ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Ephedrine, Acetylation of, in Dilute Aqueous Solution. L. H. Welsh. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 545.) Because it had been shown previously that *N*-acetylephedrine could be obtained in 98 to 99 per cent. yield by the action of acetic anhydride on a dilute (*ca.* 1 per cent.) aqueous solution of ephedrine in the presence of sodium bicarbonate, the reaction was studied in some detail in an effort to make the reaction quantitative. Yields of 96 to 97 per cent. were obtained by using approximately 3·6 moles of anhydride per mole of ephedrine, but for the reaction to proceed quantitatively, 4 times that amount of anhydride was required, and the reagent had to be added in portions. The presence of sodium acetate or a large excess of bicarbonate adversely affected the yield. The factors affecting acetylation in an aqueous medium are discussed. A. H. B.

Neoprotoveratrine and Protoveratrine, Isolation from *Veratrum viride* Ait. M. W. Klohs, R. Arons, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek. (*J. Amer. chem. Soc.*, 1952, **74**, 5107.) Neoprotoveratrine, a new hypotensively active tetraester of protoverine and protoveratrine were isolated from *Veratrum viride* Ait. by the technique of Craig's countercurrent distribution of the hypotensively active "amorphous bases" obtained from the ground roots and rhizomes. Neoprotoveratrine crystallised from acetone as cubic crystals, m.pt. 255·4° to 255·8° C. (with decomposition), $[\alpha]_D^{24^\circ C.} - 39 \pm 2^\circ$ in pyridine. The analysis of neoprotoveratrine and its picrate, and equivalent weight determinations indicate a molecular formula of $C_{41}H_{63}O_{15}N$. The infra-red spectrum is recorded. Saponification yielded the alkamine protoverine as well as α-methylbutyric acid, α-methyl-α:β-dihydroxy-butyric acid, and 2 moles of acetic acid. Neoprotoveratrine and protoveratrine accounted for approximately 12 per cent. of the hypotensive activity. A. H. B.

Solamargine, a New Alkaloid from *Solanum marginatum*. L. H. Briggs E. G. Brooker, W. E. Harvey and A. L. Odell. (*J. chem. Soc.*, 1952, 3587.) From the green fruit of *Solanum marginatum* a new alkaloid, solamargine, $C_{45}H_{73}O_{15}N$, was isolated and characterised as its picrate and picrolonate. On hydrolysis with aqueous-ethanolic hydrochloric acid at 100° C. it yielded a crystalline hydrochloride, which on basification yielded solasodine identified by mixed melting points of the free base and its picrate. In the remaining solution glucose and rhamnose were detected. With 2 to 3 per cent. aqueous hydrochloric acid, partial hydrolysis occurred with liberation of rhamnose and formation of solasodine β-glucoside ($C_{33}H_{53}O_7N$) as an insoluble hydrochloride which produced solasodine and glucose upon further hydrolysis. An attempt to prepare solasodine β-glucoside by condensation of solasodine with acetobromoglucose in the presence of silver oxide, under anhydrous conditions gave solasodine hydrobromide. Since glucose and rhamnose were the only sugars formed on hydrolysis, the evidence indicates that solamargine is constituted as rhamnose-rhamnose-glucose-solasodine. A formula for solamargine is put forward.
A. H. B.

ANALYTICAL

Analgesics and Antipyretics, Paper Chromatography of. A. Jermstad and T. Waaler. (*Dansk Tidsskr. Farm.*, 1952, **26**, 205.) Paper chromatography may be used for the identification of a number of compounds. The solvent is a mixture of light petroleum (b. pt. 65° to 70° C.), 25 parts; methanol, 10 parts; benzene, 20 parts; and water 0·5 parts, all by weight. It is important that the atmosphere should be fully saturated by allowing the vessel to stand overnight. R_F values are as follows: acetanilide, 0·40; acetylsalicylic acid, 0·05; amidopyrine, 0·67; phenacetin, 0·45; phenazone, 0·32. To improve the separation of acetanilide and phenacetin, the movement of the liquid front should be slow, and only a small quantity of substance applied. The dried chromatogram may be developed with ferric chloride, which gives colours with acetylsalicylic acid, amidopyrine and phenazone; or with Millon's reagent, which gives a colour with all of the above compounds. In view of the danger of spraying a mercurial solution, the method of Wickstrøm and Salvesen (*J. Pharm. Pharmacol.*, 1952, **4**, 98) should be used. Phenazone shows an orange mark in the cold; the other compounds require warming for a few minutes at 103° C. The method has been used for a number of tablets, containing as additional constituents caffeine, codeine phosphate and diethylbarbituric acid. G. M.

Barbiturates, Determination of. L. R. Goldbaum. (*Analyt. Chem.*, 1952, **24**, 1604.) A simple, rapid, ultra-violet spectrophotometric procedure for the specific identification and quantitative determination of microquantities of barbiturates and for the differentiation of many barbiturates is described. The method is based on the fact that substituted barbiturates in strong alkali show characteristic maxima at 255 mμ and minima at about 235 mμ; in a buffer solution at *p*H 9·8 to 10·5 there occur higher maxima at 240 mμ with no minima. When the optical densities in *p*H 10·5 solution are subtracted from those in strong alkali, differences appear that are highly characteristic of all barbiturates except the *N*-methyl and thio-derivatives. By comparing the differences at various wave lengths with that at 260 mμ, ratios are obtained that can differentiate many of the commonly used barbiturates. Absorbing substances appearing in the extracts of drug-free biological materials, as well as other drugs, show no significant differences at these *p*H's and do not interfere in this procedure. The difference in optical density at 260 mμ is related to the barbiturate concentration. The method can be used to determine barbiturate concentrations as low as 0·1 mg./100 ml. of blood and 0·3 mg./100 g. of tissue. Blood levels occurring after therapeutic doses of barbiturates range from 1 to 10 mg./ml., and after toxic doses range from 15 to 100 mg./ml., the corresponding tissue levels being higher; the sample used for analysis should be such that the alkaline extract should contain about 25 mg. of barbiturates per ml. R. E. S.

Calcium and Magnesium, Determination of, with Ethylenediamine Tetra-acetate. J. Banks. (*Analyst*, 1952, **77**, 484.) An investigation has been made of the determination of calcium and magnesium in siliceous materials by titration with disodium ethylenediamine tetra-acetate. The sample is ignited to determine the loss, the silica is removed and also the iron group (by a double precipitation with ammonium hydroxide, each preceded by the addition of 5 g. of ammonium chloride) and manganese (by the bromine-ammonium hydroxide separation); the filtrate from the manganese precipitation is diluted to a known

volume and used for subsequent determinations as the bulk solution. For the joint titration of calcium and magnesium an aliquot of the bulk solution is made alkaline with ammonia, Eriochrome Black T is added, and the solution is titrated with 0·02N disodium ethylenediamine tetra-acetate until a blue colour free from any trace of pink appears, with vigorous shaking near the end-point. For the titration of calcium only an aliquot is made alkaline with sodium hydroxide, murexide indicator is added and titrated with 0·02N disodium ethylenediamine tetra-acetate to the violet end-point. The end-point is reached when the addition of 2 drops of titrant produces no further change in colour. Formulæ are given for the calculation of results. The results generally showed a satisfactory degree of accuracy over a wide range of calcium and magnesium concentrations and in the presence of various quantities of interfering elements. R. E. S.

Cobalt Compound for Precipitation of Sulphate. R. Belcher and D. Gibbons. (*J. chem. Soc.*, 1952, 4216.) Five co-ordination compounds of cobalt were examined as possible reagents for the precipitation of sulphate in order to find if they possessed any advantages over existing reagents. The most promising compound, octa-ammino-μ-amino-μ-nitro-dicobaltic nitrate was studied in detail and a gravimetric procedure for the determination of sulphate developed. Since the precipitate is more soluble than barium sulphate, careful control of the volumes of the solution is necessary to obtain quantitative results. The interfering effect of a selected number of ions was examined, and interferences shown to be few, in particular the nitrate ion had no effect. The method might be of particular use in the determination of sulphur in organic compounds containing nitrogen, by combustion on the microscale, which requires elimination of nitric acid before precipitation of sulphate as barium sulphate. A. H. B.

Copper, Iodimetric Determination of. L. Meites. (*Analyt. Chem.*, 1952, **24**, 1618.) The method of Scott (*Scott's "Standard Methods of Chemical Analysis,"* 1939) for the iodimetric determination of copper has been examined. Enough iodide was added to retain the copper in solution throughout the titration, presumably as CuI_2^-, thus eliminating all the errors generally associated with the separation of a solid phase during a titration, giving a clear colourless solution at the end-point. Experiments showed that the method was superior to the normal precipitation method using ammonium thiocyanate to prevent adsorption of iodine by the precipitate. The effects of numerous metals and radicals likely to interfere have been examined and are reported. R. E. S.

Digitoxin, Colorimetric Estimation of. E. L. Pratt. (*Analyt. Chem.*, 1952, **24**, 1325.) The determination proposed is based on the reaction of 3:5-dinitrobenzoic acid and benzyltrimethylammonium hydroxide with digitoxin in dilute ethanol to produce a bluish-red coloured solution which has maximum optical density 550 mμ. The selection of the base, benzyltrimethylammonium hydroxide, and the π acid, 3:5-dinitrobenzoic acid was made primarily from the results of rate curves obtained at room temperature after a study of bases and 5 π acids. Details are given of the method which produces approximately 50 per cent. as much colour for gitoxin as is produced by an equimolar amount of digitoxin. About 85 per cent. as much colour is given by digoxin, but the latter is not usually found with digitoxin. A procedure is described which, when used with the method for analysis of digitoxin outlined above, has proved satisfactory in the assay of digitoxin tablets. R. E. S.

Fluoride in Water, Determination of. M. J. Price and O. J. Walker. (*Analyt. Chem.*, 1952, **24**, 1593.) The bleaching of highly coloured lakes, formed by the reaction of aluminium and zirconyl salts with hæmatoxylin, by small amounts of fluoride, has previously been used as a basis for a method for the determination of fluoride in water; in the present work it was not found possible to duplicate previous results, much of the difficulty being due to the instability of the hæmatoxylin. A method was devised using a partly oxidised hæmatoxylin which could be used satisfactorily in the examination of water supplies that naturally contain fluorides as well as of those to which fluorides have been added. Details of the procedure are given together with the method for the preparation of the hæmatoxylin and the absorption spectra of the purple complex. The method allows the determination of 0 to 1·5 p.p.m. of fluoride ion with an accuracy of 0·05 p.p.m. if recrystallised hæmatoxylin, which has been oxidised with hydrogen peroxide, is used at *p*H 4·6; satisfactory colour development occurred within 4 hours. Sulphates and phosphates interfere in the method, but have less effect than on other colorimetric and photometric methods; the corrections necessary for sulphate concentrations up to 200 p.p.m., and phosphate concentration up to 8 p.p.m. are given. R. E. S.

Fluoride Ion, Determination of. H. E. Bumstead and J. C. Wells. (*Analyt. Chem.*, 1952, **24**, 1595.) A reagent suitable for the spectrophotometric determination of μg. quantities of fluoride ion was developed; it consisted of a modified zirconium-alizarin reagent which would produce colours intense enough to be read spectrophotometrically in the range 0 to 4 μg. of fluoride per ml. of sample. Concentrations as low as 0·1 μg./ml. could be satisfactorily determined. The method provides for precise measurement of the bleaching effect of the fluoride ion on the zirconium-alizarin lake and eliminates end-point and other visual variations. The method does not overcome the intereference of phosphate, sulphate, and chloride ions, and samples suspected of containing interfering amounts of these ions must be distilled to isolate the fluoride ion. R. E. S.

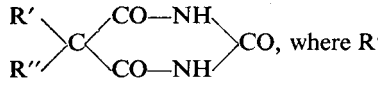
Tocopherol Mixtures, Quantitative Paper Chromatography of. F. Brown. (*Biochem. J.*, 1952, **52**, 523.) A method is given for the quantitative estimation of α, β, γ, and δ, tocopherols. Before chromatography it was necessary to remove lipids, sterols and carotenoids; the lipids were readily removed by saponification and the vitamin A and carotenoids were separated from the tocopherols by absorption on Floridin Earth; the removal of sterols was accomplished by crystallising from methanol solution at $-10°$ C. For chromatography filter paper was used after coating with vaselin and ethanol (75 per cent.) was used as the mobile phase. After developing the chromatogram for about 16 hours using the ascending method and drying, the strips were sprayed with 2:2′-dipyridyl solution (0.25 per cent. w/v in ethanol) and then with ferric chloride solution (0·1 per cent. w/v in ethanol); for quantitative estimation untreated "tocopherol zones" were cut out, extracted with 4 ml. hot ethanol, 1 ml. benzene added and the filtered ethanol-benzene solutions were then treated with 2:2′-dipyridyl and ferric chloride, the colour being measured after 2 minutes. The method permitted the quantitative separation of the α and δ compounds; a further procedure is described for the β and γ isomers. When the chromatograms were run for 16 hours the recoveries were about 80 per cent.; reduction of the time of development to 6 hours gave 90 per cent., but a clear-cut separation of the γ and δ compounds was not obtained. Results obtained by this method for

certain vegetable oils were compared with those obtained for the same oils by other workers. R. E. S.

Water, Determination of. J. Aubry and G. Monnier. (*C.R. Acad. Sci., Paris*, 1952, **235**, 1037.) Small quantities of water dissolved in ether, light petroleum, liquid paraffin, benzene, toluene, xylene, chloroform or carbon tetrachloride may be estimated by precipitation as lithium bromide hydrate, $LiBr,H_2O$. The reaction is performed in a closed apparatus fitted with magnetic stirring, in which the reagent is filtered through sintered glass before use. A saturated solution of lithium bromide in anhydrous ether is added to an ethereal solution of the water to be determined, until no further precipitation occurs. In the same apparatus the precipitate is separated by filtration with a positive pressure of dry gas, washed with anhydrous ether and dissolved in water. The quantitity of bromide present is determined by titration with 0·1N silver nitrate, and hence the quantity of water is calculated. For the determination of water in the other organic liquids, ether is added to obtain a water-ether-organic liquid solution which is treated as above. Good precision is possible with as little as 10 mg. of water, and quantities of 50 to 100 mg. may be determined with an accuracy of 1 per cent. G. B.

ORGANIC CHEMISTRY

Barbituric Acids, Sulphur-containing. H. Böhme and H. G. Greve. (*Pharm. Zentralh.*, 1952, **91**, 259.) The authors describe the general method of preparation and properties of substituted barbituric acids of the general formula



where R′ = $CH_3S.CH_2$—, $C_2H_5.S.CH_2$—, $C_2H_5.S.CH(CH_3)$—, $C_6H_5.CH_2.S.CH_2$—, and the corresponding sulphones, while R″ = CH_3—, C_2H_5—, $(CH_3)_2CH$—, C_6H_5—, $C_6H_5.CH_2$—. A few corresponding thiobarbituric acids are also mentioned. There is no mention of their physiological action. G. M.

Nitro Amines and Diamines, Some New. E. B. Hodge. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 566.) A series of new nitro amines of general formula $ArCH_2N(Ar')CH_2C(NO_2)(CH_3)_2$ were prepared by the reaction between 2-nitro-2-methyl-1-propanol and some amines of the type $ArN(CH_2Ar)H$ in the presence of a mildly basic catalyst such as sodium carbonate or sodium acetate. Reduction of these nitro amines with hydrogen in the presence of Raney nickel gave a series of diamines which showed weak antihistaminic activity. Reductive methylation of some of the nitro amines to yield ditertiary amines gave compounds which exhibited moderate to strong antihistaminic activity. A. H. B.

Steroids, Paper Chromatography of. D. Kritchevsky and M. R. Kirk. (*J. Amer. chem. Soc.*, 1952, **74**, 4484.) The preparation and use of filter paper impregnated with stearato chromic chloride for the reverse phase paper partition chromatography of steroids is described. Descending chromatography was used throughout the work. The R_F values obtained with 21 steroids (including corticosteroids, androgens and progestational hormones) using a variety of solvents are tabulated. The separations cholesterol/ergosterol, cholesterol/7-dehydrocholesterol, cholesterol/epicholesterol, cholesterol/testosterone, stigmasterol/ergosterol were achieved. A. H. B.

PLANT ANALYSIS

***Arbutus menziesii*, Antibacterial Substance from the Leaves of.** E. R. Hammarlund, D. E. Pennington and L. W. Rising. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 561.) A method is described for the extraction and concentration of a water-soluble antibacterial principle from the fresh leaves of *Arbutus menziesii*, family *Ericaceæ*. The fresh leaves were cut into small pieces, extracted with ether and these extracts discarded. The ether-insoluble material was then extracted with 85 per cent. ethanol and the extract evaporated to dryness under vacuum, and the dried extract dissolved in water to yield a clear, golden-tan extract of the antibacterial principle. Further purification was effected by passing through ion-exchange resins (anionic and cationic), extraction with acetone and chromatography using calcium hydrogen phosphate to yield a relatively concentrated but still non-crystalline product which represented about 2·5 per cent of the original weight of fresh leaves. The product, named madronin, is a slightly hygroscopic, light yellow, non-crystalline powder, which slowly darkens above 200° C. and chars at approximately 250° C. and is very soluble in water, methanol and ethanol. Various physical and chemical data are recorded. It is mainly active *in vitro* against the Gram-positive and acid-fast bacteria and shows very little activity against Gram-negative bacteria except the genera *Klebsiella*, *Shigella*, and *Alkaligenes*. It is relatively non-toxic to mice and is excreted in an active form in the urine. Various inactivating factors were investigated and it was shown not to be inactivated by cysteine or serum *in vitro*. A. H. B.

TOXICOLOGY

Barbiturates, Toxicological Detection of. H. Kaiser and W. Lang. (*Pharm. Zentralh.*, 1952, **91**, 281.) In a case of fatal phenobarbitone poisoning, material extracted from the urine gave rather indefinite reactions, somewhat improved by charcoal treatment. After two fractional microsublimations there was obtained a material showing the correct melting point and giving a characteristic crystalline form with iron iodide reagent. In another case of barbituric acid poisoning, no barbituric acid could be detected in the urine, but a portion of dried vomit, extracted with ether and purified with charcoal, gave a distinct cobalt reaction, which however was obscured by the simultaneous presence of acetylsalicylic acid. After microsublimation, characteristic crystal formations were obtained with iron and copper iodide reagents, and found to be due to cyclopal, the identity being later confirmed by the patient. This compound is evidently broken down in the body to such an extent that it cannot be detected in the urine. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Aromatic Amino-acids, Optical Resolution of, on Paper Chromatograms. C. E. Dalgliesh. (*J. chem. Soc.*, 1952, 3940.) It was found that many racemic aromatic amino-acids could be resolved into their optical enantiomorphs on paper chromatograms, using the descending technique on strips, 30 inches in length, of Whatman No. 4 paper. Racemates which were resolved showed, after travelling a short distance, an elongated spot which as it proceeded

down the paper became dumb-bell shaped and finally separated into two spots. The structural features necessary for resolution were formulated tentatively: (1) The α-amino-group (and probably also the carboxyl group) should be intact in order to be attached simultaneously to the cellulose surface to give a "three-point" attachment of the molecule required for stereochemical specificity. (2) To give this "three-point" attachment the molecule must contain some other portion, such as an aromatic ring which is also adsorbed on the cellulose surface. (3) The ring must carry one or more substituents allowing a close "fit" with the cellulose surface, and hence greater adsorption, with one of the optical isomers than with the other. It is considered likely that any phenylalanine containing a small substituent in the *ortho*-position may be resolvable on cellulose chromatograms, and that resolution would be due to steric interference of the *ortho*-substituent with some element in the cellulose surface which prevents the D- from being so strongly adsorbed as the L-isomer. (4) Although the cases showing resolution all contain hydrogen-bonding groups on the benzene ring there is no evidence as yet to show whether these groups are essential for resolution. A. H. B.

B_{12} Vitamins, Neutral, Basic and Acidic Cobalamins. E. Lester Smith, S. Ball, and D. M. Ireland. (*Biochem. J.*, 1952, **52**, 395.) Recent work on the nature of the reaction of the B_{12} vitamins is reviewed. Besides the neutral and basic classes of cobalamins, there is a third class of acidic cobalamins. They contain two acidic ions, which need not be the same, or one divalent ion like sulphite. They are unstable and only persist in solutions containing excess of the co-ordinating ion. Using radioactive carbon and sulphur it was shown that the cyano-group in vitamin B_{12} exchanges with inorganic cyanide, rapidly in alkaline solution but very slowly in acid. In high concentrations of thiocyanate in acid or alkaline, but not in neutral, solutions the cyano group is partly displaced by thiocyanate. Tracer techniques have been used to study the structures and stabilities of a series of cobalamins. R. E. S.

B_{12} Vitamins, Vitamin B_{12c} and B_{12d}. E. Lester Smith, K. H. Fantes, S. Ball, J. G. Waller, W. B. Emery, W. K. Anslow and A. D. Walker. (*Biochem. J.*, 1952, **52**, 389.) Three red, crystalline, microbiologically and clinically active B_{12} vitamins have been isolated from *Streptomyces griseus* fermentations, namely vitamin B_{12} itself, vitamin B_{12b} and vitamin B_{12c}. The yield of the new factor, vitamin B_{12c}, was enhanced at the expense of the vitamin B_{12b} by treatment with nitrous acid, which, by deaminating amino-acids and peptides, facilitated their removal from the crude concentrate. Nitrous acid appeared to have no effect on vitamin B_{12} itself, but it readily converts vitamin B_{12b} into vitamin B_{12c}; removal of the nitrite group, by treatment with sulphamic acid or distillation of an acidified solution, yields vitamin B_{12b} again. Vitamin B_{12d} previously reported was found to be identical with vitamin B_{12b}. The absorption spectra, polarography, electrical conductivity, magnetic susceptibility and optical rotation of the B_{12} vitamins are reported. R. E. S.

Vitamin B_{12}, Microbiological Synthesis of, by Propionic Acid Bacteria. A. Leviton and R. E. Hargrove. (*Industr. Engng Chem.* (*Anal.*), 1952, **44**, 2651.) Because of the discovery of vitamin B_{12} activity in cultures of propionic acid bacteria, the cultivation under various conditions which have given rise to excellent yields is described. Contrary to the impression created by the literature of industrial microbiology, the fermentation of lactic acid to propionic, acetic and carbonic acids may be carried out within a period of time comparable

to that required for most industrial fermentations. Experiments were designed in which vitamin B_{12} yields were determined under various conditions of anærobiosis and ærobiosis. The vitamin content was determined by microbiological and rat assays. The absorption spectrum was more characteristic of vitamin B_{12b} than of vitamin B_{12}. That the activity was largely due to vitamin B_{12b} was confirmed by means of partition chromatography from which it appears that approximately 80 per cent. of the activity is due to vitamin B_{12b} and 20 per cent. to the cyanocobaltammine compound. The activity developed in certain of the fermentations listed resided entirely in the cells. The harvested cells consequently furnish a highly concentrated source of vitamin B_{12} activity - 1·2 mg./g. in one instance. The active substances may easily be stripped from the cells to yield a concentrate containing 12 mg./g. with the colour and absorption spectrum in the visible region belonging largely to vitamin B_{12b}.

A. H. B.

BIOCHEMICAL ANALYSIS

***d*-Amphetamine in Body Fluids, Determination of.** R. E. Keller and W. C. Ellenbogen. (*J. Pharmacol.*, 1952, **106**, 77.) A method is described for the estimation of *d*- or *dl*- amphetamine in body fluids. It is based on the extraction of the free amine, liberated in alkaline solution, with benzene containing a small amount of amyl alcohol, and its subsequent coupling with methyl orange to form a complex in the presence of a small amount of sulphuric acid in methanol. The colour is concentrated by extraction with a small amount of hydrochloric acid. It is quite stable and is measured on a Beckman spectrophotometer at 508 mμ. An estimation can be carried out on 5 ml. of plasma or 10 ml. of urine. *In vivo* studies in dogs showed that some tissue in the brain rapidly removed *d*-amphetamine from the blood. G. F. S.

Benzylpenicillin, Diethylaminoethyl Ester of, Determination of Rate of Hydrolysis. H. L. Dinsmore and S. D. Bailey. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 532.) Although esterified penicillin will itself be inactive, this will not be a serious limitation to its application if appreciable hydrolysis of the ester to yield free penicillin can be expected to take place at the desired site in the body. Therefore as a first approximation in establishing the therapeutic value of the diethylaminoethyl ester of benzylpenicillin, the rates of hydrolysis *in vitro* were determined in buffered media covering the *p*H range from 2 to 8. The hydrolysis was followed by removal of aliquot portions of the reacting solutions and the successive determinations of the partition coefficients using butanol as the other phase. Using ultra-violet spectroscopic assay of the partitioned layers, and from the known partition coefficients of the pure ester and benzylpenicillin, the amount of each component present was determined. In the case of the hydroiodide salt, a solubility method is reported which confirms the hydrolysis rates given by the partition coefficients. The hydrolysis of the hydrochloride and hydroiodide salts of the diethylaminoethyl ester of benzylpenicillin was shown to occur rapidly in either weakly alkaline or strongly acidic dilute solutions, with maximum stability near *p*H 5 to 6. A. H. B.

Benzylpenicillin, Diethylaminoethyl Ester of. Rate of Hydrolysis in a Pharmaceutical Suspension. R. E. Keller. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 536.) The rate of hydrolysis of the diethylaminoethyl ester of benzylpenicillin in a pharmaceutical suspension was determined by a single contact

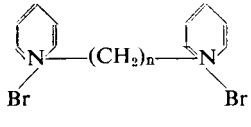
partition method depending upon a difference in partition coefficient for the ester and for sodium penicillin "G." With a chloroform and aqueous phosphate buffer pair, the ester favoured the organic phase, whereas sodium penicillin "G" favoured the aqueous phase. The partition coefficients K_e of the pure ester, K_x of the partially hydrolysed sample and K_p of sodium penicillin G were calculated from ultra-violet absorption data. From these three partition coefficients, the percentage of ester hydrolysed at any interval was calculated. The hydrolysis rate curves, obtained at temperatures of 5°, 25° and 37° C. were plotted over a 168 hour period. The maximum hydrolysis was 19 per cent., 7 per cent., and 2 per cent. at 37°, 25° and 5°, respectively. This information is of value in determining the full dosage of the intact ester after it has been stored at various temperatures. A. H. B.

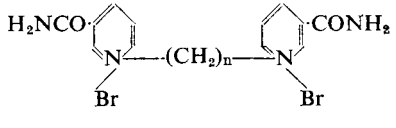
Desoxycholic Acid, Determination of. E. L. Pratt and H. B. Corbitt. (*Analyt. Chem.*, 1952, **24**, 1665.) A method is given for the determination of desoxycholic acid is the presence of other bile acids. Vanillin dissolved in 85 per cent. phosphoric acid gave with desoxycholic acid a colour having a maximum at 545 mμ; cholic acid, and apocholic acid ($\Delta^{8,14}$-3 : 12-dihydroxycholanic acid), reacted with the proposed reagent giving maxima at 465 mμ. Details of the conditions and procedure are given; the desoxycholic acid content and the cholic acid content can be obtained separately after solution of equations involving absorptiometric readings at 545 mμ and 465 mμ. The assay can be used to evaluate the content of desoxycholic acid in the presence of hydroxy- and ketocholanic acids; the quantities of cholic and desoxycholic acids are evaluated simultaneously and no colour is given with ketocholanic acids and lithocholic acids. R. E. S.

Desoxycholic Acid in Ox Bile, Determination of. C. R. Szalkowski and W. J. Mader. (*Analyt. Chem.*, 1952, **24**, 1602.) An investigation was made into the determination of desoxycholic acid in ox bile in the presence of cholic acid, cholesterol, and fatty acids; on alkaline hydrolysis the nitrogenous constituents which may be combined with cholic and desoxycholic acids are split off and the liberated desoxycholic acid is extracted with ether from an acid solution. In an attempt to find a reagent showing specificity, a series of aldehydes was investigated and salicylaldehyde in the presence of glacial acetic acid was found to be the most specific under carefully controlled conditions; curves are given for desoxycholic acid, methyl desoxycholate, cholic acid, lithocholic acid, apocholic acid, and cholesterol. Results are given for the purity of commercial desoxycholic acid samples (84 to 99 per cent.) and for the desoxycholic acid content of typical bile samples. R. E. S.

CHEMOTHERAPY

Curare-like Synthetic Derivatives of Pyridine and Nicotinamide. R. Hazard, J. Cheymol, J. A. Gautier, E. Corteggiani and E. Leroi. (*Arch. int. pharmacodyn.*, 1952, **90**, 271.) Derivatives of pyridine and nicotinamide of the type shown below were prepared, for n = 2, 3, 4, 5, 6 and 10.





Members of the pyridine series were made by heating the alkyl dibromide with 2 moles. of pyridine on a water bath. The nicotinamide derivatives were obtained by heating on a water bath or under a reflux condenser, the alkyl dibromide and 2 moles of nicotinamide in saturated ethanolic solution. For the pyridine derivatives the LD50 dose in mice indicates that the toxicity increases with the length of the polymethylene chain. The rabbit head-drop dose is usually less than the LD50 for mice, and this ratio is more favourable than with *d*-tubocurarine. Tests with the turning reflex and the rectus abdominis give an even more favourable ratio, but in the rat diaphragm test the ratio of curarising to toxic doses is less than for *d*-tubocurarine. In a comparison of respiratory arrest and cardiac arrest doses with the head-drop dose in the rabbit, compounds n = 5 and n = 10 give the best ratio. Small doses of these compounds increase the sensitivity to acetylcholine, although the compound n = 10 has only 1/50 the potency of physostigmine or neostigmine. The derivatives of nicotinamide do not show pharmacological properties regularly related to chain length, and appear to be less promising as curarising agents.

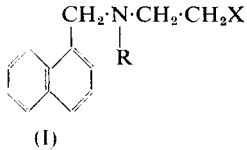
G. B.

***NN*-Dialkyl-*N'*-benzyl (or -ethyl)-*N'*-1 (or 2)-naphthyl-ethylenediamines as Potential Histamine Antagonists.** N. B. Chapman, J. W. James and J. F. A. Williams. (*J. chem. Soc.*, 1952, 4024.) Because ethylenediamines fully substituted on the nitrogen atoms have been known for many years to be active against histamine, and in particular "Antergan" ($Ph.N(CH_2Ph).[CH_2]_2.NMe_2$), the possibility of loading one aryl residue more heavily than previously, as in

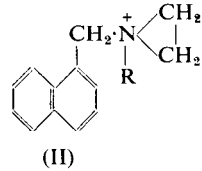
$1-C_{10}H_7.NR'.[CH_2]_2.NR_2$ (I) $\qquad$ $2-C_{10}H_7.NR'.[CH_2]_2.NR_2$ (II)

(I) and (II) R′ = CH_2Ph; R = Me, Et), in an attempt to get a more persistent action, was investigated. Eight ethylenediamines were prepared but none of them proved to be very active as histamine antagonists. A. H. B.

2-Haloethylamine Derivatives with a Naphthylmethyl Group, Chemical Reactivity and Pharmacological Activity among. N. B. Chapman, J. W. James, J. D. P. Graham and G. P. Lewis. (*Chem. Ind.*, 1952, 805.) Kinetic studies demonstrate that some of the compounds of type (I) (where X = F, Cl, Br and I, R = Me, Et and Ph), cyclise to form a substituted ethyleneimonium ion (type II) *in vitro*.



(I)



(II)

From (I) (R = Me, X = Br) was isolated the ethyleneimonium ion (II) R = Me) as picrylsulphonate. On the other hand, compounds of type (I) (R = Me, Et, X = F) and (I) (R = Ph, X = Cl, Br, I) are chemically inert, yield no ethyleneimonium ion, and are inactive as anti-adrenaline agents. The information of the behaviour of the ethyleneimonium ion (II) (R = Et) has been applied to provide solutions of it of differing but definite concentration which were assayed biologically for anti-adrenaline activity, thus correlating chemical

reactivity with pharmacological activity. The results indicate that the ion (II) is rapidly formed and relatively slowly destroyed *in vitro*, and is the pharmacologically active species. A. H. B.

Pyrazinamides and Related Compounds in the Chemotherapy of Tuberculosis. S. Kushner, H. Dalalian, J. L. Sanjurjo, F. L. Bach, S. R. Safir, V. K. Smith and J. H. Williams. (*J. Amer. chem. Soc.*, 1952, **74**, 3617.) Many pyrazinamides and related compounds, the majority of which were alkyl, aryl, acyl and heterocylic derivatives of pyrazinamides, were prepared for testing for antituberculous activity. A number of thiosemicarbazones of related pyrazines were also prepared. Pyrazinamide was found to be more active than *p*-aminosalicylic acid in the mouse test, and was found to be clinically active in humans. Pyrazinamide is stated to be comparatively non-toxic. A. H. B.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and Noradrenaline, Antidiuretic Action of. E. H. Dearborn and L. Lasagna. (*J. Pharmacol.*, 1952, **106**, 122.) Experiments carried out on trained perineotomised female dogs suggest that adrenaline and noradrenaline inhibit water diuresis by two different mechanisms (*a*) by vasoconstriction which reduces the glomerular filtration rate, renal blood flow and urine flow for a short time, (*b*) by acting on the hypothalamo-hypophyseal system producing a long lasting diuresis, due to liberation of antidiuretic hormone. The second conclusion is made because the effect was absent in dogs in which the hypothalamo-hypophyseal system was surgically damaged. G. F. S.

Amphetamine in the Treatment of Barbiturate Poisoning. H. L. H. Dick. (*Amer. J. med. Sci.*, 1952, **224**, 281.) Various analeptics have been used in the treatment of barbiturate poisoning but they have not been uniformly successful. Difficulties have arisen from the dangers of overdosage or from other causes such as delay in producing an effect, as with picrotoxin, or short duration of action, as with metrazol. The author has successfully treated 11 cases of severe barbiturate poisoning by intravenous injection of amphetamine sulphate and confirms earlier reports of the value of amphetamine for this purpose. The dose recommended is from 40 to 100 mg. every ½ to 1 hour, basing the choice on close clinical observation. These doses are larger than those proposed by previous workers but are safe and more effective than smaller amounts; no convulsions occurred even with the largest dosage. Routine procedure should include the maintenance of an adequate airway with oxygen under positive pressure if necessary, treatment of shock and dehydration with plasma or whole blood and intravenous fluids, gastric lavage if the drug has been taken within 2 hours of admission but not allowing this procedure to delay the administration of amphetamine, penicillin by intramuscular injection, and provision of an indwelling urethral catheter. Frequent observation for increasing signs of coma is also essential. In the author's series no deaths occurred although 2 of the patients were in a state of respiratory arrest and circulatory collapse when first seen. H. T. B.

Isoniazid in Leprosy. J. Lowe. (*Lancet*, 1952, **263**, 1012.) Up to 300 mg. of isoniazid daily was given to 20 patients with newly diagnosed leprosy, showing easily visible lesions in which improvement could readily be detected. Of 10 patients with tuberculoid leprosy, treated for 8 to 19 weeks, only 1 showed

definite improvement and the same result might well have been obtained with no treatment at all. 10 lepromatous cases in which the disease was not too severe were treated for up to 23 weeks. None of the patients showed distinct improvement, and in no case was the number of bacilli definitely decreased or distinct morphological changes in the bacilli observed. 7 patients with complications in the form of repeated reactions with fever, erythema nodosum, neuritis, etc., probably precipitated by previous treatment, were given isoniazid. The acute symptoms subsided in 6 of them but the later results were very variable, and similar to those obtained simply by stopping chemotherapy. Isoniazid is possibly of slight benefit in leprosy, but is much less active than sulphones or thiosemicarbazones. G. B.

Isoniazid in Pulmonary Tuberculosis, With and Without Streptomycin. C. L. Joiner, K. S. MacLean, E. K. Pritchard, K. Anderson, P. Collard, M. B. King and R. Knox. (*Lancet*, 1952, **263**, 843.) After a preliminary trial in which isoniazid was shown to have a clear advantage over a placebo (lactose), patients were divided into 2 groups, paired for weight, age and progress of the disease. Both groups received 250 mg. of isoniazid daily and group B received in addition 1 g. of streptomycin sulphate, intramuscularly, 6 times a week. Both groups progressed during the first 6 weeks and there was a sense of well-being accompanied by gain in appetite and weight. In group A (isoniazid alone) the improvement did not continue but was eventually followed by deterioration, associated with the excretion of resistant strains of tubercle bacilli in the sputum. In the group receiving streptomycin in addition, the initial improvement was continued throughout the 18-week trial period. Weight continued to increase while the erythrocyte sedimentation rate fell and the sputum count and clinical condition showed improvement. A further series of 18, including a greater proportion of more toxic patients, were divided into 2 similar groups, C and D. Group C, treated with isoniazid and streptomycin for 14 weeks showed a weight increase greater than group B; group D treated with streptomycin and *p*-aminosalicylic acid showed a steady but smaller weight gain. The improvement in sputum counts and erythrocyte sedimentation rate was about the same in the two groups C and D. The haphazard use of isoniazid alone is strongly condemned since patients may deteriorate during treatment and also transmit isoniazid-resistant strains of the organism to others. G. B.

Isoniazid in the Control of Experimental Corneal Tuberculosis. R. Goulding and J. M. Robson. (*Lancet*, 1952, **263**, 849.) Experiments were made on groups of mice and rabbits inoculated intracorneally with human or bovine strains of *Mycobacterium tuberculosis*. In such animals the results of treatment with antituberculous drugs show significant agreement with clinical observations. In mice, isoniazid (12 mg./kg./day) had a suppressive effect only and a combination with *p*-aminosalicylic acid was only slightly more effective. A combination of isoniazid with streptomycin gave a more nearly complete control of the disease. In rabbits with established corneal lesions there was no significant difference in effect between isoniazid, streptomycin and both together. In rabbits, freshly inoculated intracorneally, isoniazid had a greater suppresive action than streptomycin and there was no recrudescence of the disease after the drug was withdrawn as observed in the experiments with mice. A temperature difference is suggested as an explanation for the differing action of isoniazid, which appears to be bactericidal in rabbits and bacteriostatic in

mice. Corneal temperatures have not been accurately measured, but the body temperature of rabbits is on average 4 C. degrees higher than that of mice.

G. B.

Milontin: A New Drug in the Treatment of Petit Mal. J. G. Millichap. (*Lancet*, 1952, **263**, 907.) Milontin (*N*-methyl-α-phenylsuccinimide) was selected for trial because it can prevent leptazol convulsions in laboratory animals in non-depressive dosage. Tests were made in 20 unselected patients, including 17 children, 10 being cases of pure petit mal, 5 the akinetic form of petit mal and 5 having grand mal combined with one or both types of petit mal. Complete control was obtained in 26 per cent. of patients and attacks were reduced by 80 per cent. or more in 37 per cent. of patients, the duration and severity of seizures being diminished. Of 21 patients, 13 showed toxic effects and 10, glomerulo-tubular damage. This rate of toxicity, higher than previously encountered, could be explained by the large doses used in a series consisting mainly of children. A table of effective daily doses according to the age of the patient is included, by the use of which the more severe toxic effects could be avoided. G. B.

Penicillin; Lack of Influence on Blood Coagulation. D. C. Triantaphyllopoulos and B. A. Waisbren. (*Arch. intern. Med.*, 1952, **90**, 653.) Experiments were conducted, *in vitro* and *in vivo*, on the effect of varying concentrations of crystalline penicillin on the prothrombin time, coagulation time, thrombin, prothrombin consumption, labile factor, clot retraction, and protamine titration of normal blood. It was shown for the first time that penicillin did not affect prothrombin consumption, labile factor, thrombin, and protamine titration. In addition, studies that had failed to show an effect of penicillin on the coagulation and prothrombin times, and on clot retraction of normal blood were confirmed. It appears probable that effects on blood coagulation attributed to penicillin were due to impurities present in early preparations of the drug.

S. L. W.

Phenylacetylurea (Phenurone), Pharmacological Studies of. G. M. Everett and R. K. Richards. (*J. Pharmacol.*, 1952, **106**, 303.) Phenylacetylurea is an anticonvulsant drug effective in various types of human epilepsy including psychomotor seizures. It has a low toxicity, the oral LD50 to mice being 5 g./kg., while dogs and cats survived single oral doses up to 2 g./kg. High doses produced ataxia and impaired righting reflexes, while lethal doses caused respiratory failure. A dose of 300 mg. of this drug had strong anticonvulsant properties against supramaximal and "psychomotor" electroshock seizures in mice as well as against metrazol, strychnine and thujone convulsions. The effects lasted 4 to 5 hours. Double nephrectomy did not increase the duration of action, but it was prolonged in mice in which liver damage had been induced by carbon tetrachloride. The drug had no antipyretic properties and in anæsthetised cats intravenous doses of 20 mg./kg. had little or no cardiovascular actions. Absorption studies showed phenylacetylurea to be absorbed from the small intestine while excretory studies showed that it was not excreted in the urine but possible metabolic products were present. Chronic toxicity studies in rats, dogs and cats over a period of 3 years revealed no significant changes in the organs. The structural activity relations of acetylureas are also discussed. G. F. S.

(ABSTRACTS *continued on p.* 280.)

(*c*) lergigan base (as used for the infra-red spectrum determination), dissolved in dilute hydrochloric acid. Assays were carried out on the isolated guinea-pig ileum. The pharmacological effects of lergigan and promethazine were indistinguishable, and both could be readily differentiated from *iso*promethazine. Thus lergigan was equally as active as promethazine in antagonising the stimulant effects of both histamine and acetylcholine. But though lergigan and promethazine had the same activity as *iso*promethazine against the effects of histamine, either of the former compounds was 20 to 24 times more active than *iso*promethazine in antagonising the effects of acetylcholine. This formed a ready means of distinguishing between them.

N. D. EDGE.
W. R. WRAGG.

Research Laboratories,
May and Baker, Ltd.,
Dagenham, Essex.

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ABSTRACTS (*continued from p.* 278).

BACTERIOLOGY AND CLINICAL TESTS

Skin Disinfectants, Testing of. P. Story. (*Brit. med. J.*, 1952, **2**, 1128.) Strains of *Staphylococcus pyogenes*, *Bacterium coli*, *Pseudomonas pyocyanea* and *Proteus vulgaris* isolated from wound swabs were used as test organisms. 6 circular areas 4 cm. in diameter were outlined on the forearm or thigh and 1 drop of bacterial suspension spread over 5 of these areas, which were left to dry. The first 4 circles and the uninoculated area were treated with disinfectant, applied fairly freely. The areas were sampled after ½, 1, 2 and 5 minutes, by placing a glass ring over the test area, adding 5 ml. of sterile water, rubbing for 15 seconds with a glass spreader and taking 1 ml. of the fluid for viable counting. All the test organisms were penicillin-resistant, and penicillin was added to the culture medium to suppress growth of normal skin flora. In experiments on quaternary ammonium compounds the recovered organisms were mixed with sterile milk before cultivation, to neutralise the bacteriostatic action of the chemical. A disinfectant was considered satisfactory when not more than 1 colony grew per plate, and since at least 10,000 organisms were recovered from each staphylococcal control area, this involved the death of at least 99·9 per cent. of bacteria. Solutions in industrial methylated spirit containing 1 per cent. of iodine, 1 per cent. of cetrimide or 0·1 per cent. of dynium chloride killed all bacteria within 30 seconds. Industrial methylated spirit alone was a satisfactory disinfectant in 30 seconds and 1 per cent. of aqueous iodine was satisfactory against *Ps. pyocyanea* in 30 seconds. A 1 per cent. aqueous solution of domiphen bromide (bradosol), 2 per cent. aqueous cetrimide and 0·1 per cent. zephiran in 50 per cent. ethanol were not consistently effective even when left in contact with the bacteria on the skin for 5 minutes. G. B.