REVIEW ARTICLE

A CRITICAL ACCOUNT OF THE SPECTROPHOTOMETRIC ESTIMATION OF VITAMIN A

By H. H. Bagnall, B.Sc., F.R.I.C., and
F. G. Stock, B.Pharm., Ph.C., A.R.I.C.

City Analyst's Laboratory, Birmingham

It has been recognised for some time that, because of the presence of variable amounts of irrelevant absorption, the value obtained for the vitamin A content by merely multiplying the extinction coefficient at 328 mμ by a factor cannot be regarded, in the case of many oils, as a correct measure of their activity. A pure, reproducible primary standard, and some means of correcting the initial spectrophotometric measurements were required before the latter could be translated accurately into "units" of vitamin A.

These conditions were fulfilled when, in August, 1949, the Expert Committee on Biological Standardisation appointed by the World Health Organisation issued a report[1] of its Sub-Committee on Fat-Soluble Vitamins, which recommends the use of crystalline vitamin A acetate as the International Standard and outlines the requirements for a suitable physico-chemical method of assay. The new factor of 1900 specified for the conversion of $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 328 mμ to international units is to be used only after a suitable correction for irrelevant absorption has been made.

The spectrophotometric methods outlined in the B.P. Addendum 1951[2] and in the U.S.P. XIV[3] comply with these requirements and recognise in suitable cases the use of a geometrical correction procedure as a means of estimating that portion of the gross absorption at 328 mμ attributable to vitamin A.

The geometrical correction procedure originates in the publications of Morton and Stubbs[4,5,6] being based upon the assumed linearity of the irrelevant absorption over a narrow range on either side of $\lambda_{max.}$ and more specifically on the linearity of three points termed the fixation points; the absorption in between these chosen points may vary irregularly. The absorption curve of pure vitamin A being known, determination of the actual absorption at the three fixation points is sufficient to enable that due to vitamin A to be calculated. By subtracting the "corrected" absorption from the gross the so called "irrelevant absorption" or the absorption due to substances other than vitamin A is obtained.

Before discussing the fundamental question of the absorption curve of pure vitamin A, the attention of the reader should be called to two publications, one by Gridgeman[7] which reviews the earlier literature on the estimation of vitamin A up to about 1944, and another by Morton[8] which brings us up to the time of the introduction of the new International Standard in 1949. The 1951 edition of "Methods of Vitamin Assay"[9]

includes an extensive bibliography and discusses the U.S.P. XIV method in detail. The present year has seen the publication of further important papers, which have introduced several complications.

Over the last few years the accepted standard curve has been that published by Morton and Stubbs.[4,6] The fixation points recommended for a *cyclo*hexane solution of vitamin A acetate were 313 mμ, 328 mμ, and 338·5 mμ and $E_{313\ m\mu} = E_{338\cdot5\ m\mu} = 6/7\ E_{328\ m\mu}$. The absorption curve of vitamin A alcohol differs slightly but significantly from that of the acetate and furthermore the fixation points vary with the solvent used. Chatain and Debodard[10] have suggested further modifications and their curves indicate that their vitamin A was purer than that of Morton and Stubbs.

Cama, Collins and Morton,[11] in what is probably one of the most important publications to date, derive new standard curves for the ester and the alcohol using both synthetic and natural vitmin A, and alter the positions of the fixation points. The spectroscopic properties of all-*trans* vitamin A alcohol and acetate are fully dealt with and the data given should become the accepted standard for these substances. The new fixation points for vitamin A acetate dissolved in *cyclo*hexane are 312·5 mμ, 327·5 mμ and 337·7 mμ, and $E_{312\cdot5\ m\mu} = E_{337\cdot7\ m\mu} = 6/7\ E_{327\cdot5\ m\mu}$. These are the points specified in the B.P., Addendum, 1951, and in addition the old fixation wavelengths, together with an entirely new set with equal wavelength intervals are given, with accompanying equations for correction of the gross E value, both based on the new absorption curve. Attention is drawn to the differences in magnitude of the extinction coefficients for pure vitamin A in different solvents, these being sufficiently great to necessitate the use of different conversion factors according to the solvent used. For vitamin A ester dissolved in *cyclo*hexane the factor becomes 1920.

At this stage it will be profitable to discuss the differences between the methods of the B.P., Addendum, 1951, and the U.S.P. XIV, for it is noteworthy that while the U.S.P. method specifies the saponification of all preparations, so estimating vitamin A *via* the alcohol, the B.P. requires saponification only in the case of cod-liver oil, and the determination is made on the oil itself, i.e., via the ester, in the case of halibut liver oil and dilutions of vitamin concentrates. The unqualified inclusion of all vegetable oil dilutions of vitamin A concentrates under the same heading as halibut liver oil is undoubtedly a weakness of the B.P. method, for reasons which will appear shortly.

Vitamin A concentrates may be prepared by a number of methods:— (i) saponification followed by molecular distillation to give the alcohol; (ii) treatment of this product with a fatty acid to give the ester; (iii) direct molecular distillation of the vitamin A esters; (iv) the Solexol process for the production of ester concentrates (see Stubbs,[12] Neale-May[13] and Hilditch[14]) and (v) synthetic preparations.

One of the largest manufacturing houses in this country prepares a concentrate by method (i), i.e., vitamin A alcohol, which is sold as such. Now if this concentrate were utilised to prepare "Concentrated Solution of Vitamin A, Solution of Vitamin A and D and products of similar

properties" and the correction equation specified under that heading used to estimate the vitamin A, erroneous figures would be obtained. Before attempting, therefore, to apply a geometrical correction procedure in the case of a diluted concentrate of unknown origin, it is important to know whether the vitamin is present in the form of alcohol or ester.

There are several ways of determining the relative proportions of alcohol and ester in a preparation and these are discussed by Kascher and Baxter,[15] who recommend the determination of the distribution between light petroleum and aqueous ethanol. The relative stability of vitamin preparations derived from ester and alcohol concentrates has incidentally been fully discussed by Lindholm and Terp,[16,17,18,19] and they show that the ester is without doubt far superior to the alcohol form in this respect. It is therefore rather surprising that the alcohol should still be commercially available for use in pharmaceutical preparations.

On account of this instability, vitamin A alcohol is seldom encountered in pharmaceutical preparations in the U.S.A., and the manufacturers offer a fair proportion of their concentrates in the form of the acetate. Synthetic vitamin A concentrates are also available in the same form. This complicates vitamin A analysis because vitamin A acetate has properties in between those of the alcohol and the esters of the higher fatty acids. Spectrophotometrically, however, the acetate behaves like the other esters.[20] It is on account of the possibility that vitamin A alcohol might be encountered in pharmaceutical products, some of which are of necessity saponified during the assay as part of the purification process, that the U.S.P. XIV has required saponification of all samples.

The U.S.P. XIV differs from the B.P. Addendum 1951 in using *iso*propanol as solvent, instead of *cyclo*hexane, and it also includes an identity colour test with antimony trichloride, the vitamin A being estimated from a standard curve prepared by the use of the saponified International Standard. The ratio between the values for vitamin A obtained from the colour test and the ultra-violet absorption test should be between 1·00 and 1·30. Most good quality fish oils and their concentrates give a ratio of about 1·15, the systematic discrepancy between the two methods apparently being due to the absence of the so-called neovitamin A from the standard. An allowance of $\pm 0{\cdot}15$ from the mean represents the variability inherent in the two methods.[21] Though less precise than ultra-violet absorption methods, blue colour assays are more specific and more sensitive, and for these reasons have wider applicability. The reproducibility of the method is from 3 to 10 per cent., depending upon the product tested.[9]

Some of the less satisfactory features of the U.S.P. XIV method are discussed by Cama *et al.*[11] For example, the conversion factor for vitamin A acetate in *cyclo*hexane is 1920 and for the alcohol in the same solvent 1910, but for the corresponding *iso*propanol solutions they are 1895 and 1820 respectively. The $E^{1\,\text{per cent.}}_{1\,\text{cm.}}$ $\lambda_{\text{max.}}$ value of the International Standard Preparation using *iso*propanol as solvent would be 5·28, and that of an equivalent solution of vitamin A alcohol 5·49. If this rise in the E value is ignored a solution actually containing 9620

I.U./g. would be accepted as containing 10,000 I.U./g. Cama *et al.*[11] further state that, although saponification in many cases removes most of the irrelevant absorption, with high potency material the process is much less effective, and stress that the more elaborate the chemical manipulation the greater will be the risk of loss. The correction equations can be applied much more successfully to untreated oils in *cyclo*hexane than to their unsaponifiable fractions. Moreover *cyclo*hexane is a more suitable solvent than *iso*propanol because the difference between the spectra of vitamin A and neovitamin A is least in the hydrocarbon solutions. Application of the correction procedure to the unsaponifiable fraction leads to low results, the irrelevant absorption is not strictly linear and there is reason to suspect that isomerisation takes place during saponification. Accordingly Cama *et al.*[11] recommend the use of unsaponifiable extracts for low potency oils of good quality which should then give a "normal" absorption curve, the *E* value of which needs to be corrected only for vitamin A_2. The "corrected" figure on the oil direct will agree closely if adjusted for the presence of neovitamin A. This implies a criticism of the application of correction procedures to cod-liver oil after saponification. For higher potency oils they advise that two or more of the correction formulae should be used. The results, when adjusted for the presence of neovitamin A, will be practically the same as those resulting from the most laborious elimination one by one of interfering substances.

The presence of neovitamin A, mentioned above, further complicates the problem. This isomer of vitamin A, discovered by Robeson and Baxter,[22] differs from vitamin A itself only in the spatial configuration about the double bond nearest the hydroxyl group. Its estimation is based on the more rapid formation of an adduct of maleic anhydride with all-*trans* vitamin A than with neovitamin A and the subsequent use of antimony trichloride; the all-*trans* isomer gives very little colour after this treatment.

Robeson and Baxter[22] report that neovitamin A occurs to the extent of 30 to 40 per cent. of the total vitamin A in a large number of oils examined by them. Meunier and Jouanneteau[23] give figures for the neovitamin A content of only 8 oils, which range from 18 to 65 per cent.; 5 of these vary only from 40 to 50 per cent. Figures of 55 per cent. and 65 per cent. were obtained on a tunny fish liver oil, the former on the oil direct and the latter on the unsaponifiable fraction of the same oil.

Cawley, Robeson, Weisler, Shantz, Embree and Baxter,[24] announcing the commercial synthesis of vitamin A, showed that the synthetic concentrates contain neovitamin A. Since there are a number of possible geometrical isomers of vitamin A it is of interest to note that only the naturally-occurring forms actually resulted. Assays of two synthetic concentrates by the maleic anhydride method indicated that the proportions of vitamin A and neovitamin A present were 1·5 : 1 and 2 : 1. These ratios closely approximate to those earlier reported for fish liver oils. The authors suggested that vitamin A either *in vivo* or *in vitro* is converted, in part, by catalytic agents into neovitamin A, and that therefore the occurrence of neovitamin A in fish liver oils is not necessarily indicative of any

peculiar requirements of the fish for this isomer. Instead, it appears that "vitamin A," physiologically speaking, must be considered as a mixture of the two geometrical isomers. Schwarzkopf, Cahnmann, Lewis, Swidinsky and Wüest[25] in their synthesis of vitamin A reported 36·9 to 40·9 per cent. of neovitamin in their products.

Dalvi and Morton[26] state that the absorption curves for neovitamin A esters closely resemble those of the all-*trans* isomer, except that on the long wave side of 310 mμ they are displaced by 2 to 3 mμ. They state that their experience shows that many natural products contain about 25 per cent. of the total vitamin A in the neo-form, and if this is confirmed it seems likely that for ester concentrates "over correction" need not reach 5 per cent. If for example, the fixation points appropriate to all-*trans* vitamin A acetate be used to "correct" the curve for 100 per cent. neovitamin A esters the result would be about 20 per cent. too low, using *iso*propanol as solvent, and 14 per cent. too low using *cyclo*hexane.

Until very recently it had been assumed that biologically neovitamin A and vitamin A were equivalent. Harris, Ames and Brinkman,[27] however, discussing the biopotency of neovitamin A in the rat, say that repeated bioassays of the two vitamin A isomers in pure form, both free and esterified, have indicated a significant difference in their biological potencies of the order of 20 to 28 per cent., the all-*trans* form having the higher activity. This is in line with recent researches on the carotenoids, the change from a *trans* to a *cis* configuration resulting in a decrease of physiological activity ranging from 25 to 75 per cent. The conversion factor, therefore, of a product containing 1 part of neovitamin A to 2 parts of all-*trans* vitamin A, would be about 5 per cent. to 8 per cent. lower than the conversion factor of a similar preparation of all-*trans* vitamin A. On this basis the correction procedure in the spectrophotometric estimation discounts neovitamin A to about the same extent as does the rat bioassay. More work needs to be done, involving large scale inter-laboratory assays using many more animals, before this statement can be universally accepted. It does, however, indicate that there is a distinct probability that neovitamin A is not so potent biologically as all-*trans* vitamin A.

The question of the so-called "irrelevant" absorption, i.e., that exhibited by other constituents of oils, apart from vitamin A, can now be considered. The principal contributions to this are made by vitamin A_2, kitol, anhydrovitamin(s) A and, most important from the point of view of the public analyst, oxidation products of the vitamin itself. The properties of the first three will be briefly considered.

Vitamin A_2 is present to a greater or lesser extent in most fish liver oils. Its presence gives rise to some distortion of the spectral absorption curves resulting in a "shoulder" effect on the long wave side of 328 mμ. Vitamin A_2 cannot be separated by chromatography but the absorption curve of the blue colour formed with antimony trichloride has a maximum at 693 mμ, which can be used for its estimation. The ultra-violet absorption at 328 mμ due to vitamin A_2 can then be calculated and allowed for. It is eliminated by the correction procedure as "irrelevant" absorption. Shantz[28] reported the isolation of pure vitamin A_2 and gave an account of

its spectroscopic characteristics. Recently Shantz and Brinkman[29] determined its biological activity and concluded that it has approximately 40 per cent. of the activity of crystalline vitamin A as determined by the U.S.P. rat growth procedure. It is stored in the liver less effectively than is vitamin A but does not appear to be converted to vitamin A *in vivo*. The B.P. Addendum 1951 states that the simple form of correction procedure may be affected by the presence of vitamin A_2 in unusual amounts. An indication of its presence is given by its maximum at 351 mμ in the subtraction curve; the amount is usually small and may be neglected, but oils containing abnormal amounts are occasionally met with and care should then be taken. The analyst may in this case be well advised to estimate it and allow for its biological activity, and Cama *et al.*[11] have suggested that the determination of vitamin A_2 may well have to be undertaken as a routine estimation.

Kitol is a major constituent of whale oil and its separation by chromatographic methods is necessary before the vitamin A content of an oil containing it can be estimated. The B.P. monograph on halibut liver oil specifies that the absorption at 300 mμ must not be greater than 75 per cent. of that at 328 mμ (absence of whale-liver oil). It should be noted that in a badly oxidised oil the absorption at 300 mμ may well exceed 75 per cent. of that at 328 mμ even in the absence of whale-liver oil. Gridgeman, Gibson and Savage[30] described a method for the estimation of vitamin A in whale-liver oil, involving a chromatographic separation after saponification, while Barua and Morton[31] preferred to work on the original oil. Kitol is not normally encountered in halibut-liver oil and the two references mentioned are possible methods to follow if a chromatographic method is indicated for reasons other than the presence of kitol.

Anhydrovitamin A_1 is characterised by its three maxima at 351, 371 and 392 mμ. The subsidiary band at 392 mμ is always well defined, but the maximum at 351 mμ is in some samples a pronounced peak and in others only a sharp inflexion. If present to any great extent it causes a "shoulder" effect on the long wave side of 328 mμ but is readily identified by its 392 mμ maximum. It is not removed by saponification and is eliminated by the correction procedure. It is biologically practically inactive.[32]

The vitamin A content of an oil decreases on storage, and this fact is reflected in a corresponding decrease in the gross $E^{1\text{ per cent.}}_{1\text{ cm.}}$ 328 mμ and an increase in absorption on the short wave side of $\lambda_{max.}$ It is believed that vitamin A epoxide is formed, having an absorption maximum in the 270 to 280 mμ region. An interesting feature of the oxidative process, which may more clearly be demonstrated by its artificial acceleration, is the appearance of a so called isosbestic point, or point of constant absorption, at about 290 mμ. Bolomey[33] has already called attention to this phenomenon, which in our opinion might conceivably be used to give an approximate idea of the original vitamin A in an oxidised oil. In some cases in our experience, it would have been extremely interesting to be able to say whether or not the amount of vitamin declared had ever been

present in an oil before oxidation. As oxidation proceeds, vitamin A epoxide in its turn decomposes and at the same time the isosbestic point becomes indefinite.

A rough indication of the quality of an oil is given by the "persistence," defined as the difference between $\lambda_{max.}$ and $\lambda_{min.}$ on the short wave side. As the irrelevant absorption due to oxidation increases, the persistence decreases, and since most of the oils found by a public analyst to be deficient in vitamin A also show evidence of oxidation, it follows that the possibility of accurate analysis is least when it is most important that it should be greatest. This fact must be borne in mind when the respective merits of the two alternative methods of calculation of vitamin A in high potency oils ($E_{1\,cm.}^{1\,per\,cent.}$ 328 mμ greater than 4·0), viz.: gross $E_{1\,cm.}^{1\,per\,cent.}$ 328 mμ $\times$ 1600 and corrected $E_{1\,cm.}^{1\,per\,cent.}$ 328 mμ $\times$ 1900, are considered.

It has been shown empirically that, within the limits of error of the animal experiments, $E_{1\,cm.}^{1\,per\,cent.}$ 328 mμ $\times$ 1600 gives a figure for potency which agrees with the biological value, and Morton[8] states that subsequent work has shown that no better factor for fish-liver oils in general could even to-day be chosen for converting gross $E_{1\,cm.}^{1\,per\,cent.}$ values to international units, although oils showing little irrelevant absorption would be somewhat undervalued and oils exhibiting more irrelevant absorption would be a little over-valued.

The points of view of the manufacturers and the public analyst on this question of potency are essentially different. The former deal with great numbers of oils which are normally fresh and unoxidised, and the gross $E_{1\,cm.}^{1\,per\,cent.}$ 328 mμ $\times$ 1600 will *on average* give the same results as the corrected $E_{1\,cm}^{1\,per\,cent.}$ 328 mμ $\times$ 1900. They will therefore not need to introduce the question of correction of the gross $E_{1\,cm.}^{1\,per\,cent.}$ 328 mμ at all. On the other hand, a large number of the samples submitted to a public analyst under the Food and Drugs Act will have deteriorated owing to oxidation of the vitamin and gross $E_{1\,cm.}^{1\,per\,cent.}$ 328 mμ = 1600 will therefore give a distorted picture, and cannot be recommended for checking label claims, or for testing the compliance of a sample with B.P. requirements. Furthermore, a public analyst has to give a reasonably accurate opinion on a single sample and this would be difficult, not to say impossible, without the use of some sort of correction procedure or separation.

We have discussed this question with a number of manufacturers and find that they prefer to use the gross $E_{1\,cm.}^{1\,per\,cent.}$ 328 mμ $\times$ 1600, partly because of its simplicity and partly on account of the controversy which has arisen over geometrical correction procedures in connection with the basic assumption of the effective linearity of the "irrelevant" absorption curve over the critical range, not to mention the further complications which have been discussed above. In any case, a manufacturer dealing with fresh oils would, taking into account the large quantity passing through his hands, arrive at approximately the same figure for the total vitamin content expressed in international units in the whole bulk whichever method of calculation he used. The consumer, however, with whose interests the public analyst is vitally concerned, purchases a small quantity

of oil, the potency of which calculated from gross $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 328 mμ × 1600 might be either greater or less than the actual value.

The problem is to ascertain whether the correction procedure introduces a larger error or greater uncertainty than is implicit in the multiplication of gross $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 328 mμ by an arbitrary factor, and on balance the present position seems to be that if the new official B.P. Addendum 1951 process using a geometrical correction procedure can be shown to give the same result, within reasonable limits, when carried out on the same oil in different laboratories, then it will become acceptable. If, however, an operator is thinking in terms of a possible variation of ±15 per cent.[34] he will probably conclude that the method is not worth the trouble. On the other hand the public analyst has need of a method which, in the case of a partially oxidised sample, would not necessitate multiplying absorption due to oxidised vitamin by 1600 and returning the quotient as units of vitamin A, but rather would enable him to arrive at a reasonably accurate figure for the vitamin content, which can be compared with the claim made on the label, and with the requirements of the B.P.

As has already been mentioned, the validity of geometrical correction procedures depends on the assumption that the irrelevant absorption at the fixation points is linear, and the controversy over this question arises on account of the fact that this assumption cannot be tested experimentally. Gridgeman,[35] in a very recent publication, discusses the theory of the method, and deals fully with the effect on the reliability of the method of departures from the basic assumptions. It is unfortunate in some respects that the new knowledge made available by Cama *et al.*[11] in their recent publication, and now used as the basis of the method given in the B.P. Addendum 1951 was not accessible when this paper was published. Gridgeman,[35] however, deals with the method as outlined by Morton and Stubbs in 1946-8 and although Cama *et al.*[11] put forward a number of alternative equations and the spectroscopic properties of all-*trans* vitamin A acetate and alcohol given in their paper became standard data, the basic theory of the method remains unaltered. Gridgeman[35] discusses possible errors under the headings of "observational" and "assumptive." Dealing with observational errors, he states that a result which is a function of three *E* values will clearly have wider limits of error than a result depending on one, proceeds to a statistical analysis of the problem, and concludes that for every 1 per cent. of observational error in a gross reading, an error of the order of 8 to 10 per cent. is to be expected in a corrected reading. Assumptive errors are defined as those introduced by departures from the assumed shapes and positions of the two absorption curves, those of vitamin A and the irrelevant material. The departures from the assumed characteristics of the vitamin A curve are discussed under "displacement" and "distortion." The displacement may be artificial due to maladjustment of the wavelength scale or, on the other hand, the natural ester may be slightly different chemically or stereoisometrically, and therefore spectrophotometrically, from pure all-*trans* vitamin A acetate. In our own experience, the wavelength scale can easily get out of adjustment and it is important frequently to check the position of the 4861 Å hydrogen line.

Gridgeman[35] finds that displacement of 1 mμ to the longer wavelengths results in a corrected *E* value 4 per cent. above the true value, while displacement to the shorter wavelengths is not so serious, as 1 mμ implies a corrected *E* value just over 1 per cent. less than the truth. The question of stereoisometric differences and the natural occurrence of neovitamin A have now been dealt with by Dalvi and Morton[26] and can be allowed for. Distortion effects would be produced by errors in engraving on the wavelength scale and we are of course entirely in the makers' hands in this respect, although some check can be made by the use of the mercury lines. The bulk of the evidence on this point, however, indicates it to be very unlikely that the wavelength at any particular point on the scale of a modern photoelectric instrument over the range 310 to 340 mμ will be more than ±0·1 mμ in error. Gibson and Balcom[36] of the U.S.A. National Bureau of Standards publish data on the Beckman instrument and state that the errors are so small that they may probably be neglected in most of the work for which the instrument is used, and can moreover be determined only by the most careful work and after installing an auxiliary indicator line over the wavelength scale to eliminate parallax.

The question as to whether the irrelevant absorption over the critical range is effectively linear can still not be decided, but the effect of possible departures from linearity may be minimised by the use of a number of correction equations instead of only one. Gridgeman[35] discusses this possibility and Cama *et al.*,[11] as mentioned above, deduce a number of equations.

In a consideration of the mechanism of the correction, Gridgeman[35] compares the irrelevant absorption curves obtained from a Solexol concentrate and a halibut-liver oil, and notes the similarity in their general shape. He says that this seems surprising since one is a natural oil and the other a processed concentrate and goes on to discuss the possibility of some degree of error in the assumption made in the correction method, that the absorption curves of the vitamin A fatty acid esters in these oils are identical with the published vitamin A acetate curve. The consensus of opinion seems to be that variations in the fatty acid portion of the molecule will not materially alter the absorption curve. Saponification would avoid any possibility of error in this respect but would introduce disadvantages which have already been discussed. Stereoisomerism causes bigger differences, and the effect of neovitamin A and the method of making allowance for it were described by Dalvi and Morton[26] as mentioned before. Although vitamin A has 4 possible geometric isomers only 2 have been reported as occurring naturally, and it seems highly significant that it is these 2 only that are present in the synthetic vitamin, and furthermore in approximately the same proportions as in the naturally occurring vitamin. Gridgeman[35] concludes that the reliability of the method is *sub judice* and its use requires caution. We should not like to comment on this statement beyond expressing our opinion that the reliability is indeed closely linked with the caution mentioned in the latter half of the statement. Cama *et al.*[11] mention that the simple correction procedure of Morton and Stubbs (1946-1948) has been very widely used,

sometimes with less caution than is required by the plainly stated assumptions on which it rests.

Adamson, Elvidge, Gridgeman, Hopkins, Stuckey and Taylor[34] discuss the precision of the three point correction method. 7 laboratories assayed each of 5 vitamin A oils, readings being made in duplicate on photoelectric instruments. The gross $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 328 mμ values were geometrically corrected for irrelevant absorption, and the conclusion arrived at from a statistical analysis of the results was that the limits of error of a determination of vitamin A content in duplicate by any one of the 7 laboratories were about ± 15 per cent. for $P = 0{\cdot}05$, the corresponding figures for gross E values being ± 2 per cent. As the authors state that the paper is concerned with the reproducibility of the method, the apparent lack of precision implied by this conclusion is, from the public analyst's point of view, somewhat disconcerting, since any statement of vitamin A content which he gives on his official certificate is open to challenge by the defendant's analyst.

Some doubt, however, may be felt about the value of this inter-laboratory test from the point of view of assessing the *possible* precision of the method, if we consider some of the material that was put into the statistical machine in relation to the meaning or value to be attached to what emerges from it. As a practical example of material of very doubtful value, may be mentioned a sample of halibut-liver oil which was submitted to each of the 7 laboratories, the average percentage of irrelevant absorption of which was found to be 10·7 per cent. The figure submitted by one of the laboratories, however, was *minus* 4 per cent., and we cannot help feeling that any results obtained by a laboratory which returns such a figure for an oil which may, on the available evidence, be assumed to show about 10 per cent. irrelevant absorption, would be somewhat unreliable. In any case the inclusion of a nonsense result is unjustified since in practice no conclusions would be drawn from such a result. Possibly insufficient attention was paid to the variability of the results from the laboratory furnishing the nonsense result and from one other. While a precise statement cannot be made in the absence of the original data, it would seem that an analysis based on the more consistent results of the 5 other laboratories might yield a precision estimate significantly lower than that given. As an experiment carried out by a number of laboratories in order to discover *what would happen* if particular oils were analysed by the use of the Morton and Stubbs (1946-48) correction procedure for their vitamin A content, the test is fair enough, but the ± 15 per cent. limits of error in no way indicate the ultimate precision attainable by the use of geometrical correction procedures, since at this stage laboratories differ widely in care, expertise and experience. What in fact the paper of Adamson *et al.* does strongly suggest is the need for certain laboratories to re-examine their methods, with particular attention given to rigid standardisation of technique. In this connection it may be mentioned that the use of three different correction equations as suggested by Cama *et al.*[11] and an increase in the number of replicates would materially add to the accuracy of the estimation and would in fact of itself reduce the limits of error.

The calibration of the spectrophotometer is a necessary basis for all reliable work, and its importance cannot be over emphasised; greater attention paid to this side of the picture will result eventually in errors being reduced to a minimum and the attainment of far better agreement between laboratories. The discussion of results obtained using geometrical correction procedures should have particular reference to the precautions which a laboratory has taken to ensure accuracy.

Gibson and Balcom[36] give an excellent account of the Beckman instrument and the precautions to be taken to attain results of high reliability. Rawlins and Wait[37] also discuss factors affecting its reliability and precision and conclude that by careful observance of all the variations the determination of the *E* value may be made with considerable precision, in their case, for example, with a standard deviation of 0·22 per cent. They suggest the use of control charts for laboratories doing large numbers of vitamin A determinations, which not only serve as a check on the technique of the operator but are invaluable in detecting incipient trouble in the equipment.

The best known and most popular solutes in use for calibration purposes are potassium chromate and dichromate. The United State Department of Commerce National Bureau of Standards, however, discusses methods of checking the calibration of spectrophotometers in Letter Circular L.C.929[38] and the reader is alse referred to Mellon's[39] very useful book which contains much valuable information.

The reproducibility in any one laboratory can be made to approach the ±0·4 per cent. mentioned in the B.P. Addendum 1951 by giving careful attention to all the relevant factors. The trouble begins with interlaboratory assays, and the results obtained by the Photoelectric Spectrometry Group,[40] using 28 Beckman instruments on two solutions of potassium nitrate, make rather startling reading, but it does seem that with improved techniques the variation can be reduced. Preliminary reports[41] of the second P.S.G. collaboration test, using potassium dichromate, reveals slightly lower errors than in the first test, but the position is still far from satisfactory. For vitamin A work it is advisable that the instrument be checked against the International Standard Preparation of vitamin A acetate.

The results obtained by us on our Unicam instrument are given in Table I.

These results were obtained without previous knowledge of the figures of Cama *et al.*,[11] which were not available at the time. The International Standard Preparation, using the diluent oil as compensator and *cyclo*hexane as solvent would be expected to have an $E^{1\%}_{1\,cm.}$ 328 mμ value of 5·21 (× 1920 = 10,000 I.U./g.). Three determinations were made, using separate weighings from different capsules and, rather than assume that each capsule contained exactly 0·250 g. of material, we weighed a known amount from each capsule and compensated with the same concentration of diluent oil. The results were 5·104, 5·099 and 5·112, having a mean of 5·11 which agrees closely with the value of 5·09 on a weighed amount, obtained by Cama *et al.*[11] The International Standard Preparation

TABLE I

λ mμ	Vitamin A acetate in *cyclo*hexane (Cama, Collins and Morton, 1951) $\frac{E\lambda}{E\lambda_{max.}}$	International Standard Preparation Compensator: cotton seed oil Solvent: *cyclo*hexane $\frac{E\lambda}{E\lambda_{max.}}$
295	0·448	0·447
300	0·555	0·555
305	0·670	0·667
310	0·806	0·806
311	0·830	0·829
312	0·846	0·846
312·5		0·857
313	0·867	0·867
315	0·894	0·890
317·5		0·916
320	0·935	0·937
322·5		0·965
325	0·985	0·988
326	0·993	0·995
327	1·000	0·998
328	1·000	1·000
330	0·989	0·991
335	0·915	0·914
338	0·853	0·857
338·5		0·843
340	0·811	0·814
345	0·695	0·700
350	0·556	0·562

according to these figures is apparently 2 per cent. deficient in activity. There is certainly something abnormal about it, because continuation of the absorption curve over the short wave side of 300 mμ shows a very marked departure from the Standard vitamin A acetate curve. In these circumstances the International Standard cannot be considered as an absolute reference standard for spectroscopic purposes and it would not be feasible to consider the use of a factor to raise the apparent 98 per cent. to 100 per cent. Nevertheless the Standard over the range 300 to 340 mμ is very useful as a means of checking the ratio $E\lambda/E_{max.}$ at a particular wavelength and the values obtained agree very closely with the figures of Cama *et al.*[11] for pure all-*trans* vitamin A acetate.

In our view the spectroscopic procedure given in the B.P. Addendum 1951 is not to be regarded as an absolute standard method, but rather as indicating the broad principles which must be followed. One thing is certain—the analyst must allow for irrelevant absorption before using the 1900 factor; whether he does this by geometrical correction or by a separation method is left to his own particular fancy. In point of fact the onus is now upon the analyst to ascertain the true vitamin A content by using the method appropriate to the sample in hand, for each one may exhibit its own peculiar problems and must be treated on its own merits.

We believe the method has actually been indirectly official for some time. The B.P. 1948 stated that "an expression of the content of vitamin A . . . in Units per g. is obtained by multiplying the *ultra-violet absorption* $E^{1\text{ per cent.}}_{1\text{ cm.}}$ by the factor declared by the Permanent Commission on Biological Standardisation of the Health Organisation of the League of Nations as the factor to be used for this purpose." Since the World Health Organisation adopted the 1900 factor in August, 1949, it indirectly became official in the B.P. from that date, conditions under which the

factor is applicable and an indication of how, in principle, irrelevant absorption may be allowed for, including the use of geometrical correction procedures, being embodied in the report of the W.H.O.

For the estimation of vitamin A in halibut-liver oil and vegetable oil dilutions of concentrates we have followed the B.P. Addendum 1951 method with the following modifications, viz., all 3 correction equations recommended by Cama *et al.*[11] were used with each of 3 separate weights of oil dissolved in *cyclo*hexane so obtaining 9 "corrected" values for $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 327·5 mμ. The use of more than one set of fixation points goes some way to meet Gridgeman's criticism that the irrelevant absorption may not be strictly linear, for departures from linearity will be reflected in an increase in the fiducial limits of the mean, and so the effect will be minimised to some extent. The P = 0·05 fiducial limits of the mean of the 9 values obtained for $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 327·5 mμ were then calculated.

We found the reproducibility of a gross *E* value on our Unicam instrument to be about $\pm$0·5 per cent. and the P = 0·05 fiducial limits of the mean of 9 corrected *E* values in the region of $\pm$1 to 2 per cent.

The correction procedure eliminates the effects of irrelevant absorption and the resulting corrected *E* value only needs to be adjusted for neovitamin A by the use of a correction factor of 1·04. It is not possible to be dogmatic about this factor, but it has a reasonably factual basis. If, of course, it is subsequently shown, as seems possible, that neovitamin A is less potent biologically than vitamin A, the correction would no longer be necessary. Since we used *cyclo*hexane as solvent the conversion factor of 1920 was employed and our figures are therefore about 5 per cent. higher than those obtained by the strict use of the B.P. Addendum 1951 method, but we feel that we should in fairness to manufacturers give them every possible advantage. On the other hand, they will probably find it advisable to use the B.P. Addendum 1951 procedure, secure in the knowledge that they have a possible additional overage in reserve.

It should be noted as a matter of strict accuracy, that vitamin A_2 has some biological activity, but that its effect on the *E* value is eliminated by the use of the method described. If therefore it be present in abnormal amounts it should be estimated and its equivalent in terms of vitamin A determined and included in the final figure.

Finally we should like to express our thanks to Professor R. A. Morton, F.R.S., and his colleagues at Liverpool University, for their kindness in allowing us access to the recent paper by H. R. Cama, F. D. Collins and R. A. Morton on all-*trans* vitamin A and another by P. D. Dalvi and R. A. Morton on neovitamin A, in both cases prior to publication.

References

1. *World Hlth. Org. techn. Rep. Ser.*, 1950, 3.
2. *Addendum* 1951 *to the British Pharmacopœia*, 1948, *Appendix XVA*, pp. 92–93.
3. *United States Pharmacopœia XIV*, Vitamin A Assay—Spectrophotometric Method, p. 784.
4. Morton and Stubbs, *Analyst*, 1946, **71**, 348.
5. Morton and Stubbs, *Biochem. J.*, 1947, **41**, 525.
6. Morton and Stubbs, *ibid.*, 1948, **42**, 195.
7. Gridgeman, *The Estimation of Vitamin A*, Lever Bros., and Unilever Ltd., 1945.

8. Morton, *J. Pharm. Pharmacol.*, 1950, **2**, 129.
9. The Association of Vitamin Chemists, Inc., *Methods of Vitamin Assay*, Interscience Publishers, Inc., New York, 1951.
10. Chatain and Debodard, *C.R. Acad. Sci., Paris*, 1950, **231**, 1102.
11. Cama, Collins and Morton, *Biochem. J.*, 1951, **50**, 48.
12. Stubbs, *The Propane Segregation of Glyceride Oils, South African Industrial Chemist*, January, 1951.
13. Neale-May, *Production and Processing of Marine Drying Oils, ibid.*, August, 1950.
14. Hilditch, *Industrial Chemistry of Fats and Waxes*, Ballière Tindall and Cox, London, 1949.
15. Kascher and Baxter, *Ind. Engng. Chem., Anal. Ed.*, 1945, **17**, 499.
16. Terp, *Arch. Pharm. Chem.*, 1948, **55**, 513.
17. Nilou, Lindholm and Terp., *ibid.*, 1950, **57**, 332.
18. Lindholm and Terp, *ibid.*, 1950, **57**, 480.
19. Lindholm and Terp, *ibid.*, 1950, **57**, 661.
20. Lehman, *private communication.*
21. "*U.S.P. XIV and the Vitamin A Business*," *Distillation Products Industries N.Y.*, December, 1950.
22. Robeson and Baxter, *J. Amer. chem. Soc.*, 1947, **69**, 136.
23. Meunier and Jouanneteau, *Bull. Soc. chim. biol. Paris*, 1948, **30**, 260.
24. Cawley, Robeson, Weisler, Shantz, Embree and Baxter, *Science*, 1948, **107**, 346.
25. Schwarzkopf, Cahnmann, Lewis, Swidinsky and Wüest, *Helv. chim. Acta.*, 1949, **32**, 443.
26. Dalvi and Morton, *Biochem. J.*, 1951, **50**, 43.
27. Harris, Ames and Brinkman, *J. Amer. chem. Soc.*, 1951, **73**, 1252.
28. Shantz, *Science*, 1948, **108**, 417.
29. Shantz and Brinkman, *J. biol. Chem.*, 1950, **183**, 467.
30. Gridgeman, Savage and Gibson, *Analyst*, 1948, **73**, 662.
31. Barua and Morton, *Biochem. J.*, 1949, **45**, 308.
32. Shantz, Cawley and Embree, *J. Amer. chem. Soc.*, 1943, **65**, 901.
33. Bolomey, *J. biol. Chem.*, 1947, **169**, 331.
34. Adamson, Elvidge, Gridgeman, Hopkins, Stuckey and Taylor, *Analyst*, 1951, **76**, 445.
35. Gridgeman, *ibid.*, 1951, **76**, 449.
36. Gibson and Balcom, *J. opt. Soc. Amer.*, 1947, **37**, 593.
37. Rawlings and Wait, *Oil and Soap*, 1946, **23**, 83.
38. *U.S. Dept of Commerce, National Bureau of Standards, Letter Circular*, LC929, November 26, 1948.
39. Mellon, "*Analytical Absorption Spectroscopy*," John Wiley & Sons, Inc., New York, 1950.
40. *Photoelectric Spectrometry Group Bulletin*, No. 1, April, 1949.
41. *Ibid.*, No. 3, October, 1950.

RESEARCH PAPERS

THE ANALYSIS OF PHARMACOPŒIAL SAMPLES OF ADRENALINE; A LIMIT TEST FOR NORADRENALINE IN ADRENALINE

By G. B. West

From The Department of Pharmacology and Therapeutics, University of St. Andrew's Medical School, Dundee

Received October 17, 1951

It is now well established that noradrenaline is present with adrenaline in the suprarenal glands of most mammals, although its relative amount varies from species to species. Tullar[1] in 1949 was the first worker to isolate *l*-noradrenaline from suprarenal extracts, and in the same year Goldenberg and his co-workers[2] showed by paper chromatography that preparations of adrenaline complying with the requirements of the United States Pharmacopœia may contain up to 36 per cent. of noradrenaline. The biological assay of adrenaline in the United States Pharmacopœia is based on the pressor effect in dogs, and since noradrenaline is about 1·5 times more pressor than adrenaline, these two amines would not be differentiated by this test. The monograph on adrenaline in the British Pharmacopœia, 1948, states "it may be prepared from an acid extract of the suprarenal glands of certain mammals, or by synthesis." The difficulties of synthesis and the search for cortisone-like activity in the adrenal cortices of mammals make the natural product still a practicable proposition in this country.

Two of the chief therapeutic uses of adrenaline are in circulatory collapse and in asthma. Whereas noradrenaline as well as adrenaline has already proved valuable in combating low blood pressure in operational shock, noradrenaline is considerably less active than adrenaline in producing relaxation of the circular fibres of the bronchioles in the treatment of asthma. Inaccurate dosage may result, therefore, when a natural adrenaline (containing much noradrenaline) is used in the treatment of these two conditions. For these reasons, we have investigated the nature of four pharmacopœial samples of natural adrenaline purchased on the open market in this country and of one similar sample from the United States. The standard material used was a sample of pure synthetic adrenaline. The results suggest that a limit should be made on the amount of noradrenaline present in samples of natural adrenaline so as to achieve accurate dosage.

Experimental

All samples were dissolved in 0·1N hydrochloric acid and diluted with water to give a 1 in 1000 solution in 0·01N hydrochloric acid. Biological assays for adrenaline and noradrenaline contents were completed on the spinal cat by the method of Burn, Hutcheon and Parker.[3] The chemical assays were performed by the method of Euler and Hamberg,[4] which is based on the formation of coloured compounds with iodine. Paper chromatography was carried out using the ascending method with

butanol-acetic acid-water as solvent and potassium iodate or ferricyanide[5] as developer. In each experiment, duplicate strips of paper were used but these were not developed after drying. Instead, the areas on the paper corresponding to the position of the standard adrenaline (R_F value 0·36) and noradrenaline (R_F value 0·28) spots were each cut out, extracted with 0·01N hydrochloric acid, and tested on the isolated rabbit ileum against standard solutions of the two amines. Determination of the specific rotation and melting-points of all specimens gave values lying within the pharmacopœial limits.

TABLE I

BIOLOGICAL AND CHEMICAL DATA ON SAMPLES OF ADRENALINE OF NATURAL ORIGIN, EXPRESSED AS PERCENTAGE ACTIVITY COMPARED WITH STANDARD SYNTHETIC ADRENALINE AND NORADRENALINE

Sample	Colour	Adrenaline per cent.		Noradrenaline per cent.		
		Biological	Chemical	Biological	Chemical	Chromatography
1. British	Light brown	100	89	0	11	3
2. British	Pale buff	92	84	8	16	9
3. British	Light brown	90	88	10	12	10
4. British	Almost white	100	93	0	7	0
5. American ..	Very pale buff	81	73	19	27	20

RESULTS

The results of the assays on the samples of natural adrenaline are shown in Table I. The values for noradrenaline percentages obtained by biological assay and by paper chromatography showed good agreement, but chemical assays gave raised values, suggesting the presence of an interfering substance. The one American sample (U.S.P. Reference Epinephrine Standard—1949) contained much more noradrenaline than that found in any of the four British samples. We suggest that a limit of 10 per cent. of noradrenaline be imposed on samples of adrenaline; by this means, adequate dosage would be achieved in the treatment of both circulatory collapse and asthma. A suitable method of testing samples is described below.

Limit test for noradrenaline in adrenaline. The chromatogram is carried out by the ascending method in a glass tank of suitable size (e.g., 15 × 7 × 22 in.) containing the solvent to a depth of $\frac{1}{2}$ in. The solvent is prepared by shaking *n*-butanol (4 vol.), glacial acetic acid (1 vol.) and water (5 vol.) together and discarding the lower layer. The butanol and acetic acid are of ordinary reagent quality and need not be purified before use. Sheets of Whatman No. 4 filter paper (up to 12 in. in width) are suspended from horizontal glass rods placed 18 in. above the liquid surface, the paper being kept taut by means of a thin glass tube (up to 12 in. in length), closed at both ends and threaded through vertical slits $\frac{3}{8}$ in. long and 3 in. apart cut near the bottom of the sheet. The top of the tank is sealed (e.g., by a vaselined glass plate).

Test solutions are applied from a graduated glass syringe as single drops each 0·01 ml., 1 in. apart, along a line 2 in. from the foot of the paper and at least 2 in. from the lateral edges of the paper where the flow tends to be erratic. When the drops have dried, chromatography is carried out at

room temperature for 18 hr. during which time the solvent travels approximately 12 in. from the starting line. After drying for 15 minutes in a cabinet through which a current of warm air circulates, the paper is sprayed with a 1 per cent. w/v aqueous solution of potassium iodate. On development in an air oven at 100° to 110° C. for not more than 2 minutes, adrenaline and noradrenaline are rendered visible as pink and violet spots respectively. Adrenaline (R_F value 0·36) travels slightly faster than noradrenaline (R_F value 0·28) so that separate spots are usually seen. Care must be taken however to avoid overheating which causes both spots to become brown and indistinguishable by colour. At room temperature, the spots assume a uniform brown colour within a few hours. By this technique, spots containing 1 μg. of adrenaline or 2 μg. of noradrenaline can be readily detected.

For the test, 0·01 ml. of a 1 in 1,000 solution of the sample and of synthetic adrenaline in 0·01N hydrochloric acid (i.e., 10 μg. each) are chromatographed. On developing the paper, no violet spot at R_F 0·28 should be visible (indicating under 1 μg. of noradrenaline). In addition, controls of 0·01 and 0·02 ml. of a 1 in 10,000 solution of standard synthetic noradrenaline (i.e., 1 and 2 μg.) may be used to indicate the sensitivity of the method and the correct position and colour of the noradrenaline spot.

Discussion

The results of the assays on samples of adrenaline of natural origin clearly indicate that one-fifth of the activity may be due to noradrenaline. Since this latter amine is much less effective in relaxing bronchial muscle and in causing glycogenolysis than is adrenaline, it is advisable to restrict its concentration. Besides, noradrenaline predominates in the adrenal glands of whales, of young calves and bullocks, and of most other young mammals, and all of these are possible sources of pharmacopœial adrenaline.

Concerning the chromatographic limit test, mention should be made of the fact that separation of adrenaline and noradrenaline by a similar technique was reported[5] in 1948. In the original ferricyanide method, the spots are viewed against a yellow background; in the suggested limit test using potassium iodate, the spots are viewed against a white background and sensitivity is thereby increased.

Summary

1. 5 samples of pharmacopœial adrenaline of natural origin have been subjected to chemical, biological, and chromatographic examination. Noradrenaline was found in 4 of the samples, in amounts representing up to 20 per cent. of the total activity.

2. A limit test for noradrenaline in adrenaline is described using paper chromatography. It is suggested that pharmacopœial adrenaline should contain not more than 10 per cent. of noradrenaline.

References

1. Tullar, *Science*, 1949, **109**, 536.
2. Goldenberg, Faber, Alston and Chargaff, *Science*, 1949, **109**, 534.
3. Burn, Hutcheon and Parker, *Brit. J. Pharmacol.*, 1950, **5**, 142.
4. Euler and Hamberg, *Science*, 1949, **110**, 561.
5. James, *Nature, Lond.* 1948, **161**, 851.

THE SEPARATION AND IDENTIFICATION OF SOME BARBITURATES BY PAPER PARTITION CHROMATOGRAPHY

By Alf Wickström and Bjarne Salvesen

From the Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Oslo

Received October 31, 1951

When barbiturates are extracted from biological materials, micro-methods are usually required for their identification. Many workers have contributed to the technique of micro-identification of the barbituric acid derivatives that are used in therapeutics. Among the more recently described methods should be mentioned optical crystallography[1] and the observation of X-ray diffraction powder patterns.[2] The purpose of the present paper is to report some results obtained by application of the technique of paper partition chromatography[3] to the problem of micro-identification of barbituric acid derivatives. In view of the success of this technique in other fields, there was *a priori* good reason to believe that results of interest would emerge. Moreover, the difficulties which may occur in crystallographic analysis or in melting-point determinations because of the polymorphism of some barbituric acid derivatives, should be eliminated by the use of partition chromatography. As far as the chemical literature is known to the authors, the paper chromatographic separation of the barbiturates commonly used in therapeutics has not as yet been made the subject of a general experimental study. A short outline of the paper partition chromatography of barbiturates and other pharmaceutical products has been given by Alvarez de la Vega[4] and Raventós[5] has described the separation of barbiturates from thiobarbiturates by chromatography on alumina column and successive elution.

The authors have developed chromatograms of 8 commonly used barbiturates and 2 thiobarbiturates with different organic solvents saturated with water. When chromatograms of barbiturates are run with a neutral solvent and with water as the stationary phase, no distinct spots can be obtained because of the ionisation of the acids. This difficulty, well known from previously reported paper chromatographic studies of other organic acids, may be overcome by adding a stronger acid[6,7,8] or ammonia[9,10,11] to the solvent system. To eliminate the ionisation effect in the chromatograms of barbituric acid derivatives, we have used formic acid or acetic acid, ammonia or pyridine. Most of the solvent systems, acid or alkaline, which have been examined in the present work, prove to give a quite unsatisfactory separation of the barbituric acid derivatives. Some of these unsuccessful solvent systems are shown in Table I, as the negative results should be considered useful for further investigation in this field. A good separation of the barbituric acid and thiobarbituric acid derivatives that have been subjects for this study, is obtained in toluene-acetic acid-water (100 : 40 : 50), and in chloroform-10 per cent. ammonia (100 : 50); the separation is fairly good for some of the substances in *n*-butanol—10 per cent. ammonia (100 : 35). The R_F values observed

TABLE I

Solvent system, alkaline mobile phase	Toluene-water-pyridine 100 : 30 : 50	Toluene, 10 per cent. ammonia 100 : 50	Chloroform (B.P.)-water-pyridine 100 : 20 : 40
R_F values at 18° to 20° C. for substances 1, 2, 3, 4, 9, 10	All about 0·95	All about 0, except No. 4	All about 0·92
Solvent system, acidified mobile phase	*n*-Butanol-water-acetic acid (95 per cent.). 40 : 50 : 10	Chloroform (B.P.)-methanol-water-formic acid (90 per cent.). 100 : 80 : 60 : 20	Chloroform (B.P.)-water-formic acid (90 per cent.). 100 : 50 : 20
R_F values at 18° to 20° C. for substances 1, 2, 4, 5, 6, 8	All about 0·90	All between 0·85 and 0·96	All between 0·75 and 0·96

TABLE II

Substance number	Barbituric acid derivatives. Substituents			R_F values at 19° ± 1° C.		
	5	5	1	Toluene-water-acetic acid 100 : 50 : 40	*n*-Butanol-10 per cent. ammonia 100 : 35	Chloroform-10 per cent. ammonia 100 : 50
1	Allyl	Allyl	—	0·52	0·64	0·07
2	Allyl	*iso*propyl	—	0·61	0·69	0·18
3	Ethyl	*Cyclo*hexenyl	—	0·69	0·69	0·22
4	Methyl	*Cyclo*hexenyl	Methyl	0·90	0·75	0·90
5	Ethyl	Ethyl	—	0·26	0·50	0·03
6	Ethyl	Phenyl	—	0·50	0·61	0·06
7	Ethyl	1-Methylbutyl	—	0·74	0·79	0·64
8	Ethyl	Phenyl	Methyl	0·90	0·75	0·82
	Thiobarbituric acid derivatives. Substituents					
9	Allyl	*iso*propyl	—	0·86	0·77	0·36
10	Ethyl	*Cyclo*hexenyl	—	0·89	0·80	0·50

on chromatograms developed with these three solvent systems are shown in Table II. The R_F values reported in Table II are mean values from series of chromatograms where 25 μg. of the barbiturates have been employed and the temperature has been maintained at 18° to 20° C. during the experiments. For the detection of the spots 4 different spraying reagents have been used. Thiobarbituric acid derivatives will appear as black spots on spraying with ammoniacal silver nitrate and heating, and as green spots on spraying with a solution of diethylamine and copper sulphate in methanol. Barbituric acid derivatives with unsaturated substituents will reduce a dilute solution of potassium permanganate sprayed on the chromatogram. The positions of the saturated barbituric acid derivatives have been revealed by means of a precipitating reagent, mercuric sulphate solution.

In some cases two barbiturates, whose R_F values under identical conditions do not differ sufficiently to make it possible to confirm their separation, may be differentiated by spraying with the various reagents described in this paper.

For the unambiguous identification of the barbiturates listed in Table II replicate chromatograms should be run with at least 2 of the different

solvent systems and further replicate chromatograms should be sprayed with the various reagents. Since absolute R_F values are not given in this paper, measurements of R_F values should not be considered as sufficient for the definite identification of a barbituric acid derivative, but reference substances should be included in the chromatograms of the unknown substance.

Experimental

Apparatus and Technique. The apparatus for descending chromatograms and the technique utilised in the present work, have been described in detail by Partridge[12] and later by other authors.[8] The same technique has been employed in chromatographic work previously published by this laboratory.[13,14]

Each barbituric acid derivative is dissolved in chloroform to obtain a 0·5 per cent. solution. Melting-point determinations under the microscope are used to control the purity and identity of the samples employed. 5 μl. or 10 μl. of the 0·5 per cent. solutions, corresponding to 25 or 50 μg. of the barbiturates were applied on the "starting line," drawn on Whatman filter paper No. 1, cut in rectangular sheets, 20 × 56 cm. Two of the sheets thus prepared are then suspended from the steel trough (without solvent), which is supported in the glass chamber. At the bottom of the chamber is placed a liberal volume of each phase of the solvent system, that is to be used. The paper and the atmosphere within the carefully closed, airtight chamber are now brought into vapour equilibrium with the solvent system, by allowing it to stand for 15 to 20 hours (over-night) at 18° to 20° C. After this equilibration period the non-aqueous phase of the solvent system is added to the trough and this mobile phase is allowed to flow down the paper till about 45 cm. from the "starting line." This movement will require at 18° to 20° C. about 3 hours for toluene, 18 hours for *n*-butanol, and 4 hours for chloroform. The solvent systems are prepared by shaking for 1 minute in a separating funnel the following mixtures, which are then allowed to separate by standing for 15 to 20 hours at 18° to 20° C.: (1) Toluene (b.pt. 109° to 110° C.)—water—acetic acid (95 per cent.) (100 : 50 : 40 by weight). (2) *n*-Butanol (b.pt. 116° to 177° C.)—ammonia (10 per cent.) (100 : 35 by weight). (3) Chloroform—ammonia (10 per cent.) (100 : 50 by weight).

Commercial chloroform, stabilised with ethanol, may be used, if reference substances are included in the chromatograms. The observed R_F values will then depend upon the ethanol content of the sample of commercial chloroform used in the experiment. A greater reproducibility of the experiments is obtained when ethanol-free, anhydrous chloroform is used. The R_F values reported in Table II are obtained by using alcohol-free, anhydrous chloroform, which should be freshly prepared, since alteration products of this unstabilised chloroform may undergo reactions with ammonia. It should be mentioned that the chloroform-ammonia system seems to be very sensitive to temperature variations during the equilibration period for the chamber atmosphere, and a shorter equilibration period (4 to 6 hours) may be used before running the chromatograms,

if temperature variations may thus be prevented. The chromatograms are dried in the air at room temperature and the spots are then revealed by the following spraying reagents.

Spraying reagents. (1) Thiobarbiturates will appear as black spots with ammoniacal silver nitrate. The paper is sprayed with a mixture (10 ml.) of equal volumes of 0·1N silver nitrate and 10 per cent. ammonia, and dried for 15 minutes at 95° C. Barbiturates may now appear as white spots (silver salts) on a yellow background; thio-barbiturates will usually not appear. The paper is then sprayed with more of the same reagent. Thiobarbiturates will now appear as black spots, immediately or after 5 minutes at 95° C.

(2) The paper is sprayed with a mixture of equal volumes of diethylamine 10 per cent. in methanol (99 per cent.) and a saturated solution of anhydrous copper sulphate in 90 per cent. methanol. Green spots indicate the position of the thiobarbiturates. The colour is best observed in daylight and will be visible for at least 20 hours. This colour-reaction has been described as fairly specific for thiobarbiturates by Raventós.[5]

(3) The paper is sprayed with a 0·02N solution of potassium permanganate. Yellow spots on a red background will appear immediately for barbituric acid derivatives with substituents containing a double carbon to carbon bond in open chain. If the reducing effect is produced by the hexenyl group, the spots will appear 1 to 2 minutes after spraying and will be well developed after 3 minutes.

(4) The saturated barbituric acid derivatives will appear as white spots (precipitation) when the paper is irrigated with a solution of mercuric sulphate. 5 g. of mercuric oxide is dissolved in 100 ml. of water and 20 ml. of concentrated sulphuric acid (the mercuric sulphate solution of the Danish Pharmacopeia IX). This solution is diluted with an equal volume of water before use. The reagent should not be sprayed on the chromatogram, since inhalation of the finely dispersed drops of this reagent will be dangerous. The spots are therefore revealed by the following method. A sheet of filter paper (Whatman No. 1) is dipped into the reagent and then placed on a clean glass plate. Another sheet of filter paper is pressed gently against it to absorb any excess of the reagent. The chromatogram is then placed between the two irrigated paper sheets on the glass plate and pressed gently with the hand until the reagent has penetrated it completely. The saturated barbituric acid derivatives (substances No. 5, 6, 7, 8) will appear as white spots that are well defined and easy to observe. The unsaturated barbituric acid derivatives, except substance No. 3, will not appear, probably because of the greater solubility of their mercuric complex in excess of the reagent.

Other spraying reagents which have been tried, failed to give satisfactory results. Detection of spots by means of an indicator proved to be difficult because of the weak acid character of the barbituric acid derivatives. The colour reaction of barbiturates with cobalt acetate and ammonia or an amine was examined under varying conditions. A spraying reagent based upon this reaction, that will give distinct coloured spots with less than 100 μg. of barbiturate in the chromatograms, has not as yet been found.

Summary

1. Separation of 8 barbituric acid derivatives and 2 thiobarbituric acid derivatives by paper partition chromatography with 3 different solvent systems is described and the observed R_F values are reported.

2. The preparation of various spraying reagents, which may be used to differentiate substances showing approximately the same R_F value under identical conditions is described.

3. The results are considered useful for micro-identification purposes, when barbiturates are isolated from biological materials.

References

1. Castle, *J. Amer. pharm. Ass. Sci. Ed.*, 1949, **38**, 47.
2. Tso-Yueh Huang, *Acta Pharm. Internat.*, 1951, **2**, 43.
3. Consden, Gordon and Martin, *Biochem J.*, 1944, **38**, 224.
4. Alvarez de la Vega, *Galenica Acta*, 1949, **2**, 85. *Chem. Abstr.*, 1949, 8094.
5. Raventós, *Brit. J. Pharmacol.*, 1946, I, 210. *Chem. Abstr.*, 1947, 1269.
6. Lugg and Overell, *Nature, Lond.* 1947, **160**, 87.
7. Lugg and Overell, *Austral. J. Sc. Res.*, 1948, **1**, 98.
8. Jermstad and Briseid Jensen, *Pharm. Acta Helvet.*, 1950, **25**, 209.
9. Brown, *Nature, Lond.* 1951, **167**, 441.
10. Brown and Hall, *Nature, Lond.* 1950, **166**, 66.
11. Kennedy and Barker, *Anal. Chem.*, 1951, **23**, 1033.
12. Partridge, *Biochem. J.*, 1948, **42**, 238.
13. Courtois and Wickström, *Bull. Soc. Chim. Biol.*, 1950, **32**, 759.
14. Hérissey, Wickström and Courtois, *Bull. Soc. Chim. Biol.*, 1951, **33** (in press).

A NOTE ON THE GRAVIMETRIC DETERMINATION OF SANTONIN

By N. A. Qazilbash

From the Department of Botany, Islamia College, Peshawar

Received July 27, 1951

Santonin is extracted from the leaves and unopened flower-heads of a number of santonin-containing species of Artemisia. The most important santonin-containing species are :—*Artemisia Cina* (Berg) Willkomm, *Artemisia pauciflora* Weber, *Artemisia brevifolia* Wall and *Artemisia kurramensis* Qazilbash.[1] They are confined to different geographical areas, and differ from one another as regards ecological, morphological and physiological features. Santonin in the plant tissue is closely associated with resinous, oily and fatty substances. The relative proportion of santonin, oils, fats and resins varies remarkably in different species. The synthesis of chemical constituents in the plant is dependent upon its physiological activities. The normal physiological functions are greatly influenced by various ecological factors such as temperature, solar radiation, altitude, atmospheric humidity, soil composition and water-availability. Prolonged influence of abnormal ecological conditions bring about remarkable changes in the nuclear structure of the cells, and these changes invariably are followed by notable morphological and physiological changes. Under the influence of ecological factors, a particular santonin-containing species may show a remarkable variation in its santonin content. Individuals of the same species, when growing under different ecological conditions, show great differences in the relative proportions of santonin, oils and resins. Some of them contain a very high proportion of resinous and oily substances. The complete separation of santonin from these substances is rather a difficult task for which different workers have employed special techniques. The important gravimetric methods fall into two main groups:—(1) methods by which a soluble santonate is formed; (2) methods using a solvent for the primary extraction of santonin. The numerous suggested methods yield results of varying accuracy as judged by the amount and the purity of the final santonin product. The purity of the final product is determined by the colour and the melting-point. Pure santonin is white, and the m.pt. varies from 171° to 174° C. The efficiency of a particular method can easily be ascertained by taking into consideration the weight and purity of the final santonin-product. The weight should be consistent with the theoretical values of the control experiments. The purity of the final product can be determined by noting the colour and the m.pt. Impure and contaminated product is a coloured material, with m.pt. lower than 171° C.

Methods by which a Soluble Santonate is Formed

Trommsdorff,[2] Miahle and Claud,[3] Calloud,[4] Wettstein,[5] Grosschopff,[6] Hirsch,[7] Busch,[8] Soteria,[9] Sestine[10] and Massagetov,[11] extracted santonin from the plant as calcium santonate after treating it with a mixture of calcium

oxide or calcium hydroxide in water, dilute ethanol or ethanol. The solution is acidified with hydrochloric acid, acetic acid, or carbon dioxide and the santonin liberated. Massagetov, Sestine and Soteria extracted the liberated santonin from the acidified solution with chloroform. Other workers dissolved the liberated santonin in ethanol, and the ethanolic solution, after treatment with a suitable purifying reagent (animal charcoal, lead acetate, sodium carbonate, or zinc sulphate), was filtered, the fatty and resinous substances being removed. Santonin crystals were recovered from dilute ethanolic solution.

Sestine pretreated the drug with light petroleum, which not only removes the oily substances but also extracts a portion of the santonin. If the light petroleum extract is evaporated in a porcelain dish on a steam bath, and the residue is treated with a few drops of potassium methoxide, a deep orange red or carmine red colour is produced. This indicates an appreciable amount of santonin present in the light petroleum extract.

Massagetov and Soteria removed the oily and resinous substances by shaking the chloroform extract with 4 per cent. sodium hydroxide solution and dilute ammonia solution respectively. Soteria obtained a syrupy residue of santonin by evaporating the chloroform. Massagetov dissolved the dried chloroform extract in 1 or 2 ml. of ethanol, to which was added 100 ml. of boiling water, the mixture was evaporated to 50 to 70 ml., and then placed in a cool place for crystallisation. Santonin crystals were collected after 16 to 24 hours, and a solubility correction was applied.

Dragendorff[12] and Neuman[13] used sodium hydroxide and extracted the santonin in the form of sodium santonate. On treatment with hydrochloric acid, santonin is liberated and extracted with chloroform.

The oldest methods of extraction of santonin are included in this group. These methods suffer from the following disadvantages:—(i) the methods require several manipulations, are long and time consuming; (ii) large volumes of liquids are used; this is definitely a great disadvantage, when dealing with large quantities of material; (iii) the santonin finally obtained is not quite pure. The different methods yield final products of varying purity. In this group, Massagetov's method on the whole gives very good results, especially when dealing with artemisia containing a high percentage of santonin. It must, however, be pointed out that some santonin is also removed along with resinous substances when chloroform extract is shaken with 4 per cent. sodium hydroxide solution. The presence of santonin in the sodium hydroxide solution can easily be detected with potassium methoxide.

Methods Using a Solvent for the Extraction of Santonin

A number of solvents have been used for this purpose. The more important ones are: chloroform, ether, benzene, ethanol, acetone.

(i) *Chloroform.* In the methods of Fromme,[14] Van den Berg,[15] Nelson,[16] Katz-Nelson[17] and Qazilbash[18] chloroform is used as the primary solvent. The chloroform extract is treated with an aqueous solution of barium hydroxide and the santonin is converted into barium santonate.

The latter is acidified and the liberated santonin is extracted with chloroform. The residue obtained from the chloroform solution is dissolved in ethanol and the santonin is crystallised from 15 per cent. ethanol. The methods employed suffer from the following disadvantages:—(i) numerous extractions are necessary; (ii) chloroform is not a suitable primary solvent; under the action of light and moist air, chloroform readily undergoes decomposition into phosgene and hydrochloric acid and the decomposition is accelerated during the refluxing process for extraction, also during the distillation for recovery of the solvent; (iii) the santonin crystals finally obtained contain impurities, as is shown by the colour and the m.pt.; (iv) a definitive solubility correction is applied, but the solubility is variable at different temperatures.

(ii) *Ether.* Thatter,[19] Katz,[20] Goerlich,[21] Caspari[22] and Feldhoff[23] employed ether as the primary solvent. Kariyone and Kimura[24] also employed ether as the primary solvent in their volumetric method. Lime, barium hydroxide, and carbonic acid, aluminium acetate and magnesium oxide, or basic lead acetate were employed as decolorising and purifying agents and for the removal of resinous impurities. The santonin is finally crystallised from 15 per cent. ethanol. Thatter's method gives an impure final product, which is contaminated with some aluminium acetate and magnesium oxide. Katz's method is very long and tedious, and the final santonin product is impure and contaminated with resinous impurities. Goerlich adopted Katz's method of extraction but modified his method of purification. Goerlich's method requires several manipulations and is very lengthy and on this account is not of much practical utility. The final product is impure and contaminated with resinous impurities. Feldhoff's method gives fairly good results. The final product is slightly coloured on account of the presence of some resinous impurities. The percentage of santonin is also somewhat lower than that obtained by the other reliable gravimetric methods.

(*III*) *Ethanol.* Burlage and Smith[25] employed ethanol for the extraction of santonin from the drug. The powdered drug is first defatted by boiling with light petroleum saturated with santonin. The drug thus treated is extracted with ethanol, the combined extracts are boiled with lead acetate solution and filtered hot. The filtrate is allowed to stand for 24 hours and the crystals are collected and dried to constant weight at 100° C. A solubility correction is applied.

The use of light petroleum saturated with santonin is objectionable, since some additional santonin is introduced into the plant tissues, and is not removed prior to extraction with ethanol; this leads to high results. The final product of santonin is contaminated with resinous and other impurities from the lead acetate. Light petroleum removes the oily substances but very little of the resinous substances is eliminated. The m.pt. of the final product varies from 166° to 168° C.

(*IV*) *Acetone.* Palkin[26] used acetone for the extraction of santonin. The concentrated extract is treated with alkali, the santonin is converted to the alkali salt, the resins are precipitated by calcium chloride and the solution is filtered. The filtrate is acidified, the acidified solution is

extracted with chloroform, the chloroformic extract is washed with alkali and evaporated to dryness. The residue is converted to calcium santonate and the solution is then acidified and the liberated santonin is dissolved in chloroform. The residue on evaporation represents the yield of santonin.

Palkin's method is long and involves several time-consuming manipulations, and the results are not concordant. The residue of santonin is contaminated as judged by its colour and m.pt. The m.pt. varies from 165° to 168° C. Palkin's method therefore cannot be employed as a reliable practical method.

(*V*) *Benzene.* Eder and Schneiter,[27] Mouton,[28] Janot and Mouton,[29] Janot and Esteve,[30] Coutts,[31] Farnandez and Socias[32] and Qazilbash[33] employed benzene for the extraction of santonin from the plant. Butzelman[34] used a mixture of benzene 90 per cent. and chloroform 10 per cent. for the primary extraction. Benzene is given preference over chloroform and ether, as it dissolves less of the resinous substances. This group includes the latest gravimetric methods; the more important are given below in outline:—

(i) *Eder and Schneiter's method.* The finely powdered drug is shaken frequently during half an hour with benzene. An aliquot portion of the filtered extract is evaporated to dryness. The dry residue is boiled with 15 per cent. ethanol under a reflux condenser for 15 minutes and filtered. The cooled filtrate is boiled with kaolin, and filtered hot. Santonin crystallises on cooling and standing for 24 hours. A solubility correction is added.

(ii) *Janot and Mouton's method.* The powdered drug is made into a paste with ammonium hydroxide solution. The paste is dried and powdered. The powdered material is shaken with benzene for half an hour and an aliquot portion of the extract is evaporated and dried at 100° C. The residue is boiled with 15 per cent. ethanol. The santonin separates from filtrate on cooling and standing for 24 hours. A solubility correction is applied.

(iii) *Janot and Esteve's method* This is an adaptation of Janot and Mouton's method. The dry residue obtained from the benzene extract is treated with a saturated solution of barium hydroxide, and filtered; the filtrate is acidified with dilute hydrochloric acid and after 48 hours the crystals of santonin are collected.

(iv) *Coutts's method.* The coarsely powdered drug is shaken frequently during 6 hours with benzene and the extract is agitated with 8 per cent. sodium carbonate solution. An aliquot portion of the benzene extract thus obtained is evaporated to dryness on a water-bath. The residue is heated with a saturated solution of barium hydroxide, filtered and the cold filtrate acidified with dilute hydrochloric acid. The santonin crystals are collected after 24 hours from the acid solution, and dried to constant weight.

(v) *Butzelman's method.* The powdered material is first treated with 10 per cent. hydrochloric acid and then shaken with a mixture of benzene 90 per cent. and chloroform 10 per cent. After 12 hours the extract is boiled with 5 g. of barium hydroxide and 100 ml. of water and filtered; the filtrate is acidified with hydrochloric acid. The solution is shaken with

chloroform, the extract washed with 1·5 per cent. sodium hydroxide solution and water, and evaporated to dryness. The residue is boiled with 15 per cent. ethanol, filtered and allowed to cool. After 24 hours the crystals of santonin are collected, washed with 15 per cent. ethanol alcohol and dried at 105° C.

(vi) *Qazilbash's method.* The finely powdered drug is thoroughly mixed with anhydrous sodium carbonate, and the mixture shaken with 15 per cent. ammonia solution. Benzene is added and the mixture thoroughly shaken at frequent intervals during 3 hours. After 24 hours, an aliquot portion is dried, and then heated at 60° to 70° C. with 5 per cent. w/v solution of barium hydroxide, and filtered. The filtrate is acidified with dilute hydrochloric acid. The cooled solution is shaken with chloroform, and the extract evaporated to dryness. The residue is boiled with 15 per cent. ethanol under a reflux condenser and filtered. The filtrate is heated with a mixture of equal parts of animal charcoal and kieselguhr, and filtered. The filtrate is allowed to crystallise in the dark at 15° to 17° C. for 24 hours. The santonin crystals are dried at 100° to 105° C. and placed in a dessicator over sulphuric acid for 24 hours. A solubility correction is applied.

The method of Eder and Schneiter needs very little material and the number of manipulations is much reduced. The method is simple, precise and rapid, and on the whole gives very good results, when dealing with *Artemisia Cina* (Berg) Wilkomm and *Artemisia kurramensis* Qazilbash, containing more than 1·5 per cent. of santonin. The final product is lightly coloured, and is not quite pure as judged by the colour and the m.pt. The m.pt. of santonin, obtained from artemisias containing more than 1·5 per cent. varied from 166° to 168° C. With *Artemisia brevifolia* Wall, and the induced polyploids of *Artemisia kurramensis* Qazilbash, the results are unsatisfactory. With artemisias containing a low percentage of santonin, the final product is much contaminated with resinous impurities. In Eder and Schneiter's method, santonin is not completely extracted during the period of half an hour, the final result on this account is somewhat lower than the result obtained by the methods of Massagetov and Qazilbash. Eder and Schneiter agitated the powdered drug for half an hour. Coutts considered 6 hours' agitation necessary for adequate extraction of santonin. Farnandez and Socias digested the drug with benzene for 24 hours. Coutts's method gives low results. Some santonin is also removed with the sodium carbonate solution used to remove the oily and resinous substances. Moreover all the santonin is not crystallised out during a period of 24 hours. Complete separation of all the santonin as crystals takes a much longer time. Janot and Esteve collected the santonin crystals after 48 hours. The acidified solution, however, still contained some santonin. The santonin crystals finally obtained by the methods of Coutts and Janot and Esteve are contaminated.

The methods of Janot and Mouton, and Buzelman give satisfactory results when dealing with artemisias containing a good percentage of santonin. With artemisias containing a high proportion of resinous and oily substances, the final product of santonin is impure. Various forms

of *Artemisia brevifolia* Wall, growing at high altitudes under extreme xerophytic conditions in the Himalayas, contain a high proportion of oily and resinous substances.

Qazilbash's method is of general application. Santonin is adequately extracted, and the final product of santonin is pure as judged by the colour and the m.pt. The m.pt. is 171° to 173° C. and the product is white.

Summary

1. The important santonin-containing species of Artemisia are: *Artemisia Cina* (Berg) Willkomm, *A. pauciflora* Weber, *A. brevifolia* Wall and *A. kurramensis* Qazilbash. They represent distinct species, differing from one another ecologically, morphologically and physiologically.

2. Santonin is closely associated with oily and resinous substances in the plant tissue. The complete separation from these oily and resinous substances is a difficult task.

3. A review of important gravimetric methods is given.

4. The gravimetric methods are useful in determining santonin in artemisias containing a good amount of santonin. Manufacturers are not interested in artemisias with low santonin-content.

References

1. Qazilbash, *J. Ind. bot. Soc.*, 1950, **29**, 190.
2. Trommsdorff, *Jahresber. Phar.*, 1834, **3**, 309.
3. Miahle and Claud, *J. Pharm.*, 1843, **4**, 387.
4. Calloud, *J. prakt. Chem.*, 1949, **15**, 106.
5. Wettstein, *Amer. J. Pharm.*, 1858, **30**, 124.
6. Grosschopff, *Arch. Pharm.*, 1866, **2**, 128, 210.
7. Hirsch, *British Pharmacopœia*, 1885, 352.
8. Busch, *J. prakt. Chem.*, 1887, **35**, 322.
9. Soteria, *J. Soc. chem. Ind.*, 1822, **13**, 521.
10. Sestine, *Gazz. Chem. Ital.*, 6, 357.
11. Massagetov, *Arch. Pharm.*, 1932, **270**, 392; *Quart. J. Pharm. Pharmacol.*, 1932, **5**, 681.
12. Dragendorff, *Arch. Pharm.*, 1878, **12**, 300.
13. Neuman, *Russ. Z. Pharm.*, 1884, **23**, 747.
14. Fromme, *J. Amer. pharm. Ass.*, 1914, **3**, 634; *Merck's Report*, 1914, **23**, 201.
15. Van den Berg, *Pharm. Weekbl.*, 1923, **60**, 858.
16. Nelson, *J. Assoc. Off. Agric. Chem. Wash.*, 1916, **2**, 82.
17. Katz-Nelson, *ibid.*, 1916, **2**, 79.
18. Qazilbash, *Bull. Sci. Pharm.*, 1935, **42**, 132; *Quart. J. Pharm. Pharmacol.*, 1942, **15**, 323; *Ind. J. Pharm.*, 1943, **5**, 57.
19. Thatter, *Arch. Pharm.*, 1897, **235**, 401; *J. chem. Soc.*, 1898, **74** (ii), 59.
20. Katz, *Arch. Pharm.*, 1899, **237**, 245; *J. Soc. chem. Ind.*, 1899, **18**, 952.
21. Goerlich, *Chem. Abstr.*, 1911, **5**, 1163.
22. Caspari, *ibid.*, 1914, **8**, 1949; *Pharm. J.*, 1914, **91**, 462.
23. Feldhoff, *Pharm. Ztg.*, 1925, **70**, 661; *Chem. Abstr.*, 1925, **19**, 2387.
24. Kariyone and Kimura, *J. pharm. Soc.* (*Japan*), 1920, **405**, 927; *Chem. Abstr.*, 1921, **15**, 375.
25. Burlage and Smith, *J. Assoc. Off. Agric. Chem. Wash.*, 1932, **15**, 495.
26. Pakin, *ibid.*, 1926, 9, 326.
27. Eder and Schneiter, *Schweiz. Apoth. Ztg.*, 1925, **63**, 405; *Chem. and Drugg.*, 1925, **103**, 423.
28. Mouton, *These Doct. Pharm. Univ. Paris*, 1930, 46.
29. Janot and Mouton, *Bull. Sci. Pharm.*, 1930, **37**, 337.
30. Janot and Esteve, *ibid.*, 1933, **40**, 280.
31. Coutts, *Quart. J. Pharm. Pharmacol.*, 1932, **5**, 375.
32. Farnandez and Socias, *J. Pharm. Chim.*, 1932, **16**, (8), 49.
33. Qazilbash, *J. Pharm. Pharmacol.*, 1951, **3**, 105.
34. Butzelman, *Chem. Abst.*, 1934, **28**, 574.

A NEW METHOD FOR THE DETERMINATION OF BROMINE IN THE α-BROMOACYLCARBAMIDES, CARBROMAL AND BROMVALETONE

By R. B. Hardwick, Mary V. Johnston and C. W. Picard

From the Laboratories, Amber Pharmaceuticals Ltd., Batley, Yorkshire

Received May 31, 1951

Introduction

The methods in general use for estimating bromine in α-bromoacylcarbamides are based on the Volhard method. Many foreign pharmacopœias include this method in their specifications, e.g., the Danish Pharmacopœia for bromisovalerylurea, carbromal and acetylcarbromal, the Swedish and German Pharmacopœias for bromisovalerylurea, and the Finnish Pharmacopœia for carbromal. The British Pharmaceutical Codex 1949, in its monographs on bromvaletone and carbromal, does not call for a quantitative assay, but here also the Volhard procedure is generally accepted whenever a bromine assay is required to determine the purity of such substances. The general procedure involves hydrolysis of the test sample with hot aqueous alkali, acidification of the solution with nitric acid, and estimation of bromide by the normal Volhard procedure. The Swedish Pharmacopœia XI and the German Pharmacopœia VI specify that the initial alkaline hydrolysis is carried out by boiling; the Danish Pharmacopœia 1948 requires heating on a steam-bath for 15 minutes. Detailed study of these variations has led us to conclude that the results obtained by these methods tend to be inaccurate and erratic.

When applied to the α-bromoacylcarbamides, the method appears to be liable to some specific sources of error, in addition to those which are generally known to be associated with this method, viz., the usual disadvantages inherent in the use of back titrations, the effect of traces of nitrous acid present in the nitric acid used, the risk of decomposition of the thiocyanate solution to give cyanate, difficulties in attaining adsorption equilibrium, etc. (cf. Kolthoff and Lingane,[1] and others). It was, therefore, considered desirable to develop a simple alternative method, which is at once more rapid and more accurate.

Alternative Methods

We investigated various direct titration procedures, using an adsorption indicator. It was at first thought that, for this purpose, titration would have to be performed in a strongly acid solution, and the procedure developed by Lang and Messinger[2] was therefore tried, using oxidised diphenylamine as adsorption indicator. However, when applying this method, difficulties were experienced, due to fading of the indicator.

Attention was next turned to a procedure involving alkaline hydrolysis, followed by acidification with acetic acid, and silver nitrate titration with eosin as indicator. The use of eosin as indicator for the estimation of bromine generally has been described by Kolthoff and Van Berk.[3] Its application to bromine estimation in carbromal was described by Hök.[4]

It was found that the end-point obtained with this method was somewhat indefinite. This was thought to be due to interference by some material produced during the hydrolysis, and an attempt was, therefore, made to apply the alkaline ashing procedure of Francis and Harvey[5] to the product obtained after alkaline hydrolysis. Unsatisfactory results were at first obtained, again owing to a somewhat doubtful end-point. The cause of this was traced to the formation of cyanide in the ashing, the presence of which was confirmed qualitatively. In view of this finding, the product obtained from the alkaline ashing procedure was dissolved in water and subjected to oxidation by means of hydrogen peroxide (100 vols.).[6] The solution, after boiling to destroy excess of peroxide, was then titrated as before, using eosin as indicator, and very sharp end-points were obtained. This method, though giving reproducible and accurate results, suffers from one serious drawback. It involves a very lengthy procedure, one estimation requiring between 3 and 4 hours for completion.

The next method to be examined was based on an alkaline hydrolysis of the test sample, followed by oxidation of the resulting bromide to bromate, by means of sodium hypochlorite in a phosphate buffer (Kolthoff and Yutzy[7]), and iodimetric determination of the bromate. However, it was found that the oxidation of bromide to bromate was incomplete, owing to the presence of organic matter which would have to be destroyed completely before this procedure might be applied with success.

Since cyanide was known to be one of the hydrolytic products of carbromal (Newbery[8]), attention was redirected to an alkaline hydrolysis procedure, followed by oxidation with hydrogen peroxide (100 vols.) to destroy the cyanide[6] and, after removal of excess of hydrogen peroxide by boiling, acidification with acetic acid and subsequent titration with silver nitrate solution, using eosin as indicator. It is essential to destroy any cyanide produced on hydrolysis, since its presence is known to interfere with the end-point in titrations using eosin. This method gave a sharp and reproducible end-point and was ultimately adopted as our method of choice.

Method Recommended

Reagents. (i) 0·1N silver nitrate; (ii) 0·5 per cent. eosin solution (sodium salt in water); (iii) 5N acetic acid; (iv) sodium hydroxide (pellets); (v) hydrogen peroxide (100 vol.); (vi) phenolphthalein, 0·2 per cent. solution in 60 per cent. ethanol.

Procedure. About 0·5 g., accurately weighed, is washed into a 250-ml. conical flask with about 50 to 100 ml. of water. About 0·2 g. of solid sodium hydroxide (1 pellet) is added and the mixture is boiled on an electric hot plate for 10 minutes under reflux (e.g., "cold finger" inserted in the mouth of the flask). The flask is removed from the hot plate, allowed to cool slightly, and 5 to 10 ml. of hydrogen peroxide (100 vol.) is then added and the mixture is again boiled gently to destroy excess of peroxide. After cooling to room temperature, 1 drop of phenolphthalein indicator solution is added, and the contents are acidified with acetic acid. A further 10 ml. of acetic acid is then added, followed by 10 drops

of eosin indicator, and the solution is titrated with 0·1N silver nitrate, as quickly as possible and with vigorous shaking. Just before the end-point is reached the solution assumes a rose-pink colour, and thin strings of precipitate may appear on the surface and on the sides of the flask. The titration is now completed very carefully and with vigorous shaking until, at the end-point, the precipitate coagulates and turns intensely red.

1 ml. of 0·1N silver nitrate = 7·992 mg. of Br; 22·31 mg. of $C_6H_{11}O_2N_2Br$ (bromisovalerylurea); 23·71 mg. of $C_7H_{13}O_2N_2Br$ (carbromal); 27·92 mg. of $C_9H_{15}O_3N_2Br$ (acetylcarbromal).

The tables which follow will serve to illustrate the types of results that have been obtained in our laboratories over the past 12 months.

Results

Table I shows typical results obtained with highly purified samples of bromisovalerylurea and carbromal. The values were obtained for separate weighings and illustrate the high degree of accuracy which is attained by the new method of assay. In Tables II and III, typical results obtained by the new method are compared with those given by the Swedish and Danish Pharmacopœia modifications of the Volhard method. Commercial samples of bromisovalerylurea and carbromal were used in this series of experiments.

For the repeat determinations shown in Tables II and III for samples No. 1, 2, 6 and 7, each determination has been carried out on a freshly weighed sample of material. The "average deviation" shown in these cases refers to average deviation of a single result.

TABLE I

DETERMINATION OF BROMINE IN PURE BROMISOVALERYLUREA AND CARBROMAL, BY THE PEROXIDE/EOSIN METHOD

Bromisovalerylurea, m.pt. 152° to 153° C. (Theoretical Br-content: 35·82 per cent.)			*Carbromal*, m.pt. 118° to 119° C. (Theoretical Br-content: 33·70 per cent.)		
Assay	Bromine per cent.	$C_6H_{11}O_2N_2Br$. per cent.	Assay	Bromine per cent.	$C_7H_{13}O_2N_2Br$. per cent.
1	35·84	100·06	4	33·71	100·03
2	35·82	100·00	5	33·74	100·12
3	35·84	100·06	6	33·74	100·12
Average value	35·83	100·04	Average value	33·73	100·09

The new procedure has also been used successfully for assaying acetylcarbromal and can be applied also to other bromoacylcarbamide derivatives. The results of some typical assays of acetylcarbromal are shown in Table IV.

TABLE IV

DETERMINATION OF BROMINE IN ACETYLCARBROMAL BY THE PEROXIDE/EOSIN METHOD (28·63 PER CENT. OF Br. = 100 PER CENT.)

Sample	M.pt. °C.	Bromine per cent.	$C_9H_{15}O_3N_2Br$. per cent.
11	109 to 110	28·69	100·20
12	108 to 110	28·25	98·65
13	110 to 112	28·69	100·20
14	109 to 110	28·67	100·10

TABLE II

DETERMINATION OF BROMINE IN BROMISOVALERYLUREA
COMPARISON OF RESULTS OBTAINED BY THREE DIFFERENT METHODS OF ASSAY

Sample	M.pt.	Peroxide/Eosin Method		Volhard (Swedish Pharmacopœia)		Volhard (Danish Pharmacopœia)	
		Bromine per cent.	$C_6H_{11}O_2N_2Br$. per cent.	Bromine per cent.	$C_6H_{11}O_2N_2Br$. per cent.	Bromine per cent.	$C_6H_{11}O_2N_2Br$. per cent.
1	147° to 148° C. (A)	35·24	98·37	34·18	95·43	34·58	96·54
	(B)	35·24	98·37	34·13	95·30	31·35	87·52
	(C)	35·23	98·35	34·50	96·32	33·78	94·30
	(D)	35·25	98·40	34·45	96·18	32·28	90·14
	Average value	35·24	98·37	34·32	95·83	33·00	92·12
	„ deviation	0·005	0·013	0·16	0·44	1·18	3·30
2	153·5° to 154° C. (A)	35·88	100·19	36·13	100·90	35·84	100·06
	(B)	35·90	100·20	36·35	101·50	35·69	99·63
	(C)	35·88	100·19	35·46	99·00	35·88	100·19
	Average value	35·89	100·2	35·98	100·50	35·80	99·95
	„ deviation	0·01	0·007	0·44	0·97	0·08	0·22
3	147° to 148° C.	35·39	98·81	34·47	96·25	34·45	96·18
4	148° to 149° C.	34·89	97·40	33·90	94·64	35·33	98·65
5	149° to 150° C.	34·98	97·66	34·65	96·74	35·02	97·77

TABLE III

DETERMINATION OF BROMINE IN CARBROMAL

COMPARISON OF RESULTS OBTAINED BY THREE DIFFERENT METHODS OF ASSAY

Sample	M.pt.	Peroxide/Eosin Method		Volhard (Swedish Pharmacopœia)		Volhard (Danish Pharmacopœia)	
		Bromine per cent.	$C_7H_{13}O_2N_2Br$. per cent.	Bromine per cent.	$C_7H_{13}O_2N_2Br$. per cent.	Bromine per cent.	$C_7H_{13}O_2N_2Br$. per cent.
6	117° to 119° C. (A)	33·81	100·32	33·71	100·03	30·64	90·93
	(B)	33·82	100·36	31·28	92·83	32·60	96·74
	(C)	33·80	100·30	33·72	100·06	32·64	96·85
	(D)	33·81	100·32	30·32	89·97	30·74	91·22
	Average value	33·81	100·32	32·26	95·72	31·66	93·96
	„ deviation	0·005	0·015	1·46	4·32	0·97	2·86
7	118° to 118·5° C. (A)	33·40	99·10	33·19	98·49	33·11	98·24
	(B)	33·40	99·10	33·43	99·19	33·13	98·31
	(C)	33·42	99·17	33·12	98·28	33·11	98·24
	Average value	33·41	99·13	33·25	98·65	33·12	98·28
	„ deviation	0·01	0·03	0·12	0·36	0·01	0·04
8	118° to 119° C.	33·40	99·10	32·59	96·72	33·59	99·68
9	118° to 119° C.	32·72	97·09	31·60	93·78	31·73	94·17
10	117° to 119° C.	33·30	98·81	32·26	96·03	32·25	95·72

Summary

1. In the determination of bromine in bromoacylcarbamides the values obtainable by the Volhard method are unreliable and inconsistent. Of the two modifications which have been examined, and which differ from each other only by the method of hydrolysis to which the test sample is subjected, neither has been found to give consistently high or low results, although more often both are inclined to be low.

2. There was a tendency for both Volhard modifications to be rather more reliable with extremely pure samples of the bromocarbamides, e.g., Nos. 2 and 7. These samples had been purified by repeated recrystallisations and were of a much higher degree of purity than that demanded by any of the official specifications in this country or abroad.

3. An adsorption indicator method, using eosin, has been found to give accurate and consistent results.

4. The advantages of the method may be summarised as follows: (*a*) It requires only one standard solution, instead of the two required by the Volhard procedures; (*b*) it is rapid and much simpler than the Volhard method; (*c*) it gives results of a degree of accuracy far superior to the old Volhard procedures which, in our experience, tend to be inaccurate and unreliable when applied to this class of substances. These difficulties are entirely eliminated by the new procedure.

References

1. Kolthoff and Lingane, *J. Amer. chem. Soc.*, 1935, **57**, 2126.
2. Lang and Messinger, *Ber. dtsch. chem. Ges.*, 1930, **63B**, 1429.
3. Kolthoff and Van Berk, *Z. anal. Chem.*, 1927, **70**, 369.
4. Hök, *Svensk. Farm. Tidkr.*, 1930, **34**, 121, 141; *Chem. Abst.*, 1930, **24**, 4899.
5. Francis and Harvey, *Biochem. J.*, 1933, **27**, 1545.
6. Schulek, *Z. anal. Chem.*, 1938, **112**, 415.
7. Kolthoff and Yutzy, *Ind. Engng. Chem., Anal. Ed.*, 1937, **9**, 75.
8. Newbery, *J. chem. Soc.*, 1925, **127**, 295.

THE ACTION OF *N*-BROMOSUCCINIMIDE ON ALCOHOLS AND AMINES

By M. Z. Barakat and G. M. Mousa

From the Biochemistry Department, Faculty of Medicine, Abbassia, Ibrahim Pasha El-Kebir University, Cairo, Egypt

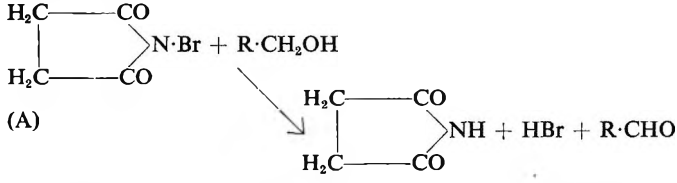
Received May 25, 1951

N-Chlorosuccinimide has been shown to react vigorously with benzyl alcohol to give benzldehyde in 74 per cent. yield. Benzhydrol similarly gives the corresponding ketone in 77 per cent. yield.[1]

Nothing seems to be known about the reaction mentioned in the title. *N*-Bromosuccinimide reacts readily at room temperature on primary alcohols, e.g., methanol, ethanol and benzyl alcohol, yielding the corresponding aldehydes, formaldehyde, acetaldehyde and benzaldehyde respectively (compare scheme A). In the case of secondary alcohols, e.g., *iso*propanol, the reaction proceeds rapidly at room temperature giving the corresponding ketone, e.g., acetone. The formation of succinimide and hydrobromic acid has been proved in each case.

When *N*-bromophthalimide was allowed to react with benzyl alcohol in aqueous medium, it was found necessary to warm the reaction mixture to effect rapid oxidation.

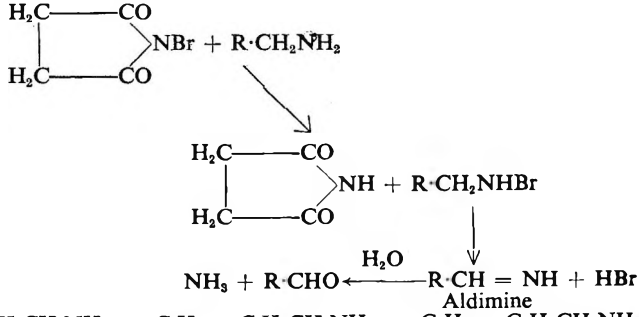
(A)



$R = H \rightarrow CH_3OH$; $= CH_3 \rightarrow C_2H_5OH$; $= C_6H_5 \rightarrow C_6H_5CH_2OH$

N-Bromosuccinimide is stable towards water and crystallises from hot water.[2] This fact eliminates the assumption that hypobromous acid is first formed which subsequently oxidises the alcohol. The aldehydes produced cannot be detected easily by odour, but they are isolated in the form of the 2:4-dinitrophenylhydrazones and identified by the m.pt. and mixed m.pt.

(B)



$R = CH_3 \rightarrow CH_3CH_2NH_2$; $= C_2H_5 \rightarrow C_2H_5CH_2NH_2$; $= C_6H_5 \rightarrow C_6H_5CH_2NH_2$

Similarly, it has been found that *N*-bromosuccinimide reacts readily with primary amines (e.g., ethylamine, *n*-propylamine and benzylamine) at room temperature in an aqueous medium giving the corresponding aldehydes, acetaldehyde, propionaldehyde and benzaldehyde respectively (compare scheme B). The ammonia liberated in the reaction is present as ammonium bromide.

In the case of amines, the aldehydes have also been isolated as the 2:4-dinitrophenylhydrazones and identified by the m.pt. and mixed m.pt. The formation of succinimide and ammonia has been proved.

The fact that *N*-bromosuccinimide reacts readily on alcohols and primary amines at room temperature provides a new reagent for the detection of these compounds.

Experimental

Action of N-*Bromosuccinimide on Alcohols.* *N*-Bromosuccinimide (2 g.) and methanol (5 ml.), or ethanol (6 ml.) or *iso*propanol (7 ml.), or benzyl alcohol (4 ml.) were mixed together at room temperature for 10 minutes with occasional shaking; a vigorous reaction took place with the formation of an orange red colour. The mixture must be cooled as the reaction is exothermic, especially in the case of *iso*propanol and benzyl alcohol. After cooling, the reaction mixture was filtered from any unchanged *N*-bromosuccinimide. To the clear filtrate excess of 2:4-dinitrophenylhydrazine hydrochloride in aqueous ethanol was added, giving respectively formaldehyde 2:4-dinitrophenylhydrazone 1·1 g.), acetaldehyde 2:4-dinitrophenylhydrazone (1·3 g.), acetone 2:4-nitrophenylhydrazone (1·2 g.) and benzaldehyde 2:4-dinitrophenylhydrazone (2·2 g.).

Similar experiments were carried out using the similar quantities of *N*-bromosuccinimide and each alcohol and, after the reaction was complete, the filtrate was concentrated to a small bulk (about 2 ml.). After cooling, a white crystalline precipitate was formed, which was filtered and proved to be succinimide (0·8 g.). The presence of hydrobromic acid in the filtrate of the reaction mixture was proved by the silver nitrate test.

Action of N-*Bromophthalimide on Benzyl alcohol.* *N*-Bromophthalimide (0·50 g.), benzyl alcohol (0·50 g.) and water (10 ml.) were heated gently under a reflux condenser at about 90° C. for 3 minutes, whereupon a vigorous reaction took place. The mixture was then allowed to cool with continuous shaking and was filtered, yielding a filtrate (A) and a solid (B). (B) was crystallised from ethanol and proved to be phthalimide. (A) was treated with excess of 2:4-dinitrophenylhydrazine hydrochloride in aqueous ethanol, whereupon orange crystals were formed. The mixture was allowed to stand in ice for 1 hour, and the deposit was crystallised from ethanol and proved to be benzaldehyde 2:4-dinitrophenylhydrazone; yield about 30 per cent.

Action of N-*Bromosuccinimide on Primary Amines.* *N*-Bromosuccinimide (2 g.) and ethylamine 33 per cent. aqueous solution (3 g.), or *n*-propylamine (1·2 g.), or benzylamine (0·5 g.) in 6 ml. of water were

mixed together at room temperature (18° C.) for 10 minutes with frequent shaking. A vigorous reaction took place with the formation of a red colour. The mixture was cooled in ice during the gradual addition of *N*-bromosuccinimide because the reaction was exothermic. After cooling, the mixture was filtered from any unchanged *N*-bromosuccinimide and to the clear filtrate, excess of 2:4-dinitrophenylhydrazine hydrochloride in aqueous ethanol was added, whereupon an orange precipitate was formed, allowed to stand in ice for ¼ hour, filtered off and crystallised from ethanol giving acetaldehyde 2:4-dinitrophenylhydrazone, propionaldehyde 2:4-dinitrophenylhydrazone and benzaldehyde 2:4-dinitrophenylhydrazone respectively. Yield about 40 per cent.

Similar experiments using similar quantities of *N*-bromosuccinimide and each amine were carried out and the clear filtrate concentrated to a small bulk (2 ml.) and allowed to cool. On standing, colourless crystals separated which proved to be succinimide (1 g.). The formation of ammonia during the reaction was also proved, after rendering the filtrate alkaline with sodium hydroxide (20 per cent.), by the dense white fumes obtained when a glass rod moistened with a drop of concentrated hydrochloric acid was held at the mouth of the test-tube containing the reaction mixture.

Summary

(1) *N*-Bromosuccinimide converts primary alcohols at room temperature into the corresponding aldehydes, e.g., ethanol into acetaldehyde. Secondary alcohols are similarly oxidised to the corresponding ketones, e.g., *iso*propanol gives acetone.

(2) *N*-Bromosuccinimide reacts vigorously with primary amines in an aqueous medium at room temperature to give the corresponding aldehydes, e.g., benzylamine yields benzaldehyde.

(3) *N*-Bromosuccinimide is a new reagent for the detection of primary alcohols, secondary alcohols and primary amines. *N*-Bromophthalimide behaves similarly with primary alcohols, e.g., benzyl alcohol, but requires warming to effect oxidation.

References

1. Hebbelynck and R. H. Martin, *Experientia*, 1949, **5**, 69.
2. Lamchen, *J. chem. Soc.*, 1950, 747.

A NOTE ON THE PREPARATION OF *m*-HYDROXYPHENYLPROPIONIC ACID

By M. Dombrow and W. H. Linnell

Received September 6, 1951

Methods hitherto used for the preparation of *m*-hydroxyphenylpropionic acid have involved several stages. *m*-Hydroxybenzaldehyde, obtained by reduction of *m*-nitrobenzaldehyde and diazotisation of the amino compound, is converted by a Perkin or Knövenagel condensation into *m*-hydroxycinnamic acid,[1,2] which is then reduced by means of sodium amalgam[3] or catalytic hydrogenation.[2] Alternatively, the *m*-nitrobenzaldehyde is converted into *m*-nitrocinnamic acid, using the Perkin method,[4,5] and reduced to *m*-aminocinnamic acid by stannous chloride in hydrochloric acid,[4,6] diazotised and hydrolysed to *m*-hydroxycinnamic acid,[4] which is then reduced to the phenylpropionic derivative by sodium amalgam.[3]

It is now shown that *m*-nitrocinnamic acid can be reduced to *m*-aminophenylpropionic acid in a single operation, thereby eliminating one stage of the above sequence. Furthermore, the troublesome diazotisation of the very insoluble *m*-aminocinnamic acid is replaced by the straightforward diazotisation of the *m*-aminophenylpropionic acid. The reduction is carried out (*a*) by catalytic hydrogenation, using Adam's catalyst, (*b*) by nickel-aluminium alloy in sodium hydroxide solution, using the Schwenk-Papa technique.[7] Hydrogenation at atmospheric pressure using Raney nickel fails to reduce the cinnamic group.

Experimental

m-*Nitrobenzaldehyde.* This was prepared by nitration of benzaldehyde using the methods of Friedlander and Henriques[8] and Ehrlich,[9] m.pt. 58° C.

m-*Nitrocinnamic acid.* *m*-Nitrobenzaldehyde (20 g.), acetic anhydride (24 ml.) and pyridine (1 ml.) were heated in a 500-ml. round-bottomed flask fitted with a wide air condenser, to 120° C. on a wax bath. Potassium carbonate (3·6 g.) was added as rapidly as possible without the solution foaming over. The temperature was raised to 180° C. and maintained for 25 minutes. The solution was cooled, diluted with water, and the precipitated acid dissolved in dilute ammonia, boiled with charcoal and filtered. The acid was re-precipitated with dilute sulphuric acid, washed, dried and recrystallised twice from ethanol. Yield 16 g., m.pt. 196° C.

m-*Hydroxyphenylpropionic acid.* (*a*) *Schwenk-Papa Method.* *m*-Nitrocinnamic acid (150 g.) was dissolved in a solution of sodium hydroxide (600 g. in 3 l. of water) in a 5-l. wide-necked flask. The solution was automatically stirred at 90° C. and nickel-aluminium alloy (450 g.) added in small portions, care being taken to avoid foaming. The contents of the flask were then boiled for 2 hours and filtered whilst hot. The precipitate of nickel was washed with 100-ml. quantities of hot water, about 500 ml. being required to remove all the amino acid. The filtrate and washings, whilst still warm, were acidified by running in a mixture of

concentrated sulphuric acid (1·25 l.) and water (2 l.) with continuous stirring. The mixture was heated until all the precipitated aluminium hydroxide had redissolved, cooled to 5° C. and diazotised with sodium nitrite (30 g.) dissolved in water (50 ml.). After 15 minutes, urea was added until no nitrite could be detected, and the solution boiled as rapidly as possible with the addition of copper sulphate solution (30 ml.) as catalyst. When evolution of nitrogen had ceased, the flask was cooled and the solid matter, consisting of a mixture of *m*-hydroxyphenylpropionic acid and tar, was filtered out. The filtrate was extracted with ether and the mixture of tar and acid was washed with ether, in which the tar was insoluble. The bulked ether extract was evaporated. and the residue, after solution in ethanol and treatment with charcoal, gave 90 g. of brown crystals, which recrystallised from benzene/ligroin or chloroform in the form of light brown crystals. Yield 83 g. (65 per cent.), m.pt. 112° C. (Lit. 111° to 112·5° C.).

Benzyl ether. From sodium derivative and benzyl chloride in ethanol. M.pt. 84·5° C. Colourless plates. Found: C, 75·23; H, 5·96. $C_{16}H_{16}O_3$ requires C 75·00; H, 6·25 per cent.

(*b*) *Catalytic hydrogenation—Adam's catalyst.* Ethyl *m*-nitrocinnamate (10 g.), suspended in ethanol (150 ml.) containing Adam's catalyst (0·2 g.), was hydrogenated at atmospheric pressure and room temperature. Six equivalents of hydrogen were taken up rapidly (within an hour) and a further 2 equivalents in 24 hours. The ethanol was removed, the product dissolved in acid, diazotised and boiled as above. The ester was extracted with ether and after solution in ethanol and treatment with charcoal, evaporated to give an oily residue, which was hydrolysed giving 7 g. of *m*-hydroxyphenylpropionic acid, m.pt. 112·5° C. Benzyl ether, m.pt. 84·5°. Mixed m.pt. with product from (*a*), 84·5° C.

(*c*) *Catalytic hydrogenation—Raney nickel.* Method as (*b*), using glacial acetic acid as solvent, Raney nickel catalyst (0·5 g.) and hydrogenating at 95° C. and atmospheric pressure. Six equivalents were absorbed in 5 hours, after which absorption ceased. The product, treated as above, gave 5 g. of *m*-hydroxycinnamic acid, m.pt. 191° C. Mixed m.pt. with material prepared by method of Ingold and Piggot,[4] 191° C.

Summary

By an adaptation of the Schwenk-Papa method, or by catalytic hydrogenation, *m*-nitrocinnamic acid is reduced to *m*-aminophenylpropionic acid, which is converted without isolation into *m*-hydroxyphenylpropionic acid. Advantages are claimed for this method.

References

1. Tiemann, *Ber. dtsch. chem. Ges.*, 1882, **15**, 2049.
2. Johnson *et al*, *J. Amer. chem. Soc.*, 1944, **66**, 218.
3. Tiemann, *Ber. dtsch. chem. Ges.*, 1882, **15**, 2043.
4. Ingold and Piggot, *J. chem. Soc.*, 1923, 1469.
5. Organic Syntheses, Coll. Vol. 1, 390.
6. Gabriel, *Ber. dtsch. chem. Ges.*, 1882, **15**, 2297.
7. Schwenk, Papa *et al.*, *J. Organic Chem.*, 7, 587; **9**, 1; **14**, 366.
8. Friedlander, *Ber. dtsch. chem. Ges.*, 1881, **14**, 2802.
9. Ehrlich, *ibid.*, 1882, **15**, 2010.

A NOTE ON SODIUM PROPIONATE

By W. W. Heseltine

From The Technical Division, Harker Stagg Limited, London

Received August 16, 1951

It is known that the normal skin can combat pathogens such as streptococci and staphylococci and an interesting account of its autosterilising action is provided by Bigger,[1] who states that few bacteria are able to survive on the human skin owing to the presence of bactericidal substances. The antibacterial and antifungal properties of sodium propionate have been discussed in a previous communication[2] and it is evident that the compound is effective against a wide range of pathogens, although its activity against viruses is doubtful. Peck and Rosenfeld[3] were apparently the first workers to show that the human perspiration is fungistatic owing to its content of fatty acids, although the literature contains earlier references to the inhibitory action of these acids against micro-organisms. The normal sweat exuded from the glands exhibits the slight alkalinity of plasma, but the reaction becomes acid after admixture with sebum and substances excreted by the epidermis. Propionic acid, a constituent of human body fluids, is probably produced chiefly by the breakdown of higher fatty acids; it is found in the sweat as free acid or as salts. These substances are metabolised in the body and Lorber and his associates[4] gave isotopic sodium propionate to fasted rats, isolated the resulting liver glycogen and hydrolysed the latter to glucose. Their results indicate that propionate may give rise to pyruvate by a process involving loss of orientation of the α- and β-carbon atoms of the propionate, the pyruvate acting as intermediate in glucose and glycogen formation. Unlike acetates, propionates are not used by the body in synthesising cholesterol.[5]

Since glycogen is present in the sweat glands, it may be suggested that a portion of the propionate in perspiration could be formed locally by the reverse of the process proposed by Lorber *et al.*, the mechanism thus resembling that whereby glycogen is converted into lactic acid. Further quantities, produced in the liver during the metabolism of fat, may escape oxidation in the extra-hepatic tissues and pass into the sweat.

Some Pharmacological Properties

Sodium propionate is a white solid, soluble in about two parts of water. Specimens of the substance available commercially were found to have a variable, alkaline reaction, but neutral products can be prepared for pharmaceutical use.

The acute toxicity is apparently so low that it was considered neither practicable nor necessary to determine it in terms of LD50. Daily oral doses of 6 g. administered to an adult male rendered the urine faintly alkaline but caused no appreciable diuresis, catharsis or other effects. It would seem that sodium propionate is absorbed fairly readily, so producing insignificant osmotic effect in the bowel, and the greater part of a dose is probably oxidised in the tissues with the formation of carbonate.

Theodore[6] reported that 20 per cent. solutions caused no deleterious effects when instilled into the eyes of rabbits and that 10 per cent. solutions appeared to facilitate the healing of experimental lesions. This investigator employed sodium propionate in the treatment of about 1200 cases of ocular diseases and concluded that the drug is particularly suitable for ophthalmic use. Application of a 10 per cent. solution of *p*H 7·2 to the conjunctiva and nasal mucosa of human subjects has been found to cause only slight, transient stinging and no appreciable irritation of the intact skin was produced by 20 per cent. solutions with *p*H values of 7 to 8·5. Alkaline preparations appeared to irritate the skins of dogs and cats, but this effect was probably due solely to the *p*H factor, since similar irritation was caused by sodium bicarbonate solutions of *p*H 8·4 and since a neutral propionate preparation was well tolerated by these animals. Chronic topical use did not result in sensitisation in any member of a group of subjects and extensive clinical trials have proved sodium propionate to be hypoallergenic. It has no apparent anticoagulating effect and this observation is supported by Hecht's report[7] that fatty acids have no action on the coagulation of chicken plasma. A 10 per cent. solution of *p*H 7·2 was diluted to provide a range of concentrations and the osmotic pressures were determined by the freezing-point technique and by means of a semi-permeable membrane/manometer apparatus. The results indicate that the concentration of sodium propionate which is isotonic with serum and lachrymal fluid is approximately 2·4 per cent.

Antihistaminic Activity. One drop of a 0·1 per cent. sterile solution of histamine acid phosphate was allowed to remain in contact with uniformly scarified areas on the left arms of male and female volunteers for a period of 5 minutes. After removing the histamine, one weal was left untreated as control and the others were treated respectively with 2 per cent. diphenhydramine hydrochloride, 2 per cent. antazoline hydrochloride and 15 per cent. sodium propionate. Measurement of the weals after a further interval of 10 minutes demonstrated the following order of decreasing antihistaminic activity in each case:—

Subject	*Order of increasing weal-size*
F. 1	Diphenhydramine, Antazoline, Propionate, Control.
F. 2	(Propionate, Diphenhydramine), Antazoline, Control.
M. 1	Propionate, Diphenhydramine, Antazoline, Control.
M. 2	Diphenhydramine, Propionate, Antazoline, Control.

Uniformly scarified areas on the right arms of the same subjects were then treated with (*a*) 0·05 per cent. histamine acid phosphate, (*b*) 0·05 per cent. histamine acid phosphate plus 1 per cent. diphenhydramine hydrochloride, (*c*) 0·05 per cent. histamine acid phosphate plus 1 per cent. antazoline hydrochloride, (*d*) 0·05 per cent. histamine acid phosphate plus 7·5 per cent. sodium propionate. The weals were then measured after an interval of 15 minutes.

Subject	*Order of increasing weal-size*
F. 1	(Diphenhydramine, Antazoline, Propionate), Control.
F. 2	Propionate, Diphenhydramine, Antazoline, Control.

Subject	*Order of increasing weal-size*
M. 1	Propionate, Diphenhydramine, Antazoline, Control.
M. 2	Diphenhydramine (Antazoline, Propionate), Control.

Note.—Brackets indicate only slight differences in weal-size.

These results demonstrate the variation of activity of antihistaminic drugs in different persons which has been observed by a number of investigators, but it is apparent that sodium propionate exhibits some local antihistaminic effect. Although its potency is less than that of similar concentrations of recognised histamine antagonists, this property is likely to be of practical advantage when sodium propionate is used as an antiseptic in the treatment of burns and certain diseases of the skin and mucous membranes. The value of this effect, moreover, may be particularly apparent when the compound is substituted for penicillin, sulphonamides or other drugs in patients who have become sensitised to these agents.

Summary

1. Sodium propionate has a low systemic and local toxicity.
2. It is hypoallergenic and virtually non-irritant in appropriate therapeutic concentrations.
3. The concentration of sodium propionate isotonic with serum and lachrymal fluid is approximately 2·4 per cent.
4. The drug has some local antihistaminic activity.

References

1. Bigger, *Handbook of Bacteriology*, 1949, Baillière, Tindall & Cox, London.
2. Heseltine and Galloway, *J. Pharm. Pharmacol.*, 1951, **3**, 581.
3. Peck and Rosenfeld, *J. Invest. Dermat.*, 1938, **1**, 237.
4. Lorber, Lifson, Sakami, Wood, Cook and Bordeaux, *J. biol. Chem.*, 1950, **183**, 531.
5. Bloch and Rittenberg, *J. biol. Chem.*, 1942, **145**, 625.
6. Theodore, *J. Amer. med. Ass.*, 1950, **143**, 226.
7. Hecht, *Le Sang*, 1950, **21**, 486.

A NOTE ON THE RAPID ASSAY OF EXTRACT OF IPECACUANHA AND OF IPECACUANHA ROOT

By Donald P. Lowdell

Received August 16, 1951

Extract of Ipecacuanha

The B.P. method for the assay of extract of ipecacuanha consists in the initial removal of colouring matter with chloroform, the washing of this chloroform with acid and the extraction of the alkaloids from the combined acid liquors with chloroform after the addition of excess of ammonia. The solvent is then distilled off, the residual alkaloids are dissolved in excess of standard acid and the solution back-titrated with standard alkali using methyl red as indicator. In practice this method is often very long and tedious owing to the formation of very stable emulsions, which cannot be broken down even by the addition of large volumes of chloroform. Time is also wasted during the distillation, while again there is the danger that the delicate alkaloids may be partly decomposed during the final evaporation and drying.

These difficulties may all be overcome by at once making alkaline with ammonia, extracting the alkaloids with ether, washing the ether free from ammonia with water, shaking with excess of standard acid, running out the acid and back-titrating with standard alkali.[1] In this way the necessity for distillation and removal of colouring matter is avoided and no troublesome emulsions are produced, so that the whole assay can be completed in 30 to 40 minutes, the results being identical with those given by B.P. method, which often takes several hours.

Details are as follows:—Transfer 5 ml. of the extract to a separator with a pipette. Add 3 ml. of strong solution of ammonia. Shake with successive quantities of about 50 ml. each of ether until the ethereal extract is free from alkaloid. About four extractions are usually sufficient. Wash the combined ethereal extracts with successive quantities, each of about 10 ml., of water, shaking each after separation with the same quantity of about 50 ml. of ether until the ether-washed water no longer turns red litmus paper blue. Mix the ethereal extracts. Add 10 ml. of 0·1N sulphuric acid, shake well and run the acid into a beaker. Shake the ethereal layer with further quantities of water until the last washing no longer turns blue litmus paper red, adding these washings to the acid solution in the beaker. Titrate with 0·1N sodium hydroxide using methyl red as indicator.

Ipecacuanha Root

By using a procedure similar to the above it is possible to dispense with the extraction of colouring matter and the distillation of solvent in the B.P. assay of this substance, thus saving a considerable amount of time and tedious work. The modified method is as follows. Extract the powdered root, as in the official method, using ammonia and a mixture of chloroform and ether. Shake the solvent with successive quantities,

each of about 15 ml., of water, shaking each water extract after separation with the same 50 ml. of ether, contained in a second separator, until the ether-washed water no longer turns red litmus paper blue. Add 25 ml. of 0·1N sulphuric acid, shake well and run into a beaker. Wash the ether with further quantities of water until the washings no longer turn blue litmus paper red, adding each washings to the acid solution in the beaker. Titrate with 0·1N sodium hydroxide using methyl red as indicator.

My thanks are due to the Directors of Messrs. Whiffen & Sons, Ltd., for permission to publish this note.

Reference

1. Lowdell, *Pharm. J.*, 1946, **156**, 141.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Acetylsalicylic Acid, Phenacetin, Caffeine and Bromvaletone in Admixture, Determination of. M. Langejan and J. A. C. van Pinxteren. (*Pharm. Weekbl.*, 1951, **86**, 508.) 1·5 g. of the mixture is dissolved in 5 ml. of ethanol and titrated with 0·5N sodium hydroxide, with phenolphthalein as indicator. This gives the content of acetylsalicylic acid. The titrated liquid is shaken out with 3 × 50 ml. of chloroform, the aqueous phase being rejected. The chloroformic solution, after washing with a little water, is evaporated, and the residue is evaporated with 5 ml. of ethanol. The residue is refluxed for 30 minutes with 10 ml. of 4 N sulphuric acid, cooled, neutralised with sodium bicarbonate (litmus paper) and shaken out with 3 × 25 ml. of chloroform, the chloroform extracts being washed with a little water. The aqueous solution is warmed to remove chloroform, treated with 10 ml. of 4 N sodium hydroxide and boiled down to about 25 ml. After cooling 35 ml. of water, 10 ml. of N potassium cyanide solution and 40 ml. of hydrochloric acid (38 per cent.) are added; 0·1N potassium bromate solution is added drop by drop until there is no yellow colour, followed immediately by a mixture of 20 ml. of phenol solution (8 per cent.), 10 ml. of N potassium bromide solution and 1 g. of potassium iodide. The iodine is then titrated with 0·1N thiosulphate. The number of ml. used × $\frac{22.3}{3}$ represents mg. of bromvaletone. The chloroformic solution is concentrated to 10 ml. and shaken out with 2 × 5 ml. of 4 N sulphuric acid, then with 5 ml. of water. The chloroformic solution is evaporated, the residue being re-evaporated twice with a little ether. The residue is dried at 100° C., and represents the caffeine (anhydrous). The aqueous solution from the last chloroform extraction is diluted with 500 ml. of water and treated with 30 ml. of concentrated hydrochloric acid and 25 ml. of 25 per cent. solution of potassium bromide. The mixture is cooled to about 4° C. and titrated with 0·1M sodium nitrite until, after standing for 5 minutes, the mixture turns starch iodide paper blue. The rate of titration should be, for a titration of up to 4 ml., 2 ml. per minute; for 4 to 8 ml., 1 ml. per minute; and for 8 to 14 ml., 0·5 ml. per minute. The sodium nitrite is standardised against pure phenacetin (1 ml. = 17·9 mg. of phenacetin). G. M.

Alcohols, Paper Partition Chromatography of, using the Potassium Xanthogenates. Tatsuo Kariyone and Yohei Hashimoto. (*Nature, Lond.* 1951, **168**, 511.) Alcohols are converted to the corresponding xanthogenates by treatment with pure carbon disulphide and pure powdered potassium hydroxide and the residual solid product dissolved in water. The aqueous xanthogenate solution is submitted to paper partition chromatography by an ascending method using alkaline butanol as the developing solvent. The xanthogenate spots are detected either by their dark brown luminescence under ultra-violet light or by spraying with Grote's reagent. With solid alcohols the xanthogenates are dissolved in formamide to give the corresponding ammonium xanthogenate. The method has been successfully applied to the detection of small quantities of methanol (0·1 per cent.) in samples of ethanol. Both spots can be identified under ultra-violet light and give colours with Grote's reagent, both of which are

yellow initially, though that due to ethanol becomes brown within half an hour. R_F values for the following alcohol xanthogenates are given:—methanol 0·23, ethanol 0·35, *iso*propanol 0·44, *iso*butanol 0·54, *n*-butanol 0·55, *iso*amyl alcohol 0·62, benzyl alcohol 0·45, octyl alcohol 0·15, *cyclo*hexanol 0·04, ethylene-chlorhydrin 0·91. J. B. S.

Alkaloids in Tissues, Colorimetric Determination by Methyl Orange. A. O. Gettler and I. Sunshine. (*Anal. Chem.*, 1951, **23**, 779.) The authors have modified the methyl orange reaction developed by Brodie and Udenfriend (*J. biol. Chem.*, **158**, 705) to apply it to the quantitative determination of alkaloids in human organs. The main steps in the method were the extraction of alkaloids from tissue with boiling acidified water; the extraction of these alkaloids from the filtered aqueous solution by means of chloroform; and the formation of a chloroform-soluble coloured compound of the alkaloids with methyl orange. Experimental details are given and results are quoted for the analysis of tissues containing known amounts of various alkaloids. The recoveries of most of the alkaloids and other organic compounds including antihistamines were good. Low recoveries were experienced with cocaine probably due to decomposition during the steam distillation, since aqueous solutions of cocaine that were not heated gave good recoveries. Pontocaine and nupercaine were not satisfactorily estimated by the process and experiments with morphine gave poor results. R. E. S.

Alkaloids, Precipitation of, in Ethereal Solution. H. Wachsmuth. (*J. Pharm. Belg.*, 1951, **6**, 86.) The precipitation of alkaloids by silicotungstic acid in aqueous solution is a reaction of low sensitivity, and the composition of the products is variable. On the other hand, by precipitation in ether the sensitivity is high and the precipitate has a constant composition corresponding to 1 molecule of the acid to 3 of alkaloid. The alkaloid, if a base, is dissolved in ether and precipitated with an ethereal or ether-ethanolic solution of the acid. Alkaloidal salts are dissolved first in ethanol. In any case the final concentration of ethanol should be about 12 per cent. The precipitate is washed with ether containing 20 per cent. of ethanol, under slight suction, and dried at 50° C. The results are in general slightly high. As an alternative the alkaloid may be determined by difference iodimetrically, a portion of the filtrate being evaporated to dryness, taken up in water, and treated with iodide-iodate, the free iodine being then titrated with thiosulphate. Results obtained by this latter method are somewhat higher. The sensitivity of the precipitation is high, opalescence being visible at a dilution of 1 in 2 millions. G. M.

***p*-Aminosalicylic Acid, Detection of Impurities in.** O. E. Neufeld. (*Med. J. Austral.*, 1951, **1**, 727.) Several tests are given for the detection of impurities in commercial samples of *p*-aminosalicylic acid. Readily carbonisable substances are tested for by dissolving 10 mg. sample in 2 ml. cold sulphuric acid when no more than a light yellow colour is produced without a bluish fluorescence. The colour produced by a 3 per cent. solution with 5 per cent. ferric ammonium sulphate solution is used for comparison purposes. Ether-soluble matter should not exceed 0·05 per cent. The melting-point is regarded as unreliable, but a limit test for *m*-aminophenol (less than 0·1 per cent.) is given, based on the fact that *p*-aminosalicylic acid as an acid forms an ether-insoluble salt with sodium bicarbonate, whereas meta-aminophenol at this *p*H (7·5 to 7·8) remains the free phenol and can be extracted by an organic solvent like ether. R. E. S.

Antihistamine Preparations, Spectrophotometric Assay of. D. Banes. (*J. Assoc. off. agric. Chem. Wash.*, 1951, **34**, 703.) The antihistaminic compounds studied included thonzylamine, pyranisamine, tripelennamine, and methapyrilene of the ethylenediamine group. Substances examined as being likely to interfere with the estimation were ephedrine sulphate, amphetamine sulphate, naphazoline hydrochloride, benzocaine, butacaine sulphate, dibucaine hydrochloride, procaine hydrochloride, aminophylline, caffeine, phenacetin, and acetylsalicylic acid. The ultra-violet absorption spectra of the various antihistaminics were determined in 0·1N sulphuric acid; the results are given in the table.

Antihistamine salt	Distinctive $\lambda_{max.}$ mμ	Extinction coefficient $E^{1}_{1\ cm.}$ per cent.
Thonzylamine hydrochloride	313	104
Methapyrilene hydrochloride	315	269
Pyranisamine maleate	314	196
Tripelennamine hydrochloride	314	274
Prophenpyridamine maleate	265	212
Chloroprophenpyridamine maleate ..	264	219
Doxylamine succinate	262	227
Diphenhydramine hydrochloride ..	258	16·5

Various procedures are given for the isolation of the antihistamines prior to spectrophotometric estimation, involving four variations of immiscible solvent extraction. In addition to solvent extraction, chromatographic methods were necessary for some of the compounds examined; a 1·0M solution of monobasic potassium phosphate adsorbed on celite was found to constitute a convenient immobile phase for the isolation of prophenpyridamine, chloroprophenpyridamine, doxylamine, or pyranisamine, when chloroform was used as the mobile solvent. Diphenhydramine was quantitatively split into a neutral fragment and an amine fragment by heating with moderately strong acid, the neutral product being isolated from basic substances and compared spectrophotometrically with standards for assay purposes; the procedure permitted an accurate estimation of diphenhydramine in the presence of ephedrine, amphetamine, naphazoline, aminophylline, caffeine, phenacetin, acetylsalicylic acid, benzocaine, or dibucaine. Recoveries using the suggested procedures ranged from 96 to 103 per cent. R. E. S.

Ascaridol, Determination of. H. Bohme and K. van Emster. (*Arch. Pharm. Berl.*, 1951, **284**, 171.) The reagent is the leuco base of 2·6-dichlorphenolindophenol, prepared by reduction of the latter with ascorbic acid. The base should give a practically colourless solution in alcohol. For the determination, 10 to 12 mg. of the sample is mixed with 1·00 ml. of pure toluene: 0·3 to 0·5 ml. of this solution is treated with 0·2 ml. of 1·5 per cent. solution of the leuco base in absolute alcohol, and 5 ml. of toluene containing 5 per cent. of acetic acid, and heated on the water-bath for 20 minutes by the side of a blank test. After cooling, the volumes are made up to 10 ml. with toluene-acetic acid and the extinction is determined using filter S53. It is important that the solvents should be pure—the extinction coefficient of the blank should not be greater than 0·1. G. M.

Barbiturates, Cobalt Reaction for Identification of. H. Gomahr and H. Kresbach. (*Scientia Pharm.*, 1951, **3**, 148.) Cobalt compounds of barbitone and narconumal were prepared in the crystalline form: in both cases the compounds contained 2 molecules of barbituric acid to 1 of cobalt. Both of these cobalt compounds gave a compound with pyridine. The authors conclude that

the compound is a complex in which the barbituric acid is bound to the cobalt by main valencies, and the basic component is linked to the first co-ordination sphere. The exchange of these bonds is the cause of the change of colour observed when using different bases. The necessity for using an optimum proportion for the reaction mixture is explained by an excess of inorganic base giving basic cobalt salts, while excess of organic base forms compounds of the type of dipyridine cobaltochloride. It is thus necessary to use a reaction mixture corresponding to the stoichiometric composition of the complex, i.e., cobalt (base)n (barbiturate)$_2$. The difference in the behaviour of N- and C-substituted derivatives is that in the latter two co-ordinative valencies of the cobalt are already saturated on the imino groups of the barbituric acid in the form of an inner complex salt. These views do not agree with those expressed by other workers. It is concluded that, in spite of the value of the method for identification purposes, it is for structural reasons unsatisfactory as a quantitative test, especially with N-substituted compounds, while qualitatively it is not reliable for very small quantities of the di-imide barbiturates. G. M.

Barbiturates, Copper Reaction for Detection of. H. Gomarh and H. Kresbach. (*Scientia Pharm.*, 1951, **19**, 154.) The reagents used are a 1 per cent. aqueous solution of copper sulphate, and a mixture of 1 volume of pyridine with 9 volumes of chloroform. A few mg. of the substance is dissolved in 1 ml. of the solvent and treated with 1 ml. of copper sulphate solution; barbiturates give a violet colour in the chloroform solution; thiobarbiturates a green one. The colour is stable for several hours. The barbituric acid may be recovered by adding a little ammonia and shaking out into the aqueous layer. The reaction may be applied directly to sodium salts, and to tablets. Hydantoins give a blue colour under these conditions, while carbamides, urethanes, sulphones remain colourless. While caffeine and theobromine give no colour, blue is given by caffeine sodium benzoate, and green by caffeine sodium salicylate, theobromine sodium salicylate, theophylline sodium salicylate, methyl- and propylthiouracil, and phenylcinchoninic acid. G. M.

Barbiturates, Identification of, by X-ray Analysis. T. Y. Huang. (*Acta Pharm. Internat.*, 1951, **2**, 443.) X-ray diffraction patterns may be used for the micro-identification of crystalline barbiturates. Data are recorded for allobarbitone, hexobarbitone, and hexemal, and for 4 different modifications of phenobarbitone. G. M.

Benzocaine, Cocaine and other Local Anaesthetics, Chromatographic Separation of. F. Jaminet. (*J. Pharm. Belg.*, 1951, **6**, 81.) Benzocaine, amylocaine, cocaine, amethocaine and procaine may be separated by paper chromatography. About 20 to 50 μg. of the substance, in the form of hydrochloride, is used, and the solvent is prepared by shaking 50 ml. of *iso*butanol with 7·5 ml. of concentrated hydrochloric acid and 13·5 ml. of distilled water. After separation into two phases, the upper one is used as solvent and the lower one for saturating the atmosphere. The chromatogram may be observed in screened ultra-violet light (procaine and benzocaine), or developed with Dragendorff's reagent (with which benzocaine does not react), or by spraying the paper first with a solution of sodium nitrite in acetic acid, then with an ammoniacal solution of α-naphthol. G. M.

Cardiac Glycosides, Colour Reactions with Antimony Trichloride. F. Jaminet. (*J. pharm. Belg.*, 1951, **6**, 90.) Colour reactions with antimony trichloride may be used for the determination of various heterosides and their genins. *Characterisation of digitoxin and gitoxin.* About 3 mg. of the material is treated with

1 ml. of a solution of 50 per cent. of antimony chloride in acetic anhydride. After solution is complete, the mixture is warmed for exactly 3 minutes at 75° C., then cooled quickly. The resulting solution shows two maxima at 490 and 590 mμ due respectively to digitoxin and gitoxin. *Determination of ouabain and k-strophanthoside.* From 0·5 to 2 mg. of the material is treated as above, the solution being finally diluted with 5 ml. of acetic anhydride. The absorption is determined at 490 mμ in a 1 cm. cell. It is important to adhere exactly to the prescribed conditions in both cases. It may be noted that the colour obtained with ouabain and strophanthoside increases with the time of heating, that with gitoxin decreases. *Fluorimetric determination of ouabain.* The sample (0·1 to 1·4 mg.) is treated as before, the final solution being diluted with 10 ml. of acetic anhydride. The fluorescence is compared against a standard prepared with 2 mg. of ouabain, using filters U.V. and P.C. and a Coleman spectrophotometer. G. M.

Cinchona Alkaloids, Separation by Paper Partition Chromatography. D. J. Lussman, E. R. Kirch and G. L. Webster. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 368.) A method for the separation of the major alkaloids of cinchona by means of paper partition chromatography, using *cyclo*hexanol or *cyclo*hexanone with hydrochloric acid as the solvent mixture is described. The position of the individual alkaloids upon the chromatogram was identified by means of R_F values determined with pure samples, coupled with the observation of fluorescence in the cases of quinine and quinidine and the development of a coloured compound by cinchonidine and cinchonine when the chromatogram was treated with a potassium iodoplatinate reagent. The most efficient separations were obtained using *cyclo*hexanol saturated with distilled water as solvent, but more reproducible results were obtained when the *cyclo*hexanol was saturated with dilute acid. *cyclo*Hexanone gave a quicker development of the chromatogram than *cyclo*hexanol, but the efficiency of the separation was greater using the latter solvent. Using this method as little as 3 μg. of quinine or quinidine, 25 μg. of cinchonidine, and 100 μg. of cinchonine may be detected. A. H. B.

Digitalis, Chemical Assay of. M. Langejan. (*Pharm. Weekbl.*, 1951, **86**, 593.) As a preliminary to working out a chemical process for the assay of digitalis, a study was made of digitoxin. This gives on complete hydrolysis 1 molecule of genin and 3 of digitoxose. A semi-micro titrimetric method is given for the latter, and also a colorimetric one based on the reaction of Bial. By modifying this reaction it has been made more specific for digitoxose: 1 ml. of the digitoxose solution is mixed with 2 ml. of Bial's reagent, and the mixture is heated for exactly 1 minute in a water-bath, and cooled quickly. The liquid is transferred to a 25-ml. measuring flask, washed in with 5 ml. of 25 per cent. hydrochloric acid, then made up to the mark with water. The extinction is measured at 620 mμ. This reaction can be carried out without interference from relatively large quantities of other sugars, while digitoxose in glycosides may be determined without previous hydrolysis. Free and combined digitoxose may be distinguished by shaking the latter into chloroform. Digitoxin is not easily hydrolysed, and it was not found possible to hydrolyse it completely without decomposition of the sugar. The aglycone content (free and combined) may be determined colorimetrically using 3 : 5-dinitrobenzoic acid by the method of Kedde. The extinction coefficient in this reaction is increased after boiling with acid. Treatment of the glycosides or aglycones with alkali decreases this extinction, indicating the production of alterations in the molecule. G. M

Formaldehyde, Acidimetric Determination of. W. Krause. (*Pharm. Zentralh.*, 1951, **90**, 218.) About 2 g. of formaldehyde solution is diluted with 50 ml. of water and treated with 4 drops of a mixture of equal parts of 0·1 per cent. ethanolic solution of dimethyl yellow and 0·1 per cent. ethanolic solution of methylene blue. The mixture is neutralised to a grey colour, and a neutralised solution of 2·5 g. of hydroxylamine hydrochloride in 50 ml. of water is added. After standing in a closed flask for 1 hour, the mixture is titrated with N potassium hydroxide, 1 ml. of which corresponds to 30·03 mg. of formaldehyde. For smaller amounts of formaldehyde, 1 g. of hydroxylamine hydrochloride and 0·1N alkali may be used. G. M.

Glacial Acetic Acid and other Non-Aqueous Solvents, Titrations in. M. E. Auerbach. (*Drug Standards*, 1951, **19**, 127.) The methods used for the titration of weak bases and weak acids in non-aqueous solvents are described, special mention being made of the titration of weakly basic substances by dissolving in glacial acetic acid and titrating with perchloric acid—the so called "acetous-perchloric" titration. A general method for this determination is given. A standard solution which is 0·1N in respect to perchloric acid is prepared by mixing the requisite amount of 70 per cent. perchloric acid with glacial acetic acid, and adding enough acetic anhydride to react with the water necessarily added with the perchloric acid. This solution is standardised by such primary standards as sodium acetate, sodium carbonate, guanidine carbonate, potassium acid phthalate, or sodium salicylate. The standard, or the sample, is dissolved in 25 ml. of acetic acid, 5 drops of 0·1 per cent. crystal violet indicator (in glacial acetic acid) are added, and the solution titrated with the standard acetous-perchloric acid. Crystal violet shows a series of colour changes in the vicinity of *p*H 0 to 1. Before the end-point, the colour becomes blue, at the end-point a definite clear green, and further addition of perchloric acid produces a yellow colour. Amino acids, alkaloids, pyridine, β-naphthylamine, salts such as sodium potassium tartrate, etc., and salts of organic bases such as picrates, citrates, oxalates, etc., could be titrated directly by the above method. Apparently any basic substance stronger than anthranilic acid can be titrated in this way using crystal violet as indicator. The method is particularly useful where the advantages of a good organic solvent, and the ability to titrate a really weak base can be combined. For example, benzocaine ointment may be assayed by dissolving in chloroform, diluting with acetic acid, and titrating directly. A. H. B.

Morphine, New Colour Reaction of. R. Castagnou and C. Paoletti. (*Bull. Soc. Pharm. Bordeaux*, 1951, **89**, 91.) By warming 5 ml. of morphine solution with 0·5 ml. of formaldehyde and 5 drops of ammonia for 30 minutes on the water-bath, a yellow colour is produced. The sensitivity is 1 mg. The reaction is given also by diacetylmorphine, but not by ethylmorphine. G. M.

Nitrites in the Presence of Nitric Acid, Iodopermanganate Determination of. R. C. Brasted. (*Anal. Chem.*, 1951, **23**, 980.) The iodopermangante method consists of adding an aliquot portion of a nitrite solution to a known volume of standard 0·1N permanganate solution acidified with 5N sulphuric acid, and after the completion of the relatively slow reaction between the nitrite and permanganate ions, reducing the excess of permanganate with iodide and titrating the liberated iodine with standard 0·1N thiosulphate. The present investigation studies the effect on nitrite determination when the permanganate solution is acidified with varying amounts of nitric acid instead of sulphuric acid, the effect

of impurities likely to be found in commercial concentrated nitric acid, the means of removing interfering substances from concentrated nitric acid and the effect of titrating thiosulphate solution in highly acidified nitric acid solutions. In the presence of concentrated nitric acid, added to standard permanganate prior to the addition of sodium nitrite solution, accurate results are obtained only if the nitric acid has been pretreated with crystals of sulphamic acid to remove nitrous acid, or freshly boiled. Successful titrations were carried out with less than 0·1 g. of sodium nitrite in 48 g. of concentrated nitric acid, and thus the method is suitable for the determination of nitrites in the presence of nitrates. It is shown that iodine may be titrated with thiosulphate in solutions containing approximately 8M concentration of nitric acid, and that the oxidation of iodide to iodine is insignificant within the time required for a thiosulphate titration. Solid sodium nitrite was found to lose less than 0·1 per cent. of its original weight on heating at 115° C. for 28 hours, and solutions of sodium nitrite were stable over 90 days' standing. A. H. B.

Pepsin Assay, Comparison of Methods. H. J. Anderson, D. M. Findlay, M. Targy, H. H. Wiesman and F. W. Wheeler. (*Drug Standards*, 1951, **19**, 135.) A comparison of the official National Formulary procedure and a "milk curdling method" for the determination of pepsin is made. The "milk curdling method" is applied as follows. The milk for the assay is prepared by siphoning the lower layer from 1 quart of pasteurised whole milk, adding 20 ml. of 0·5N hydrochloric acid, mixing quickly and rapidly filtering. Portions (25 ml.) of this milk are pipetted into 1 inch diameter tubes and the tubes placed in a 30° C. bath for 10 minutes. To each tube is added 1·0 ml. of the pepsin standard, or sample, and the contents quickly mixed by inversion of the tubes. The time of addition is noted to the nearest second. The setting time for each tube is noted and the strength of the sample obtained by comparison with the standard solution. The results by this method check well with those obtained by the National Formulary method, and the former is a quick assay involving no expensive or complicated apparatus. Although other enzymes such as rennin and papain curdle milk, tests are available for distinguishing pepsin from these enzymes. A. H. B.

Sulphonamides, Identification of, by Paper Partition Chromatography. R. Robinson. (*Nature, Lond.* 1951, **168**, 512.) The method of ascending paper chromatography described by Williams and Kirby (*Science*, 1948, **107**, 481) has been used for the identification of sulphonamides in small volumes of biological fluids. Although no single developing solvent is suitable for the separation of all sulphonamides, it is shown that these substances fall into two classes, separable with basic and acidic solvents respectively. R_F values for sulphaguanidine, sulphathiazole, sulphanilamide, sulphadiazine, sulphamethazine, sulphamerazine, sulphacetamide and sulphapyridine have been determined using *n*-butanol-ammonia and *n*-butanol-acetic acid respectively. Solutions (0·01 ml.) containing 5 to 15 μg. of sulphonamide in N/1 hydrochloric acid are spotted on the base line, sprayed with Ehrlich's reagent and developed for 6 to 12 hrs. according to the standard technique. Irrigation with the basic developing solvent caused the spots to fade and a second spraying with Ehrlich's reagent may be necessary. J. B. S.

Vanillin, Assay of. L. K. Sharp. (*Analyst*, 1951, **76**, 215.) An examination has been made of various methods available for the assay of vanillin. It was found that the usual volumetric methods including that of the B.P. 1948 and

the hydroxylamine hydrochloride method gave inconsistent results, mainly because of indefinite end-points in coloured solutions. A modified gravimetric procedure was worked out which gave satisfactory results; the procedure involved precipitation with dinitrophenylhydrazine hydrochloride under carefully specified conditions, the resulting precipitate being filtered off, washed, dried at 110° C., and weighed; other gravimetric procedures gave results that were too high. The ultra-violet absorption curve of pure vanillin in 0·01N aqueous hydrochloric acid was determined; it gave the following points of inflexion, $\lambda_{max.}$ 279 mμ, $E_{max.\ 1\ cm.}^{1\ per\ cent.}$ 680·0; $\lambda_{max.}$ 307·5 mμ, $E_{max.\ 1\ cm.}^{1\ per\ cent.}$ 596·2; $\lambda_{min.}$ 296 mμ; $E_{min.\ 1\ cm.}^{1\ per\ cent.}$ 549·5; the figures so obtained were found to be satisfactory criteria of purity. A satisfactory volumetric oxidation procedure was worked out in which 0·4 g. was dissolved in 20 ml. of N sodium hydroxide, 40 to 60 ml. of solution of hydrogen peroxide (20 vol.) were added, and the mixture was then heated on a water-bath until effervescence ceased and then cooled to room temperature; the excess of sodium hydroxide was titrated with N hydrochloric acid, using phenolphthalein as indicator. A blank determination was necessary and an arbitrary factor of 1 ml. of N hydrochloric acid equivalent to 0·2954 g. of $C_8H_8O_3$ was used. R. E. S.

ORGANIC CHEMISTRY

Alginic Acid, Organic Derivatives of. A. B. Steiner and W. H. McNeely. (*Industr. Engng. Chem.*, 1951, **9**, 2073.) Alginic acid reacts relatively rapidly under mild conditions with alkylene oxides, to give water-soluble esters by esterification of the carboxy group of each anhydro-β-D-mannuronic acid residue in alginic acid, the reaction of greatest importance being with propylene oxide. The propylene oxide penetrates the water-soluble fibres of the acid, reaction taking place slowly at room temperature but considerably faster at 75° C. The reaction rate of alginic acid and propylene oxide being high, 50 to 75 per cent. esterification was readily obtained, resulting mainly in 2-hydroxypropylene alginate. Derivatives of reactions with ethylene oxide and short and long chain oxides were also prepared. The decrease in solubility and mole per cent. esterification which was found to occur with increasing chain length of the oxide was to some extent overcome by the addition of glycerol and by partially neutralising the alginic acid when a minimum mole per cent. esterification of 20 mole per cent. could be obtained. Under these conditions the main course of the reaction takes place through the alginate ion rather than the alginic acid when esterification is very slow. When 10 to 20 mole per cent. of the carboxyl groups are neutralised with a base the reaction becomes strongly exothermic, *p*H rises rapidly and a practicable esterification is obtained in a few hours. The stability and emulsifying and thickening properties of these derivatives, and their uses in pharmacy, are reviewed. J. R. F.

Nicotinic Acid Derivatives, Paper Chromatography of. E. Kodicek and K. K. Reddi. (*Nature, Lond.* 1951, **168**, 475.) Rapid colour and fluorescent tests are described for the detection of tertiary and quaternary pyridinium compounds. The colour test is based upon the formation of coloured compounds when tertiary pyridine derivatives are treated with cyanogen bromide and a primary aromatic amine. The fluorescence test, which allows of the detection of quaternary nicotinamide derivatives, depends on the formation of a bluish-white fluorescence, when quaternary pyridine compounds with a side chain CO–NHR in the β position are treated with methyl ethyl ketone and ammonia. These tests were applied to various nicotinic acid derivatives after descending chromatograms had been run on Whatman paper with different solvent systems. R_F

values of nicotinamide and coramine are more or less constant irrespective of the solvents used, whereas the more acidic compounds, such as nicotinic acid moved slower in neutral or alkaline solutions than in acid solutions.

J. B. S.

PLANT ANALYSIS

***Ammi Visnaga*, Pyrone Content of.** E. Steinegger. (*Phar. Acta Helvet.*, 1951, **26**, 291.) Ammi visnaga is known to contain a number of γ-pyrones related to furochrome. The fruits of the related *Ammi majus* L. contain α-pyrones, and it appeared possible that such compounds might occur also in *Ammi Visnaga*. In order to decide this point, 125 g. of the fruit from a botanically identified culture (of Swiss growth) was exhausted with ethanol (50 per cent.), and the extract was treated with 20 g. of lead acetate in 60 ml. of water. The precipitate was removed by centrifuging, and a solution of 4 g. of monosodium phosphate was added. After centrifuging and concentrating to half bulk, the solution was shaken out with chloroform. The residual solution was dried in vacuo and boiled out with ether, the ethereal solution being washed with 10N sulphuric acid, then with water, and evaporated: 1·173 g. of semi-fluid greenish residue was obtained (α-pyrone fraction). The sulphuric acid extract was nearly neutralised, and the precipitate obtained taken up in chloroform, giving 2·556 g. of semi-solid greenish residue (γ-pyrone fraction). From the latter a total of 1 g. of crystalline products was obtained. The α-pyrone fraction was treated by chromatography on alumina. Two crystalline products were isolated, melting respectively at 97° C. to 100° C., and 187° C. to 188° C. The latter compound was a coumarin, but not identical with any of the three coumarins which have been isolated from *Ammi majus*. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenotrophic Hormone, Inactivation by Plasma. M. Reiss, F. E. Badrick, I. D. K. Halkerston and C. Plaice. (*Nature*, 1951, **168**, 206.) The rapid inactivation of an adrenotrophic hormone preparation during incubation with heparinised plasma from rats, rabbits and humans was observed. Parallel incubation of the same preparation with the solvent containing an equivalent amount of heparin showed no measurable inactivation when assayed by the Sayers adrenal ascorbic acid depletion assay. R. E. S.

***p*-Aminobenzoic Acid, Metabolism of.** C. W. Tabor, M. V. Freeman, J. Bailey and P. K. Smith. (*J. Pharmacol.*, 1951, **102**, 98.) *p*-Aminobenzoic acid is rapidly absorbed and excreted. In the rat the concentrations in the plasma are appreciably higher than in brain, muscle and erythrocytes. In normal man very little of the drug is excreted as free *p*-aminobenzoic acid and only a small amount is excreted in the acetylated form. Most of it is excreted either as a conjugation product with glycine or as the glucuronate. A small amount of the glucuronate derivative is also acetylated. S. L. W.

Dextran and its Derivatives, Chemistry of. C. R. Ricketts. (*Proc. Roy. Soc. Med.*, 1951, **44**, 558.) Dextran is a polysaccharide formed during the growth of *Leuconostoc mesenterioides* on a medium containing sucrose. The organism produces an enzyme which polymerises the glucose portion of the sucrose to form dextran; the fructose portion is liberated. The glucose units are joined through 1 : 6-glucoside links, and the main chains so formed have

short branches at frequent intervals, probably joined through 1 : 4-glucoside links. The dextrans produced by the various strains of *Leuconostoc* differ in the extent of the branching. In their native state, the chains are composed of about 200,000 glucose units, with a molecular weight of about 40 million and comparable in size to viruses. These large molecules are unsuitable for use as a plasma substitute and are broken down by partial hydrolysis. The hydrolysate contains molecules varying in molecular weight from 1 million to 10,000. On adding alcohol or acetone and cooling, fractional precipitation occurs and makes possible the selection of a sample containing the desired distribution of molecular size. Viscosity and osmotic pressure measurements indicate that dextran molecules are longer and thinner than proteins of comparable molecular weight. Dextran sulphate is prepared by treating dextran with chlorosulphonic acid and pyridine. The free ester is only stable in solution, but solid sodium, calcium and other salts can readily be prepared. These compounds have an anticoagulant action similar to that of heparin, which is itself the sulphuric acid ester of a polysaccharide. G. R. K.

Folic Acid, Stability in Solutions of the B Group Vitamins. A. R. Biamonte and G. H. Schneller. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 313.) The solubility of folic acid in aqueous media increases with increase in *p*H. At *p*H 5·6 and above it is completely dissolved in a concentration of 1 mg./ml.; in the range *p*H 3 to 4 it is substantially undissolved. The dissolved folic acid at *p*H 6 and above is stable for one year at room temperature; at *p*H 3 to 4 the dissolved folic acid is unstable, although mixtures containing any undissolved folic acid at this *p*H range exhibit good stability and are pharmaceutically practicable and useful. In the presence of riboflavine and, to a lesser extent, aneurine, dissolved folic acid is rapidly decomposed, particularly at *p*H 6 to 7; at lower *p*H levels, where folic acid is considerably undissolved, good stability is exhibited. Pyridoxine and pantothenyl alcohol cause some decomposition of dissolved folic acid but none of undissolved folic acid. Nicotinamide has no effect at any *p*H level. The stability of folic acid in syrup is essentially the same as that in aqueous media, but in mixtures of propylene glycol and syrup, the decomposition is considerably increased. The decomposition of folic acid at low *p*H levels and also in the presence of other vitamins of the B group involves the formation of a pteridine and *p*-aminobenzoylglutamic acid. G. R. K.

Malt Extract, Fermentation of. M. P. English. (*Nature, Lond.* 1951, **168**, 391.) An investigation of a sample of fermented malt extract yielded certain yeasts capable of fermenting the yeast-free fresh malt when pure cultures were inoculated back into it. With one exception, these yeasts were found to be species of *Zygosaccharomyces*, a genus which contains many osmophilic species. 5 of the isolates were identified by the Centraal-bureau voor Schimmelcultures Baarn, Holland, as *Z. japonicus* Saito, though they differed slightly in minor cultural characteristics. As the extract originally contained about 50 per cent. of sugars, mostly maltose, any organism capable of fermenting must be highly osmophilic; the organism can grow on substrates of considerably higher osmotic pressure than malt extract as shown by its power of fermenting almost saturated solutions of maltose, and glucose solutions of as high a concentration as 90 per cent. w/v. R. E. S.

Organic Acids, Partition Paper Chromatography of. J. Opienska-Blauth, O. Saklawska-Szymonowa and M. Kanski. (*Nature, Lond.* 1951, **168**, 511.) Acids known to be metabolites in living bacterial cells have been examined. The standard Consden, Gordon and Martin technique (*Biochem. J.*, 1944, **38**,

224) is employed using phenol saturated with water as the developing solvent. The atmosphere in the chromatographic cabinet is saturated with formic acid vapour to prevent the ionisation of the organic acids. The position of the spots is revealed by treatment with an alcoholic solution of bromophenol blue. No apparent influence on R_F values was observed when the concentration varied between 0·5 and 5·0 per cent. and, in general, the size and intensity of the spots was proportional to the concentration of acid used, though some exceptions were noted. Volatile organic acids were not detected and uric acid could not be recovered, probably owing to its insolubility in water. Halogenated derivatives of acetic acid did not give consistent R_F values. In an attempt to find a numerical relationship between chemical constitution and R_F values of organic acids, the theoretical ΔR_F values for a number of different groups have been calculated by the direct comparison of the R_F values for various pairs of related acids. J. B. S.

Vitamins D, Separation by Condensation and Irradiation. J. Green. (*Biochem. J.*, 1951, **49**, 54.) An investigation has been made into the analytical purification of the vitamins D by the methods of maleic and citraconic anhydride condensation and by selective ultraviolet irradiation. The use of selective condensation with maleic or citraconic anhydrides as an analytical step in the assay of irradiation products and fish-liver oils for vitamins D is ineffective in quantitatively removing vitamin A or, alternatively, in removing enough vitamin A to eliminate interference in the vitamin D determination. The condensation procedure of Milas *et al.* (*Industr. Engng. Chem., Anal. Ed.*, **13**, 227) completely destroyed large amounts of vitamin D_3 and over 60 per cent. of calciferol, even when large quantities of the latter are used. The use of ether for the condensation gave good recoveries of calciferol and vitamin D_3 but complete elimination of vitamin A is not possible with a reasonable reaction time. Condensation in benzene gave up to 20 per cent. loss of calciferol and about 40 per cent. loss of vitamin D_3. Selective ultraviolet irradiation to remove vitamin A from vitamin D was not suitable for routine use. Although vitamin A could be smoothly destroyed by filtered radiation of wavelength 300 to 400 mμ, the irradiation, under similar conditions, of small amounts of vitamin D resulted in inevitable losses. R. E. S.

BIOCHEMICAL ANALYSIS

Adrenaline in Blood and Adrenal Gland, Colorimetric Determination of. N. C. Ghosh, C. Deb and S. Banerjee. (*J. biol. Chem.*, 1951, **192**, 867.) In view of the fact that established methods for the colorimetric estimation of adrenaline are unsuitable for its estimation in blood, a new procedure has been worked out. The blue colour formed by the action of Folin's reagent on adrenaline is measured in such a way that the contributions to the total colour of the solution due to the reaction of such substances as ascorbic acid, cysteine, glutathione, ergothionine and uric acid, with the reagent, are eliminated. It has been observed that a 10 per cent. solution of sodium bicarbonate completely destroys a dilute solution of ascorbic acid and cysteine, and partly destroys glutathione, without at the same time affecting adrenaline. On treatment of the resulting solution with 5 per cent. sodium hydroxide only adrenaline is destroyed, uric acid, ergothionine and the remaining glutathione being stable under these conditions. This observation forms the basis of the method used for the estimation of adrenaline in trichloracetic acid extracts of blood and adrenal glands. Two identical samples are treated simultaneously at 30° C. for 30 minutes with 10 per cent. sodium bicarbonate and a mixture of 10 per cent.

sodium bicarbonate and 5 per cent. sodium hydroxide respectively. The blue colour which developed on the addition of the reagent was measured after 1½ minutes in a Lumetron photoelectric colorimeter. The difference of the two colorimeter readings represents the colour due to adrenaline. The adrenaline content of the blood from rats, rabbits, guinea-pigs, monkeys and man, and of the adrenal gland of rabbits, has been determined by this method. J. B. S.

Aminoacids and Peptides, Spectrophotometric Analysis of. J. R. Spies and Dorris C. Chambers. (*J. biol. Chem.*, 1951, **191**, 787.) A simple and rapid spectrophotometric method is based on the colour obtained when combined copper is converted to the copper salt of alanine. Since the colour intensities of equimolar concentrations of amino acid copper complexes vary, solutions of the copper complexes of the various amino acids are treated with alanine, thus largely converting them to the highly soluble alanine copper complex, which is used as the standard. 17 amino acids, 9 dipeptides and 5 tripeptides were studied. Dipeptides, in general, react with copper as though the component amino acids were free, while only two of the amino acids of tripeptides react. The restriction of copper-binding property of peptides to the "end group" (free NH_2 adjacent to peptide-linked carboxyl or free carboxyl adjacent to peptide-linked nitrogen) amino acids, increases the usefulness of the method in that protein hydrolysis can be followed. Excellent agreement was obtained between the values for chromogenic nitrogen found and calculated for (*a*) complete acid hydrolysates of casein, (*b*) β-lactoglobulin and (*c*) fraction CS-54R (cottonseed allergenic protein) based on their amino acid contents. J. B. S.

Progesterone in Blood, New Method for Determination of. W. R. Butt, P. Morris, C. J. O. R. Morris and D. C. Williams. (*Biochem. J.*, 1951, **49**, 434.) The initial separation of steroid hormones from plasma is effected by a solvent partition method. The first ethanol-ether extracts are concentrated and the steroid fraction partitioned between water and ethyl acetate and between water and light petroleum in turn. The solid residue from the light petroleum extract is partitioned on a column of Hyflo grade Supercel with methanol (70 per cent.) as the stationary phase, and *n*-hexane as the mobile phase. Extensive details of column dimensions and packing are described and under those conditions progesterone appears in the second and third ml. of eluate. The eluate is evaporated, the residue is dried over phosphorus pentoxide and treated with a solution of Girard's reagent T in anhydrous acetic acid for 2 minutes at 100° C. Sodium chloride, sodium hydroxide and water are added, the solution is deoxygenated and examined polarographically. The method has been used to examine the concentration of progesterone in the circulation after injection into normal and partially hepatectomised rats. Examination of human pregnancy blood samples has shown that the progesterone level is less than 0·1 μg./ml. In two cases progesterone has been detected in human placental blood and in two further cases a substance giving a polarographic wave resembling that of a Δ^4-3-keto-steroid, but showing no 20-ketosteroid wave, was detected. J. B. S.

Sugars, Detection of, by Paper Chromatography. R. J. Bayly, E. J. Bourne, M. Stacey. (*Nature, Lond.*, 1951, 168, 510.) Some observations of unusual phenomena encountered during the paper chromatography of sugar mixtures are reported. Paper chromatograms of sugars in diabetic urines showed three spots when developed with a mixture of *n*-butanol, ethanol, water and ammonia, and sprayed with certain primary aromatic amines. The R_F value of one of the spots was identical with a reference glucose spot, while the R_F values of the other two were respectively 37 per cent. and 78 per cent. of that of the reference spot.

The slowest moving substance stained with naphthoresorcinol but not with ammoniacal silver nitrate; the middle spot stained with ammoniacal silver nitrate. In *n*-butanol saturated with water the slowest spot moved only 11 per cent. of the distance moved by the glucose spot, while a mixture of *n*-butanol, acetic acid and water as the developing solvent only the glucose spot was observed. Two dimensional chromatography of the urine samples, developed first with butanol-ammonia followed by butanol-acetic acid, showed the complete reversion, in the second solvent, of all spots to glucose. Acid hydrolysates of dextran and starch, subsequently neutralised and submitted to paper chromatography using butanol-ammonia as the developing solvent, indicated the presence solely of glucose, when 20 μg. quantities of sugar were used. A tenfold increase in quantity, however, indicated the presence of a spot, identical in R_F value and reaction to spraying reagents, with the slowest one observed in the urine samples. The same spot was also observed when 200 to 300 μg. of pure glucose was dissolved in water and run on a chromatogram. Other aldoses, including both pentoses and hexoses, behaved similarly, though no additional spots were observed with the ketoses fructose and sorbose. Similar, but less regular, phenomena have also been noted with some partially methylated sugars. J. B. S.

Uric Acid in Blood and Urine, Determination of. D. S. Bidmead. (*J. clin. Path.*, 1951, **4**, 370.) The Folin method was found to give too variable results when used for the determination of uric acid in the blood of the same patient 3 or 4 times a day and an alternative colorimetric procedure was developed, based on precipitation of proteins and treatment of the filtrate with urea-cyanide and an arsenophosphotungstate reagent. The urea-cyanide reagent is prepared by dissolving 25 g. of pure sodium cyanide in 400 ml. of distilled water and adding 75 g. of urea and water to 500 ml. The arsenophosphotungstate reagent is made by dissolving 50 g. of molybdate-free sodium tungstate in 300 ml. of distilled water, adding 25 g. of arsenic pentoxide and when solution is complete adding 12·5 ml. of syrupy phosphoric acid and 10 ml. of hydrochloric acid; boiling for 20 minutes, cooling and diluting to 500 ml. The stock solution of uric acid is prepared by Folin's method. In applying the test to blood, 1 ml. of plasma is centrifuged with 7 ml. of water, 1 ml. of 0·67N sulphuric acid and 1 ml. of 10 per cent. sodium tungstate solution. 5 ml. of the clear supernatant liquid is mixed with 2 ml. of urea-cyanide solution, 0·2 ml. of arsenophosphotungstate solution and water to 10 ml. After 5 minutes, the colour is read in a photoelectric absorptiometer. For urine, a 1 per cent. dilution of the sample is made and 5 ml. of the dilution is used for the test, which is similar to that adopted for plasma. A standard curve is prepared from the results obtained with known amounts of uric acid. Good recoveries were obtained when the procedure was applied to samples both of blood and urine to which additional uric acid had been added. H. T. B.

Vitamin A, Assessment of Potency by Spectrophotometry. T. Boldingh, H. R. Cama, F. D. Collins, R. A. Morton, N. T. Gridgeman, O. Isler, M. Kofler, R. J. Taylor, A. S. Welland and T. Bradbury. (*Nature, Lond.* 1951, **168**, 598). Because of the variations of absorption spectra of many organic solutes upon varying the solvent, the $\epsilon_{max.}$ and λ_{max} values were determined for pure all-*trans* vitamin A alcohol and acetate in *iso*propanol, ethanol, *cyclo*hexane and light petroleum (40° to 60° C.). The figures recorded are the means of the results from 5 laboratories. Analysis of variance of the 40 individual results indicated that the coefficient of variation between laboratories was 1·3

and that the residual coefficient of variation of the one determination was 1·1. Appropriate factors are given for the conversion of values at $\lambda_{max.}$ for vitamin A alcohol or acetate to I.U./g. A. H. B.

Vitamin A, Spectrophotometric Assay of, Geometric Correction of Absorption Curves. N. T. Gridgeman. (*Analyst*, 1951, **76**, 449.) The theory and the reliability of the Morton-Stubbs geometric correction of absorption curves by separating the compound curve into two parts (the major part being the characteristic curve and the minor part due to "irrelevant" absorption) is discussed in its application to the analysis of vitamin A in fish-liver oils and concentrates. The accuracy of the method depends upon the valid scope of two assumptions, (1) that the absorption curves of natural forms of the vitamin are indistinguishable from that of pure all-*trans* vitamin A; (2) that the ultra-violet absorption curves of materials other than vitamin A in natural oils have three linear points at certain wave-lengths. Evidence is presented to show that these assumptions are not always correct, and that comparatively small departures from these conditions may cause appreciable loss of accuracy, and an attempt is made to correlate degrees of departure with accuracy of the result. The influence of the normal observational errors of spectrophotometry on the precision of the result is considered. A. H. B.

Vitamin A, The Precision of the 3-point Correction Method of Spectrophotometric Assay of. D. C. M. Adamson, W. F. Elvidge, N. T. Gridgeman, E. H. Hopkins, R. E. Stuckey and R. J. Taylor. (*Analyst*, 1951, **76**, 445.) The reproducibility of the Morton-Stubbs 3-point geometric method of correcting for irrelevant absorption in the spectrophotometric assay of vitamin A was investigated. For the whole oil in *cyclo*hexane the formula is:—E at 328 mμ (corr.) = 7 × E at 328 mμ − 2·882 × E at 313 mμ − 4·118 × E at 338·5 mμ. Seven independent laboratories determined by photo-electric spectrophotometry $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ at 313 mμ, 328 mμ and 338·5 mμ, each in duplicate (separate weighings), on 5 oils, the solvent being *cyclo*hexane. The geometrically corrected $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ at 328 mμ results were then statistically analysed and it was concluded that the limits of error of a determination in duplicate from any one laboratory are about ±15 per cent. for P = 0·05. The corresponding figure for uncorrected E values is about ±2 per cent. A. H. B.

Vitamin B_{12}, Assay of. Interference with the *Escherichia coli* Response. W. F. J. Cuthbertson, H. F. Pegler, C. Quadling and V. Herbert. (*Analyst*, 1951, **76**, 540.) The effects of various substances on the *E. coli* plate assay of vitamin B_{12} were investigated. Different concentrations of the reagents studied were placed on the assay plates in the absence or presence of the vitamin; when the reagents were tested in its presence the concentration of the vitamin used was always kept at 0·2 μg. per ml. (the same as that used in the provisional assay technique). The growth zones were compared qualitatively with those caused by the vitamin at concentrations of 0·02 and 0·2 μg. per ml. on the same plate; if the test mixtures produced growth qualitatively similar to that obtained with the vitamin alone then the diameters of the growth zones were measured and the apparent concentrations of vitamin B_{12} in the test mixtures were calculated. The allowable concentration of interfering substances may be expressed in the form C = KN per cent. where C is the permitted concentration in the test solution expressed as a percentage, N is the vitamin B_{12} concentration (in μg. per ml.) and K is a constant depending on the interfering substance. Tables are given showing the values of K at levels leading to a 2 per cent. or a 5 per cent. error and showing whether the interfering agents cause an increase or a decrease in the

apparent vitamin B_{12} activity of the solution. Substances studied were ethanol, acetone, ethylene glycol, propylene glycol, ascorbic acid, formalin 40 per cent. (response reduced); butanol, toluene, sodium formate, manganese sulphate, ferrous sulphate, choline, betaine, ammonium sulphate (response unaltered); methionine, thioglycollic acid, phenol, copper sulphate, sodium chloride (response increased); homocystine, potassium cyanide (effect variable). R. E. S.

Vitamin B_{12}, Cup-Plate Assay of, Using *Lactobacillus lactis* Dorner 10697. F. E. Larkin and R. E. Stuckey. (*Analyst*, 1951, **76**, 150.) The routine use of tube assays of vitamin B_{12} with a variety of organisms was abandoned in favour of a cup-plate assay with *Lactobacillus lactis* Dorner 10697 using a modification of the method of Foster, Lally, and Boyd Woodruff (*Science*, 1949, **110**, 507). The effects of variation of the *p*H of the test liquid and of variation in the concentration of cresol present were studied. The most reliable results were obtained on solutions containing crystalline vitamin B_{12}, although considerable variations were experienced; the microbiological assay was found to be particularly useful in determining the stability of samples of vitamin B_{12} of differing purities. With other vitamin B_{12} preparations the microbiological assay was always interpreted in conjunction with organic cobalt determinations since although the organic cobalt assay gave a maximum figure, the microbiological assay often exceeded this result. Concentrated liver preparations gave, in general, concordant results but on autoclaving there was often an increase in microbiological activity, sometimes as much as 70 per cent. Assays of vitamin B_{12} concentrates obtained from streptomyces fermentation liquors gave results that were difficult to interpret, anomalous growth zones being present; it was considered necessary to use the assay in conjunction with a chromatographic procedure, although some difficulty was experienced in getting a quantitative elution of the small amounts present on the chromatogram. R. E. S.

Vitamin B_{12}, Microbiological Determination of. B. Noer. (*Dansk Tidsskr. farm.*, 1951, **25**, 222.) Four organisms were compared for suitability for vitamin B_{12} assay: *Lactobacillus lactis* Dorner, *L. Leichmannii*, *Thermobacterium lactis* and *T. Yogurth*. The most reproducible results were obtained with *T. lactis*, which also has the advantage that it requires the simplest substrate. The methods usually adopted for the assay are not altogether satisfactory, for the following reasons. It is not possible to be certain that all the other growth factors required are present, since many of them are of an unknown nature and their stability to heat and other conditions is unknown: this applies especially to hydrolysed casein. Growth-hindering substances may be present, and slight alterations in *p*H or *r*H may affect the growth. In order to compensate for these factors it is proposed to use for the test three solutions: a normal standard solution, a test solution, and a test solution to which has been added the optimal quantity of vitamin B. The amount of growth is determined turbidimetrically, using a photo-electric colorimeter. The corrected values obtained by this "compensation method" allow for the extra growth-promoting substances which may be lacking in the culture solution, and for growth-inhibiting substances in the test solution. However, such tests can not alone give a certain figure for the vitamin B_{12} content. Growth resulting from desoxyribosides can be distinguished from that due to the vitamin, as the latter is unstable to heat or light and comparative tests before and after autoclaving may show the presence of such factors. Paper chromatography is also of value for this purpose. When applied to liver extract, agreement between the different methods was satisfactory. An animal food addition product, prepared by fermentation, was

shown to contain both vitamin B_{12} and desoxyribosides. Extract of rye bread gives a misleading result by direct methods, but by combining the results of the "compensation method" before and after autoclaving it was shown that no vitamin B_{12} was present. With products containing a large proportion of the vitamin, a simple activity determination can be used with good approximation, as other substances which affect growth have no appreciable effect at the dilutions used. By the use of the various control methods determinations can be carried out satisfactorily in solutions containing 0·0001 μg. of vitamin B per ml.

G. M.

CHEMOTHERAPY

Antispasmodics. Esters of β-Alkyltropic Acids. A. W. Weston and R. W. DeNet. (*J. Amer. chem. Soc.*, 1951, **73**, 4221.) The synthesis is reported of a series of basic esters of β-substituted tropic acids of general formula $C_6H_5CH(C(OH)R_1R_2)\cdot COO\cdot(CH_2)_nNR_2HCl$ (I). Intermediate β-alkyl-and β-dialkyltropic acids (II) were synthesised by treating either phenylacetic acid or sodium phenylacetate with *iso*propyl magnesium halide and condensing the resulting Grignard complex with the appropriate aldehyde or ketone. The required basic esters (I) were obtained by reaction of the acids (II) with dialkylaminoalkyl chlorides in boiling *iso*propanol. An alternative preparation of the esters was attempted, in which the sodium β:β-pentamethylenetropate, formed by the reaction of the free acid with sodium hydride, was heated with diethylaminoethyl chloride in benzene. The method was unsuccessful, the only basic product isolated being diethylaminoethyl phenylacetate, a tendency which was particularly marked when the substituted tropic acid contained a β-aryl group. Preliminary results indicate that basic esters of type (I) have a pronounced antispasmodic action.

J. B. S.

Antithyroid Substances, 2-Mercaptoglyoxalines, Mercaptothiazoles and Thiohydantoins. C. E. Searle, A. Lawson and H. V. Morley. (*Biochem. J.*, 1951, **49**, 125.) The antithyroid activity has been determined of a further series of 2-mercaptoglyoxalines, several 2-thiohydantoins and a number of 2-mercapto- and 2-aminothiazole derivatives. For assay the screening test of Searle *et al.* (*Biochem J.*, 1950, **47**, 77) was used; all drugs were administered by stomach tube, usually at a level of 0·05 m. mol/kg. body weight. The dose of radioactive iodide (approx. 1 μc. in 0·2 ml. of 0·9 per cent. sodium chloride without added carrier) was injected intraperitoneally after 1 hour and the rats killed after a further 4 hours. The ^{131}I uptakes of the thyroids of the dosed and control animals were then compared using a liquid counter, the results being expressed as the percentage depression of the mean ^{131}I uptake of the control animals. Results are given for 10 glyoxaline derivatives, for 5 thiohydantoins, and for 15 thiazoles; of the mercaptoglyoxalines the 3 most active were 2-mercaptoglyoxaline, and its 1-methyl and 1-ethyl derivatives these being found to be only half as active as thiouracil. The figures for acute toxicity determinations in mice carried out with three representative mercaptoglyoxalines are given. No 2-mercapto- or 2-aminothiazole tested showed activity comparable with the most active thiouracils and mercaptoglyoxalines, the largest depression of ^{131}I uptake being produced with 5-amino-2-carbethoxythiothiazole. 3 simple thiohydantoins had a fairly high activity.

R. E. S.

Nitroparaffins, Antitubercular and Antirickettsial Properties of Derivatives. T. Urbanski. (*Nature, Lond.* 1951, **168**, 562.) A series of nitrocompounds was tested against saprophytic mycobacteria (six strains) *in vitro* in Youman's medium and *in vivo* against *Mycobacterium tuberculosis* ($H_{37}Rv$ strain) in white mice

inoculated intravenously. They were also tested *in vivo* against *Rickettsia prowazeki*. These nitro compounds included 1:3-tetrahydro-oxazines and 1-oxa-3-aza-*cyclo*octanes formed by the interaction of 1-nitropropane with formaldehyde and ammonia and open chain amines derived from these compounds. Condensation products from 2-nitropropane and nitroethane, formaldehyde and ammonia, and also 1-nitropropane, formaldehyde and methylamine were also tested. Most of the compounds were shown to possess a relatively low toxicity. Details of toxicity and activity are tabulated.

A. H. B.

Open-chain Aminoketones Related to Morphine. J. H. Burckhalter and S. H. Johnson, Jr. (*J. Amer. chem. Soc.*, 1951, **73**, 4832.) A series of α-alkyl-α(2-dialkylaminoalkyl)-phenylacetonitriles having the general formula $C_6H_5C{\cdot}(CH_3){\cdot}(CN){\cdot}CH_2CH(R){\cdot}N(CH_3)_2$ with various substituents in the aromatic ring have been prepared from the appropriate phenylacetonitrile and 2 dialkylaminoalkyl halide by means of a sodamide condensation. α-Methyl-α-(2-dimethylaminoethyl)-phenylacetonitrile with ethylmagnesium bromide gave 6-dimethylamino-4-methyl-4-phenyl-3-hexanone (I) in 76 per cent. yield. 6-Dimethylamino-4-methyl-4-(2:3-dimethoxyphenyl)-3-hexanone (II) was obtained by a similar route in 35 per cent. yield. The ketone II could not be reduced catalytically to the corresponding alcohol; attempted demethylations of II were also unsuccessful. Both I and II failed to exhibit analgesic activity in guinea-pigs. This lack of activity is attributed to the 4-methyl group, which replaces the 4-phenyl group in the analogous methadone, the methyl group being too small to effect the necessary steric hindrance which locks the aliphatic chains of methadone into a morphine-like spatial arrangement. J. B. S.

Thiosemicarbazones in the Chemotherapy of Tuberculosis. E. Hoggarth and A. R. Martin. (*Brit. J. Pharmacol.*, 1951, **6**, 454.) Some 60 compounds derived from thiosemicarbazones by a variety of oxidative and reductive processes, or closely related to such compounds, were examined in tuberculous mice. Two groups of "active" compounds were found, namely, 1-benzylthiosemicarbazides and 1-amino-4-phenyl-2:3-diazabuta-1:3-diene sulphonic acids. The activity was of the same order as that found in the parent series of thiosemicarbazones. S. L. W.

PHARMACY

NOTES AND FORMULÆ

Chlorprophenpyridamine Maleate (Chlor-Trimeton Maleate). (*New and Nonofficial Remedies*; *J. Amer. med. Ass.*, 1951, **147**, 128.) Chlorprophenpyridamine maleate is 1-(*p*-chlorophenyl)-1-(2-pyridyl)-3-dimethylaminopropane maleate and occurs as a white crystalline solid, m.pt. 130° to 135° C., soluble in water (1 in 3·4), ethanol (1 in 10) and chloroform (1 in 10), and slightly soluble in benzene and ether; a 1 per cent. solution has *p*H 4·8. When heated in a Bunsen flame on copper wire, the flame turns green (distinction from prophenpyridamine). The yellow crystalline picrate obtained by adding an ethanolic solution of trinitrophenol to a chloroform solution of the base melts at 197° to 200° C. after drying *in vacuo* for 4 hours and then at 105° C. for 4 hours. On adding chloroform and potassium hydroxide to a solution in ethanol and heating, a red colour develops but no odour of isonitriles (absence of primary amines). Chlorprophenpyridamine maleate loses not more than 0·5 per cent. of its weight after drying at 105° C. for 4 hours; residue on ignition, 0·15 per cent. A 0·003 per cent. solution in water exhibits an ultra-violet absorption maximum at 2620 Å ($E_{1\,\text{cm.}}^{1\,\text{per cent.}}$, 143 ± 3), an inflection at about 2680 Å and a

minimum at about 2430 Å. It contains 7·0 to 7·4 per cent. of N, equivalent to 97·5 to 102·5 per cent. of chlorprophenpyridamine maleate. It is used as an histamine antagonist in a dose of 2 to 4 mg. G. R. K.

Cyclopentamine Hydrochloride (Clopane Hydrochloride). (*New and Non-official Remedies*; *J. Amer. med. Ass.*, 1951, **147**, 128.) Cyclopentamine hydrochloride is 1-*cyclo*pentyl-2-methylaminopropane hydrochloride and occurs as a white, odourless, crystalline powder, with a mild characteristic odour, m.pt. 113° to 116° C., soluble in water (1 in 1), ethanol (1 in 1·8), benzene (1 in 23·8) and chloroform (1 in 1·3), and slightly soluble in ether; a 1 per cent. solution has *p*H 6·2. When boiled with sodium nitrite and treated with hydrochloric acid, a yellowish orange oily material separates; on the addition of more hydrochloric acid the oily layer disappears and a white precipitate which is soluble in water forms. When heated on a steam bath with sulphuric acid and potassium cyanate, it yields a white precipitate which melts at 126° to 129° C. after recrystallisation from hot water and drying at 80° for 4 hours. Cyclopentamine hydrochloride yields no odour of isonitriles when heated with chloroform, ethanol and potassium hydroxide; it loses not more than 0·4 per cent. of its weight when dried at 80° C. for 3 hours and leaves not more than 0·05 per cent. of residue on ignition. It is assayed by Kjeldahl determination and contains 98·0 to 102·0 per cent. of cyclopentamine hydrochloride. It is used as a sympathomimetic agent. G. R. K.

Phenacemide (Phenurone). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1951, **147**, 60.) Phenacemide is phenylacetylurea, $C_6H_5CH_2{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}NH_2$. It is a white to creamy white, odourless, tasteless, crystalline solid, m.pt. 212° to 216° C., slightly soluble in alcohol, benzene, chloroform, ether, and water. When heated with potassium nitrate and sulphuric acid, treated with water and zinc powder and again heated, cooled, diluted and filtered, the filtrate gives a light purple colour when treated as follows: add an equal volume of a 20 per cent. solution of toluenesulphonic acid, dilute with water, and add a solution of sodium nitrite; after 3 minutes add a solution of sodium sulphamate, allow to stand for 2 minutes, add an ethanolic solution of *N*:*N*-dimethyl-1-naphthylamine and allow to stand for 15 minutes. The phenylacetic acid obtained in the assay melts between 75° and 77° C. Phenacemide loses not more than 1 per cent. of its weight when dried at 105° C. for 4 hours, and leaves not more than 0·05 per cent. of residue on ignition. It is assayed by hydrolysing with sulphuric acid, extracting the phenylacetic acid with chloroform, removing the chloroform, dissolving the residue in ethanol and titrating with sodium hydroxide, using phenolphthalein as indicator. It contains 74·9 to 77·9 per cent. of phenylacetic acid, equivalent to 98·0 to 102·0 per cent. of phenacemide. Phenacemide is used as an anticonvulsant in doses of 0·5 g. 3 times a day. G. R. K.

Phenacetin, Acetchloranilide as Impurity in. J. Hald. (*Acta Pharm. Internat.*, 1951, **2**, 27.) Cyanosis has been observed after the taking of impure phenacetin, which was contaminated with a considerable quantity of acet-4-chloranilide, a compound which produces methæmoglobinæmia. This compound is produced in the synthesis of phenacetin and, if present in quantities of not more than 1·5 per cent., the material may still pass pharmacopœial requirements. The permissible limit of this impurity would appear to be about 0·15 per cent.: i.e., 6 mg. would be present in a daily dose of 4 g. of phenacetin. Doses of 10 mg. of acetchloranilide do not in general convert more than 2 per cent. of the total hæmoglobin to methæmoglobin. G. M.

Phethenylate Sodium (Thiantoin Sodium). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1951, **147**, 129.) Phethenylate sodium is sodium 5-phenyl-5-(2-thienyl) hydantoinate. It is a white, odourless, hygroscopic, microcrystalline powder which absorbs carbon dioxide with the liberation of 5-phenyl-5-thienyl hydantoin; in aqueous solution it gradually dissociates. It is soluble in water (1 in 2), and ethanol (1 in 5), very slightly soluble in ether, and practically insoluble in benzene and chloroform; a 1 per cent. solution has *p*H 10·0. When shaken with thiophene-free benzene and a solution of isatin in sulphuric acid, the acid layer becomes green and turns blue after about an hour (distinction from phenytoin sodium). The precipitate formed when hydrochloric acid is added to a solution in water melts at 251° to 259° C. after drying at 105° C. for 4 hours. Phethenylate sodium contains not more than 30 p.p.m. of lead and loses not more than 1·5 per cent. of its weight when dried at 105° C. for 24 hours. It is assayed by extracting an acidified solution with ether, evaporating the ether and drying the residue at 105° C. for 4 hours, and also by determining the nitrogen content. It contains 97·0 to 103·0 per cent. of phethenylate sodium. It is used in the treatment of epilepsy. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

Absorbable Gelatin Sponge in Experimental Surgery. G. Blaine. (*Lancet*, 1951, **261**, 427.) Gelatin sponge is essentially a dried gelatin foam. By heating or whipping a sterile and highly formalised gelatin solution a foam of uniform porosity is produced, which, when dried under suitable conditions, retains its original porous structure. The dried material is cut into required shapes and sizes and packed and sterilised at 160° C. Although gelatin sponge is insoluble in water it is completely digested by proteolytic enzymes. Dry gelatin sponge is soft, springy and light; it can be cut with ease and has sufficient tensile strength to withstand normal handling. Wet gelatin sponge rapidly absorbs moisture and becomes jelly-like. It can be used safely in conjunction with penicillin. Experimental surgical studies were carried out on rabbits. In some rabbits gelatin sponge measuring 3·0 × 2·0 × 0·1 cm. was introduced into the abdominal cavity; in others, gelatin sponge of the same size was introduced into the muscular tissue in the anterior triangle of the neck; and in the remainder the liver was slit for ¾ in. on the anterior surface and gelatin sponge inserted into the wound. The rabbits were killed on the 5th, 11th, 16th, 23rd, 28th and 42nd post-operative days for necropsy, and histological studies were made. From the findings it would appear that gelatin sponge is absorbed or "organised" in about 4 to 6 weeks. It may therefore be considered safe for implantation as an absorbable hæmostatic in surgery. It produces hæmostasis promptly and adheres readily to bleeding surfaces. If too much is implanted it does not become absorbed normally, since it prevents access of phagocytes to the centre of the implant. S. L. W.

Cortisone and Related Hormones; Effect on Responses to Analgesic Drugs. C. A. Winter and L. Flataker. (*J. Pharmacol.*, 1951, **103**, 93.) Cortisone was found to antagonise all the actions of morphine and amidone that were studied in rats and mice. These include: (1) analgesic effect, measured by the D'Amour Smith technique, (2) toxicity of amidone, (3) hyperactivity in mice, (4) hypnosis and catalepsy in rats. The effects of deoxycortone were the opposite of those produced by cortisone, while adrenocorticotrophic hormone produced effects similar to those of cortisone. All the results obtained in these experiments indicate that cortisone has a stimulant effect on the central nervous system. S. L. W.

Heparin, Sublingual Administration of. J. Litwins, J. J. Vorzimer, L. N. Sussman, M. Applezweig and A. D. Etess. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **77**, 325.) Sublingual wafers containing 125 mg. of sodium heparin were given to 10 subjects. Absorption was usually complete within 10 minutes. A therapeutic level was obtained within ½ hour and maintained for 4 hours. The authors suggest that this may become the method of choice for the early anticoagulant treatment of myocardial infarction, pulmonary embolism, and thrombophlebitis, and in vascular surgery. It would also appear to be of value in the early treatment of frostbite in soldiers before hospitalisation. S. L. W.

Morphine, Intravenous, in Ocular Surgery. E. A. Johnson. (*Canad. med. Ass. J.*, 1951, **64**, 429.) A series of 101 unselected ophthalmic surgical cases was operated on under local anæsthesia, using a morphine-scopolamine-ephedrine solution intravenously. The proportions of the drugs used were morphine sulphate ¼ gr., ephedrine sulphate ⅙ gr., scopolamine hydrobromide 1/200 gr., with chlorbutol 0·5 per cent., dissolved under aseptic conditions in 4 ml. of triple distilled water. Premedication consists of the administration of amylobarbitone sodium 3 gr. before retiring, repeated 2 hours before operation. The intravenous injection of the anæsthetic is made very slowly, at an approximate rate of 1 ml. in 2 minutes. The maximum depressant effect occurs in from 7 to 10 minutes, the maximum psychic sedative effect begins in about 10 minutes, and the maximum analgesic effect in about 20 minutes. Apart from mild dizziness in 2 cases, mild tachycardia in 2 cases, and respiratory depression with mild cyanosis in 3 cases, there was a striking absence of severe side-effects. Postoperative reactions such as headache, nausea and vomiting, were also mild and occurred in less than 10 per cent. of patients. S. L. W.

NPH Insulin. K. A. Swallow and A. L. Chute. (*Canad. med. Ass. J.*, 1951, **65**, 23.) The chief distinction between protamine zinc insulin and NPH insulin is that the former is an amorphous material containing a variable quantity of protamine (1·25 to 1·5 mg. per 100 units) which is sufficient to provide an excess, while NPH insulin is a crystalline product in which the protamine is completely combined with insulin. After injection the action of NPH insulin commences in about 2 hours, reaches a maximum in 10 to 20 hours, and is complete in 28 to 30 hours. In 10 out of 18 diabetic juvenile patients, whose ages ranged from 3 to 14 years, and whose total daily insulin requirements varied from 10 to 80 units, as good or better control was achieved by a single injection of NPH insulin given before breakfast as was possible with the double injection of unmodified insulin and protamine zinc insulin. It must be emphasised, however, that each patient's requirement of insulin must be considered as an individual problem, both as regards the type of insulin and the amount required. Since NPH insulin does not significantly alter the effect of added unmodified insulin the use of mixtures of the two insulins has also proved satisfactory by the single injection technique. S. L. W.

NPH Insulin; Composition and Properties. M. Jameson, A. H. Lacey and A. M. Fisher. (*Canad. med. Ass. J.*, 1951, **65**, 20.) Late in 1950 there was made generally available in Canada and the United States a new preparation of insulin under the name "NPH insulin." This was introduced because of the requirement by clinicians for a protamine-insulin preparation acting more quickly than protamine zinc insulin and for only 22 to 26 hours rather than 36 hours as is sometimes the case with protamine zinc insulin. NPH insulin is a buffered aqueous suspension of crystals containing insulin (40 or 80 units per ml.), protamine and zinc. Each 100 units contains 0·03 mg. of zinc and 0·4 mg.

of protamine plus sodium chloride; it has a *p*H of 7·2. In order to distinguish it from other insulins it is distributed only in vials with a square cross section. NPH insulin has the advantage over protamine zinc insulin that when mixed with unmodified insulin for simultaneous administration much of the quick action of the unmodified insulin is retained, whereas with simultaneous administration of protamine zinc insulin and unmodified insulin the quick-acting effect of the insulin is lost. S. L. W.

Phenobarbitone, Mode of Absorption of. M. R. Fabre, M. T. Regnier and M. E. Grasset. (*Ann. pharm. franc.*, 1951, **9**, 98.) The absorption of phenobarbitone is greatly influenced by the type of preparation used. When administered directly into the stomach of dogs, the concentrations (mg. per 100 ml.) in blood and chyle after 8 hours were as follows:

Type of preparation	Phenobarbitone in	
	blood	chyle
Gummy suspension	5.3	0
Oily emulsion	4.2	2.3
Phenobarbitone-sodium, in water	11.6	8.9

Differences were also observed in the concentrations in the organs of the body, and in the urine. The blood level rises much more rapidly when the drug is administered to fasting animals, than when the stomach is full, and the period before death supervenes is correspondingly less, but the final blood concentration is approximately the same. G. M.

Phenol, Cutaneous Absorption of. M. V. Freeman, J. H. Draize and E. Alvarez. (*J. Lab. clin. Med.*, 1951, **38**, 262.) Albino rabbits subjected, under anæsthesia, to various procedures involving trauma of the skin (scalds, dry heat burns, ultra-violet burns) were exposed to topical applications of 2·3 per cent. of phenol in corn oil, and phenol-camphor (a mixture of 5 per cent. of phenol and 10 per cent. of camphor in corn oil). Urinary analyses showed that phenol is rapidly absorbed and excreted following topical application. A single application of 2·3 per cent. phenol in corn oil mixture does not appear to affect the ability of the skin to absorb phenol. Animals whose skin was irradiated with ultra-violet rays to a point of erythema and slight œdema formation exhibited a retarded excretion of phenol. Urinary phenol values were shown to be in direct ratio to the phenol content of the preparations employed. After treatment, animals with severely burned skin showed much higher values of urinary phenol than animals with intact skin, indicating a potential danger in the indiscriminate use of phenol compresses in severe burns. S. L. W.

α-Phenyl Succinimides, Anticonvulsant Activity of. G. Chen, R. Portman, C. R. Ensor, A. C. Bratton. (*J. Pharmacol.*, 1951, **103**, 54.) This report deals with the laboratory evaluation of some derivatives of α-phenyl succinimides with respect to their actions on the central nervous system. The material, in solution or in suspension with acacia, was administered perorally to fasting rats, a convulsive dose of metrazol solution being injected subcutaneously half an hour later and the animals observed for the time of onset and severity of convulsions for 36 minutes. The anti-electroshock effect was measured either in cats or in mice. The α β-methyl and ethyl substituted compounds were more effective against metrazol than against electrically-produced convulsions. Methylation of the heterocyclic nitrogen of most of these succinimides resulted

in an increase of anti-metrazol potency, this enhancing effect becoming less with the increasing length and branching of the alkyl chain. The αα-diphenyl and αβ-diphenyl succinimides were, however, more effective in suppressing electrically-induced than metrazol-induced convulsions. Methylation of the heterocyclic nitrogen of these compounds diminished their anti-electroshock activity. S. L. W.

Podophyllin, Toxicology of. M. Sullivan, R. H. Follis and M. Hilgartner. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **77**, 269.) In mice podophyllin and podophyllotoxin administered parenterally were shown to be respectively 1/15 and 1/6 as toxic as colchicine. Death usually occurred within 15 to 18 hours after administration of fatal doses of podophyllin. Diarrhœa, rapid and laboured respiration and a dragging gait were usually observed during the 8 hours after dosing. After 9 hours there was a period of excitation resulting in spastic convulsions. This was followed by flaccidity, dragging gait and slow, shallow, laboured respiration. The most constant finding on microscopic study was acute enteritis. There were increased numbers of polymorphonuclear leucocytes in intestinal sub-mucosa and a striking breaking up of their nuclei, small masses of deeply staining chromatin being found in profusion. Necrosis of the lymphoid tissue beneath the mucosa was also prominent. Increased mitotic activity was present in the epithelium of the tongue. There were no arrested mitoses in the skin. A comparison of the median lethal doses of podophyllin and podophyllotoxin in young and adult rats showed a greater degree of toxicity for young rats. Cumulative toxic effects were produced in rats by giving podophyllin and podophyllotoxin several times a day in individual doses that were in the range of 1/6 and 1/5 of the LD50. S. L. W.

Polymyxin in the Treatment of Burns. D. M. Jackson, E. J. L. Lowbury and E. Topley. (*Lancet*, 1951, **261**, 137.) Local application of 0.1 per cent. polymyxin-E cream every other day to burns colonised by *Ps. pyocyanea* in a controlled trial significantly reduced the incidence of this organism; by the 4th day the organism persisted in 26 per cent. of 19 polymyxin-treated burns compared with 81 per cent. of 16 control burns. Routine local application of this cream protected burns from colonisation by *Ps. pyocyanea* and some coliform bacilli; 7 per cent. of 162 burns so treated acquired *Ps. pyocyanea* compared with 24 per cent. of 207 control burns. Polymyxin-E locally was used for 6 weeks on all burns with no local or general toxic effects, and there was no evidence of acquired resistance by *Ps. pyocyanea*. The use of polymyxin in a controlled prophylactic trial was associated with a significant reduction in the healing time of full-thickness skin-loss burns; 57 per cent. of 28 polymyxin-treated patients were healed in 4 weeks in contrast to 19 per cent. of 43 control patients. The average healing times were 5·2 and 8·5 weeks respectively. There was also a significant increase in the incidence of complete graft takes, and a slight reduction in the incidence of anæmia, pyrexia and death. From the data presented the authors conclude that *Ps. pyocyanea* and some coliform bacilli act as pathogens on burns, and that the routine application to burns of a cream containing penicillin, to combat Gram-positive cocci, and polymyxin, to combat many Gram-negative bacilli, would be more valuable than the penicillin cream widely used to-day. S. L. W.

Pressor Substance in Urine. J. Dekanski. (*Brit. J. Pharmacol.*, 1951, **6**, 351.) Human urine, from which the gonadotrophins had been removed by absorption on kaolin, was treated for the preparation of antidiuretic concentrates, and these concentrates were then tested for other forms of activity.

They were found to have a pressor action in rats which was not due to adrenaline, noradrenaline, *iso*amylamine, tyramine, piperidine, or nicotine, since the action survived the injection of dibenamine, ergotoxine, and piperoxane. It is suggested that the substance responsible for this action is vasopressin. A test for oxytocic activity of the urine concentrates on the guinea-pig's uterus gave negative results and, in terms of posterior pituitary extracts, was certainly less than 1/20th of their pressor activity. S. L. W.

Salicylates: Effect on the Pituitary and Suprarenal Glands. B. S. Hetzel and D. C. Hine. (*Lancet*, 1951, **261**, 94.) This investigation was stimulated by a report of the development of Cushing's syndrome in a patient with rheumatic fever under treatment with aspirin 5 g. a day. Experiments were designed to show whether salicylates, in therapeutic dosage, had an effect on the pituitary and suprarenal glands that was manifested by removal of ascorbic acid from the suprarenal glands and could be abolished by hypophysectomy or by preliminary treatment with suprarenal cortical hormone. The initial dosage was based on the daily dose of sodium salicylate estimated to maintain in the rat an adequate blood-salicylate level of 30 to 40 mg./100 ml.; the dosage of sodium *p*-aminosalicylate was based on similar considerations. These doses were 0·2 g./kg. of bodyweight and 0·3 g./kg. of bodyweight for sodium salicylate and *p*-aminosalicylic acid respectively. The drugs were given in saline solution intraperitoneally. The suprarenal glands were excised 100 to 130 minutes after the treatment. It was shown that in these doses the salicylates cause a significant depletion of the ascorbic acid content of the suprarenals, the effect being directly proportional to the dose. This response can be abolished by hypophysectomy and tends to be inhibited by preliminary treatment with suprarenal cortical hormone. It is concluded that the therapeutic effects of salicylates are mediated by the pituitary and suprarenal glands. The beneficial results of salicylate therapy in rheumatic fever may be due to the production of cortisone-like steroids due to the activation of these glands and salicylate therapy has a beneficial effect in rheumatic carditis according to the dosage and consequent degree of stimulation. S. L. W.

Salicylic Acid, Fatty Acid Esters of. A. Lespagnol, J. Batteur and C. Lespagnol. (*Therapie*, 1951, **6**, 125.) Certain derivatives of salicylic acid, such as diethylacetylsalicylic acid and diethylacetyldi-*iso*propylsalicylic acid, show a resistance to hydrolysis considerably greater than that of acetylsalicylic acid, and it is possible that salts of such compounds would be free from the objections to salts of acetylsalicylic acid, while retaining their advantages. On the other hand, such a substitution might increase the hypnotic activity. With both the acids mentioned, the solubility in oil is considerably greater than that of acetylsalicylic acid. It may further be noted that diethylacetic acid is degraded to methylpropylacetone and the corresponding secondary alcohol, and this is liable to increase the hypnotic power. Further tests are being carried out on these acids. G. M.

Sparteine as Antagonist of Eserine. R. Hazard, E. Corteggiani and A. Cornec. (*C. R. Acad. Sci. Paris*, 1951, **223**, 211.) In view of the antagonistic action of procaine to eserine, sparteine, which resembles procaine as a ganglionary inhibitor, was also tested. The muscular contractions, induced by eserine in the heart of a chloralosed dog, disappeared completely under the influence of sparteine. As with procaine, the action of acetylcholine on the heart is suppressed without affecting the hypotensive action. The action of the two compounds is similar, with some secondary points of difference. G. M.

Tigloidine as a Substitute for Atropine in Parkinsonism. E. M. Trantner. (*Med. J. Austral.*, 1951, **1**, 751.) Tigloidine, the tiglic ester of pseudo-tropine, was tried as a substitute for atropine in 4 cases of parkinsonism, after it had been found that its effect on the action potential and contraction of the muscle-stimulated isolated sartorius of the toad was identical with that of atropine. The drug was employed in the form of the hydrobromide, commencing with a dose of 0°5 mg. 3 times daily. This dose was gradually increased to 4 mg., then to 16 mg., and finally to 64 mg. 3 times daily. The optimum beneficial effect of the drug appeared to be reached with a dosage of 40 to 48 mg. 3 times daily. The therapeutic effect on the symptoms of the disease was found to be identical with that of atropine, but tigloidine does not produce the undesirable side-effects of the latter, namely, excessive mydriasis, dryness of the throat, gastric disturbances and severe headache. The author discusses the clinical and chemical significance of these findings, and the common chemical groupings of atropine, tigloidine and procaine are compared with those of the antihistamine drugs found to be beneficial in parkinsonism. S. L. W.

Toxiferines, Pharmacology of. W. D. M. Paton and W. L. M. Perry. (*Brit. J. Pharmacol.*, 1951, 6, 299.) The toxiferines are alkaloids extracted from the bark of *Strychnos toxifera*, which is known to be one of the principal ingredients of calabash curare. The object of these experiments was to discover the main properties of the compounds and to analyse the type of block produced at the neuromuscular junction and at the ganglionic synapse. Toxiferines I, II, IV, V, VI, IX, XI and XII were investigated. In most respects the toxiferines share the pharmacological properties of *d*-tubocurarine. The paralysis is such that the muscle is always capable of a full contraction if excited directly, and a typical endplate potential can be recorded, thus demonstrating that toxiferine causes a true neuromuscular block. Further features common to both drugs are the smooth onset of paralysis, antagonism by anticholinesterases, post-tetanic relief of block, and depression of the respiration. The paralysis must therefore be due to a raising of the threshold of the motor endplate to acetylcholine, and the toxiferines must thus be classed with *d*-tubocurarine rather than with drugs such as decamethonium which cause neuromuscular block by a prolonged depolarisation of the endplate. Three important deviations, however, from *d*-tubocurarine are noted: (1) a muscle blocked with toxiferine often responds to a tetanus with a sustained contraction; (2) post-tetanic potentiation seems greater in muscle exposed to toxiferine than in that exposed to *d*-tubocurarine; (3) neostigmine not only reverses block due to toxiferine but also potentiates the normal twitch tension. In their actions on other organs toxiferines show a qualitative resemblance to *d*-tubocurarine. The salient features of the pharmacology of the toxiferines are their high activity and the uniformity with which that activity is displayed in different species. Weight for weight they are more active than any other neuromuscular blocking agent.
S. L. W.

Treburon, a new Heparin-like Anticoagulant. C. N. Mangieri, R. Engelberg and L. O. Randall. (*J. Pharmacol.*, 1951, **102**, 156.) Treburon is a synthetic sulphated polygalacturonic acid methyl ester methyl glycoside which has many properties in common with heparin. It has one-half the toxicity of heparin in mice and one-fourth to one-half the anticoagulant activity. Like heparin, it appears to exert its anticoagulant activity chiefly by virtue of its antithrombin activity. It has very little, if any, antithrombin activity at low

doses but measurable activity at high doses. Treburon produces no agglutination of platelets, no change in sedimentation rates and no precipitation of fibrinogen. Like heparin, it is readily neutralised by protamine sulphate, and the clotting-time of the blood which is prolonged can be returned to normal by the injection of an equal weight of protamine. The duration of action of equi-active doses of treburon and heparin is similar. Large doses produce infinite clotting times and the rates of return of the clotting times towards normal are parallel. A dose of 10 mg./kg. intramuscularly in rabbits has an intensity and duration of action at least as great as 5 mg./kg. of heparin. In preliminary clinical trials these findings have been confirmed in human subjects; 150 mg. of treburon had the same anticoagulant activity as 50 mg. of heparin intravenously in 14 cases. The intravenous injection of 500 mg. produced significant prolongations of clotting times. No toxic manifestations were observed in any of the patients. S. L. W.

Triethanolamine Trinitrate; Cardiovascular Effects of. K. I. Melville and F. C. Lu. (*Canad. med. Ass. J.*, 1951, **65**, 11.) In the isolated perfused rabbit heart triethanolamine trinitrate is shown to be an effective coronary vasodilator, comparing favourably with glyceryl trinitrate and exerting a more prolonged action. On repeated injection both substances give rise to the development of a slight tolerance. Glyceryl trinitrate exerts a more marked vasodepressor action, dose for dose, in anæsthetised animals, than does triethanolamine trinitrate. In small doses, both substances exert little significant action on the electrocardiogram or cardiac output (in heart-lung preparation); larger doses of both, however, depress cardiac output and slow the heart rate. Acute toxicity studies on rats and rabbits show both substances to be relatively non-toxic when injected intravenously. S. L. W.

Triethanolamine Trinitrate in Angina Pectoris. J. H. Palmer and C. G. Ramsey. (*Canad. med. Ass. J.*, 1951, **65**, 17.) Triethanolamine trinitrate (metamine) was administered to 5 patients with angina pectoris over 3-week periods. They alternated with 3-week control periods using placebos, (*a*) of enteric-coated chalk, and (*b*) of lactose. The tablets were taken 4 times daily; 3 times before meals and at bedtime. Each dose of the drug was 2 mg. While taking the drug all patients showed a reduction in the daily number of attacks. They were not informed which of the tablets contained the drug and which the placebos. S. L. W.

Tromexan (bis-3:3′-(4-oxycoumarinyl) ethyl acetate), Pharmacology of. M. Stirling and R. B. Hunter. (*Lancet*, 1951, **261**, 611.) Weight for weight tromexan has one quarter of the activity of dicoumarol in the production of hypoprothrombinæmia, but the action is quicker and excretion more rapid, giving greater control by oral administration and less danger of prolonged hæmorrhage. A wide variation of dosage is necessary to induce and maintain hypoprothrobinæmia, indicating that dosage must be decided individually. Divided doses are more effective than a single daily dose. The drug is of relatively low efficiency when compared with heparin therapeutically; its place is essentially in prophylaxis and not in the treatment of thrombosis. Water-soluble naphthaquinones given by mouth counteract tromexan hypothrombinæmia; proof is lacking that they produce any significant effect on that induced by dicoumarol. Sometimes vitamin K oxide was found to produce a rapid rise in prothrombin concentration. J. R. F.

BOOK REVIEWS

THE VITAMIN B COMPLEX, by F. A. Robinson. Pp. xi + 629 and Index. Chapman and Hall, London. 1951. 60s.

The author has written a valuable and readable textbook on the vitamins of the B complex. Beginning with aneurine, he goes on to deal with riboflavine, nicotinic acid, pyridoxine, pantothenic acid, biotin, the folic acid group, vitamin B_{12}, *p*-aminobenzoic acid, inositol and, finally, choline. Chapter XIII is concerned with miscellaneous water-soluble growth factors. The concluding chapter (XIV) makes an attempt to show the close biological relationship existing between the B-vitamins by indicating the different stages of metabolism in which each participates. The arrangement of each chapter follows the general plan: introduction, isolation, structure and synthesis of the vitamin, properties, stability, biological, microbiological and chemical assay, effects of deficiency and overdosage, metabolism, intestinal synthesis, animal and human requirements, pharmacological action, functions, nutritional requirements of micro-organisms, effects on higher plants, requirements of insects and synthetical analogues. Certain features of the book deserve special mention. Industrial methods of production are indicated, together with references to the patent literature. Due importance is attached to the role of intestinal bacteria in the endogenous production of the vitamins. Pharmaceutical interests are remembered in the sections dealing with stability, dosage and assay. It is, therefore, a pity that the author has not seen fit to include any drawings or photographs of the deficiency states, and it is hoped that a future edition may remedy this defect.

The sections dealing with the more established members of the B-complex are well documented and up to date, and give excellent reviews of current status and thought. Progress in the vitamin B_{12} field, however, has been so rapid in the past year that this part is already in need of revision. The name erythrotin for vitamin B_{12} has not received general acceptance, whilst the biological relationship between vitamin B_{12} and folic acid has still to be established. Apoerythein is spelt apoerythrein. It now appears that this component of gastric juice is not identical with Castle's intrinsic factor. *Citrovorum factor*, the newest addition to the folic acid group, is not mentioned, but will presumably be included in a later edition, together with the synthetical factor, folinic acid-SF (tetrahydroformyl pteroyglutaric acid) of like biological activity. Vitamin B_{13} is now thought to be identical with vitamin B_{12}. The latter is probably the active component of zoopherin. Few errors have crept into the book. Certain minor points, however, can with advantage be corrected. Thus the formula for activated pyruvic acid on p. 103 is wrong and is not reproduced from ref. 70 as stated. The phraseology on pp. 406–407 dealing with yields of biotin isolated from natural materials is ambiguous and should be revised. Such minor errors are, however, inseparable from the trials of authorship. In conclusion, the author may be congratulated on an excellent volume, which should prove of value to workers in diverse fields. V. PETROW.

PAPIERCHROMATOGRAPHIE, by Friedrich Cramer. Pp. 81 (including 47 illustrations). Verlag Chemie GMBH, Weinheim. 1952. Paper cover, DM.9.80.

Since its discovery seven years ago, paper chromatography has proved to be a tool of unprecedented power in microanalytical chemistry; the technique has been applied with success in many branches of chemistry but its greatest

efficacy has been demonstrated in the chemistry of natural products. With appropriate attention to the effects of controllable variables, the R_F value may now be recorded as a simple analytical character of many substances, such as carbohydrates and amino-acids, for which certain of the usual physical constants may be of little analytical significance. Dr. Cramer's monograph provides a concise, well-documented survey of the experimental techniques and applications of paper chromatography, together with a valuable series of tables of R_F values published up to early 1951. Most of the important procedures for qualitative and quantitative work are described and brief mention is made of retention analysis. The necessary information is given for the laboratory operation of the chromatopile in preparative paper chromatography and of paper electrophoresis. The illustrations are excellent. More attention could well have been given to the extremely valuable extension of the range of paper chromatography achieved by the use of paper impregnated with buffers and surface-active adsorbents.

The chapters on applications include information on developing solvents, spraying reagents and other methods of location for amino-acids, carbohydrates, phosphoric esters, purines, nucleic acid derivatives, pterins, phenols, organic acids and bases, vitamins, antibiotics, porphyrins, steroids, dyestuffs and inorganic compounds. Although the R_F values of simple substances are recorded, the utility of certain tables of R_F values is reduced by the lack of information on the exact composition of mixed developing solvents. It is not normally advisable to correct the R_F value of an unknown substance for the departure of the value of a known substance from its standard R_F value by more than $\pm$ 0·02. It cannot be said that this section is free from errors; for example, a publication ascribed to the reviewer and a colleague was not in fact concerned with paper chromatography; the R_F values of phenols tabulated on p. 59 as being determined in cresol were observed with a mixture of *m*-cresol and acetic acid; incorrect figures are given for the R_F values of the cyanidin glucosides and l-epicatechin. This monograph is nevertheless a very useful compilation of a mass of widely scattered information and with its 283 references will be welcomed by experimental workers in many branches of chemistry.

M. W. PARTRIDGE.

BOOKS RECEIVED

Clark's APPLIED PHARMACOLOGY (8th Ed.). Revised by Andrew Wilson and H. O. Schild. Pp. x + 670 including 120 illustrations and Index. J. and A. Churchill, London, 1952. 37s. 6d.

HISTORY OF PHARMACY (2nd Ed.) by Edward Kremers and George Urdang. Pp. xiv + 622 including 30 illustrations. J. B. Lippincott Company, London, 1951. 60s.

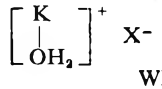
ORGANIC CHEMISTRY by A. F. Holleman, revised by J. P. Wibaut, translated from the 16th Dutch Edition by Samuel Coffey. Pp. xiv + 626 and Index. Cleaver-Hume Press, London, 1951. 55s.

THE PLANT GLYCOSIDES by H. J. McIlroy. Pp. 125 and Index. Edward Arnold, London, 1951. 18s.

LETTER TO THE EDITOR

Some Observations on Vitamins B_{12a}, B_{12b} and Chlorocobalamin

SIR,—The material obtained by catalytic reduction of vitamin B_{12} followed by aerial oxidation of the product has been designated vitamin B_{12a} by Kaczka *et al.*[1] Whatever may be the structure of this compound in the solid state (see Cooley *et al.*[2]) it is evident that in aqueous solution it undergoes changes leading to the formation of aquocobalamin hydroxide (I; X = OH).[3,4]



Where K = cobalamin

(I)

The basic aquocobalamin cation present in such solutions is characterised by the truly remarkable facility with which it undergoes combination with suitable anions. The products formed, however, fall into two main groups. The first of these, to which such compounds as cyanocobalamin (vitamin B_{12}), nitritocobalamin (vitamin B_{12c}) and thiocyanatocobalamin belong, do not dissociate appreciably in aqueous solution. The second group of complexes are clearly ionic in character and give the normal reactions of the entrant anion. Thus treatment of an aqueous solution of "sulphatocobalamin" with barium acetate leads to the formation of a precipitate of barium sulphate.[5] Such complexes, in our view, are more accurately formulated as aquocobalamin salts, e.g., aquocobalamin sulphate (I; X = $\frac{1}{2}SO_4$) rather than "sulphatocobalamin." Similarly, the substance termed "chlorocobalamin" by Kaczka *et al.*[5] is best regarded as aquocobalamin chloride (I; X = Cl). The latter compound, moreover, is necessarily identical with the substance obtained by Veer *et al.*[6] by photolysis of vitamin B_{12} in dilute hydrochloric acid, and termed by them and subsequently by Cooley *et al.*[3] vitamin B_{12b}. The exact nature of the product isolated by Pierce *et al.*[7] from cultures of *Streptomyces aureofaciens* and from liver and originally assigned the designation vitamin B_{12b}, however, has not yet been revealed. In these circumstances, we should like to propose that, pending elucidation of the nature of the vitamin B_{12b} isolated from natural sources, reactions in aqueous solution effected with this compound, as well as with B_{12a} and "chlorocobalamin." be regarded as taking place between the aquocobalamin cation and the reagent involved.

B. ELLIS.
V. PETROW.

Research Department,
The British Drug Houses, Ltd.,
London, N.1.

December 27, 1951.

REFERENCES

1. Kaczka, Wolf and Folkers, *J. Amer. chem. Soc.*, 1949, **71**, 1514.
2. Cooley, Ellis, Petrow, Beaven, Holiday and Johnson, *J. Pharm. Pharmacol.*, 1951, **3**, 607.
3. Cooley, Ellis, Petrow, Beaven, Holiday and Johnson, *ibid.*, 1951, **3**, 271.
4. Buhs, Newstead and Trenner, *Science*, 1951, **113**, 625.
5. Kaczka, Wolf, Kuehl and Folkers, *J. Amer. chem. Soc.*, 1951, **73**, 3569.
6. Veer, Edelhauser, Wijmenga and Lens, *Biochim. Biophys. Acta*, 1950, **6**, 225.
7. Pierce, Page, Stokstad and Jukes, *J. Amer. chem. Soc.*, 1949, **71**, 2952.