ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Digitalis Glycosides, Colorimetric Assay of. (F. H. L. van Os and D. H. E. Tattje. (*Pharm. Weekbl.* 1951, **86,** 104.) In the Soos modification of the Keller-Kiliani method for digitalis glucosides, the aqueous extract of the leaves, after treatment with lead acetate, is shaken out with chloroform. By this method lower results were obtained from stabilised leaves than from those dried in the ordinary way. This is due to the fact that the primary glycosides (purpurea glucosides A and B) do not enter quantitatively into the reaction. The third digitoxose molecule in these glucosides is combined with dextrose, and this linkage is not broken by treatment with strong acid, so that in fact purpurea glucoside A gives a colour corresponding to only 65 per cent. of its digitoxin equivalent. Secondly, the solubility of the glycoside in chloroform is low, and only about 20 per cent. is recovered by shaking out. G. M.

Carboxylic Acids, Non-volatile, Paper Chromatography of. F. Brown. (*Nature,* 1951, **167,** 441.) It was found that, in the separation of non-volatile carboxylic acids by paper chromatography, the use of ethyl alcohol-ammonia overcame the difficulty that the acids possessing more than one carboxy group did not move from the starting line when *n*-butyl alcohol saturated with 1·5 N ammonium hydroxide was used as the mobile phase. The water content of the solvent could be adjusted to any desired level, the flexibility of the method being greatly increased; the excursions of the acids became greater as the proportion of water in the solvent was increased, so that a pair of acids possessing similar R_F values at low concentrations of water (for example, malic and tartaric acids) could be separated completely by increasing the proportion of water in the solvent. Development of the papers after drying (5 minutes at 95°C.) was carried out by spraying with chlorophenol red (40 mg. in 100 ml. of water adjusted to *p*H 7) revealing the anions as bright yellow spots on a mauve background, or by spraying with ammoniacal silver nitrate solution (equal volumes of 0·1 N silver nitrate and 5 N ammonium hydroxide) and heating at 95°C. for 5 minutes, the citrate, tartrate, maleate and lactate ions then being revealed as intense yellow fluorescent spots under an ultra-violet lamp. The adipate and oxalate ions gave less intense white fluorescent spots. The acids being non-volatile were applied directly to the paper; in general, spots containing approximately 10 μg. of each acid were introduced on the starting line, giving readily detectable spots on spraying, although amounts as low as 5 μg. could be detected. R. E. S.

Morphine, Colorimetric Determination of. J. S. N. Cramer and J. G. Voerman. (*Acta pharm. int.,* 1950, **1,** 219.) The method described by Guarino is modified by the substitution of nickel sulphate for ferric chloride. The 5 principal alkaloids of opium (apart from morphine), as well as meconic acid, do not give a colour in this test. The method is:— dissolve 2 to 8 mg. of morphine or an equivalent of morphine-containing extract in 15 ml. of water. Add 15 ml. of 0·1 N hydrochloric acid and 2 ml. of 5 per cent. iodic acid. Allow the solution to stand for 2 minutes, add

5 ml. of a saturated solution of ammonium carbonate and fill up to 50 ml. with a solution of ammonium carbonate (5 per cent.). Allow the solution to stand for 30 minutes, add 1 ml. of a solution of nickel sulphate (1 per cent.). After mixing, the solution is allowed to stand for 90 minutes and the light transmission is measured in a suitable instrument. Adjust the zero of the instrument by means of a solution prepared in exactly the same way as described above but omitting the solution of iodic acid and replacing it by 2 ml. of water. A. H. B.

Resorcinol in Pastes and Ointments, Determination of. L. A. Welsh. (*J. Amer. pharm. Ass. Sci. Ed.*, 1950, **39**, 686.) The following method avoids mechanical difficulties which are inherent in the assay of the U.S. National Formulary. Place a sample containing 100 mg. of resorcinol in a separating funnel, and 10 to 15 ml. of light petroleum and swirl until the paraffins have been extracted and the solid ingredients have separated as a powder. Add 30 ml. of 5 per cent. hydrochloric acid, shake for a few minutes, allow to separate, and filter the aqueous layer. To a 20 ml. aliquot add 20 per cent. sodium hydroxide solution until neutral methyl red. Add 50 ml. of 0·1 N bromine and complete the determination by titration with sodium thiosulphate in the presence of potassium iodide as in the U.S.P. XIII assay for resorcinol. The method is suitable for strong resorcinol paste (20 per cent.) and resorcinol ointment (5 per cent.), and analytical results for these preparations are given. G. B.

Rutin, in Tablets, Determination of. E. B. Dechene. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 93.) The powdered tablets were extracted with hot ethanol for 8 to 10 hours, and the resulting solution assayed by treating with aluminium chloride and potassium acetate, allowing to stand for 40 minutes and measuring the transmittance of the yellow colour at a wavelength of 415 mμ spectrophotometrically. The concentration of rutin was obtained from a standard curve prepared by treating various dilutions of a standard solution of rutin containing 50 μg./ml. in a similar manner. The yellow colour reached a maximum intensity after 30 minutes and appeared to be stable for several hours. 95 to 105 per cent. of the expected amount of rutin was recovered and there was no interference from other ingredients of the tablet, including aminophylline, phenobarbitone, mannitol hexanitrate and ascorbic acid. Rutin and quercetin were separated by paper partition chromatography using a mixture of 4 parts of *n*-butanol, 5 parts of water and 1 part of glacial acetic acid. The position of the fluorescent spots was found by spraying with aluminium chloride solution and potassium acetate solution and examining by ultra-violet light. When the alcoholic extracts were treated thus there was no evidence that the rutin had hydrolysed in any of the tablets examined. G. R. K.

Silver Nitrate as a Test for *ortho* and *para* Dihydric Phenols. B. S. Wildi. (*Science*, 1951, **113**, 188.) It is shown that in general aromatic compounds containing two or more phenolic hydroxyl groups, which are *ortho* and *para* to each other, readily react with neutral silver nitrate in alcoholic solution to produce a silver mirror and give a red or gold coloured solution. Aromatic compounds possessing a single phenolic hydroxyl group, or two or more hydroxyl groups *meta* to each other, do not react. Substances which form insoluble silver salts in the alcoholic solution do not give a positive test even though they possess the requisite structural features. The test is carried out by dissolving 5 to 10 mg. of the compound in 5·0 ml.

of 95 per cent. ethanol in a clean test tube. 3 drops of the silver nitrate test reagent (1·0 g. of silver nitrate in 8.0 ml. of distilled water) are added, and the solution shaken for 15 minutes. If a negative or very faint test is only apparent after 10 minutes' shaking, the test tube is warmed in a water-bath at 60°C. for 1 minute. A. H. B.

Thyroxine in Thyroid Gland, Determination of. K. E. Gronkvist and H. Hellberg. (*Farm. Revy.*, 1951, **50,** 189.) The paper reports the results of unsuccessful attempts to isolate and determine thyroxine in thyroid gland. The thyroxine-containing protein was hydrolysed by the method of Blan (*J. biol. chem.*, 1933, **102,** 269; 1935, **110,** 251) using barium hydroxide solution followed by acidification and extraction with *n*-butanol. The final thyroxine determination was made by two methods, the classical volumetric iodine method and also by a photometric procedure based on the nitrosation of thyroxine. Although good agreement was obtained between both methods, several signs were found which indicated that the agreement was fortuitous and that the butanol fraction did not contain thyroxine as the only reacting constituent. Examination of the butanol fraction by partition chromatography using kieselguhr as the supporting medium showed that only a minor part of the apparent thyroxine determined by the two methods employed was really free thyroxine. This was confirmed by the fact that the absorption spectrum of the colour produced on nitrosation differed markedly from that obtained using pure thyroxine. Details are given of the nitrosation method and of the chromatographic procedure. R. E. S.

ESSENTIAL OILS

***Artemisia vulgaris* L. Root, Essential Oil of.** K. Stavholt and N. A. Sørensen. (*Acta chem. scand.*, 1950, **4,** 1567.) Steam distillation of the root yielded 0·02 per cent. of a yellow brown oil which deposited crystals on cooling. The crystals were purified from light petroleum and were found to be a methyl-*n*-decene-triynoate, $C_9H_5COOCH_3$ (dehydromatricaria ester). Chromatographic analysis of acetone extracts of the root showed that this unsaturated compound occurred as such in the plant. The root also contained an unsaturated hydrocarbon with an ultra-violet spectrum very similar to that of "Centaur X," a substance discovered by Löfgren in *Centaurea* species, and a crystalline acetylenic carbonyl compound, $C_{12}H_{10}O$, m.pt. 52° to 52.5°C. G. R. A. S.

Ascaridole, Synthesis of. A. Halpern. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40,** 68.) Ascaridole was prepared by passing oxygen for 6 hours through a solution of α-terpinene in alcohol and benzene irradiated by ultra-violet light from a mercury vapour lamp. The solvent was evaporated at reduced pressure and the residual oil fractionated in vacuo. The α-terpinene was obtained from cardamom oil by distillation. Among the by-products in the preparation of ascaridole were *p*-cymene, 1:4-oxido-2-*p*-menthene, a polymeric fraction and an unidentified ketone; the yield of ascaridole was about 25 per cent. Its identity was confirmed by rearrangement; it was shaken with ferrous sulphate solution, extracted with ether, the ether evaporated and the residue benzoylated. The ascaridole-glycol monobenzoate so obtained was converted to the dibenzoate by treating in *p*-cymene solution with benzoic anhydride at 130°C. Hydrolysis of the dibenzoate and oxidation with potassium permangate yielded 1:4-cineolic acid. Since α-terpinene has been

reported to be present in oil of chenopodium in small amounts it may be regarded as the precursor of ascaridole in biogenesis in the plant. Similarly the formation of *p*-cymene in the reaction is also consistent with its presence in whole chenopodium oil. G. R. K.

***Erigeron*, Essential Oils of Some Species of.** N. A. Sørensen and K. Stavholt. (*Acta chem. scand.*, 1950, **4**, 1575.) The essential oils of *Erigeron acre* L, *E. boreale* (Vierh.) Simm, *E. canadense* L, *E. politum* Fr., and *E. uniflorum* L. all contained enzyme derivatives of methyl caprate. Lachnophyllum ester $C_9H_9COOCH_3$ was present to the extent of 81 per cent. in the flower oil and almost 100 per cent. in the oil from the non-floral parts of *E. acre* L; this ester was also found in *E. uniflorum* L. Matricaria ester was present in the oils of *E. boreale* (Vierh.) Simm, *E. canadense* L, *E. politum* Fr. and *E. uniflorum* L. Dehydro-matricaria ester $C_9H_5COOCH_3$ and "Centaur X" of Löfgren were found in *E. canadense* L only. Hexahydro-matricaria ester ("Composite cumulene I") was found in *E. uniflorum* L. only. G. R. A. S.

FIXED OILS, FATS AND WAXES

***Macadamia ternifolia*, Seed Fat of.** R. E. Bridge and T. P. Hilditch. (*J. chem. Soc.* 1950, 2396.) The evergreen tree of *Macadamia ternifolia* (Proteaceæ) is cultivated in New South Wales, Southern Queensland, Hawaii and California. It bears hard woody nuts, containing a single seed with a high content of a fatty oil resembling olive oil in general character. The analytical characteristics of the oil have been determined and are in close agreement with earlier data, as follows: iodine value 74·8, saponification value 292·0, free fatty acid (as oleic) 0·5 per cent., unsaponifiable matter 1 per cent. The mixed fatty acids of the oil were found to contain a considerable proportion of an ethenoid acid other than oleic acid which was characterised as hexadec-9-enoic (palmitoleic) acid. Detailed examination of the mixed fatty acids led to the following composition, myristic 1·6, palmitic 8·0, stearic 3·3, arachidic 2·2, behenic 0·8, hexadecenoic 20·4, oleic 59·3, linoleic 2·2 and eicosenoic 2·2 per cent. (wt.). The high proportion of hexadecenoic acid is unusual, though it has been reported in fats from other botanical sources.

ORGANIC CHEMISTRY

Chloroform-*d*. A New Preparation of. W. M. Boyer, R. B. Bernstein, T. L. Brown and V. H. Dibeler. (*J. Amer. chem. Soc.*, 1951, **73**, 770.) A new synthesis of chloroform-*d* by the reaction of trichloroacetophenone with sodium deuteroxide gave a product of 99·2 per cent. isotopic purity. It was shown that isotopically pure $CDCl_3$ is not obtained by the reaction of chloral with sodium deuteroxide, and that the chloroform dilution of the product does not occur through the mechanism of protium exchange with the solvent. Mass spectrometric, infra-red spectrophotometric, and density measurements were used in the quantitative investigation of the products and the reactions. A. H. B.

Chloroform-*d*. A New Synthesis of. M. H. Earing and J. B. Cloke (*J. Amer. chem. Soc.*, 1951, **73**, 769.) Chloroform-*d* was prepared in good yields by the action of deuterium oxide on anhydrous calcium trichloroacetate as follows.

$$(Cl_3C.COO)_2Ca + D_2O \longrightarrow 2\ CDCl_3 + CaCO_3 + CO_2.$$

Dehydration under diminished pressure was used to prepare anhydrous calcium trichloroacetate from the air-dried material. The infra-red absorption spectrum of the product of the above reaction was measured, and the purity determined by this means and by the density increment. A. H. B.

PLANT EXTRACTS

Rutin, Isolation, Purification and Derivatives of Plant Pigments related to. T. J. Haley and M. Bassin. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 111.) Morin (3:5:7:2′:4′-pentahydroxyflavone) was obtained from sawdust from *Morus tinctoria* by extraction with a large volume of boiling distilled water for about 26 hours, concentrating to low volume *in vacuo* at 35°C., and separating the amorphous yellow precipitate by centrifuging. The powder was dried *in vacuo*, extracted with boiling ethanol, filtered and the filtrate diluted with water. The morin which separated was removed and dried over anhydrous calcium chloride. The yield was further increased by extracting the original solution with ether. The total yield from 300 g. of sawdust was 2·5 g. after purification by extraction with water, solution in boiling ethanol and precipitation by the addition of water. The morin was obtained as silky, almost colourless needles subliming at 289° to 290·5°C. and melting in a sealed tube at 303° to 304°C. Maclurin (2:4:6:3′:4′-pentahydroxybenzophenone) was obtained from the aqueous mother liquor after the removal of the morin by allowing to stand in a refrigerator for 7 days, centrifuging, dissolving the residue in boiling water, filtering and allowing to stand in the refrigerator overnight. The maclurin was recrystallised from ethanol and obtained as faintly yellow needles, m.pt 222° to 222·5°C.; yield 1·45 g. Iridin was obtained in a yield of 10 g., m.pt. 208·4° to 208·8°C. by extracting 1 kg. of orris root with four 2-litre quantities of boiling ethanol, and allowing the combined extract to stand at 5°C. for 3 days. The crude precipitate thus obtained was purified by extraction with ethanol, ether and finally water, and the milky white suspension of iridin filtered, washed with cold water and dried. The preparation of irigenin by hydrolysis of iridin, and its demethylation to irigenol, are also described, together with the preparation of hesperetin and naringenin by the hydrolysis of hesperidin and naringin respectively. The pentamethyl ethers of maclurin and morin, the trimethyl ether of narigenin and the dimethyl ether of hesperetin were obtained by treating the flavonoid in acetone solution with methyl sulphate and potassium carbonate. Irigenol hexa-acetate was prepared by boiling irigenol for 4 hours with an excess of acetic anhydride containing a few drops of pyridine. G. R. K.

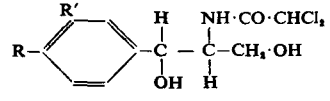
BIOCHEMISTRY

GENERAL BIOCHEMISTRY

***p*-Aminobenzoic Acid, Glucuronic Acid in Urine of Patients Treated with.** C. J. D. Zarafonetis and J. P. Chandler. (*J. Lab. clin. Med.*, 1951, **37**, 425.) Previous observations having shown that a reducing substance is present in the urine of patients treated with *p*-aminobenzoic acid, investigations have been carried out on 2,000 specimens of urine collected from patients undergoing treatment with the sodium or potassium salt. Dosage ranged from 0·8 g. per day to 48 g. per day orally, the total quantity being given in divided doses. The samples were tested with Benedict's solution, and a number

of determinations of total glucose were carried out. Some samples were subjected to yeast fermentation tests, osazone reactions, the resorcinol test for fructose and Tollens test for glucuronic acid. Patients receiving 18 g. daily, or more, usually gave a positive response within 24 hours. The yeast fermentation test gave a negative response owing to the inhibitory action of acid excreted as such, and negative responses were also obtained in the test for fructose. Strongly positive responses were obtained in the Tollens test and the presence of glucuronic acid was confirmed by the reaction with phenylhydrazine, the osazone crystallising out on cooling the reaction mixture whereas glucosazone is precipitated when the reaction mixture is heated on the water-bath. The authors have observed hypoglycæmia in patients undergoing treatment with *p*-aminobenzoic acid but doubt whether the production of glucuronic acid, whether from body glucose or liver glycogen, is sufficient to account for it. H. T. B.

Chloramphenicol, Analogues of. N g. P h. B u u - H o i, N g. D. X u o n g, and N g. K. K h o i. (*J. chem. Soc.*, 1951, 255.) Three analogues of chloramphenicol (I) were synthesised, namely II, III and IV.



(I) : R = NO_2, R′ = H.
(II) : R = Me , R′ = H.
(III) : R = OMe, R′ = H.
(IV) : R = R′ = Cl.

The usual synthetic approach was adopted. Screening bacteriological tests showed compounds (II) and (III) to have an extremely low degree of activity against *Escherichia coli* and *Staphylococcus aureus*. Compound (III) showed notable activity against *Shigella paradysenteriæ* and *Staph. aureaus*. A. H. B.

Foot and Mouth Disease Virus, Compound with Serum Albumen and Protamine. A. H a n s e n and P. H o l m. (*Dansk. Tidsskr. Farm.*, 1951, **25**, 72.) Virus of foot and mouth disease, treated with serum albumin, shows an increased resistance to drying and temperature effects. In the compound formed with protamine the virus is not stable, and soon loses its virulence. Both albumen and protamine form compounds which are insoluble in water at low concentrations of electrolytes. By the addition of both protamine and albumen to the virus, precipitation occurs more slowly than with protamine alone. The compound is soluble in N sodium chloride solution, and the solution shows the full activity of the virus. The danger of excess of protamine, which rapidly destroys the activity, is greatly reduced by this addition of albumen, which combines with the excess of protamine. The method of production is as follows: 100 ml. of a 10 per cent. extract of virulent material is diluted to 500 ml. with M/1500 phosphate buffer (*p*H = 7·5), poured into 500 ml. of 1 per cent. bovine albumen solution at *p*H 7·5, and filtered through hard paper. Precipitation is carried out in stages, first with 0·150 g. of clupeine sulphate in 150 ml. of water, then after 15 minutes with 0·086 g. of clupeine sulphate in 100 ml. of water, and finally, after 18 hours, at 4°C., with 0·044 g. of clupeine sulphate in 50 ml. of water. After standing for 24 hours at 4°C., the precipitate is separated by decantation and centrifuging, washed with buffer solution and suspended in 1000 ml. of phosphate buffer with the aid of a Waring Blendor. This preparation contains the virus at full potency. By irradiation with ultraviolet light it is detoxicated without losing its antigenic effect. The addition of an equal volume of aluminium hydroxide gel to the albumen-protamine

virus increases the antigenic effect, and a dose of 1 ml. of the mixture (equivalent to 5 mg. of virulent material) per head of horned cattle produces satisfactory immunity. G. M.

Fradicin. R. H. Hickey and P. H. Hidy. (*Science*, 1951, **113**, 361.) An antifungal agent, presumably fradicin, produced by *Streptomyces fridiæ*, is described. It is obtained as light, greenish-yellow crystals, fairly soluble in propylene glycol, very sparingly soluble in methanol, ethanol and water, and practically insoluble in light petroleum, cyclohexane and xylene. Fradicin is a weak base, which forms a hydrochloride obtained as needles. The ultra-violet absorption spectrum is recorded. Antifungal activity is strong at *p*H 7·0 and higher, but below this *p*H activity is greatly reduced. Activity against several yeasts is present, but activity against bacteria is poor. The LD50 using mice (intraperitoneal injection and orally) is approximately 4 mg./kg. Skin tests employing rabbits have shown that, in a hydrophilic ointment, irritation is produced. A. H. B.

Streptomycin, Decomposition of. D. Pramer and R. L. Starkey. (*Science*, 1951, **113**, 127.) Soils that had been treated with streptomycin and also slime from a disposal system where waste from a streptomycin plant was treated were inoculated into a mineral salts medium containing streptomycin as the only organic constituent. Bacteria developed in the medium and continued to grow in the same medium through several transfers. The crude cultures were planted on a nutrient agar medium containing streptomycin 1,000 μg./ml. Various colonies were isolated from the plates and inoculated into the specific streptomycin medium. Several of the cultures grew and inactivated the streptomycin. The fact that the cultures were able to grow in the medium where streptomycin was the only organic constituent indicates that the streptomycin molecule must have been decomposed and that the inactivation was not due to some product of growth. There was little or no loss of streptomycin from the solution medium supporting growth of crude and pure cultures for the first few days, but after 10 to 14 days all streptomycin activity had disappeared. All the active bacterial cultures were alike. The bacterium is a motile, non-sporulating, Gram-negative rod producing a greenish-yellow pigment on nutrient agar; it is probably a *Pseudomonas*. S. L. W.

BIOCHEMICAL ANALYSIS

***p*-Aminosalicylic Acid and Sulphonamides in Body Fluids, Determination of.** E. I. Short. (*Biochem. J.*, 1951, **48**, 301.) The difficulties which arise in the estimation of *p*-aminosalicylic acid in the presence of diaminodiphenylsulphone derivatives and sulphonamides have been studied. Dimethylaminobenzaldehyde reacts with free aryl amino groups yielding characteristic lemon-yellow compounds, which can be used for the estimation of *p*-aminosalicylic acid. The sulphonamides give a similar reaction, and comparison of the visible absorption spectra of the coloured solutions at equivalent concentration showed that the intensities of the colours were approximately equal. Sulphetrone also gave a colour with *p*-dimethylaminobenzaldehyde, but it was much less intense, and concentrations in the coloured solution of less than 0·2 mg./ml. did not differ significantly from the blank; at the dilutions necessary for the estimation of *p*-aminosalicylic acid (generally 1/100), this would correspond to concentrations of sulphetrone in the blood (at least 20 mg./100 ml.) much higher than those normally

encountered. This method could therefore be used for the estimation of *p*-aminosalicylic acid in the presence of sulphetrone but not in the presence of sulphonamides. For the estimation of *p*-aminosalicylic acid in the presence of sulphonamides the red colour of diazotized *p*-nitraniline when added to a solution of *p*-aminosalicylic acid followed by sodium hydroxide was used; a comparison of the visible absorption spectra for sulphathiazole, sulphanilamide, sulphaguanidine, and *p*-aminosalicylic acid after coupling with diazotized *p*-nitraniline and making alkaline with sodium hydroxide, was made and indicated that solutions of 16 mg./100 ml. of the sulphonamides showed absorptions which did not differ significantly from that of the blank solution. This method was suitable for general purposes and could be used to determine *p*-aminosalicylic acid in the presence of sulphonamides, although its sensitivity was only two-fifths of that in the determination employing *p*-dimethylaminobenzaldehyde. Details are given for the estimation of the drugs quoted in blood, urine, and tissue filtrates, together with the results of recovery experiments. R. E. S.

Antibiotics, Turbidimetric Assay of. N. J. Berridge and J. Barrett. (*Nature*, 1951, **167**, 448.) The method described enables assays to be carried out within 30 minutes by the use of a culture of a test organism *Streptococcus agalactiæ* in its logarithmic phase of growth. By subculturing twice daily, a rapidly growing strain is maintained and by limiting the incubation period during the night a vigorous culture is obtained on the following morning. Serial dilutions of the antibiotic are prepared in sterile broth by a special technique employing a syringe with a spring-activated plunger. It was found possible to prepare sets of serial dilutions with 7 dilutions in each set at the rate of one set per minute using the technique outlined, a hand-operated ampoule-filling machine was used for adding rapidly and with sufficient mixing the required amount of logarithmic culture of test organism to each dilution of the antibiotic. After 30 minutes' incubation, 0·02 per cent. thiomersalate solution was added as violently as possible to each tube, again using a spring-operated syringe. Readings of turbidities on a photo-electric absorptiometer followed by the usual graphical comparison between standard and unknown solution enabled the concentration of the latter to be calculated. The method has proved satisfactory for " nisin " preparations and preliminary experiments indicate that it might be successful with penicillin, streptomycin, aureomycin and gramicidin. R. E. S.

Benzoic and Hippuric Acids, Micro-estimation of. F. Dickens and J. Pearson. (*Biochem. J.*, 1951, **48**, 216.) The method used is based on the nitration of benzoic acid at room temperature, reduction of the resulting mononitrobenzoic acid to aminobenzoic acid, diazotisation of the latter and coupling with a colour-forming base. The estimation is completed by colorimetric estimation of the resulting dye and is here described for samples containing 0·05 to 10 mg. of benzoic acid or equivalent amounts of hippuric acid. After evaporation to dryness of the sample and nitration with 10 per cent. potassium nitrate solution in sulphuric acid, the nitrated sample is diluted with water and extracted with *iso*-amyl alcohol; the nitro-compounds are extracted with aqueous alkali and are reduced with titanous chloride after acidification. After diazotisation and coupling with *N*-(1-naphthyl)-thylene-diamine the resulting colour is measured photoelectrically using a green light filter (Ilford green No. 604, 5,000 to 5,400 A.). Details of the procedure are given together with a specimen calibration curve that is required in the

estimation. The mean percentage errors in the estimation of pure solutions of benzoic and hippuric acids were: over the range 0·1 to 1·0 mg. benzoic acid, −6 per cent. (19 observations); hippuric acid +3 per cent. (10 observations); over the range 1 to 10 mg. benzoic acid, +4 per cent. (23 observations); hippuric acid, −0·1 per cent. (12 observations). In dealing with samples of blood the proteins are precipitated with ethyl alcohol, while for urine, owing to the presence of phenols and other interfering substances preliminary treatment with bromine is necessary. Interference is caused by the presence of any aromatic substance capable of undergoing nitration in the cold to give an acidic nitro-compound. R. E. S.

Magnesium in Body Fluids, Microestimation of. M. Orange and H. C. Rhein. (*J. biol. Chem.*, 1951, **189**, 379.) A modification of the Titan yellow method for the accurate estimation of magnesium on 0·1 ml. of serum is described; substantially the procedure is that reported by Garner (*Biochem. J.*, 1946, **40**, 828) and Heagy (*Canad. J. Res., Sect. E.*, 1948, **26**, 295), except that the final volume is reduced and the light absorption path in the spectrophotometer is increased. The use of trichloracetic acid as a protein precipitant is retained and the method has been adapted for the estimation of magnesium in whole blood, red blood cells and in urine. As a dispersing agent for the final colour measurement, polyvinyl alcohol was preferred in which case the absorption was measured at 560 mμ; if gum ghatti was used the absorption maximum was at 540 mμ. In recovery experiments specimens of serum, whole blood, red blood cells, and urine were mixed with equal volumes of 0, 1, 3, and 5 mg. per cent. of Mg standards respectively; 0·1 ml. portions were then analysed, the average recovery for serum being 100·88±2·09 per cent.; for whole blood 99·91±2·74 per cent.; for red blood cells 100·47±2·33 per cent.; and for urine 99·82±1·77 per cent. Calcium, oxalate, and citrate did not interfere and a method for eliminating interference from iron is given. Normal values for the magnesium content of human serum, whole blood, and red blood cells are given. R. E. S.

Penicillin, Iodimetric Titration of. K. Ilver, O. I. Johansen and F. Reimers. (*Acta pharm. int.*, 1950, **1**, 225.) In an earlier communication it was shown that the concentration of potassium iodide in 0·01 N iodine, used in the iodimetric titration of penicillin, is of decisive importance. The present communication gives the results of the examination of the significance of the *p*H value and the iodide concentration on the following. 1. The size of the blank and its dependence on the reaction time. 2. Consumption of iodine on titration of the hydrolysed penicillin after different reaction times. 3. The dependence of the experimental results on the temperature. For the experiments, purified crystalline benzylpenicillin sodium, commercial crystalline benzylpenicillin sodium, impure crystalline benzylpenicillin calcium, and commercial crystalline procaine benzylpenicillin were used. The experiments show that, for these salts, it is most advantageous to use 0·01 N iodine, 0·05 N with respect to iodide, and to carry out the determination at *p*H 4 to 6, as small changes in the *p*H or iodide concentration under these conditions cause the least alteration in the experimental results. The temperature must be fixed, e.g. 19° to 21°C. An iodide concentration of 0·05 N and a constant temperature should be chosen for the titrations where the blank is determined at *p*H about 5 to 6, and the hydrolysed penicillin is titrated at *p*H about 2. A. H. B.

Stilbœstrol in Urine, Determination of. R. S. Teague and A. E. Brown. (*J. biol. Chem.*, 1951, **189**, 343.) A method is given for the quantitative determination of stilbœstrol and its glucuronide in urine using the colour reaction between stilbœstrol and antimony pentachloride. The urine is acidified, extracted with ether, the extract purified and re-extracted with sodium bicarbonate solution. Hydrolysis of the glucuronide in the sodium bicarbonate solution is accomplished by acidification, extraction with ether and evaporation of the ether extract; the residue is taken up in alkali, acidified to *p*H 3·45 and then autoclaved for 90 minutes at 180°C. The resulting solutions are extracted with ether and purified before determination with antimony pentachloride. Details of all procedures are given together with a calibration curve of the red colour produced. A study of the colours given by compounds likely to interfere has been made; the solutions should be free from indicators of the phthalein series, phenolic compounds and lipides, although these substances are mainly removed in practice. Results are reported on experiments made to determine the distribution of stilbœstrol and its glucuronide between immiscible solvents; the conditions for the hydrolysis of stilbœstrol glucuronide were also studied. The method could be applied to the estimations in urine and in blood. For blood, 81 to 102 per cent. of added stilbœstrol was recovered; for urine good recoveries were obtained with concentrations of stilbœstrol above 2 μg. per ml., the precision being 97·8 per cent. ±1·1, while above 20 μg. per ml. the glucuronide precision was 95·7 per cent. ±1·4. R. E. S.

PHARMACY

GALENICAL PHARMACY

Bentonites, Cation-saturated, as Constituents of Ointment Bases. M. Barr and E. P. Guth. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 13.) Ointment bases of comparable consistency may be prepared by stirring Volclay bentonite (20 g.) sodium bentonite (13 g.), potassium bentonite (19 g.), hydrogen bentonite (40 g.), calcium bentonite (38 g.) or magnesium bentonite (35 g.) with 10 g. of glycerin and sufficient water to produce 100 g. The brownish colour and alkalinity of the bases decrease in the order Na, K, Mg, Ca, H. The following medicaments have been incorporated: sulphathiazole, ammoniated mercury, boric acid, phenol and iodine. There is a loss of consistency and fall in *p*H when boric acid is incorporated into sodium, potassium or Volclay bentonites. When tested for bacteriological activity by the F.D.A. cup-plate method, the ointments in bentonite bases show greater antibacterial activity than those with fatty bases. There is no difference in antibacterial activity between Volclay, Na, K, Ca and Mg bentonites, but sulphathiazole, ammoniated mercury and phenol show greater antibacterial action in the hydrogen bentonite base. G. B.

NOTES AND FORMULÆ

Cyclamate Sodium (Sucaryl Sodium). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1951, **145**, 823.) Cyclamate Sodium is sodium *cyclo*hexylsulphamate, $C_6H_{11}NH.SO_3Na$, and occurs as a white, crystalline, practically odourless powder with a very sweet taste. It is freely soluble in water and practically insoluble in alcohol, benzene, chloroform and ether; a 10 per cent. aqueous solution has *p*H 5·5 to 7·5, and gives a white precipitate on the

addition of hydrochloric acid, sodium nitrite and barium chloride and on the addition of silver nitrate. Cyclamate sodium contains not more than 20 p.p.m. of heavy metals; when dried at 105°C. for one hour, it loses not more than 1·0 per cent. of its weight. It contains 6·8 to 7·1 per cent. of nitrogen, equivalent to 98·0 to 102·0 per cent. of cyclamate sodium, and is assayed by semi-micro Kjeldahl; the ammonia is distilled into boric acid solution and titrated with 0·02N sulphuric acid, using a mixture of methyl red and bromocresol green as indicator. Cyclamate sodium is a sweetening agent. G. R. K.

Hexachlorophene (Gamophen, Hex-O-San). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1951, **145**, 563.) Hexachlorophene is 2:2′-methylenebis-(3:5:6-trichloro)phenol and occurs as a white to light tan crystalline powder which is odourless, or has a slight, phenolic odour; m.pt. 161° to 167°C.; soluble in acetone, alcohol, chloroform and ether; insoluble in water. When heated it melts to a colourless or amber liquid which on further heating becomes green, blue and finally purple. The addition of ferric chloride to an alcoholic solution gives a transient purple colour. A yellowish orange oil which is soluble in benzene, chloroform and ether separates when a 20 per cent. solution in acetone is shaken with a 5 per cent. solution of titanium trichloride. When a solution in pyridine is treated with benzoyl chloride and poured into water, an oily residue separates; after washing with sodium carbonate solution and water, extracting with ether, drying and evaporating the ether, the residue melts at 145° to 158°C. after recrystallisation from alcohol and drying at 105°C. for one hour. Hexachlorophene loses not more than 0·1 per cent. when dried at 105°C. for 2 hours; the residue on ignition is not more than 0·1 per cent. It is assayed spectrophotometrically; the solution is prepared by diluting 1 ml. of a 0·2 per cent. solution in acetone to 100 ml. with a 0·025 per cent. solution of sodium carbonate, treating aliquots of 5, 10, 15 and 20 ml. with 1 ml. of a 2 per cent. solution of 4-aminoantipyrine, and diluting each to 50 ml. with the sodium carbonate solution. Before measuring the optical density at 4800 Å, using water as a blank, each solution is mixed with 0·5 ml. of an 8 per cent. solution of potassium ferricyanide and allowed to stand for 5 minutes. At a concentration of 0·2 mg. of hexachlorophene per 50·5 ml., the optical density should be between 0·422 and 0·448, equivalent in transmission to 38·0 to 35·8 per cent. and equivalent to 97 to 103 per cent. of hexachlorophene. Hexachlorophene is incorporated into soaps as an antibacterial agent in skin sterilisation. G. R. K.

Sodium *p*-Aminosalicylate (Pasem Sodium). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1951, **145**, 905.) Sodium *p*-aminosalicylate, $C_7H_6NNaO_3$, $2H_2O$, is a white to pale yellow, almost odourless, crystalline powder, freely soluble in water, sparingly soluble in alcohol and practically insoluble in ether; a 2 per cent. aqueous solution is clear and colourless and has *p*H 7·0 to 7·5. When an aqueous solution is acidified to bromophenol blue a precipitate of the free acid is obtained. A 0·0006 per cent. solution of the anhydrous salt exhibits ultraviolet absorption maxima at about 2650 Å ($E_{1\,cm.}^{1\ per\ cent.}$, about 748) and 2990 Å, with minima at 2440 and 2850 Å. The ratio of the optical densities at 2650 and 2990 Å is 1·50 to 1·56; sodium *p*-aminosalicylate contains not more than 25 p.p.m. of heavy metals and not more than 7·5 p.p.m. of arsenic, and loses 15·8 to 18·5 per cent. of its weight when dried at 105°C. for 5 hours. It yields sulphated ash equivalent to 12·8 to 13·8 per cent. of sodium. The amount of anhydrous salt present is

96·0 to 102·0 per cent. It is determined by measuring the optical density of a 0·0006 per cent. buffered solution at 2650 Å; multiplying the result by 74·8 gives the number of mg. per ml. Sodium *p*-aminosalicylate is used for the same purposes as *p*-aminosalicylic acid. G. R. K.

PHARMACOGNOSY

***Datura stramonium*, Alkaloid formation in.** R. Hegnalier. (*Pharm. Weekbl.*, 1951, **86**, 321.) It has been suggested that with Solanaceous plants, alkaloid formation occurs only in the roots. Half leaves of *Datura stramonium* were supplied with sugars and nitrogen by the vacuum infiltration method of Mothes, but no increase in alkaloidal content was detected. This is possibly associated with the absence of the midrib, which is especially rich in alkaloids. *Datura* grafted on tomato contained small amounts of mydriatic alkaloids in all parts; whereas in tomato grafted on *Datura* the amount of such alkaloids was considerably greater, but the distribution was quite different from that in normal *Datura* plants. The interpretation of these results is somewhat difficult, as it is not known whether tomato plants contain traces of mydriatic alkaloids. In the young plants of *Datura*, scopolamine is formed almost exclusively; as the plant gets older, the hyoscyamine predominates. G. M.

Water-soluble Embedding Materials for Botanical Microtechnique. R. L. V. Horne and L. C. Zopf. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 31.) The use of "Carbowaxes" has the advantage of somewhat quicker infiltration and embedding. The following process is less liable to cause distortion or shrinkage than dehydration with alcohol and embedding in paraffin. Specimens are dehydrated by passing them through graded mixtures of polyethylene glycol and water, finishing with pure polyethylene glycol 400 W. The embedding medium is prepared by melting together "Carbowax" 4000 (15 to 25 per cent.), "Tween" 20 (1 to 2 per cent.) and "Carbowax" 1540, and allowing the mixture to congeal without hardening. The proportions of the "Carbowaxes" may be varied in order to obtain a medium of suitable hardness and melting-range. Embedding requires 12 hours (more for woody specimens), and the blocks, after cooling rapidly in a refrigerator, are best sectioned at 10° to 15°C. Special precautions are necessary to obtain flattening of tissue when mounting; delicate tissues may have to be fixed to the slide with celloidin. "Carbowaxes" are water-soluble, and care must be taken to avoid excessive humidity. G. B.

PHARMACOLOGY AND THERAPEUTICS

Aureomycin in the Treatment of Bacterial Endocarditis. H. W. Spies, H. F. Dowing, M. L. Lepper, C. K. Wolfe and E. Caldwell. (*Arch. int. Med.* 1951, **37**, 66.) Aureomycin either alone or in combination with penicillin is a valuable agent in the treatment of endocarditis. 9 cases of endocarditis caused by α- and β-hæmolytic streptococci, pneumocci and staphylococci are reported. 2 patients recovered with aureomycin therapy, 1 recovered under combined penicillin and aureomycin therapy, and 1 failed to improve with aureomycin but recovered when given penicillin. 2 patients with acute bacterial endocarditis died of cardiac complications rather than from the infection itself. In acute endocarditis therefore aueromycin may

bring the infection under control, but there remains the grave danger of death from a ruptured valve cusp or from congestive heart failure. Aureomycin may be used in place of penicillin in the treatment of acute bacterial endocarditis, when the organism is resistant to the latter antibiotic, or in conjunction with penicillin when *in vitro* studies show that the 2 antibiotics exert a synergistic action on the infecting organism. No definite conclusions can be reached regarding the place or aureomycin in the treatment of subacute bacterial endocarditis. It is probably the best policy to reserve aureomycin for those cases in which the streptococcus is resistant to penicillin but very sensitive to aureomycin. If the organism is not sensitive to aureomycin alone, *in vitro* tests should be made to determine sensitivity to combinations of antibiotics and that combination used which shows greatest promise of success. G. R. B.

Intracorneal Infection in Rabbits for Testing Antituberculous Substances. P. A. Gardiner, R. J. W. Rees and J. M. Robson. (*Brit J. Pharmacol.*, 1949, **4**, 209.) The paper describes an *in vivo* method, which is simple to perform and permits observation of the tuberculous lesion and comparison with a control lesion throughout the chemotherapeutic trials. The experiments were performed on mature rabbits of both sexes, using a bovine strain of *Mycobacterium tuberculosis*. It was found that an inoculum containing approximately 300 tubercle bacilli consistently produced a suitable progressive tuberculous lesion of the cornea. The lesions were produced by intracorneal injection with a tuberculin syringe into rabbits deeply anæsthetised with ether. A white bleb was produced in the cornea, and, by restricting the bleb to about 5 mm. in diameter (which gives an inoculum volume of about 0·03 ml. of a suitably diluted standardised suspension of organisms) uniformity in size of inoculum and lesion was obtained. Single or multiple minute white primary lesions appear in the needle track and rapidly progress in size. At the same time caseation occurs at the centre of the lesion which, at about the 13th day, breaks down to form an ulcer. It was found that with a standard inoculum the lesion is preceded by a reasonably constant latent period and when the development of the lesions is studied quantitatively a remarkably equal rate of progress is observed. The standard lesion is therefore constant and reliable enough to enable the effects of antituberculous substances to be studied. The substances to be tested are given by intravitreous injection. Streptomycin and *p*-aminosalicylic acid both give results with this method comparable with those obtained with other *in vivo* tests which are more laborious and time-consuming. 30 days would be sufficient to give a preliminary indication whether a drug was active or not against a developed lesion. S. L. W.

Isopropyl Chloride, Anæsthetic Properties of. J. E. Elam and M. L. Newhouse. (*Brit. med. J.*, 1951, **1**, 13.) The use of isopropyl chloride in 50 cases showed it to have many of the properties requisite to an ideal anæsthetic. It appeared equally satisfactory for both major and minor surgery; induction was pleasant and smooth, good muscular relaxation was provided and recovery was rapid. Unfortunately, a high incidence of cardiac irregularities was noted and there was one death from complete circulatory failure. A study of electrocardiographic records obtained during administration of the anæsthetic to two healthy young men gave evidence that isopropyl chloride exerts a direct toxic action on the myocardium and it should be used only with extreme caution. S. L. W.

Œstrogenic Effect of Extract of Cortex Cinnamomi Ceylanici. A. Nöding, K. Fr. Stöa and A. Nordal. (*Acta pharm. int.*, 1950, **1**, 243.) Because of the reputed action of cinnamon preparations as emmenagogues, four extracts of cinnamon bark were investigated for œstrogenic effect. Castrated female mice and rats were used. Two of the extracts produced positive vaginal smears in mice. The effect was not due to ether-soluble substances in the drug because, when an ethereal extract from cinnamon bark was divided into three different fractions all, when dissolved in arachis oil, gave negative results. A 1 per cent. solution of cinnamal in sesame oil also gave a negative result. A. H. B.

Penicillin: Oral Administration in Children. S. A. Doxiadis, J. L. Emery and S. M. Stewart. (*Brit. med. J.*, 1951, **1**, 16.) 76 children aged 2 to 15 years were given penicillin in tablets each containing 200,000 units, 2 or more hours after a light breakfast. The dose varied from 1000 to 7000 units/lb. of body weight. Sodium citrate, 3 gr. in 1 oz. of flavoured water, was given as an antacid immediately before or after the penicillin. The blood and serum penicillin levels were estimated by Fleming's capillary-tube method, using whole blood inoculated with Richard's strain of *Streptococcus pyogenes*. The serum used for assay was from capillary blood collected in Wright's capsules. Blood was taken before the penicillin was given, and 3 hours after. A total of 208 test doses were given, involving 632 penicillin assays. It was shown that to attain a reliable bacteriostatic level of 0·06 unit per ml. 3 hours after the ingestion the single dose of penicillin should be not smaller than 6000 units/lb. of body weight, i.e. 48,000 units/lb. body weight/24 hours. Simultaneous administration of an antacid may allow a reduction of this dose by approximately 50 per cent. S. L. W.

Pilocarpine, Actions of Certain Related Amines. E. Brochmann-Hanssen, G. L. Jenkins and J. B. Data. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 61.) The synthesis of the hydrochlorides of the following amines is described: dimethyl-(±)-*iso*pilopylamine, diethyl-(±)-*iso*pilopylamine, *N*-(±)-*iso*pilopyl-*N*:*N'*:*N'*-trimethylethylenediamine, dimethyl-(±)-homo*iso*pilopylamine, diethyl-(±)-homo*iso*pilopylamine and *N*-(±)-homo-*iso*pilopyl-*N*:*N'*:*N'*-trimethylethylendiamine. The substances are structurally related to pilocarpine in possessing the same lactone ring; they differ in that the imidazole ring in pilocarpine is replaced by different side chains. Preliminary pharmacological tests on rabbit intestine showed that only dimethyl-(±)-*iso*pilopylamine and dimethyl-(±)-homo*iso*pilopylamine possessed parasymphathomimetic action. Subsequent tests of these two on the blood pressure of the intact cat, on the frog's heart, on the pupil of the cat's eye, on the salivary and lachrimatory glands of rats and for antispasmodic activity indicated that their activity was considerably weaker than that of pilocarpine. The results showed that the lactone ring appears to be important for the pilocarpine-like action whereas the imidazole is less specific.

G. R. K.

Sodium Citrate, Intravenous Toxicity of. R. Charonnat and P. Lechat. (*Ann. pharm. franc.* 1950, **8**, 795.) Experiments with rabbits showed that the toxic action of trisodium citrate is a function of the speed of injection and concentration of the solution, and can be avoided by the use of a solution of lower concentration, and slow injection. It is useless to attempt to state a toxic dose for a substance of this type without indicating the speed and the concentration of the injection. G. M.

Streptomycin *p*-Aminosalicylate and Streptomycin Resistance. J. D. Adcock, W. F. Fiddler, W. A. Meier and C. R. Owen. (*Amer. J. med. Sci.*, 1951, **221**, 149.) In view of the known effect of oral *p*-aminosalicylic acid in delaying the emergence of streptomycin resistant tubercle bacilli, an investigation was carried out of the development of resistance by the infecting organisms when streptomycin *p*-aminosalicylate was given by intramuscular injection twice daily in tuberculosis, each dose containing the equivalent of 0·5 g. of streptomycin and 0·4 g. of *p*-aminosalicylic acid. In 10 out of 12 patients there was a significant increase in the resistance, and in 6 of these the resistance had increased by the 45th day. The patients all had advanced cavitation and caseation, conditions in which the incidence of resistance would be likely to be high, so that it cannot be said that oral *p*-aminosalicylic acid would have prevented or delayed the development of resistance. Nevertheless in this series the resistant organisms appeared as promptly as could have been expected with streptomycin alone and the authors suggest that if the compound is used clinically, oral *p*-aminosalicylic acid in the usual dosage should be added. Although all the patients were in the "near hopeless" class, 3 showed remarkable clinical and X-ray improvement, and further trial of the compound seems warranted. H. T. B.

Vasodilators in Peripheral Vascular Conditions, Comparative Effects of. W. J. Reedy. (*J. Lab. clin. Med.*, 1951, **37**, 365.) In many patients with peripheral arterial insufficiency a functional element of vasoconstriction superimposed on the organic occlusive changes makes it difficult to compare the effectiveness of different vasodilators. A closely associated factor is that the area of the lumen of an artery can be greatly reduced before an appreciable reduction in the blood flow occurs. Tests were carried out on 16 patients suffering from arterial insufficiency in the upper or lower extremities, the drugs used and the doses, which were given intravenously, being priscoline (benzylimidazoline), 75 mg.; tetraethylammonium, 500 mg.; alcohol and ether, 25 ml., in 500 ml. of 5 per cent. glucose solution or physiological solution of sodium chloride. The temperature of the room was kept constant within 2°C. Temperature readings of the skin of the dorsum of all digits proximal to the nail were taken by means of the Dermalor since this area reflects changes in the blood supply to the skin of the toes. Readings were taken repeatedly, before administration of the drug, to obtain a basal temperature when a condition of stability had been reached, and at 5 to 10 minute intervals after injecting the drug for a period of one hour or until the temperature reached a peak and began to fall. Blood pressures and pulse rates were also determined in some of the patients. With ether, only 1 out of 6 patients showed increased blood flow, the remainder showing a decrease. In contrast to the commonly accepted view, alcohol produced no definite vasodilating effects in 5 out of 6 patients, although the unresponsive subjects gave an excellent response to benzylimidazoline. Tetraethylammonium produced a normal vasodilatation response in only 1 out of 11 patients; small responses were obtained in 5 of the other patients. Benzylimidazoline produced a marked response in 11 out of 15 of the subjects; the rise in temperature was slower than with tetraethylammonium and its duration considerably longer. If there is no surface temperature response to the intravenous administration of benzylimidazoline, the arterial insufficiency is probably due to organic occlusion of the vessels and the compound can also be used as a test agent to distinguish vasoconstriction from mechanical obstruction of the lumen.

H. T. B.