ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Mescaline, New Synthesis of. M. U. Tsao. (*J. Amer. chem. Soc.*, 1951, **73**, 5495.) The cactus alkaloid, mescaline, β-(3:4:5-trimethoxyphenyl)-ethylamine was synthesised by methylating gallic acid to produce the methyl ester of 3:4:5-trimethoxybenzoic acid, which was then reduced to 3:4:5-trimethoxybenzyl alcohol by means of lithium aluminium hydride. The –OH group was replaced by –Cl and then –CN to produce 3:4:5-trimethoxyphenyl-acetonitrile, the reduction of which by lithium aluminium hydride gave mescaline. A. H. B.

ANALYTICAL

Antioxidants in Lard, Estimation of. J. H. Mahon and R. A. Chapman. (*Anal. Chem.*, 1951, **23**, 1116.) Preliminary experiments indicated that a ferrous tartrate reagent for propyl gallate and a ferric chloride plus 1:1′-bipyrine reagent for butylated hydroxyanisole, nordihydroguaiaretic acid, and tocopherol were satisfactory and these reagents were studied more intensively. Procedures were developed for the extraction and spectrophotometric determination of propyl gallate using the absorption at 540 mμ after treatment with ferrous tartrate; butylated hydroxyanisole, nordihydroguaiaretic acid, and tocopherol were determined from the colour produced (at 515 mμ) with ferric chloride 1:1′-bi-pyrine reagent; the methods permitted the determination of all combinations of these four antioxidants except when propyl gallate and nordihydroguaiaretic acid were present in the same sample. Results are given for the extraction and colorimetric procedures recommended; the lower limits of the determinations, were propyl gallate 0·003 per cent., butylated hydroxyanisole 0·005 per cent., nordihydroguaiaretic acid 0·005 per cent., and tocopherol 0·015 per cent.; recoveries ranged from 96·4 per cent. for nordihydroguaiaretic acid in combination with butylated hydroxyanisole, to 98·4 per cent. for nordihydroguaiaretic acid alone. R. E. S.

Ascorbic Acid in Pharmaceutical Preparations, Estimation of. D. G. Chapman, O. Rochon and J. A. Campbell. (*Anal. Chem.*, 1951, **23**, 1113.) Several methods for the estimation of ascorbic acid in the presence of interfering materials such as iron and copper salts have been investigated with special reference to the limitations of these procedures as applied to preparations which may contain other interfering substances. The method of Roe *et al.* (*J. biol. Chem.*, 1948, **174**, 201) based on the reaction between dehydroascorbic acid and 2:4-dinitrophenyl-hydrazine gave a measure of the reduced ascorbic, dehydroascorbic acid, and diketo-l-gulonic acid; results obtained indicated that none of the added materials present in commercial multivitamin products caused interference in the recovery of ascorbic acid, the precision of the method as shown by the small standard deviation being good. The U.S.P. method gave an extremely high recovery of ascorbic acid when ferrous iron was present; cuprous chloride resulted in a low recovery, whereas cupric sulphate caused no interference. The method of Gawron and Berg (*Ind. Engng. Chem., Anal. Ed.*, 1944, **16**, 757) using 8 per cent. acetic acid to extract the ascorbic acid was not affected by the presence of iron but gave low recoveries in the presence of cuprous chloride, cupric sulphate, and

ferrous sulphate plus cupric sulphate. The procedure of Brown and Adam (*J. Sci. Food Agr.*, 1950, **1**, 51) who used a sodium acetate-hydrochloric acid mixture buffered to *p*H 0·65 to overcome the interference due to ferrous iron was satisfactory in the presence of ferrous sulphate and cupric sulphate separately but not if these substances were present together. In the presence of ferrous iron the method of Brown and Adam proved to be most suitable for routine analyses; if both ferrous iron and copper were present, the method of Roe *et al.* was most reliable. R. E. S.

Cadmium Iodide Linear Starch Reagents. J. L. Lambert. (*Anal. Chem.*, 1951, **23**, 1247.) The cadmium iodide-linear starch reagent reported previously was studied to determine its usefulness and potentialities for the colorimetric determination of trace amounts of oxidising substances. The intensity of the starch-iodide blue colour developed in solution was found to be directly proportional to the concentration of the oxidising agent over relatively wide ranges. Potassium bromate was selected as the oxidising agent and the rate of colour development was studied and the extinction coefficient determined. The blue linear starch-iodide colloid was little affected by weak acids, and by strong acids only at high concentrations. High concentrations of polyvalent cations tended to precipitate the blue colloid except in the presence of complexing agents such as tartrates and phosphates. In applying the reagent to a particular procedure it is suggested that the rate of colour development in the solution used should be determined by using the extinction coefficient as described, whereby the straight-line plot of optical density versus concentration can be obtained without redetermining a number of points. The reagent is colourless, unaffected by age, and capable of giving highly reproducible results. This reagent makes possible the first satisfactory utilisation of the extremely delicate starch-iodine reaction for colorimetric work, and has the added convenience of stability. It should make possible the development of a number of new analytical procedures.

R. E. S.

Calcium, Magnesium and Mercury Compounds, Application of the Schwarzenbach Method to Analysis of. H. R. Hernandez, U. Biermacher and A. M. Mattocks. (*Bull. nat. form. Comm.*, 1950, **18**, 145.) Ethylenediaminetetraacetic acid reacts with bivalent metals to give a complex with a low degree of ionisation. Magnesium, however, has less affinity for the reagent than other metals. Magnesium also forms a stable red complex with a dye, Eriochrome black-T, at *p*H 8 to 9 while other polyvalent metallic ions do not affect the blue colour of the dye. In the assay of calcium and magnesium salts the equivalents of about 40 to 50 mg. of Ca. and 30 mg. of Mg. are required. The salts are brought into solution, if necessary with dilute hydrochloric acid. An excess (40 ml.) of a standardised 1 per cent. solution of the disodium derivative of the reagent is then added to the solution and the *p*H is adjusted to about 9 with 10 ml. of an ammonium chloride 6·75 per cent. and concentrated ammonium hydroxide 57 per cent. buffer solution. 6 drops of a methanolic solution containing 5 per cent. of a mixture of the dye (1 part) and hydroxylamine hydrochloride (9 parts) are then added and the excess of reagent titrated with a standardised 0·75 per cent. solution of magnesium chloride. Combination with the dye to give a sharp blue/red colour change occurs when all the reagent is used. For mercury compounds it is more convenient to use reagent and magnesium chloride solution of twice the strength described, using the equivalent of about 40 to 50 mg. of Hg. Digestion of these compounds to obtain solubility can be carried out in most cases in a 250 ml. Erlenmeyer flask, but it was found more convenient to use a 250 ml. Kjeldahl flask with some compounds. In either

case the subsequent titration was performed as described above, in the flask used, about 15 ml. more of buffer solution being required to neutralise any excess of acid used in digestion. J. R. F.

Chloride in Presence of Iodate, Determination of. L. S. Stanton. (*Anal. Chem.*, 1951, **23**, 1331.) The determination of chloride by the Volhard method was found to be unsatisfactory in the presence of iodate, apparently because of the progress of a slow side reaction between iodic acid and sodium thiocyanate. An experimental study was carried out which showed that accurate results were obtained by the Volhard method if iodate was previously removed by the use of barium nitrate. Barium iodate was free filtering, but carbonates and sulphates decreased the filtration rate; more rapid filtration was obtained if carbonates were destroyed by acidification prior to barium iodate precipitation and if the presence of sulphates was avoided. R. E. S.

Chromium, Separation of, from Vanadium. R. K. Brookshier and H. Freund. (*Anal. Chem.*, 1951, **23**, 1110.) The determination of small amounts of chromium in vanadic oxide was investigated; titration procedures were found to be inaccurate and the perchromic acid extraction method gave erratic results. A study of the extraction procedure using ethyl acetate was made and gave the following optimum conditions: *p*H at equilibrium $1{\cdot}7 \pm 0{\cdot}2$, concentration of hydrogen peroxide 0·02 mole/l., temperature 20° C. or less, and number of extractions 3. The blue perchromic acid decomposed rapidly in aqueous solution and its immediate extraction with ethyl acetate was necessary; the blue substance was stable in ethyl acetate solution for 30 minutes. The following elements did not interfere: iron, mercury, vanadium, titanium, nickel, molybdenum. A standard sample containing 0·68 per cent. of chromium was examined by this procedure; parallel analyses using 0·10 and 0·25 g. samples gave a value of 0·65 per cent. chromium. R. E. S.

Citric Acid and Acetone, New Reaction for. A. di Giacomo and G. Rispoldi. (*Boll. chim.-farm.*, 1951, **90**, 311.) If to a solution of citric acid a few crystals of anthraquinone and twice its volume of concentrated sulphuric acid are added and the whole heated, the anthraquinone dissolves with the production of a yellow colour. On further heating, gas is evolved and then a yellowish-orange to blood-red colour, according to the amount of citric acid present, is formed. As this colour is due to acetone derived from the citric acid, acetone will also give it. A yellowish-orange colour can be obtained with 0·05 mg. and a blood-red colour with 5 mg. Using the dry acid a blood-red colour can be obtained with 1 mg. Tartaric, malic, lactic, benzoic and salicylic acids do not give the reaction. It is given by 0·5 mg. of acetone. H. D.

Methanol in Distilled Spirits, Spectrophotometric Estimation of. G. F. Beyer. (*J. Ass. off. agric. Chem.*, 1951, **34**, 745.) The method of Boos (*Anal. Chem.*, 1948, 20, 964) using chromotropic acid has been investigated. Previously no effort has been made to regulate the amount of ethanol that should be present in order to produce the maximum amount of colour; this amount was found to be 22·0 to 24·0 per cent. in the sample to be oxidised. It was found that heating the solution after the addition of sulphuric acid tended to destroy the violet colour. Spectrophotometric curves are given for the colour produced, the maximum being at 580 mμ. It is claimed that the proposed method is at least as accurate as the modified Denigè's method, and the colour is more stable.

R. E. S.

Nitroglycerin Tablets, Rapid Assay of. J. H. Cannon and R. F. Heuermann. (*J. Assoc. off. agric. Chem.*, 1951, **34**, 716.) The assay is a small scale operation with simple apparatus which gives quick results. Powdered tablets are mixed with water and extracted with ether, the ether extract being evaporated after the addition of ethanolic potassium hydroxide solution. Reduction is accomplished with Devarda's alloy and the resulting ammonia is distilled off and determined by titration against 0·01N sulphuric acid. Advantages claimed for the assay are the use of ethanol in the ammonia distillation to prevent frothing, and the absorption of the distilled ammonia in ice-water. The results obtained in 18 assays showed a mean of 6·83, standard deviation from the mean 0·011 or 0·16 per cent. R. E. S.

Phenacetin, Colorimetric Determination of. D. Horn. (*Pharm. Zentralh.*, 1951, **90**, 296.) About 0·1 g. of phenacetin is heated on the water-bath for 5 minutes with 50 ml. of 10 per cent. nitric acid. After cooling, and possibly filtering, the colour is determined at 465 mμ (filter S47). The presence of acetylsalicylic acid, quinine, caffeine, starch or sugar does not interfere with the colour production. Amidopyrine and phenazone give interfering colours, and it is necessary to heat the mixture until the blue colour produced disappears. In this case the solution becomes turbid on cooling, and must be cleared by centrifuging. If the proportion of phenazone is greater than 50 per cent. a preliminary separation is necessary; the mixture being treated with 20 ml. of water and 0·2 g. of tartaric acid and the phenacetin shaken into ether. Although some phenazone also goes into the ether solution, it is not sufficient to interfere with the reaction. G. M.

Phenacetin, Estimation of. A. Casini. (*Annali. Chim.*, 1951, **41**, 611.) Although phenacetin is included in most pharmacopœias none of them gives any method of estimation and even where, as in the U.S.P. and B.P., tablets are tested, this is only done by means of solvents, with no test to show that the residue obtained is really phenacetin. The author therefore investigated various published methods. He found that Weismann's method of determining the acetic acid set free on hydrolysis with hydrochloric acid failed to work, as even after 6 hours boiling only 74 per cent. of the phenacetin was saponified. Miller's colorimetric method using the colour produced by nitric acid in a solution in methanol was neither sensitive nor reliable. Degner and Johnson's colorimetric method, using the colour produced by the addition of chromic acid after boiling with concentrated hydrochloric acid, reading at the wavelength of 543 mμ in a Bechman spectrophotometer gave results within 1 per cent., but spectrophotometers are not usually available in pharmacies. He therefore recommends the use of tetra-iodophenacetin hydriodide, $(C_{10}H_{13}O_2N)_2I_4,HI$. Place 0·3 g. of phenacetin, 6 ml. of glacial acetic acid and 80 ml. of water in a 200 ml. stoppered graduated flask. Warm to about, but not above, 70° C. to get complete solution. Add, with continuous shaking, 50 ml. of 0·25N iodine and then 6 ml. of concentrated hydrochloric acid. Stopper immediately and shake vigorously to encourage the separation of the tetraiodophenacetin. After cooling, make up to the mark with water, mix well and allow to stand for 30 minutes. Filter off 50 ml. through a sintered glass filter and determine the excess of iodine with 0·1N thiosulphate. H. D.

Phenylmercuric or Ethylmercuric Compounds, Determination of. V. L. Miller, D. Polley and C. J. Gould. (*Anal. Chem.*, 1951, **23**, 1286.) A method is given for the direct determination of these compounds in aqueous solution without prior wet oxidation. A solution containing between 50 and

100 μg. of the compound in 0·5 to 20 ml. of water or very dilute acid or alkali is accurately measured into a separating funnel and shaken with 3·5N hydrochloric acid containing hydroxylamine hydrochloride and a definite volume of dilute dithizone solution in chloroform; the chloroform layer is separated and washed by shaking with 3N hydrochloric acid followed by shaking with water. The light absorption of the resulting solution is then determined photoelectrically. The green colour of the unreacted dithizone is determined rather than the yellow of the organic mercury dithizonate; the values for unknown samples are determined by comparison with a standard curve. Details of the procedure are given together with an adaptation for the determination of quantities of organic mercury compound as low as 1 μg. The possibility of interference from copper ions was investigated and is discussed. R. E. S.

Rotenone, Determination of, Using Mercury Acetate. I. Hornstein. (*Anal. Chem.*, 1951, **23**, 1329.) It has been found possible to determine rotenone quantitatively by adding an excess of mercuric acetate in methanol to a solution of rotenone; the *iso*propenyl double bond in the rotenone molecule reacts quantitatively with the mercuric acetate-methanol reagent and for each mole of rotenone 1 mole of acetic acid is formed. Titration with 0·1N sodium hydroxide gives a direct measure of the rotenone present. In the procedure developed, sodium chloride was used to convert excess of mercuric acetate to the chloride and thus permit direct titration of the acid with standard alkali using an excess of phenolphthalein as indicator. Results given showed that the reaction between mercuric acetate and the double bond could be carried out at room temperature and was virtually complete after 15 minutes; the volumetric method gave slightly higher results than the gravimetric method which has been shown to yield results generally about 1 per cent. lower than the correct value. The volumetric procedure appears to be as accurate as the gravimetric method. R. E. S.

Starch-Iodate Reagent, Linear. J. L. Lambert (*Anal. Chem.*, 1951, **23**, 1251.) An examination was made of a colorimetric reagent containing linear A-fraction potato starch, iodate ion, and cadmium ion which, at the proper *p*H showed a selectivity for iodide ion. The reaction of iodate ion and iodide ion to produce triiodide ion which reacts with the linear starch to form the well-known blue complex, gave reproducible and quantitative results. Details are given of the light absorption curves of the linear starch-triiodide ion blue complex and studies were made of the rate of development of the blue colour, and the effect of temperature and of various acids on the colour density. The colourless reagent prepared proved to be stable for periods up to 6 weeks and to be selective for iodide ion when used in a solution of a weak acid such as formic. The presence of small amounts of many common inorganic and organic substances can be tolerated but the peculiar interference of the bromide ion was such that its concentration must be known before using this reagent; large concentrations of bromide ion produced an orange or purplish colour. R. E. S.

Sulphate in Presence of Iron, Determination of. N. Gandolfo. (*Annali. Chim.*, 1951, **41**, 600.) For the accurate determination of sulphate by precipitation with barium, if iron is present it must be removed or some of it will be precipitated with the barium sulphate, but if the iron is precipitated as ferric oxide this is difficult to filter and may carry down some sulphate. If however the iron is precipitated as ferroso-ferric hydroxide, filtration is easy and the precipitate does not carry down sulphate. The solution containing the ferric and sulphate ions is put in a 100-ml. beaker with 2 or 3 ml. of 10 per cent. potassium iodide solution and boiled to drive off part of the liberated iodine, but leaving sufficient

to oxidise part of the reduced iron. This can be done by stopping the boiling as soon as the first violet fumes come off. If it is wished to do this quite accurately, the iron should be determined in a separate sample, and after boiling off the liberated iodine, sufficient 0·1N iodine added to convert two-thirds of the iron to the ferric condition. A freshly prepared 10 per cent. solution of sodium hydroxide is added until a slight turbidity is formed and then an excess of 10 ml. and the mixture is boiled for 2 to 3 minutes, allowed to settle and filtered while still hot. The precipitate is washed and the sulphate determined in the filtrate in the usual way. H. D.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Barley Starch, Constitution of. I. C. MacWilliam and E. G. V. Percival. (*J. chem. Soc.*, 1951, 2259.) The structure of the starch from the barley grain was investigated and found to contain 19 per cent. of amylose. By repeated precipitations with butanol highly purified amylose was obtained. It was methylated by repeated treatments with methyl sulphate and sodium hydroxide in nitrogen, the product was hydrolysed, and the resulting mixture separated on a cellulose column. The main product was 2:3:6-trimethyl-D-glucose, which was accompanied by small quantities of tetramethylglucose and a mixture of dimethylglucoses. The amount of tetramethylglucose was estimated to be 0·24 to 0·29 per cent. of the methylated glucoses produced on hydrolysis, and, corresponds to the presence of one non-reducing terminal group per 400 ± 40 glucose residues. The fractionation by precipitation with aqueous pyridine saturated with butanol to obtain barley starch amylopectin did not yield a product containing less than 2 per cent. of amylose. Methylation studies, involving the application of paper chromatography, indicated one non-reducing terminal group for 26 ± 2 non-terminal glucose residues. The application of periodate oxidation confirmed this result and indicated that over 86 per cent. of the branching linkages between the unit chains involved 1 : 6-linkages. A. H. B.

Hecogenin, A Source of. R. E. Callow, J. W. Cornforth and P. C. Spensley. (*Chem. Ind.*, 1951, **33**, 699.) The steroidal sapogenin, hecogenin, has a 12-keto group in ring C and, as a starting-point for the partial synthesis of cortisone, would be comparable with the bile acids. The sisal plant, *Agave sisalana* Perrine, widely cultivated in East Africa to produce fibre, was examined and the whole leaves were found to contain hecogenin, which can be readily extracted. It was shown that hecogenin could also be extracted from sisal waste, consisting of short fibres and debris. The extraction process is described. Hecogenin was obtained from the crude sapogenin fraction by chromatography on alumina or more simply by removal of a low melting material with hot light petroleum, followed by a Girard separation of the residue. The yield of hecogenin varied from 0·04 to 0·1 per cent. on the air-dry weight of sisal waste. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Enzymes, Separation of, by Paper Chromatography. K. V. Giri and A. L. N. Prasad. (*Nature, Lond.*, 1951, **168**, 786.) By the application of the usual paper chromatographic technique, and using aqueous acetone or sodium chloride or suitable combinations of these two as developing solvents, the separation of individual enzymes from mixtures prepared artificially, or obtained from natural sources, such as mould or tissue extracts, was accomplished. The

details of the method for the separation of amylase from the proteinase, trypsin, are described and a photograph of the chromatogram recorded. Neither aqueous acetone nor water alone was able to bring about any appreciable movement of trypsin, but the addition of sodium chloride in concentrations as little as 0·5 per cent. to the aqueous acetone or water caused considerable movement of the trypsin from the starting line. Saline solutions also arrested tailing. A. H. B.

Paper Chromatography on Borate-impregnated Paper. C. A. Wachtmeister. (*Acta chem. scand.*, 1951, **5**, 976.) The possibility of using paper impregnated with borate for the separation of phenols, phenolic aldehydes, phenolic acids and certain sugars was investigated. With any substance, or group of isomeric substances, parallel chromatograms were run using paper impregnated with a sodium borate buffer and, for comparison, a sodium phosphate buffer of approximately the same *p*H 8·7. The buffers were 0·1 M with respect to boric or phosphoric acid. Organic substances capable of forming strongly acidic complexes with boric acid would be expected to show decreased R_F values on borate-impregnated paper as compared with the values on unimpregnated or phosphate-impregnated paper. This proved to be the case, because there was a considerable decrease in the R_F values of phenols and phenolic aldehydes containing two adjacent hydroxyl groups, whereas, on the other hand, compounds with isolated hydroxyl groups as well as *o*-hydroxyaldehydes moved at approximately the same rate on phosphate- and borate-impregnated paper. Phenolic acids containing two adjacent hydroxyl groups or a hydroxyl group in *ortho*-position to a free carboxyl group also had smaller R_F values on borate- than on phosphate-impregnated paper. A good separation of glucose and sorbose was achieved using borate-impregnated paper, although these two sugars apparently move at the same rate in the systems ordinarily used. The preparation of parallel chromatograms on borate- and phosphate-impregnated paper affords a simple micromethod for the detection of borate reactive groupings. A. H. B.

BIOCHEMICAL ANALYSIS

Œstrogens, Chromatographic Separation of. J. F. Nyc, D. M. Maron, J. B. Gorst and H. B. Friedgood. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **77**, 466.) A column of pulverised rubber (vulcanised rubber powder) has been successfully adapted to the quantitatively accurate chromatographic separation of a mixture of crystalline œstrone, œstradiol and œstriol. When 20 ml. each of 20, 40 and 60 per cent. aqueous methanol (v/v) are passed successively through the column, œstriol is eluted by the 20 per cent., œstradiol by the 40 per cent. and œstrone by the 60 per cent. concentration of methanol. Known amounts of a mixture of the three natural œstrogens can be separated sharply from each other and estimated quantitatively with an experimental error of approximately ± 10 per cent. Moreover, it is possible to determine the identity of an unknown œstrogen by the concentration of methanol which will remove it from a rubber column. The method of preparation of the column and the process of selective elution are described. S. L. W.

Vitamin B_{12} and the Animal Protein Factor, Chick Assay of. M. E. Coates, G. F. Harrison and S. K. Kon. (*Analyst*, 1951, **76**, 146.) A method of assay with chicks for vitamin B_{12} and the animal protein factor is described and its accuracy discussed. Comparison of values found by chick and microbiological assay of crude materials containing vitamin B_{12} showed very clearly that the two techniques were not measuring the same thing, it being evident that

values found by the chick tests represented other growth factors as well. Difficulties in obtaining satisfactory responses to crystalline vitamin B_{12} are reported.

R. E. S.

Vitamin B_{12}, Microbiological Estimation of, using the *Lactobacillus lactis* Dorner Cup-Plate Method. W. F. J. Cuthbertson, H. F. Pegler and J. T. Lloyd. (*Analyst*, 1951, **76**, 133.) The cup-plate method for the rapid microbiological assay of aneurine and riboflavine has been found applicable to the estimation of vitamin B_{12} with a suitable strain of *Lactobacillus lactis* Dorner. The procedure involves the use of a medium similar to those found necessary for other strains of lactobacillus; ascorbic acid is needed in the assay medium but not in that for preparation of the inoculum. The effect of a number of factors on the assay have been studied including the maintenance of the organism, the preparation of the medium and the assay plates, the time of incubation, the concentration of vitamin B_{12} in the test samples, and the inoculum density. Methods for the calculation of the results are given together with typical assay figures; on average, fiducial limits of ± 15 per cent. ($P = 0{\cdot}05$) may be expected in assays employing six plates, although narrower limits can be obtained with a greater number of plates. Assays by the plate method and by a tube technique employing *L. leichmannii* 313 have given comparable results. Vitamin B_{12c} and the deoxyribosides interfere with the procedure; the effect of the latter, but not the former, can be eliminated by combining the method with paper chromatography, the presence of deoxyribosides in addition being generally apparent from the type of zone produced. The combined chromatographic and microbiological procedures make it possible, with a (2 + 2) assay design, dose ratios of 10 to 1 and incubation overnight, to attain a satisfactory degree of precision on a few μl. of vitamin B_{12} solution. R. E. S.

Vitamin B_{12}, Microbiological Estimation with *Lactobacillus leichmannii* 313 by the Turbidimetric Procedure. W. B. Emery, K. A. Lees and J. P. R. Tootill. (*Analyst*, 1951, **76**, 141.) Details are presented of a microbiological tube assay for vitamin B_{12} with *Lactobacillus leichmannii* 313 as test organism. The assay method depends on the inability of *L. leichmannii* 313 to synthesise vitamin B_{12} under defined conditions, so that there is a direct relationship between the growth of this organism and the concentration of vitamin B_{12} in the test medium over a certain range. Details of the media required, involving a modification of the process of Snell, Kitay and McNutt (*J. biol. Chem.*, 1948, **175**, 473) are given. Statistical analyses of a (3 + 3) assay and a standard response curve are given and show that the method is sufficiently sensitive and accurate for routine use. R. E. S.

Vitamin D, Analytical Purification by Differential Solubility, Precipitation and Chromatography. J. Green. (*Biochem. J.*, 1951, **49**, 45.) Methods for the quantitative separation of vitamins D from interfering sterols and vitamin A have been developed. The vitamins D could be quantitatively separated from their provitamins and other precipitable sterols by differential solubility in 72 per cent. ethanol followed by digitonin precipitation. Chromatography on floridin earth, under the conditions described, produced an efficient separation of tachysterol and vitamin A from the vitamins D. An examination of acid-washed floridin earth columns showed that, provided the columns contained no free hydrochloric acid, vitamin D could always be recovered quantitatively; tachysterol was destroyed to a large extent by single passage through a column washed with 90 per cent. ethanol, but not if the column was washed with pure ethanol. The complex destruction of vitamin A was conditioned by the acidity

and the moisture content of the column the destruction being probably due to polymerisation. For analytical purposes, the 90 per cent. ethanol-washed column was the most useful, since with one liquid chromatogram, vitamin D could be separated from tachysterol and vitamin A; β-carotene was destroyed on the column and did not interfere. R. E. S.

Vitamin D, Reaction with Iodine Trichloride. J. Green. (*Biochem. J.*, 1951, **49**, 36.) It was found that iodine trichloride in carbon tetrachloride solution reacted with substances containing systems of three or more conjugated double bonds and, in addition, a group of sterols related to the vitamins D, involving chlorination and liberation of iodine. For quantitative studies the vitamin D solution in carbon tetrachloride is placed in a spectrophotometric cell, a specially prepared solution of iodine trichloride is added from a burette followed by carbon tetrachloride to volume, when the resulting colour is measured; this procedure is continued until the addition of more iodine trichloride solution produces no increase in the colour of the resulting reaction mixture. Details of the method and of the precautions necessary to ensure accuracy are given. A number of substances related to vitamin D were subjected to the reaction with iodine trichloride; it was found that although in the case of calciferol only three bonds usually reacted and the side-chain double bond was preferentially unreactive, in ergosterol all three bonds reacted; with lumisterol, two double bonds reacted; the reagent also reacted quantitatively with vitamin A and β-carotene. The titration of many samples of calciferol and vitamin D_3 showed an overall accuracy of ±5 per cent. R. E. S.

CHEMOTHERAPY

Thyroxine, Biological Action of Substances Related to. J. H. Wilkinson, M. M. Sheanan and W. F. Maclagan. (*Biochem. J.*, 1951, **49**, 710.) It has been shown (Sheanan, Wilkinson and Maclagan, *Biochem. J.*, 1951, **48**, 188) that 3:5-diiodo-4-hydroxybenzoic acid shows a slight antithyroxine activity when tested in mice by the oxygen consumption method. Esterification produced a considerable enhancement of activity which was maximal when the *n*-butyl ester was used. A series of 15 3:5-diiodo-4-alkoxybenzoic acids and their esters have now been tested similarly, but all were markedly inferior to *n*-butyl-3:5-diiodo-4-hydroxy benzoate. 4 branched-chain alkyl esters of 3:5-diiodo-4-hydroxy benzoic acid were much less active than straight chain esters. 3:5-diiodo-4-methoxybenzonitrile showed slight antithyroxine activity, but 3:5-diiodo-4-*n*-propoxybenzonitrile and the corresponding benzamide were inactive. J. R. F.

Tubercle Bacilli, Bacteriostatic Action of Certain Compounds on. R. de Fazi and G. Berti. (*Annali Chim.*, 1951, **41**, 621.) The authors give details of the methods of preparation of a large number of esters of *p*-aminobenzoic acid and of *cyclo*hexyl esters of various aromatic acids and results of tests of their action *in vitro* on *Myco. tuberculosis*. The most active compound studied was cinnamyl *p*-aminobenzoate, next phenylethyl *p*-aminobenzoate and β-decahydronaphthyl-*p*-aminobenzoate. Then *cyclo*hexyl *p*-aminobenzoate, benzyl-*p*-aminobenzoate and *cyclo*hexyl *p*-aminocinnamate. *Cyclo*pentyl *p*-aminobenzoate and *cyclo*hexyl salicylate were next and then the di*cyclo*hexyl ester of *N'* (*p*-carboxyphenyl) glycine and the *cyclo*hexyl ester of *N* (*p*-carboxyphenyl) glycine. *p*-Amino*cyclo*hexane carboxylic acid and the amide of hexahydrobenzoic acid had slight activity and *N*-dodecyl *p*-aminobenzoate none. H. D.

Tuberculosis, Chemotherapy of. *N*-Substituted 4-Aminodiphenyls. L. Bauer, J. Cymerman and W. J. Sheldon. (*J. chem. Soc.*, 1951, 2342.) The aim of the investigation was to attempt to discover any correlation between basicity and antibacterial activity in a series of *N*-substituted derivatives of 4-aminodiphenyl and β-naphthylamine. Concurrently two other effects were to be examined: (*a*) variation in lipoid solubility, and (*b*) possession of optimal flat surface area. The compounds, *N'*-4'-diphenyl-*N N*-diethylethylenediamine, *N*-4'-diphenylyl-2-morpholinoethylamine, 4-2'-diethylaminoethoxydiphenyl, and the highly basic 4-diphenylylguanidine and -diguanide, were prepared. Other compounds were obtained by the condensation of 4-aminodiphenyl with *p*-hydroxy-*p*-dimethylamino, *p*-nitro, and *p*-methoxy-benzaldehyde, and these Schiff's bases were hydrogenated at atmospheric pressure in the presence of Adam's catalyst to give the corresponding substituted benzylamino-compounds.

A. H. B.

PHARMACY

NOTES AND FORMULÆ

Methaphenilene Hydrochloride (Diatrine Hydrochloride). (*New and Non-official Remedies: J. Amer. med. Ass.*, 1951, **147**, 862.) Methaphenilene hydrochloride is *N*: *N*-dimethyl-*N'*-(α-thenyl)-*N'*-phenylethylenediamine hydrochloride. It is a white or pale yellow, crystalline powder with a faint odour, m.pt. 184° to 189° C., soluble in water, sparingly soluble in ethanol and chloroform, and almost insoluble in ether. A 2 per cent. aqueous solution has *p*H 4·8 to 5·6 and yields the free base as an oil on the addition of alkali and a pink precipitate with Reinecke's salt. A solution in sulphuric acid is light yellow at first but becomes light orange on standing and cherry red on the addition of a 1 per cent. solution of isatin in sulphuric acid. Methaphenilene hydrochloride loses not more than 0·5 per cent. of its weight when dried at 110° C. for 4 hours, yields not more than 0·15 per cent. of sulphated ash, and complies with a limit test for heavy metals. It contains 9·2 to 9·6 per cent. of nitrogen (determined by the Kjeldahl method), and 99 to 101 per cent. of methaphenilene hydrochloride when assayed by adding an excess of silver nitrate and titrating with ammonium thiocyanate. It is also assayed by precipitating the dipicrate by the addition of a saturated solution of trinitrophenol containing sulphuric acid and contains 98 to 102 per cent. of methaphenilene hydrochloride. A 0·001 per cent. solution in ethanol exhibits ultra-violet absorption maxima at 2450 Å ($E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, 537 ± 5), and 2900 Å, and a minimum at 2840 Å. Methaphenilene hydrochloride is a histamine antagonist. The average adult dose is 50 mg.

G. R. K.

PHARMACOGNOSY

Ergot Mycelium, Effects of Nitrogen compounds on Growth and Alkaloid Biosynthesis in. S. K. Sim and H. W. Youngken. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 434.) A study has been made of the growth and alkaloidal production (as ergotoxine) in the mycelial tissues of *Claviceps purpurea*. When grown in modified culture media ergot alkaloids were found in small amounts in all the mycelial cultures examined. When these cultures were agitated mechanically the dried weight of the mycelia increased by some eightfold, but this increase in growth was accompanied by a marked decrease in alkaloidal content. On the other hand the addition of small quantities of indole, ornithine and tryptophane to a standard culture medium caused a decrease in mycelial growth with some increase in alkaloidal content. The addition of arginine, and of ammonium sulphate, caused no significant responses in either growth or alkaloidal content in mycelial tissues.

S. L. W.

PHARMACOLOGY AND THERAPEUTICS

Antidiuretic Activity: A Method of Assay. M. Ginsberg. (*Brit. J. Pharmacol.*, 1951, **6**, 411.) Groups of 18 rats, previously conditioned to water administration by stomach tube, after being deprived of food for 18 hours but denied free access to water for the last hour only, were placed in metabolism cages, and urine collected in graduated cylinders. Each rat was then given 2 doses of tepid water by stomach tube (each dose = 5 ml./100 g. of bodyweight), with 1 hour between each dose. 1 hour after the second dose the total volume of urine excreted in the preceding 2 hours was measured (V_2), and a third dose of water was then given and the test material injected subcutaneously. The urine output was then measured at intervals of 30 minutes for 120 or 150 minutes. The volume of urine, V_2 was expressed as a percentage of the amount of water administered in the first 2 doses. Those rats from which this value deviated from the mean for the whole group by more than 33 per cent. of the mean were rejected. 30 minutes was allowed for complete absorption of the material injected. The following formula was used:

$$\text{Percentage water excretion} = \frac{V_t - V_{30}}{3V_1 - (V_2 + V_{30})} \times \frac{100}{1}$$

where V_1 = the volume of water administered in each dose.
V_2 = the volume of urine excreted in the 2 hours before injection.
V_{30} = the volume of urine excreted in the first 30 minutes after injection.
V_t = the volume of urine excreted during the period of "t" minutes after injection (t = 60, 90, 120 or 150 minutes).

That is, the percentage water excretion at a given time is the volume of urine passed from 30 minutes after injection expressed as a percentage of the water administered but not excreted 30 minutes after injection. Solutions for injection are prepared in 0·9 per cent. sodium chloride solution, from 0·2 to 0·4 ml. being the amount usually injected. In each assay two dose levels of standard (vasopressin) and unknown are used; the ratio, high to low dose, being the same for standard and unknown. The error may be estimated from the results and the assay can be completed in 4 to 5 hours. "Unknown" amounts of vasopressin have been assayed against vasopressin standards; the mean standard error of 12 assays was 8·9 per cent. A total "unknown" activity equivalent to 10 milliunits is sufficient for a satisfactory assay. S. L. W.

Bacitracins A, B and C, Biological Properties of. G. G. F. Newton, E. P. Abraham, H. W. Florey, N. Smith and J. Ross. (*Brit. J. Pharmacol.*, 1951, **6**, 417.) It has been shown that crude preparations of bacitracin contain at least three polypeptides which are active against certain bacteria, as well as a number of other polypeptides showing no antibacterial activity. It has been hoped that the nephrotoxicity of commercial bacitracin might be due to impurities rather than to the antibiotic itself. The observations recorded in this paper, however, make it reasonably certain that this nephrotoxicity is due, at least in part, to the antibacterially active constituents, and that there is little hope of obtaining a preparation of bacitracin which does not damage the kidneys. For therapeutic purposes it does not appear to be worth trying to separate bacitracin A from bacitracin B, since although the latter is somewhat less toxic than the former it is less potent against certain bacteria. The data at present available suggest that bacitracin C has a greater acute toxicity than the other two bacitracins. S. L. W.

Cation Exchange Resins: Optimal Potassium Content for Clinical Use. E. W. McChesney. (*J. Lab. clin. Med.*, 1951, **38**, 199.) It has been substantiated that each g. of resin ingested by a human subject prevents the absorption of from 1 to 1·5 meq. of both sodium and potassium. The dose of resin generally used is about 50 g. per day, and when taken in conjunction with a diet of moderate sodium content this dosage usually gives prompt relief of œdema. The principal complications resulting are acidosis and potassium deficiency. The acidosis is not considered undesirable *per se*, but the potassium deficiency, which occurs quite frequently, leads to general weakness. This is particularly likely to occur on low sodium diets since the cation exchange resins have greater affinities for potassium than sodium, and large fæcal losses of potassium may occur. *In vivo* studies on rats indicate that, for clinical use, the optimal potassium content of both carboxylic and sulphonic types of resins lies between 1 and 2 meq./g. A daily intake of 12·5 g. of K-form and 37·5 g. of NH_4-form carboxylic resin (containing a total of 90 meq. of potassium) would be capable of binding about 1 g. of sodium in the intestine, would assure a positive potassium balance and would result in very little net change in acid-base balance. Such a combination would neutralise about 4 l. of gastric juice of normal acidity. A daily intake of 12·5 g. of K-form and 37·5 g. of NH_4-form sulphonic resin (containing a total of 52 meq. of potassium) would bind about 1·3 g. of sodium in the intestine, would assure a positive potassium balance and would give a small net alkali loss. Such a combination would neutralise about 1600 ml. of gastric juice of normal acidity.

S. L. W.

Cephalosporin P_1, Biological Properties of. A. C. Ritchie, N. Smith and H. W. Florey. (*Brit. J. Pharmacol.*, 1951, **6**, 430.) Cephalosporin P_1 is an antibiotic from a species of *Cephalosporium*. The cephalosporin used in this investigation was lyophil-dried material prepared by chromatography and countercurrent distribution between solvents. Cephalosporin P_1 inhibits the growth *in vitro* of staphylococci, corynebacteria and *Cl. tetani* at considerable dilution, but has little effect on streptococci, Gram-negative organisms or tubercle bacilli. Penicillin-resistant staphylococci are sensitive to the drug. Serum reduces but does not abolish its action. By repeated culture staphylococci are made resistant to its action *in vitro*. In concentrations of more than 1:200,000 it is bactericidal; in weaker concentrations it is bacteriostatic. It has the same order of activity against staphylococci as aureomycin and terramycin. Intravenously in mice it is less toxic than aureomycin or terramycin. No gross or microscopic lesions were found *post mortem* in mice given 5 mg. 12-hourly by mouth for 5½ days. The drug did not affect the blood pressure of a decerebrate cat but modified the contractions of isolated guinea-pig uterine muscle. It is well absorbed by mouth or by subcutaneous injection. It disappears from the blood rather quickly, though little is excreted in either urine or bile. After oral administration serum levels are reached and maintained greater than those necessary to inhibit the staphylococcus *in vitro*, yet mouse experiments show that while it has some protective actions against staphylococcal infections this action is much weaker than that of aureomycin or terramycin.

S. L. W.

Chloramphenicol, Serum Concentrations following Intravenous and Intramuscular Injections. J. J. Burnell and W. M. M. Kirby. (*J. Lab. clin. Med.*, 1951, **38**, 234.) This study was undertaken to test the toxicity of a new preparation of chloramphenicol (chloramphenicol 25 per cent. in aqueous acetyldimethylamine 50 per cent.) and to measure serum concentrations following

parenteral administration. When administered intravenously in the concentrated form (0·5 g. of chloramphenicol in 2 ml.), 5 of 10 patients complained of pain along the course of the vein, and in 6 patients the veins subsequently thrombosed. A fall in blood pressure occurred in 3 of the patients. When the concentrated solution was diluted in 100 ml. of saline or glucose solution, and administered by intravenous drip over a period of 15 to 30 minutes to 45 patients very few toxic reactions were observed, but in 2 patients receiving intravenous injections twice daily the antecubital veins thrombosed after 6 days. 6 patients receiving 1 g. intramuscularly tolerated the preparation well and did not complain of pain. Following a single intravenous injection of 1 g. of chloramphenicol serum concentrations averaged 20 μg./ml. at 1 hour, and gradually declined to an average of 2·4 μg./ml. at 24 hours. Following 0·5 g. intravenously concentrations fell from 10 μg./ml. at 1 hour to 3·8 μg./ml. at 12 hours. Comparing levels following oral and intravenous administration, serum concentrations were slightly higher with the intravenous route at 3 hours, but were twice as high at 12 hours, and persisted for a longer period than with the oral route. Intramuscular injections did not give adequate serum levels for therapeutic purposes. It would appear that when oral administration of chloramphenicol is not possible, 1 g. every 12 hours intravenously will provide effective therapy. S. L. W.

Hyoscine and Anti-histamine Compounds in the Prevention of Seasickness. E. M. Glaser and G. R. Hervey. (*Lancet*, 1951, **256**, 749.) A controlled and crossed over experiment was made at sea in which 68 healthy volunteers (soldiers) were in turn given 1 mg. of hyoscine hydrobromide, 25 mg. of diphenhydramine hydrochloride (benadryl), 25 mg. of promethazine hydrochloride (phenergan), and a placebo. Of those who might otherwise have vomited 96 per cent. were protected by hyoscine, 61 per cent. by promethazine and 30 per cent. by diphenhydramine. If nausea and vomiting are considered together, the figures were 77, 65 and 30 per cent. respectively. All the drugs were remarkably free from side-effects in the doses given. These findings strongly confirm previous observations that hyoscine hydrobromide effectually prevents seasickness in a large number of people and that 1 mg. is a safe dose. Hyoscine did not prevent nausea with equal success, but since it was unquestionably more effective in preventing vomiting there can be no doubt that it would be the preferred drug. Hyoscine caused a feeling of dryness in the mouth in most subjects, but headaches, dry mouth, giddiness and drowsiness are all symptoms of seasickness which were present in a number of untreated men. S. L. W.

Procaine Amide and Dibenzylmethylamine in Experimentally Produced Ventricular Tachycardia. R. Charlier and A. Klutz. (*Arch. int. Pharmacodyn.*, 1951, **87**, 241.) Administration of 500 mg. of procaine amide to 4 anæsthetised dogs with aconitine-induced ventricular tachycardia restored the sinus rhythm, but the action, lasting for a maximum of 10 seconds before remission, was of a much shorter duration than that produced by *p*-oxyphenylethanol methylamine (sympatol) of 47 minutes and by 1 : 3-oxyphenyl-1-oxy-2-ethyl-aminoethane (M.I.36) of 145 minutes duration. Experiments using 6 dogs, with aconitine-induced ventricular tachycardia, injected intravenously with dibenzylmethylamine 5 to 10 mg./kg. of body weight showed that this drug had no effect on the tachycardia in 4 of the animals, gave activity in one, and doubtful results in the other animal. J. R. F.

Procaine Amide in Cardiac Arrhythmias. H. Miller, M. H. Nathanson and G. C. Griffith. (*J. Amer. med. Ass.*, 1951, **146**, 1004.) Of 55 patients who received 250 to 500 mg. of the drug intravenously the cardiac arrhythmia was modified in 46. Of 31 with frequent ventricular systoles the ectopic beats were eliminated in 26, the effect normally occurring in 1 minute after administration. Chronic auricular fibrillation was not modified in 11 cases, in 6 of these ventricular premature beats also present, were eliminated. 3 out of 4 cases of supraventricular tachycardia were restored to sinus rhythm by the drug. The fact that the ventricular beats are more consistently affected suggests the drug has a sympathetic blocking action. No untoward effects occurred following rapid intravenous administration but no definite advantage was observed. It was demonstrated that arrhythmias could be controlled by oral administration. The drug was well tolerated and untoward reactions infrequent and of a mild nature. J. R. F.

BACTERIOLOGY AND CLINICAL TESTS

Hexachlorophen Soap, Bactericidal Properties of. H. C. Jacobsen. (*Pharm. Weekbl.*, 1951, **86**, 733.) Hexachlorophen (2:2′-dihydroxy-3:5:6:3′:5′:6′-hexachlorodiphenylmethane) satisfies all the requirements for use in the preparation of a bactericidal soap. It is stable under these conditions, non-toxic and non-irritating, and is colourless, odourless and non-volatile. At a concentration of 1 : 10^6, with an alkaline reaction, it is fatal to *Staphylococcus aureus* in 1 minute at 37° C. When soap is present, however, the action is much slower, concentrations of up to 0·5 per cent. of hexachlorophen requiring about 5 minutes in presence of appreciable quantities of soap. The lethal concentration of the compound, in water, towards *Bact. coli* is 1 : 330; and towards *Salm. typhi*, 1 : 500. In these latter cases the soap alone destroys the bacteria, and this is also the case for a number of other Gram-negative organisms. The value of this soap for disinfecting the hands thus appears somewhat dubious, since the "resident" flora of the hands, which is difficult to remove by soap, is protected by the soap from the action of the disinfectant. On the other hand, hexachlorphen is absorbed on the skin and may be detected some days after washing with liquid soap containing this compound. Regular use of the soap is therefore more effective and has been shown to reduce the number of bacteria on the skin. In carrying out culture tests with *Staph. aureus*, it is necessary to take into consideration the great sensitivity of this organism to hexachlorophen, and it is best to make an initial dilution of one drop of the solution under test with 0·2 to 0·5 ml. of sterile horse serum, which destroys the activity to a large extent. The bacteriostatic action of hexachlorophen is detectable, in ordinary bouillon, at 1 part in 8×10^6. G. M.

Octyl Cresol—A New Germicide. D. Boocock. (*Mfg. Chem.*, 1951, **22**, 308.) Octyl cresol is a mixture of octylated *meta*- and *para*-cresol with up to 10 per cent. of the *ortho* compound and containing traces of hydrocarbons and other alkyl phenols. It is a viscous transparent fluid with a faint odour, almost insoluble in water and sodium hydroxide solution, but miscible with most organic solvents. The bacteriostatic activity of octyl cresol against *Staph. aureus* and *Bact. coli* was compared with a number of other germicides. Its Rideal Walker coefficients against *Staph. aureus* and *Salmonella typhi* are 480 and 10 respectively. Its toxicity is low. Because of its powerful germicidal and fungicidal action, it has many potential uses, and its applications, in particular its uses as germicidal fogs or thermal aerosols, are described. A. H. B.