ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Pilocereine, Isolation from *Lophocereus schottii*. C. Djerassi, N. Frick and L. E. Geller. (*J. Amer. chem. Soc.*, 1953, **75**, 3632.) An examination of stems of the cactus, *Lophocereus schottii*, showed that the major part of the alkaloids resides in the green epidermis, a minor part in the cortex and practically none in the vascular cylinder and pith. The complete stems were dried, powdered and exhaustively extracted with ethanol. The crude alkaloid fraction (*ca.* 3·7 per cent.) was difficult to crystallise. After chromatography a crystalline optically inactive alkaloid with m.pt. 177° C. was obtained (0·5 per cent. yield based on dry plant). The name "pilocereine" previously given to an alkaloidal mixture was retained for the pure alkaloid of empirical formula $C_{30}H_{42}O_4N_2$. Titration and preparation of a series of crystalline derivatives (hydrochloride, perchlorate, oxalate, methiodide) demonstrated the presence of two basic nitrogen atoms, which is in marked contrast to the presently known cactus alkaloids which possess only one nitrogen atom. Pilocereine hydrochloride exhibits a hypotensive action in cats. Evidence presented indicates that, of the four oxygen atoms, two are present as methoxyl groups, one as hydroxyl group attached to an aromatic ring and the fourth one as an ether. The absence of an NH group, indicated by the various infra-red spectra, was confirmed by the formation of a dimethiodide and the detection of one *N*-methyl group. Pilocereine is thus a ditertiary base with one of the nitrogen atoms forming part of a heterocyclic ring, while the other tertiary amine bearing the *N*-methyl group may conceivably be part of a tetrahydro*iso*quinoline nucleus.

A. H. B.

ANALYTICAL

Belladonna Alkaloids, Spectrophotometric Method of Assay. L. Worrell and R. E. Booth. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 361.) Tablets of atropine sulphate may be assayed as follows. Dissolve a tablet in a mixture of 5 ml. of water and 1 ml. of solution of ammonia and extract the alkaloid by shaking with benzene. Concentrate the benzene solution on a water bath and continue heating until no more ammonia is evolved. Add 2 ml. of a 20 per cent. solution of napthenic acid in benzene, 2 ml. of a 5 per cent. aqueous solution of copper sulphate and sufficient benzene to produce 50 ml., allow to stand for 18 hours and measure the absorption in the benzene layer at 700 mμ. The quantity of atropine sulphate is calculated from a standard curve. The green coloured complex is very stable and has a maximum absorption slightly below 700 mμ, but it is an advantage to use this wavelength since the error due to the light absorption of chlorophyll in belladonna extracts is minimised. In assaying extracts of belladonna leaves, the colour may be measured against a blank prepared from the same extract, water being substituted for the copper sulphate solution used in developing the green colour, so as to compensate for the absorption due to chlorophyll. Experiments showed that maceration

overnight in a medium containing ethanol, as directed by the U.S. Pharmacopeia, is necessary for complete extraction of the alkaloids. A minimum of 3 heating periods, each of 15 minutes on a water bath was necessary for the removal of volatile bases which interfere in the assay. G. B.

Bromide, Determination of, with Nitroferroin. E. Rancke-Madsen. (*Acta chem. scand.*, 1953, **7**, 741.) The use of a redox indicator in the determination of bromide has been investigated. The indicator used must have a transition interval somewhat larger than that of starch-iodine and should be about 1·30 to 1·35 volt (somewhat dependent on the concentration of bromide); nitroferroin was found to be satisfactory although the transition interval was somewhat dependent on *p*H. In the titration 5 ml. of 4 M nitric acid, 2 drops of 0·025 M nitroferroin and 0·10 ml. of saturated bromine water are added to 20 ml. of water; a few drops of 0·1 M potassium bromide are added, and then 0·1 M silver nitrate is added drop by drop, until a transition takes place from red to nearly colourless (a faint violet). The sample containing bromide is now added and the titration is continued with 0·1 M silver nitrate until the red precipitate changes to yellow. Results from a series of experiments show that the method yields values that are about 0·1 per cent. too high. In a potentiometric titration when approaching the equivalence-point the potential became constant rather quickly until just before the equivalence-point, when it became oscillatory and unstable, probably because of the evaporation of the free bromine due to the constant stirring. R. E. S.

Carbohydrates, Colorimetric Reagent for. E. Lunt and D. Sutcliffe. (*Biochem. J.*, 1953, **55**, 122.) The use of a new colorimetric reagent, resorcinol-4:6-disulphonic acid, for the determination of hexoses and their polysaccharides, is described. The disulphonic acid was as sensitive a colorimetric reagent as the parent phenol, giving an intense golden-brown colour with carbohydrates. Under the conditions of the reaction all the isomeric hexoses tested gave a peak at 490 mμ, but the sensitivity varied with the individual hexose. For any series of determinations, therefore, the appropriate hexose must be used as standard when calibrating the reagent. The reagent has been used chiefly for determining glucose and fructose and their polysaccharides. Details of the method are given. To 5 ml. samples containing up to 250 μg. of carbohydrate in stoppered tubes, 1 ml. of 0·5 per cent. aqueous solution of resorcinol-4:6-disulphonic acid (as the Ca salt) is added, followed by sulphuric acid (10 ml.) with shaking; after standing for 1 hour in the dark to cool, the optical densities are read at 490 mμ. From 10 to 250 μg. of glucose in 5 ml. may be determined with errors of less than 1 per cent. Inulin and starch give 100 per cent. and dextrose 95 per cent. of the colour intensity given by their constituent monosaccharides. R. E. S.

Ergot Alkaloids, Identification and Separation of. H. Hellberg. (*Farm. Revy*, 1953, **52**, 535.) The partition coefficients of ergocristine, ergokryptine, ergocornine, ergosine and ergosinine between ether and benzene and 0·2 M phosphate buffer solution were determined. It was shown to be possible to separate ergotamine and ergotoxine by systematic extraction in 10 separating funnels using benzene as the stationary phase and 0·2 M phosphate buffer *p*H 3·65, as the mobile phase. Some other separations were possible, but it was not feasible to extend the same method to the separation of any arbitrary combination of ergot alkaloids. Ergometrine may be separated from a mixture by systematic extraction (at most 10 separating funnels are needed using ether and water at *p*H 8). Ergometrinine may be removed with ether and water at

*p*H 6·5 and ergosine and ergotamine with benzene and water at *p*H 3·8. The *p*H value at which an alkaloid is equally partitioned between benzene and phosphate buffer is a characteristic of the group to which it belongs. For the ergotoxine group the *p*H is 2·7 to 3·2 and for ergotamine, 4·3 to 5·5. When Craig's counter-current distribution technique was applied using benzene and 0·2 M phosphate buffer, ergocornine was partially separated from other ergotoxine alkaloids by a 30-step process, but it appeared that about 200 steps would be needed for complete separation. It was calculated that about 1000 steps would be required for the separation of ergokryptine from ergocrystine or of ergotamine from ergosine, but this number might possibly be reduced by the use of other pairs of solvents. G. B.

Essential Oils, Chromatostrips for Identification of. J. M. Miller and J. G. Kirchner. (*Analyt. Chem.*, 1953, **25**, 1107.) It was found that the chromatostrips (adsorbent-coated glass strips) could be used for the identification of compounds not only by direct comparison with known R_F values, but also from reactions performed directly on the chromatogram or from chromatography of the results of microscale reactions in test tubes. An unknown compound cannot be identified with certainty by comparison with the R_F values of known compounds although many of the compounds can be eliminated by comparison of their R_F values in a number of solvents. Hydrocarbons have an appreciable R_F value with hexane as solvent, whereas other compounds have a zero R_F value thus affording a means of identification of hydrocarbons. A number of reactions are reported whereby the pure compound is adsorbed on a chromatostrip, covered with the reagent, and chromatographed in an appropriate solvent; alternatively the reagent and compound are mixed and then applied directly to the chromatostrip. In the case of citral, in addition to the R_F values in numerous solvents, it can be oxidised to geranic acid (with 30 per cent. hydrogen peroxide and exposed to ultra-violet light) and reduced to geraniol; the R_F values of the two reaction products establish with a fair degree of certainty the identity of the original compound. R. E. S.

Mercuric Ions, Spectrophotometric Determination in Distilled Water. S. Ašperger, I. Murati and I. O. Čupahin. (*Acta pharm. Jugoslav.*, 1953, **3**, 20.) The reaction between ferrocyanide and nitrosobenzene forming a violet-coloured complex of formula $Fe(CN)_5.C_6H_5NO^{---}$, is catalysed by mercuric ions. The quantity of mercuric ions may be calculated from measurements at the absorption maximum, 528 mμ after allowing the reaction to proceed for a fixed time of 30 minutes, at 20° C. The *p*H should be adjusted to 3·5, at which the maximum reaction velocity is developed. The catalytic action is specific for mercuric ions, which can be determined in concentrations as low as 10^{-7}M. The accuracy of the method is about 20 per cent. at 10^{-7} to 10^{-6}M, and 5 per cent. at 10^{-6} to 10^{-5}M. G. B.

***iso*Nicotinyl Hydrazide, Microcrystalline Identification Test for.** E. Neuzil. (*Bull. Soc. Pharm. Bordeaux*, 1953, **91**, 122.) Isoniazid does not form hydrazones with sugars, but a white precipitate of microcrystalline needles in characteristic masses is formed on mixing 1 per cent. aqueous solutions of isoniazid and alloxan. The reaction can also be applied to solutions containing as little as 0·125 per cent. of isoniazid. Nicotinyl hydrazide gives a white precipitate of a different microcrystalline form. The white colour of these precipitates suggests that they are not hydrazones containing the chromophore group, $:C = N -$;

it is suggested that the product formed from isoniazid is *N-iso*nicotinyl-*N'*-(5-alloxanyl)hydrazine, in agreement with the results of elementary analysis. Characteristic yellow, rather irregular quadrangular plates are obtained on heating a solution of isoniazid with a saturated solution of isatin. Reaction of isoniazid with ninhydrin yields a yellow amorphous precipitate which crystallises from ethanol. With sodium β-naphthoquinone-4-sulphonate, isoniazid gives a transient red colour, followed by a yellow precipitate, which is not sufficiently characteristic for an identification test. Under the same conditions, nicotinyl hydrazide yields a precipitate of small needles. G. B.

Phosphoric Acid and Phosphates, Determination of. E. Rancke-Madsen and T. Kjærgård. (*Acta chem. scand.*, 1953, **7**, 735.) A method is proposed in which cerous nitrate is added to the sample, causing the precipitation of cerous phosphate. The precipitation of cerous phosphate at the first equivalence-point of the phosphoric acid is complete and the liberated hydrogen ions can be titrated to the *p*H of the first equivalence-point. A mixed indicator consisting of 0·02 per cent. methyl orange and 0·1 per cent. bromocresol green is added to the sample and titration to the first equivalence-point is carried out with 0·1 N hydrochloric acid; a small excess of cerous nitrate solution is then added, the resulting liquid being titrated with 0·1 N sodium hydroxide until the indicator assumes the same colour as at the first equivalence-point. Analysis of a solution of phosphate containing 1·140 per cent. of PO_4 gave results ranging from 1·136 to 1·147 per cent. using the method described. R. E. S.

Thiouracil Derivatives, Bromometric Determination of. H. Wojahn and E. Wempe. (*Pharm. Zentralh.*, 1953, **92**, 124.) In the United States Pharmacopeia process for the assay of propylthiouracil tablets, using potassium bromate, it is assumed that 1 molecule of the compound uses 10 equivalents of bromine. Experiments with pure compounds show that the figures obtained are much too high, though to a varying extent. A method which gives reliable results, and may be applied to tablets of methyl- and propylthiouracil, is as follows. About 50 mg. of tablet mass is treated in an iodine value flask with 5 ml. of 10 per cent. sodium hydroxide solution, with occasional shaking. After 10 minutes 1 g. of potassium bromide, 50 ml. of 0·1N potassium bromate and 10 ml. of 25 per cent. hydrochloric acid are added. After 60 minutes, 25 ml. of 0·1 N sodium arsenite is added, and the solution is titrated with 0·1 N potassium bromate in presence of *p*-ethoxychrysoidine hydrochloride until it is decolorised. G. M.

Trimethylphenylammonium Iodide, as Quantitative Precipitant for Bismuth. T. S. Burkhalter and J. F. Solarek. (*Analyt. Chem.*, 1953, **25**, 1125.) Trimethylphenylammonium iodide precipitates bismuth quantitatively and the precipitate can be readily filtered and dried at 120° C.; Group II and the noble metals interfere, but these can be removed by standard separation procedures. The use of the reagent depends upon the formation of an insoluble salt of the quaternary cation and a complex metal halide anion; the bismuth compound formed is $[(CH_3)_3(C_6H_5)N]^+BiI_4^-$ and contains 24·5 per cent. of bismuth. In the procedure given the tetrahydrate is isolated and weighed. The precipitation is made from a cool solution approximately 4 N in sulphuric acid from which halide ions have been removed by fuming with sulphuric acid. Bismuth ion concentrations between 0·001 and 0·1 M give satisfactory results. The results obtained in 18 determinations on a single sample which contained 0·04450 g. of bismuth, gave an average of 0·04445 g. with an average deviation from the mean of 0·00037 g. and a relative error of 0·11 per cent. R. E. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Sugars, Ion Exchange Resins in Paper Chromatography of. L. I. Woolf. (*Nature, Lond.*, 1953, **171**, 841.) The use of bio-deminrolit or bio-deminrolit F, which are mixtures of sulphonic acid type resins with strongly basic resins of the quaternary ammonium hydroxide type, for the preliminary removal of anions in dilute sugar solutions prior to paper chromatography, leads to the removal of all fructose, glucose, galactose, mannose and fucose. With amberlite IRA400 (OH form) 2 mg. of glucose was completely removed and not more than 10 per cent. was recoverable by immediate elution with dilute hydrochloric acid. Glucosamine, galactosamine and *N*-acetylglucosamine were also completely removed by the resin but were quantitatively eluted on subsequent treatment with dilute hydrochloric acid. Bio-deminrolit saturated with carbon dioxide does not remove fructose or glucose from dilute solution after 9 days contact at 0° C. J. B. S.

ORGANIC CHEMISTRY

4 : 4′-Stilbenediol, Synthesis of some Derivatives of. P. M. Bhargava and S. Husain Zaheer. (*Nature, Lond.*, 1953, **171**, 746.) A direct synthesis of *trans*-α-methyl-4 : 4′-stilbenediol (and some of its dialkyl ethers) from phenol and chloroacetone has been accomplished. Condensation of these two substances between −10° C. and 0° C. in the presence of concentrated sulphuric acid gives a nearly 75 per cent. yield of *trans*-α-methyl-4 : 4′-stilbenediol. Characteristics for a number of derivatives of this substance are also described. The reaction is a general one and dimethyl and diethyl ethers of *trans*-α-methyl-4 : 4′-stilbenediol have also been obtained in good yields by condensing chloroacetone with anisole and phenetole respectively, under similar conditions. It is thought that the *cis* compounds are formed first and are probably isomerised to the corresponding *trans* compounds on heating. J. B. S.

Sucrose, Solubility of, in Hydroethanolic Solutions. L. A. Reber. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 192.) The solubility of sucrose was determined in mixtures of water and ethanol at 25° C. The solvent was shaken with an excess of solute for 48 hours in glass-stoppered bottles sealed with paraffin in a bath maintained at 25° ± 0·01° C. and the quantity of sucrose in solution determined by evaporating a sample of solution and drying to constant weight *in vacuo* at 60° C. The specific gravities of the saturated solutions were measured and the results expressed as solubilities w/w and w/v of solution and solvent. G. B.

Thioureas, Paper Chromatography of. A. Kjaer and K. Rubinstein. (*Nature, Lond.*, 1953, **171**, 840.) A technique is described for the paper chromatography of a wide range of *N*-substituted thioureas, using chloroform-water as the mobile phase. After 16 to 18 hours for equilibration of the sheets, the solvent is allowed to rise approximately 23 cm. in the course of 110 to 120 minutes; these conditions are vital to success. The air dried papers are subsequently sprayed with Grote's reagent and then heated, when thiourea spots become deep blue. Certain variations between consecutive runs were observed even under carefully controlled conditions, and were overcome by using *N*-phenylthiourea as a reference substance in each run. The term R_{PA}-value is introduced to indicate the ratio between the distances travelled by each

component and *N*-phenylthiourea. R_{Ph}-values were reproducible within ± 0·01. Typical R_{Ph} values are given for a number of *N*-substituted thioureas.

J. B. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Benzylpenicillin, Carboxy Derivatives of. D. A. Johnson. (*J. Amer. chem. Soc.*, 1953, **75**, 3636.) A solution of triethylammonium benzylpenicillinate in methylene chloride was treated at 0° C. with ethyl chloroformate to give a mixed anhydride which was not, in general, isolated. After 30 minutes, the appropriate reagent (amine, alcohol, etc.) was added and the solution was allowed to warm to room temperature to complete the reaction. For the preparation of esters, an equivalent of triethylamine was added with the alcohol. By this convenient and efficient procedure, carboxy derivatives of penicillin of type

was prepared where D = $-NH_2$, $-HNR$, $-NR_2$, $-OR$, $-SR$.

A. H. B.

Ethyl Biscoumacetate, a Metabolic Product of, from Human Urine. J. J. Burns, S. Wexler and B. B. Brodie. (*J. Amer. chem. Soc.*, 1953, **75**, 2345.) Ethyl biscoumacetate (3 : 3′-carboxymethylenebis-(4-hydroxycoumarin) ethyl ester) had previously been shown to be almost completely metabolised in the body, and the present communication reports the isolation and characterisation of a metabolite from urine of patients receiving the drug. The metabolite was isolated by countercurrent distribution. From a consideration of ultra-violet absorption spectra, potentiometric titration curves and degradation studies it is indicated that the metabolite is derived from ethyl biscoumacetate by the introduction of a hydroxyl group into one of the benzene rings *meta* to the oxygen of the lactone bridge. Pharmacological studies indicate that, following the administration of the drug to man, 10 to 15 per cent. is excreted in the urine as the hydroxy derivative, and that the metabolite has no anticoagulant activity A. H. B.

Histamine-Metabolising Enzymes in Intact Animals. R. W. Schayer. (*J. biol. Chem.*, 1953, **203**, 787.) A study has been made of the metabolism of histamine labelled with ^{14}C in the rat. Paper chromatograms of the urine were developed in butanol, ethanol and ammonium hydroxide and the patterns of histamine metabolites measured from the radioactivity. *iso*Nicotinylhydrazine, and picolinic acid hydrazide, were used as diamine oxidase inhibitors *in vitro*, and diamine oxidase shown to be a major histamine-metabolizing enzyme in the rat. 1-*iso*Nicotinyl-2-*iso*propylhydrazine, an inhibitor of monoamine oxidase, altered the metabolism of histamine in the mouse, but the major histamine-metabolizing enzyme system remains unidentified. Studies of histamine-metabolizing activity in rat and mouse organs suggested that the site of greatest histamine metabolizing activity in rats is in the intestine, while in mice it is in the liver. G. F. S.

BIOCHEMICAL ANALYSIS

Amino-acids. A Chromatographic Colour Reagent for. G. Curzon and J. Giltrow. (*Nature, Lond.*, 1953, **172**, 356.) Vanillin followed by ethanolic potash was found to be a chromatographic reagent of considerable specificity

in the amino-acid field. One-way chromatograms were run on 20 cm. sheets, dried, dipped in a freshly prepared 2 per cent. solution of vanillin in *n*-propanol and heated at 110° C. for 10 minutes. Faint yellow colours were shown by many amino-acids at this stage. Ornithine gave a strong, and lysine a weak green-yellow fluorescence in the ultra-violet. The chromatograms were then dipped in 1 per cent. ethanolic potash and heated again at 110° C. for 10 minutes. Ornithine gave a salmon spot about 30 seconds after commencing heating; the spot quickly faded to yellow-brown on further heating. Proline, hydroxyproline, α-, β- and γ-pipecolinic acids, baikiain and sarcosine showed up as red spots after about 6 hours standing at room temperature and reached maximum intensity after standing overnight. β- and γ-pipecolinic acids gave comparatively faint colours. The complete range of commercially obtainable α-amino-acids gave either no colour or indefinite faint browns, with the exception of glycine, which gave a quite strong greenish-brown. The results were satisfactory after running in butanol-acetic acid, collidine-lutidine or acetone-urea-water solvent. A. H. B.

Barbiturates in Urine and Stomach Contents, Detection of. R. Marshall. (*Brit. med. J.*, 1953, **2**, 379.) The following method is useful in confirming suspected cases of barbiturate poisoning before treatment is begun. Quantities of 1 to 2 mg./100 ml. of material can be detected in less than 15 minutes. Acidify 50 ml. with 4 ml. of dilute sulphuric acid, and shake with 20 ml. of chloroform. Dry the chloroform solution with anhydrous sodium sulphate, shake with activated charcoal, filter and evaporate to dryness in a beaker on a water bath. Rub the residue off the vessel using a small piece of filter paper fastened over the rounded end of a glass rod so that most of the residue is transferred to an area about 2 mm. in diameter in the centre of the paper. Flatten out the paper and add 1 drop of a 1 per cent. solution of cobalt nitrate to the residue. Dry by warming over a flame and expose to ammonia vapour. The presence of barbiturates is indicated by a reddish-violet colour, best seen by transmitted light, in the residue. Long-acting barbituric acid derivatives are readily detected in the urine, but short-acting compounds which are for the most part metabolised, can be detected in the stomach contents. G. B.

Hydrogen Sulphide, Radioactive, Quantitative Paper Chromatography of Traces of Metal with the Aid of. P. C. van Erkelens. (*Nature, Lond.*, 1953 **172**, 357.) The quantitative reaction with radioactive hydrogen sulphide ($H_2{}^{35}S$) and the oxidation of the sulphides formed a difficulty; the subsequent treatment of the dried papers after chromatography to solve this difficulty is described. The application of the method to the determination of small amounts of metals in biological material is outlined. Advantages claimed for the method are: once the biological material is broken down, any metal can be detected and no concentration with oxine or similar chemical is needed; the sensitivity is very great (comparable with spectrography); the method is not costly and can be carried out on a large scale and is partly automatic.

A. H. B.

5-Hydroxytryptamine, Detection of, by Paper Chromatography. D. M. Shepherd, G. B. West and V. Erspamer. (*Nature, Lond.*, 1953, **172**, 357.) Chromatographic experiments were carried out with synthetic 5-hydroxytryptamine creatinine phosphate in order to study its sensitivity and the specificity of the golden-yellow fluorescence acquired by enterochromaffin cells after fixation in formaldehyde. A butanol-acetic acid-water solvent and a developer

of 9 parts of a solution of potassium dichromate (0·1 per cent.) and 1 part of formaldehyde solution (37 to 41 per cent.) was used. After spraying and heating at 100 to 110° C. for 5 minutes, the paper was viewed under ultra-violet light. Under these conditions, 5-hydroxytryptamine (R_F value 0·38) produced a golden-yellow fluorescence immediately after the heating and this persisted for days; quantities as small as 0·2 μg. of base could be detected. The reactions of other substances such as adrenaline, noradrenaline, histamine, histidine with the reagent are described. A. H. B.

Iodine in Blood Serum, Determination of. H. F. W. Kirkpatrick. (*Analyst*, 1953, **78**, 348.) A procedure was chosen in which organic matter is destroyed, and iodine in all forms is oxidised to iodate by digestion with a mixture of chromic and sulphuric acids; the iodine is volatilised by reducing the digest at boiling point with phosphorous acid, is trapped in an absorbing solution and is finally determined colorimetrically by the use of the catalytic action of iodine on the reduction of ceric sulphate by arsenious acid. Variations in the digestion and absorption, in the reagents, in the still, and in the final colorimetric determination are considered and working details of the chosen procedure are given. The over-all mean recovery of iodine in 40 duplicate determinations was 100 per cent.; determination of the protein-bound iodine in the sera of 40 normal healthy individuals gave values from 3·9 to 7·8 μg. per 100 ml. with a mean value of 5·9 and a standard deviation of $\pm$ 0·85. The suggested normal range is 3·5 to 8·5 μg. per 100 ml. R. E. S.

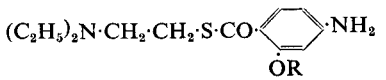
Potassium in Serum, Microdetermination of. S. Baar. (*Analyst*, 1953, **78**, 353.) A method is given for the determination of potassium in serum by precipitation with sodium cobaltinitrite to give potassium sodium cobaltinitrite without prior de-proteinisation. The precipitated complex salt is dissolved in sulphamic acid, which destroys the excess of nitrous acid, ammonia and 8-hydroxyquinoline are added and the complex of cobalt is formed; after extraction into chloroform, the absorption maximum of the solution at 403 mμ is used for a spectrophotometric determination. The coloured complex was stable after standing for 2 hours and reproducibility was good; 12 replicate determinations on a single sample of serum showed a range of 17·50 to 17·85 mg. per 100 ml, with a mean of 17·70 mg. Recovery experiments and comparison of a series of replicate determinations with colorimetric methods showed a maximum deviation of 1 per cent, from expected values. R. E. S.

Sodium in Urine, Determination of. M. O'Sullivan. (*J. Lab. clin. Med.*, 1953, **41**, 959.) A method is given in which the sodium is precipitated with zinc uranyl acetate, the precipitate is centrifuged and the volume measured in a specially constructed centrifuge tube. The sodium content of the urine is obtained by reference to a calibration curve prepared from urine samples containing known added amounts of sodium chloride. A comparison of the results obtained with values obtained by the flame photometer showed satisfactory agreement. R. E. S.

CHEMOTHERAPY

Local Anæsthetics, New Series of. F. P. Luduena, R. O. Clinton and S. C. Laskowski. (*Science*, 1953, **118**, 138.) Thiolbenzoates were prepared from 2-alkoxy-4-nitrobenzoic acids and 2-diethylaminoethane thiol, and tests for local anæsthetic activity were made by the intracutaneous wheal method in

guinea-pigs and by intraspinal injection, corneal instillation and urethral injection in rabbits. Activity ratios were calculated in relation to procaine for the intracutaneous and intraspinal tests and relative to cocaine for topical anæsthesia. Toxicity was determined in comparison with both procaine and cocaine.



The compound R = *n*-hexyl was the most active and also the most toxic, and R = *n*-propyl showed the greatest ratio of activity to irritancy as measured by the trypan blue test. Irritancy in this series of compounds was low relative to anæsthetic activity, but increased with the length of the alkoxy chain. Several compounds, including R = *n*-propyl, *n*-butyl and *n*-hexyl showed greater activity than tetracaine and butyl aminobenzoate, together with a greater margin of safety as regards irritancy and systemic toxicity. G. B.

***iso*Nicotinyl Hydrazide, Substitution Products of.** F. H. McMillan, F. Leonard, R. I. Meltzer and J. A. King. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 457.) Hydrazones were prepared by heating isoniazid with 2 or more moles of aldehyde or ketone on a water bath for 1 to 2 hours, removing the excess *in vacuo* and recrystallising the residue. N^2-alkyl derivatives of isoniazid were obtained by catalytic hydrogenation of the corresponding hydrazones. Compounds were chosen to demonstrate the effect of changes in chain length and branching and the introduction of methoxyl and carbethoxyl groups. Antitubercular activity was determined *in vitro* against *Mycobacterium tuberculosis* H37Rv and D4M3, and in mice against virulent bovine D4M3 strain. Most of the derivatives had about the same activity as isoniazid, but information about the activity conferred by the azomethine group of the hydrazones and the various substituents is awaited as a result of further study. A number of modifications of the isoniazid molecule were examined. *iso*Nicotinamide and its *iso*propyl derivative were inactive, and quaternisation of isoniazid at the pyridyl nitrogen, or introduction of two methoxyl groups in the ring produced inactive substances. Acetylation to N^1-*iso*nicotinyl-N^2-acetylhydrazine considerably reduced the antitubercular activity, which was destroyed completely by dehydration to the oxadiazole. The corresponding thiadiazole was also inactive. G. B.

PHARMACY

GALENICAL PHARMACY

Alkaloidal Drugs, Wetting Agents in Extraction of. W. J. Butler and G. A. Wiese. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 382.) Belladonna leaves, hyoscyamus, cinchona and ipecacuanha were extracted by percolation as in the preparation of liquid extracts, but a quantity of non-ionic detergent, equivalent to 20 mg./100 ml. of the final liquid extract was added to the menstruum used in the preliminary moistening. Sorbitan monolaurate, polyethylene glycol 400 monolaurate, propylene glycol monolaurate, polyethylene glycol 600 monolaurate, polyethylene glycol 400 dilaurate, glycerol sorbitol laurate and polyoxyethylene glycol sorbitol monolaurate were investigated. All increased the extraction of alkaloid, but it was not possible to decide from these results which agent would be best for a given drug. Possibly the wetting agent acts by increasing the penetration of the cells by the solvent.

The alternative explanation that the increased yield is due to increased solubility in the presence of the wetting agent is ruled out by the small concentration used in these experiments. G. B.

NOTES AND FORMULÆ

Dimethyltubocurarine Chloride (Mecostrin Chloride). (*New and Nonofficial Remedies*; *J. Amer. med. Ass.*, 1953, **152**, 920.) Dimethyltubocurarine chloride is the dimethyl ether of *d*-tubocurarine chloride, and occurs as a white, odourless crystalline powder which decomposes with evolution of gas when heated to 236° C. It is soluble in water and sodium hydroxide solution, sparingly soluble in ethanol and dilute hydrochloric acid, very slightly soluble in chloroform, and practically insoluble in benzene and ether. An aqueous solution yields a pink precipitate with ammonium reineckate and a yellow precipitate with trinitrophenol. When warmed in a water bath with Folin-Ciocalteu reagent and sodium carbonate, the resulting solution is colourless or very faintly blue (distinction from tubocurarine chloride). A 0·5 per cent. solution in water has a specific rotation, at 25° C., of 185° to 195°, and a 0·005 per cent. solution in water containing a few drops of hydrochloric acid exhibits an absorption maximum at about 2800 Å ($E^{1\%}_{1\,cm.}$, about 89). Dimethyltubocurarine chloride loses not more than 14·0 per cent. of its weight when dried in a vacuum at 60° C. over phosphorus pentoxide for 4 hours. It contains 98·0 to 102·0 per cent. of dimethyltubocurarine chloride when assayed spectrophotometrically by measuring the absorption at 2800 Å of a 0·005 per cent. solution in water acidified with hydrochloric acid. It also contains 3·60 to 3·90 per cent. of nitrogen, equivalent to 93·0 to 100·8 per cent. of dimethyltubocurarine chloride. G. R. K.

Edrophonium Chloride (Tensilon Chloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **152**, 1714.) Edrophonium chloride is dimethylethyl (3-hydroxyphenyl) ammonium chloride and occurs as a white, odourless, crystalline powder, m.pt. 163° to 169° C. (with decomposition), practically insoluble in ether, freely soluble in ethanol, and very soluble in water (a 1 per cent. solution has *p*H 4·0 to 5·0). It yields a yellow picrate, m.pt. 161° to 163° C. A 0·0025 per cent. solution in water exhibits ultra-violet absorption maxima at about 217 mμ ($E^{1\%}_{1\,cm.}$ about 291) and 273 mμ ($E^{1\%}_{1\,cm.}$ about 107), and a minimum at about 240 mμ; the ratio of the optical densities at 217 and 273 mμ is 2·6 to 2·8. It loses not more than 0·5 per cent. of its weight when dried at 105° C. for 4 hours, and yields not more than 0·5 per cent. of sulphated ash. When assayed spectrophotometrically by measuring the absorption at 273 mμ of a 0·0025 per cent. solution in water, using water as a blank, it contains 95·0 to 105·0 per cent. of edrophonium chloride. It is also assayed by titration with perchloric acid in acetic acid, using crystal violet as indicator, and contains 98·5 to 101·5 per cent. of edrophonium chloride. It is used as an antidote against the peripheral action of curariform agents and as a diagnostic agent in myasthenia gravis. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

Antihistamines and Apomorphine-induced Vomiting. E. M. Boyd and C. E. Boyd. (*Canad. J. med. Sci.*, 1953, **31**, 320.) Derivatives of antihistamine drugs, diphenhydramine-8-chlorotheophyllinate (dimenhydramine) and promethazine-8-chlorotheophyllinate (avomine) are effective in motion sickness and in

preventing vomiting due to radiation, pregnancy, labyrinthine fenestration operations and morphine. This paper reports the effects of the antihistamines diphenhydramine hydrochloride, diphenhydramine-8-chlorotheophyllinate and diphenhydramine-8-bromotheophyllinate in preventing apomorphine-induced vomiting in cats and dogs. The drugs were given orally and apomorphine injected intravenously in cats or intramuscularly in dogs. Dogs were preferred because of their greater sensitivity to apomorphine, the high doses of apomorphine required in cats producing considerable nervous excitation. Three other antihistamines, promethazine hydrochloride, methapyrilene hydrochloride and methapyrilene-8-chlorotheophyllinate were also tested. The antihistamines were given at periods of ½ to 4 hours before the apomorphine. The results showed that none of these antihistamines at therapeutic dose levels prevented vomiting in cats or dogs after apomorphine. Derivatives of diphenhydramine, and promethazine hydrochloride, partially inhibited apomorphine-induced vomiting at toxic or near toxic dose levels. G. F. S.

Aurothioglycanide in the Treatment of Rheumatoid Arthritis. R. C. Batterman. (*J. Amer. med. Ass.*, 1953, **152**, 1013.) Aurothioglycanide (lauron) contains 54 per cent. of gold and is administered by intramuscular injection in the form of a suspension in sesame oil (50 or 150 mg./ml.). 69 patients were treated, the majority beginning with 50 mg. intramuscularly at weekly intervals. This dose was given for at least 3 weekly injections, and then, if no toxicity or improvement occurred, the dosage was increased to 100 mg. weekly (or 50 mg. if the initial dose was 25 mg.). If no improvement or toxicity occurred at the end of 8 or 10 weeks the dose was again increased, and the status of the patient reviewed regularly at 8 to 10 week intervals. The maximum dose never exceeded 150 mg. weekly, and for the majority of patients 100 mg. was the maximum. The injections were discontinued if no improvement occurred within 6 months; where improvement occurred the maximum weekly dose was continued until the improvement was well established and optimum for the patient's stage of arthritis; this dose was then employed as the maintenance dose, given at 2 to 4-week intervals. The total duration of therapy varied between 5 weeks and 3 years; 12 patients were treated without interruption for over 1 year. 37·7 per cent. (26 patients) had significant improvement irrespective of the stage of the disease at the termination of initial treatment, and 56 per cent. of the patients with early (stage 1 or 2) arthritis showed complete remission or major improvement. This initial response was further enhanced by maintenance therapy, since 15 out of 17 patients so treated had a satisfactory end-result. Maintenance after initial improvement is the recommended method. In the incidence, type, severity and duration of toxic manifestations aurothioglycanide is less toxic than other available gold preparations. S. L. W.

Dextromethorphan Hydrobromide as a Cough Suppressant. L. J. Cass and W. S. Frederik. (*New Engl. J. Med.*, 1953, **249**, 132.) A test was conducted on 65 patients suffering from severe chronic cough. 3 batches of tablets, identical in appearance and taste, and containing respectively dextromethorphan hydrobromide 4 mg., codeine sulphate 17 mg., and a placebo were used. They were supplied under code numbers and the observations were made by people who were not members of the research group. The drugs were given 4 times a day at 8 a.m., 12 a.m., 4 p.m. and 8 p.m. for a period of 45 days. The medicaments were given in a random sequence. The total number of observations recorded was over 11,000, and the cough-suppressing activity of the drugs was

recorded by means of a numerical scale. The results showed that the cough-suppressing effectiveness of the 4 mg. of dextromethorphan hydrobromide was approximately half that of 17 mg. of codeine sulphate; it is anticipated that 9 to 10 mg. of dextromethorphan would be required to approximate the cough-suppressing activity of 17 mg. of codeine (further studies are under way). The incidence of side effects (constipation, drowsiness, nausea) was of the same low order for both dextromethorphan and the placebo; that for codeine was distinctly higher. S. L. W.

Diamox in the Treatment of Congestive Heart Failure. H. Belsky. (*New Engl. J. Med.*, 1953, **249**, 140.) 13 cases of congestive heart failure were treated with the oral diuretic 2-acetylamino-1 : 3 : 4-thiadiazole-5-sulphonamide (diamox) in place of mercurial diuretics. Its action is that of a specific inhibitor of carbonic anhydrase, resulting in the renal loss of bicarbonate, sodium, potassium and water. 11 of the 13 patients were kept free from œdema, with more or less constant weight, on a dose of 0·5 g. daily by mouth, together with a low-salt diet and digoxin. No serious toxic effects were noted with this dosage, but with a daily dose of 1 g. or more toxic effects were encountered, namely, a sense of numbness and pins and needles in the face and extremities, and moderate to extreme drowsiness. 8 of the patients were able to dispense with mercurial injections completely. In 2 patients with severe cardiac disease the only beneficial effect was the less frequent need for mercurial injections; it was also noted that the drug, either alone or in combination with mercurial injections, had little diuretic effect in the presence of anasarca and renal decompensation. S. L. W.

Ethanol Oxidation by the Liver, Effect of Diet on Rate of. E. Kerner and W. W. Westerfeld. (*Proc. Soc. exp. Biol. N.Y.*, 1953, **83**, 530.) The administration of relatively large amounts of ethanol to animals may reduce the amount of food eaten and the consumption of essential nutrients may be reduced to a deficiency level. Studies are reported on the influence of protein depletion or inanition on the oxidation of ethanol by the liver. The rate of ethanol oxidation by liver homogenates was studied manometrically, as was the effect of various diets on the ability of liver homogenates to oxidize ethanol. Weanling rat liver homogenates had about one half the activity of the adult rat. A protein free diet removed about 85 per cent. of this enzymatic activity from the liver, complete starvation for 7 days reduced this activity by about 50 per cent. and a 50 per cent. food restriction for 2 weeks gave less than a 20 per cent. decrease in the rate of ethanol oxidation. G. F. S.

Hexamethonium Chloride; Oral Use in Hypertension. J. H. Moyer, S. I. Miller and R. V. Ford. (*J. Amer. med. Ass.*, 1953, **152**, 1121.) Of 120 patients with hypertension treated with hexamethonium by mouth all but 17 responded with a significant reduction in blood pressure, and this reduction was maintained for follow-up periods of from 3 to 18 months. The initial dose was usually 250 mg. 4 times daily by mouth, the drug being given at meal times and at bedtime. This dose was gradually increased (by 500 mg. a day) until an adequate blood pressure response was obtained or until side reactions forced discontinuance of the use of the drug; 1 week was allowed between changes in the dose schedule and the schedule was adjusted to suit each patient's requirements. The amount of drug required was only slightly greater in the patients with diastolic blood pressures above 140 mm. Hg. than in those with pressures

less than 120 mm. but more than 100 mm. Hg. After the dose schedules were established the outstanding problems on therapy were those related to decreased intestinal peristalsis and periodic variability in the hypotensive response. The initial side-effects were seldom of serious concern and decreased as treatment continued. S. L. W.

Mercurial Diuretic, a New Oral, 3-Chloromercuri-2-methoxypropylurea, Clinical Experience with. B. M. Kaplan, I. H. Zitman, S. D. Solarz, G. Miller, J. S. Mehlman and L. G. Kaplan. (*J. Lab. clin. Med.*, 1953, **42**, 269.) 3-chloromercuri-2-methoxypropylurea (neohydrin) is a new mercurial diuretic effective by the oral route, an advantage to the ambulatory cardiac patient. The compound has been studied in fifteen ambulatory cardiac patients. It was well tolerated and caused no untoward effects. In ten patients it was possible to completely replace parenteral therapy by oral administration and partial replacement was possible in the other five. G. F. S.

Phenyl Alkane *p*-ω-Bis (Trialkylammonium) Compounds, Pharmacological Actions. R. Wien and D. F. J. Mason. (*Brit. J. Pharmacol.*, 1953, **8**, 306.) A study of an aromatic series of bis-quaternary compounds has shown several to have outstanding pharmacological actions. One of these, phenyl ethane-*p*-ω-bis (trimethylammonium iodide) was several times more active than hexamethonium. 3 times as potent as hexamethonium on the superior cervical ganglion of the cat, it was two and a half times as active in preventing the peristaltic reflex of the guinea-pig intestine. The compound had a similar mode of action to hexamethonium and was free from atropine-like actions. Acute toxicity tests showed the compound to be two and a half times as toxic as hexamethonium to the mouse. Death was due to respiratory paralysis. Small doses (3 mg./kg.) in the rabbit caused flushing of the ears, and very large doses (30 mg./kg.) caused depression of the respiration and head drop. In cats, doses of 0·5 mg. to 2 mg./kg. caused dilatation of the pupil, slowing of the heart and ataxia. 10 mg./kg. caused respiratory failure. Daily doses of 16 mg./kg. subcutaneously did not affect the growth of rats and daily doses of 3 mg./kg. intravenously to rabbits produced no ill effects to the kidneys or to the blood picture. In the anæsthetised dog 0·05 to 0·25 mg./kg. caused a fall in blood pressure characteristic of ganglionic blocking drugs. No direct effects were observed on the prefused heart of the rabbit and cat and there was no decrease in coronary flow in the dog. In the isolated perfused limb of the dog an intra-arterial injection of 3 mg. produced no effect on the vessels, while a similar dose of hexamethonium caused dilatation. The compound was effective in reducing the volume and acidity of the gastric secretion promoted by vagal stimulation in the anæsthetised dog. Excretion tests in rabbits showed from 68 to 89 per cent. of the intravenous dose to be excreted in 24 hours. In the series peaks for ganglionic and neuromuscular blockings activity were obtained, the significance of which is discussed. G. F. S.

Phenylbutazone and 4-Aminoantipyrine, Agranulocytosis Caused by. J. M. Kiely and J. M. Stickney. (*Proc. Mayo Clin.*, 1953, **28**, 341.) Two instances of agranulocytosis are reported in arthritic patients after treatment with phenylbutazone in one and 4-aminoantipyrine (1-phenyl-2:3-dimethyl-4-amino-5-pyrazolone) in the other. The first, after 2½ years on cortisone, was given 600 mg. of phenylbutazone daily. After 7 weeks he complained of aching, nausea and

soreness of the mouth. A leucocyte count showed only 600/cu. mm.; neutrophils were absent. Treatment consisted of saline irrigations of the throat, administration of aspirin, cortisone and penicillin, with dihydrostreptomycin during recurrence of fever. Neutrophils appeared in the blood smear on the 9th day and on the 18th day the leucocyte count was 2400/cu. mm. In the second instance, cortisone treatment for 18 months having failed to control the disease satisfactorily, treatment with 4-aminoantipyrine, 1200 mg. daily, was instituted. After 2 months a sore throat and fever occurred, the leucocyte count being 600/cu.mm. Treatment consisted of irrigation of the throat and administration of penicillin. By the 9th day the leucocyte count had risen to 9400/cu. mm. by which time the differential count was normal. H. T. B.

Phenylbutazone, Effect on Uric Acid Excretion in Gout and Rheumatoid Arthritis. C. Bishop and L. Beecher. (*Proc. Soc. exp. Biol. N.Y.*, 1953, **83**, 603.) Phenylbutazone (3 : 5 dioxo-1 : 2-diphenyl-4*n*-butylpyrizolidine sodium, butazolidin) is used in the treatment of gout and rheumatism. It has a simple analgesic action and it decreased the serum urate level. The urine of two patients with gout and three with rheumatoid arthritis were examined for the daily excretion of uric acid. Phenylbutazone caused a small but insignificant increase in uric acid excretion in the three arthritic patients, but in the two gouty subjects it caused a significantly increased output. G. F. S.

***iso*Propylnoradrenaline, Pharmacological Potency Ratio of Optical Isomers of.** E. Beccari, A. Beretta and J. S. Lawendel. (*Science*, 1953, **118**, 249.) Racemic *iso*propylnoradrenaline was resolved with D-tartaric acid in methanol The bitartrate of the *d*-isomer had m.pt. 110° to 120° C. (decomp.) and $[\alpha]_D^{20°\,C.} = +\ 35{\cdot}9°$, while the free base had a m.pt. 164° to 165° C. (decomp.) and $[\alpha]_D^{20°\,C.} = +\ 48{\cdot}8°$ (as hydrochloride). The *l*-isomer was obtained from the methanol solution by removing the solvent, dissolving the residue in water, liberating the free base with ammonia. This impure base was redissolved in diluted hydrochloric acid and reprecipitated with ammonia to give a product with a m.pt. 162° to 164° C. (decomp.) and $[\alpha]_D^{20°\,C.} = -\ 50°$ (as hydrochloride). For the pharmacological assays, the *d*-isomer as bitartrate, the *l*-isomer as hydrochloride and the racemate as sulphate were employed. The optical and pharmacological behaviour of *iso*propylnoradrenaline, as regards the differences between racemate and *l*-isomer, run parallel to those of adrenaline and noradrenaline. The *l*-isomer appears to be about 90 times more potent than the *d*-isomer. A. H. B.

Serotonin, Antimetabolite Action of Yohimbine and Ergot Alkaloids. E. Shaw and D. W. Woolley. (*J. biol. Chem.*, 1953, **203**, 979.) Yohimbine and the ergot alkaloids have been shown to antagonize the constrictor action of serotonin in segments of sheep carotid arteries and to reverse its action. These compounds, which are indole derivatives, are structurally related to serotonin. A series of compounds, analagous to serotonin, but more closely related to yohimbine, have been synthesised and tested. Most of these compounds were found to be active. Harman and *iso*harman were members of these series and it is suggested that these and other naturally occurring harman alkaloids owe some of their pharmacological properties to interference with the action of serotonin. G. F. S.

(ABSTRACTS *continued on p. 152*).

optical errors which are incurred in the measurement of high densities, it was not considered desirable to provide for the extension of the density range beyond the upper limit of 2·0.

SUMMARY

1. A description is given of a linear optical density potentiometer, interchangeable with the logarithmic density potentiometer incorporated in the Unicam SP500 and Beckman DU photoelectric spectrophotometers.

2. The potentiometer covers the density range 0·0 to 2·0; each of the approximately equal subdivisions of the scale throughout this range represents a density increment of 0·001.

3. The arrangement of the controls is such that, on the completion of the measurement, the density of the solution under investigation is indicated by the appearance of a horizontal row of figures, visible through an aperture in the panel.

The author is indebted to the Royal Society and to the University of London for research grants. It is proposed to demonstrate the instrument at the Exhibition of the Physical Society at Imperial College in April 1954.

REFERENCES

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(ABSTRACTS *continued from p. 147*).

Streptomycin and Isoniazid in Miliary Tuberculosis and Tuberculous Meningitis. G. M. Ritchie, R. M. Taylor and J. C. Dick. (*Lancet*, 1953, **265**, 419.) The post-mortem findings in 6 patients with both miliary tuberculosis and tuberculous meningitis treated with combined streptomycin and isoniazid are compared with those in 48 patients with either or both of these conditions not treated with isoniazid. The dosage adjusted for age corresponded to adult daily totals as follows: isoniazid, 150 mg. per day rising in 3 or 4 days to 400 mg. per day; streptomycin, intramuscular, 1 g. per day; streptomycin, intrathecal, 100 mg. per day; aminosalicylic acid, 20 g. per day. The lesions in the patients on combined therapy showed reversal of their process of formation. Caseation was absorbed, epithelioid cells had reverted to macrophages and diminished in number, a few polymorphs had appeared, there was no infiltration with leucocytes and no fibrosis had developed. In small miliary lesions, there was complete resolution. The miliary lesions in these 6 patients were in no way responsible for death. Streptomycin alone caused regression in small recent lesions but not resolution. In large and older lesions, streptomycin therapy was followed by regressive fibrosis, but with isoniazid in addition there occurred greatly increased vascularity, absorption of caseation, diminution of epithelioid cells and loosening of old fibrous tissue. The changes with isoniazid occurred even in old densely fibrosed lesions which were not affected by streptomycin. It is suggested that the effect of isoniazid is due to its more ready diffusibility. In tuberculous meningitis the addition of isoniazid led to more thorough control of the infection and changes in the lesions indicated that resolution was proceeding. In 4 patients these changes were followed by cerebral softening or fits due to swelling of tuberculomata and these harmful effects of isoniazid must be balanced against its advantages. H. T. B.