nature Lond.*, 1952, **169**, 33.) To determine whether caffeine exists in the cocoa bean free or as an L-epicatechin-caffeine complex, various extracts of fresh bean were subjected to paper chromatography using a variety of developers, and, in every case, caffeine was found only in the free form on the paper. Synthetic preparations of the complex were made (1 : 1 addition compound of caffeine and L-epicatechin), by mixing aqueous solutions of the two components. Chromatography on a paper strip caused the two components to separate and each had its usual R_F value even in neutral solvents. The caffeine could also be completely extracted from an aqueous suspension of the material by cold chloroform. The isolation of such an easily formed complex is thus not proof that it exists as such in the bean. A. H. B.

Ephedra (*Colonial Plant and Animal Prod.*, 1951, **2**, 119.) A review article dealing with the distribution and alkaloid content of the various species. Special attention is given to cultivation trials carried out in South Dakota, Kenya and Australia. In South Dakota it was found that 4 year old stems of *E. sinica* yielded the highest proportion of alkaloid and less expense was involved in handling the crop in the field. The stems were cut and dried in the sun like hay, then stacked or baled, this method giving a drug with a higher alkaloid content than oven-drying. In Kenya, one year old stems of *E. intermedia* and *E. gerardiana* dried at 50° C. had an alkaloid content varying between 1·54 and 1·69 per cent. The average ephedrine content of the total alkaloids was in the case of *E. intermedia* 30 to 40 per cent. and in *E. gerardiana* 70 to 80 per cent. Difficulties in the availability of the natural product from China stimulated interest in the synthetic production of ephedrine and as a result there has been a decline in interest in ephedra. Some manufacturers in this country favour the herb, but in order that the herb may hold its own against the synthetic alkaloid, it will be necessary to keep collection and transport costs as low as possible. G. R. A. S.

Morphine from Poppy Heads and Stalks. V. Ristic. (*Boll. chim.-farm.*, 1951, **90**, 472.) It has been suggested that morphine should be extracted from poppy heads and stalks, either to enable countries which do not produce opium to avoid the necessity of importation, or to increase the yield by extracting the heads after opium has been collected. A good deal of investigation is required before it can be said to be a commercial possibility. Various authors have reported finding between 0·02 to 0·9 per cent. of morphine in the heads, and similar amounts in the top 10 cm. of the stalks. Lower portions of the stalks are valueless. The heads lose morphine rapidly on storage. It is suggested that extraction plant should be set up in the neighbourhood of the poppy fields so that it would only be necessary to transport the extract to the factories, instead of the relatively weak and bulky raw material. H. D.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline, Noradrenaline and Methedrine; Effects on Renal Circulation during Anæsthesia. H. C. Churchill-Davidson, W. D. Wylie, B. E. Miles and H. E. de Warderer. (*Lancet*, 1951, **2**, 803.) Inulin and *p*-aminohippurate clearances were estimated on 18 occasions in 14 patients before and after the administration of adrenaline, noradrenaline or methedrine given during varicose-vein ligation or herniorrhaphy. Inulin clearance was assumed to equal the glomerular filtration-rate and *p*-aminohippurate clearance the renal plasma-flow. Renal blood-flow was calculated from the renal plasma-flow and venous hæmatocrit. The patients were anæsthetised with ether or *cyclo*propane and 10 of the patients had been made hypotensive by pentamethonium bromide. Adrenaline and noradrenaline were given by continuous intravenous drip; adrenaline at the rate of 16 to 38 μg./minute and noradrenaline at the rate of 3·5 to 37 μg./minute. Methedrine was given intravenously in divided doses up to a total of 25 to 100 mg. The renal blood-flow was reduced in all cases by adrenaline and noradrenaline, but increased in 5 out of 6 cases by methedrine. These results show that the effects of adrenaline and noradrenaline are similar in anæsthetised and in unanæsthetised persons, the rise in blood pressure being accompanied by a consistent fall in renal blood-flow, whereas methedrine produced a rise in blood pressure and an over-all increase in renal blood-flow.

S. L. W.

Cardiotonic Drugs, Chick Embryo Assay. G. H. Bryan and C. H. Waldon. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 497.) 72-hour chick embryos were exposed by removing the shell and membrane from the air pocket, giving a frontal view of the clearly defined heart. The eggs were then submerged to three-quarters of their length in water at 37° C. when the hearts continued to beat regularly but more slowly than normal for 4 to 24 hours. The drugs, in solution in normal saline solution containing 10 per cent. of ethanol, were placed in the pocket formed by the shell, egg yolk and white and the embryo, and the time taken to stop the heart measured. 3 different dose levels were used for each drug and were chosen so as to give adequate responses in 450 seconds. The 19/20 confidence limits for the ED50 of digitoxin (± 14 per cent.) and olnerin (± 12 per cent.) paralleled the limits obtained for tincture of digitalis, but those for digoxin (± 40 per cent.) and tincture of strophanthus (± 70 per cent.) were extremely high.

G. R. K.

Cortisone in Rheumatoid Arthritis, Synergistic Action of *p*-Aminobenzoic Acid on. L. L. Wiesel, A. S. Barritt and W. M. Stumpe. (*Amer. J. med. Sci.*, 1951, **222**, 243.) In view of the scarcity and cost of cortisone a search was made for a method of reducing the usual dosage in the treatment of rheumatoid arthritis. Its structural similarity to the œstrogens, whose inactivation by the liver is inhibited by the simultaneous administration of *p*-aminobenzoic acid, suggested an attempt to inhibit the destruction of cortisone in the body by a similar method. 15 patients suffering from rheumatoid arthritis were treated according to two different techniques, details of which are tabulated. The dosage of cortisone employed was, by itself, completely ineffective in controlling the manifestations of rheumatoid arthritis. On a daily intramuscular dose of 25 mg. of cortisone acetate, together with oral administration of 1·5 g. of sodium *p*-aminobenzoate every 2 hours until 12 g. had been taken, all the patients demonstrated great relief of pain and objective symptoms. None of

the undesirable side effects frequently present with the usual dosage of cortisone acetate was observed and only occasional mild heartburn resulted from administration of the sodium aminobenzoate. H. T. B.

Liquorice, Pharmacological Action of. D. Vincent. (*Thèrapie*, 1951, **6**, 448.) When a 1 in 10 decoction of dried liquorice root was injected intravenously into a chloralosed dog a rapid reduction of blood pressure was produced which lasted 20 to 30 minutes. In some dogs doses of 0·05 g. of the dry root per kg. of body weight produced a fall of 70 or 80 per cent. In other cases doses of 0·1 g. or even 0·2 g. per kg. produced less effect. Cardiac rhythm was only slightly affected and breathing was not changed. By various tests (use of physostigmine, cholesterinase, and the action on the dorsal muscle of the leech and the isolated intestine of the guinea-pig) the author showed that this action was not due to acetylcholine. Commercial ammoniated glycyrrhizin in 1 per cent. solution in corresponding doses did not produce similar falls in blood pressure. Previous injections of liquorice markedly diminish the action of acetylcholine on blood pressure and on the intestine of the guinea-pig. The drug inhibits the action of cholinesterases to an extent varying from 20 to 50 per cent. according to the source of the enzyme, so its effect in inhibiting the action of acetylcholine is not due to stimulating the enzyme. Liquorice also possesses antihistaminic properties which will be reported later. H. D.

Ouabain, Influence of Sex upon Response of Rat Heart to. A. Wollenberger and M. L. Karsh. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 637.) Langendorff preparations of the isolated hearts of 4- to 5-month old rats were perfused with glucose-Ringer-Locke solution. When the contractions had become uniform ouabain was administered at the rate of 0·25 mg. every 3 minutes until the heart stopped in contracture. The minimal lethal dose, calculated with reference to the dry heart weight was 45 per cent. higher in females than in males in May-June, and 31 per cent. in November-December. This accords with the observed sex difference in LD50 for mature rats and shows that this may be attributed to the difference in sensitivity of the heart. G. B.

Primaquine in Experimental *Trypanosoma cruzi* Infection. T. Pizzi. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **78**, 643.) Primaquine is 8-(4-amino-1-methyl butylamino)-6-methoxyquinoline and its effect in the treatment of *Trypanosoma cruzi* infection in mice has been investigated. The drug was given orally as a solution in water containing 1 mg./ml., the daily dose being 0·25 mg., and the number of trypanosomes in the blood was determined daily. During the early stages of the infection the primaquine has a strong suppressive action and most treated animals survived infection with a virulent strain. All showed a disappearance of, or a marked decrease in, the number of parasites in the blood. H. T. B.

Procaine Amide in Digitalis-induced Ventricular Tachycardia. L. I. Goldberg and M. de V. Cotten. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **77**, 741.) Auricular-ventricular block, with a slow ventricular rate, or ventricular tachycardia was induced in dogs by the administration of digitoxin to 12 and ouabain to 8 animals intravenously in divided doses. Rapid intravenous injection of a 10 per cent. procaine amide solution induced a reversion to normal sinus rhythm when administered to those in ventricular tachycardia. In most cases this was temporary and additional injections were required to maintain normal rhythm. Such injections produced no electrocardiographic signs

of cumulated toxicity. In several instances development of slow idioventricular rhythm and cardiac arrest followed the administration of the drug, demonstrating a danger of drug termination of digitalis-induced tachycardia. In agreement with other workers it was also found that the drug failed to increase the lethal dose of ouabain in an experiment with 7 cats. J. R. F.

***Veratrum viride*, Biological Estimation of.** R. Benigni. (*Boll. chim.-farm.*, 1951, **90**, 384. Craw has recommended the use of *Daphnia magna* for testing the activity of *Veratrum viride* and he calls a daphnia unit the quantity required to kill 1000 daphnias with the heart in systole in 2 hours and 22 minutes. The large number of animals, their minute size and the necessity of using the microscope to examine them, makes this a method unsuited for routine use. The author suggests the use of *Lebistes reticulatus* the "million" fish. This fish is a native of Venezuela and the West Indian islands, but has been spread over the tropics in the fight against malaria. The males are about 2·5 cm. long and the females 3·5 cm. They are obtainable commercially and are easily kept in an aquarium. They breed at the age of 3 months, are ovi-viviparous, and about once a month produce from 2 or 3 to 30 or 40 young. The method of testing is simple. In 5 or more 100-ml. beakers place 50 ml. of well-aerated water and in each place an adult lebistes, as far as possible all the same size, and add a series of doses of the preparation to be tested until a quantity is found which will kill a lebistes in 5 hours. The test is then repeated with doses more closely spaced until the minimum dose which will kill 3 fish out of 3 is found. This is called a lebistes unit (L.U.) and the author found that 5·7 of these units equals 10 daphnia units, and this is an average therapeutic dose by the mouth. Very concordant results are obtained by this method, and it is economical, as usually not more than 20 to 30 fish are used and those that survive can be used again after a sufficient interval. H. D.

BACTERIOLOGY AND CLINICAL TESTS

Boric Acid; Antibacterial Action in Tears. M. Novak and W. I. Taylor. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 430.) Bacteriostatic concentrations of boric acid in broth cultures of representative pyogenic bacterial species often found in the eye varies from 0·5 to 2 per cent. Higher concentrations, to a maximum of 4 per cent., showed a slow bactericidal action. Freshly collected as well as stored lachrymal secretions showed no inhibitory action on the antibacterial action of boric acid. The results suggest that tears actually increase the bactericidal action of boric acid to a slight degree. This action may be due to naturally occurring lysozyme. S. L. W.

Pyrogenic Properties, Attenuation of, During the Ageing of Sterile Microbial Preparations. J. Dorche and M. Castaing. (*Ann. pharm. franç.*, 1951, **9**, 583.) Sterile suspensions of *Salm. typhi*, *B. subtilis*, *E. coli*, and *Ps. æruginosa* were stored for periods of up to 2 years. They retained their pyrogenic properties with the exception of the *B. subtilis* suspension which rapidly decreased in pyrogenic power. A suspension of *Salm. typhi* appeared to be sufficiently stable for use as a standard pyrogen. G. B.

Pyrogenic Properties of Distilled Water and Microbial Cultures. J. Dorche, M. Carraz and M. Castaing. (*Ann. pharm. franç.*, 1951, **9**, 574.) Bacterial emulsions were prepared by suspending bacteria in normal saline, diluting to

[*continued on page* 344

BOOK REVIEWS

MEDICINAL CHEMISTRY (Vol. I). A Series of Reviews prepared under the auspices of the Division of Medicinal Chemistry of the American Chemical Society. Pp. vi + 466 and Index. Chapman and Hall, Ltd., London. 1951. 80s.

This volume is the first of a proposed series containing reviews, the purpose of which is to "summarise the available data on the biological properties of medicinal substances and to correlate the relationship between chemical structure and physiological activity in each area covered." The subjects dealt with in this first volume are antithyroid compounds, antispasmodics (derivatives of carboxylic acids), antibiotics from plants, benzoates and substituted benzoates as local anæsthetics, analgesics (A. aralkylamines and B. partial structures related to morphine.) Each chapter contains a discussion of the methods of synthesis and pharmacological test procedures and an examination of the relationship between chemical structure and pharmacological activity. All the compounds which have been tested for the type of pharmacological activity covered in each chapter are presented in tabular form giving the pharmacological activity and references. Each chapter has been contributed by authorities whose personal experience and knowledge in the field discussed is extensive. The style adopted in the book is similar to that used in the well known "Organic Reactions" series, produced by the same publisher, and the printing, binding and quality of paper is of the same high standard. *Medicinal Chemistry*, Vol. I, is an excellent book, and the series promises to be of great value to research chemists and pharmacologists. The only criticism is of the price—80s.

A. H. BECKETT.

A TEXTBOOK OF PHARMACOGNOSY, by T. C. Denston. Fifth Edition. Pp. xviii + 624 and Index. Sir Isaac Pitman and Sons, Ltd., London. 1951. 45s.

The fifth edition of this well-known textbook has been brought up to date by giving information on polyploidy, insect infestation, vegetable materials now being used more commonly, such as yeast, sterculia gum, derris, etc., and by revising wherever necessary the information on constituents, and the requirements of the B.P. 1948 and the B.P.C. 1949. Besides the information on individual drugs there are chapters of a general nature on drug constituents; cultivation, collection and storage; and adulteration and commerce in drugs. The relationship of the facts described to the practice of pharmacy, as well as the effect of the Second World War and the present economic situation on the commerce in crude drugs, are rightly emphasised. As the book has grown out of an earlier laboratory manual there are still retained sufficient instructions to enable suitable practical exercises to be carried out; many of the line drawings are also suitable as examples of the drawings students should make. These illustrations are supplemented by some excellent and interesting photographs. The revision of information seems to have been well done though the reference to the preparation of oil of lemon implies that the sponge and *écuelle* processes are still extensively used. According to Guenther (*The Essential Oils*, 1949, III, 9 *et seq.*) the sponge process is seldom used now and the *écuelle* process is never used in Europe; in the West Indies it is still used for the production of orange and lime oils. The book is well suited for courses such as that prescribed for the old Chemist and Druggist Examination; however, little information is given on the histology of drugs, and this omission will have to be made good if future editions are intended to cover the courses in pharmacognosy for present degrees and diplomas.

J. W. FAIRBAIRN.

LETTER TO THE EDITOR

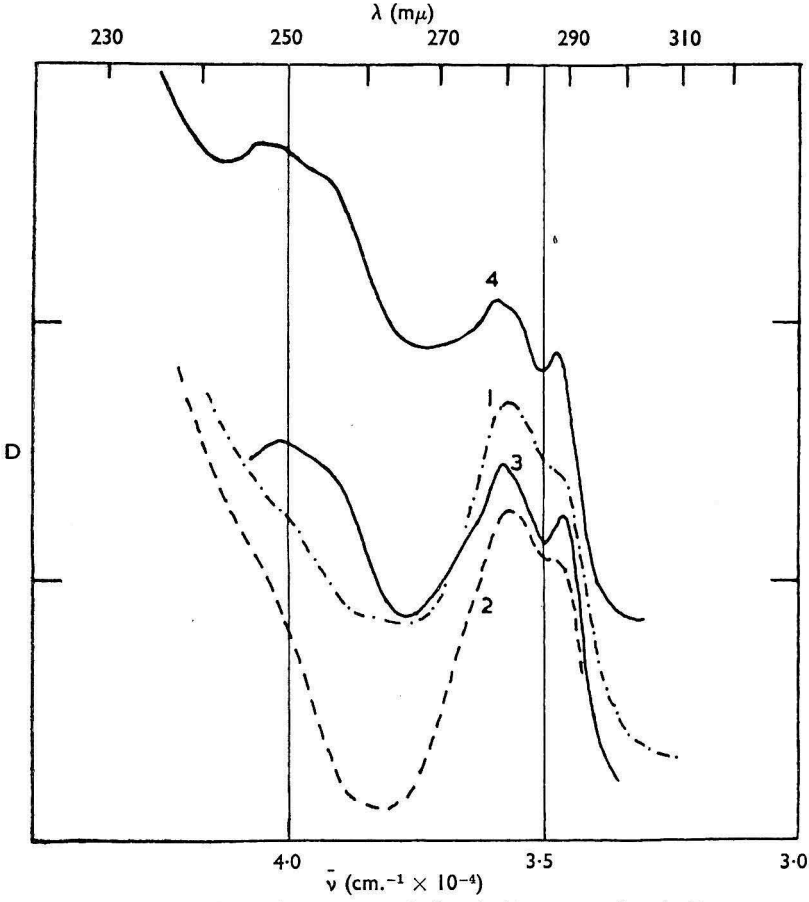
Spectroscopic Evidence for the Presence of the Benziminazole Chromophore in Intact Vitamin B_{12}.

Pfiffner[1] has reported the occurrence of pseudo vitamin B_{12} (ψB_{12}) in the harvest broth of a rumen anærobe. Vitamin B_{12} from liver and from *S. griseus* cultures yields, on acid hydrolysis, 1-aminopropanol-2, a benziminole glycoside and a red cobalt-containing fragment.[2] Pfiffner has shown, however, that ψB_{12} does not yield a benziminzole-containing fragment but an adenine glycoside. In view of a consequent necessary subclassification of vitamin B_{12}-like factors it seems to us important to establish whether the benziminazole exists intact in the B_{12} molecule or is produced under the conditions of acid hydrolysis which, so far as reported in the literature, provides the only means available for obtaining substituted benziminazoles from B_{12}. In our original communication[3] on the spectroscopic identification of 5:6-dimethylbenziminazoles in the hydrolysis products of B_{12} we gave evidence for the pre-existence of the benziminazole in the B_{12} molecule, based on the presence of some of the fine-structure features of the benziminazole chromophore in a moving-plate spectrogram[4] of an aqueous solution of B_{12}. Further more definite confirmation of this has now been obtained by examination of the difference between the spectrum of intact B_{12} and that of a specimen of the red cobalt-containing fragment obtained by acid hydrolysis of B_{12}; the preparation and properties of this fragment will be reported in a future paper.

Since the molecular weight of the red fragment (R.F.) is not yet known, it is not possible to make a simple subtraction of the molar absorption curve of R.F. from that of B_{12} and thus obtain a difference curve representing the absorption of that portion of the B_{12} molecule which is absent in R.F. It is possible to show, however, that a solution of red fragment can be found by trial whose concentration and thickness is such that the difference between its spectrum and that of a given solution of B_{12} is a "most probable" spectrum for the chromophore absent in R.F. This type of analysis has been treated theoretically by Hardy and Young.[5] The method is very laborious when using a manual spectrophotometer, since it involves the calculation or measurement of several absorption curves for different concentrations of R.F. and subtraction of these from that of the solution of B_{12}. However, with a twin-beam automatic recording spectrophotometer, a whole series of difference curves can be directly obtained by placing a solution of R.F. in a variable-length cell in the "control" beam of the spectrophotometer and the solution of B_{12} in the "sample" beam, and altering the thickness of the variable-length cell for each run. The spectrophotometer is, in effect, used as a computor by means of which, by the method of Hardy and Young, a "most probable" difference curve may be selected in a favourable case such as this turns out to be. The principles of selection will be given in a later communication in a more general context. Suffice it to say here that the absorption spectra of vitamin B_{12} and of our red fragment R.F. are, under certain conditions, very similar in the wavelength region 310 to 650 mμ and their "dicyanide" derivatives are indistinguishable in this range. The differences in their absorption characteristics are found at wavelengths shorter than 310 mμ.

The figure shows differential absorption curves between a solution of vitamin B_{12} and one of R.F. under three different conditions: (1) at *p*H 4·0, (2) at *p*H 10·0, (3) after adding a small amount of potassium cyanide to each solution at

*p*H 10. For comparison, curve (4) gives the absorption spectrum of the benziminazole fragment obtained by hydrolysis of vitamin B_{12} (component α of Beaven *et al.*[3]) in neutral or alkaline solution. From inspection of the curves the following points may be noted: (*a*) the great similarity between curve 3 and curve 4; (*b*) the lack of resolution of the 288 mμ band in curves 1 and 2; (*c*) the presence of a band at 250 mμ in curves 3 and 4; (*d*) the general similarity of all four curves in the 270 to 290 mμ region.



Differential absorption spectra of vitamin B_{12} *versus* vitamin B_{12} "red fragment"; solvent, water

No. 1 –·–·– *p*H 4
No. 2 – – – – *p*H 10
No. 3 ——— *p*H 10 plus potassium cyanide
Refer to text for conditions used to obtain spectra Nos. 1, 2 and 3.
No. 4 ——— "α Fragment" at *p*H 10 with optical zero displaced vertically for clarity.

These facts, in our opinion, support the hypothesis that the benziminazole chromophore is present in the intact vitamin B_{12} molecule, otherwise one would have to postulate a benziminazole precursor with the same absorption spectrum but with a different cyclic structure, which, in the case of this type of compound is most improbable.

LETTER TO THE EDITOR

The lack of resolved fine-structure and of acid-alkali shift in curves (1) and (2), the absence of a band at *ca.* 250 mμ in curve (2) and the presence of resolved fine-structure and the 250 mμ band in (3) are fully consistent with our previous suggestion[6] that in vitamin B_{12} the benziminazole chromophore is co-ordinated to the cobalt atom.

G. H. BEAVEN.
E. R. HOLIDAY.

Medical Research Council,
Spectrographic Unit,
The London Hospital, E.1.
March 11, 1952.

REFERENCES

1. Pfiffner, Dion and Calkins, *Fed. Proc.*, 1952, **11**, in press; *J. Amer. chem. Soc.*, 1952, **74**, in press.
2. For review, see Petrow, *App. Chem. Reports*, 1950, **35**, 339.
3. Beaven, Holiday, Johnson, Ellis, Mamalis, Petrow and Sturgeon, *J. Pharm. Pharmacol.*, 1949, **1**, 957.
4. Holiday, *J. Sci. Inst.*, 1937, **14**, 166.
5. Hardy and Young, *J. Opt. Soc. Amer.*, 1948, **38**, 854.
6. Beaven, Holiday, Johnson, Ellis and Petrow, *J. Pharm. Pharmacol.*, 1950, **2**, 944.

ABSTRACTS (*Continued from page* 340.)

about 200 million organisms per ml., as determined by opacity and autoclaving at 115° C. for half-an-hour. Organisms were also suspended in sterile water, incubated for 16 days at 37° C. and filtered or centrifuged. These preparations were injected into rabbits at the rate of 0·5 ml./kg. Emulsions, filtrates and supernatant liquids from the centrifuge were almost equally pyrogenic. The bacteria tested included 10 known pyrogenic species and some species of *Bacillus*, *Chromobacterium*, *Flavobacterium*, *Pseudomonas*, *Sarcina* and *Staphylococcus* which were isolated from pyrogenic samples of distilled water and classified. All proved to be pyrogenic in rabbits. For some species (for example *Ps. æruginosa*) there was a constant pyrogenic power, while for others (for example *B. subtilis*) it varied according to origin. No correlation was observed between pathogenic and pyrogenic power. G. B.

Pyrogens from Various Bacterial Species. L. G. Ginger, N. M. Nesset, B. Riegel and E. J. Fitzsimons. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 421.) Pyrogenic concentrates have been prepared from *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Serratia marcessens* and *Proteus vulgaris* by subjecting the cellular materials to tryptic digestion. Analysis of these concentrates has yielded information which indicates that there are essential differences in their chemical composition and biological activity; in particular, the concentrate prepared from *B. subtilis* differs distinctly from the other concentrates. At least three possible contaminants are associated with the pyrogenic polysaccharides, namely, nucleic acids, other nitrogenous material, and free lipid. The amount of contaminating nucleic acids in each concentrate is related to the initial nucleic acid content of the cellular material, while the amount of nitrogenous residues varies with the cellular species under consideration. The pyrogenic polysaccharides have been shown to consist of hexosamine, an unclassified reducing sugar, and a non-reducing fraction, but there seems to be no direct correlation between the amount of any of these constituents present in the concentrates and the pyrogenicity of the latter. It is possible that some yet undetermined component is responsible for the observed differences in biological activity. S. L. W.