ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***Dioscorea dumetorum*, a Convulsant Alkaloid of.** C. W. L. Bevan and J. Hirst. (*Chem. Ind.*, 1958, 103.) A convulsant alkaloid at first thought to be dioscorine was obtained from the fresh tubers of *D. dumetorum*, a wild plant common in West Africa, by extraction with alcoholic acetic acid. Chemically the alkaloid was found not to be identical with dioscorine, and a comparison with the infra-red spectrum of dihydrodioscorine synthesised by Pinder from dioscorine extracted from *D. hispida* Dennst left little doubt that the alkaloid was dihydrodioscorine. There are differences in the melting points of derivatives and in specific rotation between the natural and synthetic alkaloid, possibly due to the synthetic alkaloid being a mixture of stereoisomers. Pharmacologically the alkaloid showed considerable similarity to dioscorine, but is weaker in all its effects. J. R. F.

***Rauwolfia vomitoria* Afz. and Some Related African Species.** R. Paris and G. Dilleman. (*Ann. pharm. franç.*, 1957, **15**, 310.) In an investigation of the alkaloids of various species of *Rauwolfia*, a preliminary separation of the alkaloids provided two groups, a weakly basic group (including reserpine), and the remainder. These fractions were separated by paper electrophoresis under carefully controlled conditions. *R. caffra* and *R. inebrians* gave identical ionograms, which were, however, completely different from that of *R. vomitoria*. Samples of *R. vomitoria* root were similar to *R. caffra* and *R. macrophylla* in structure, but could be distinguished by certain microscopical characters, while the roots of *R. caffra* and *R. inebrians* showed only slight histological differences.

G. B.

ANALYTICAL

Ethinyloestradiol, Identification and Determination of. C. Heusghem and J.-M. Jehotte. (*J. Pharm. Belg.*, 1957, **12**, 418.) A simple method is given for the identification of ethinyloestradiol in quantities of a few mg. Tablets and other preparations are powdered, suspended in water and extracted with ether, the ethereal solution being dried over anhydrous sodium sulphate and evaporated. The residual ethinyloestradiol gives a pink colour with a green fluorescence on the addition of ethanol-sulphuric reagent (prepared by mixing 20 ml. of ethanol (95 per cent) with 80 ml. of sulphuric acid at 0°). Pink streaks appear on shaking, and after about 1 minute the ethinyloestradiol dissolves completely, imparting the maximum colour to the solution. No other steroid appears to give this reaction, but relatively large amounts of oestrone, oestradiol or methyltestosterone produce a yellow or green fluorescence which may partially mask the pink colour. Details are given of the quantitative determination of ethinyloestradiol, using the same reaction and measuring the colour at 535 mμ.

G. B.

Iproniazid and Related Compounds, Photometric Determination of. R. J. Colarusso, M. Schmall, E. G. Wollish and E. G. E. Shafer. (*Analyt. Chem.*, 1958, **30**, 62.) This method applies to drugs of general formula R′–CO–NH–NH–R″ where R′ = 4′-pyridyl and R″ = alkyl or aryl, and depends upon the formation of a red complex in acetone solution with molybdic acid. Peaks at 430 or 535 mμ are suitable for measurement, and the procedure is sensitive down to 10 μg. Since breakdown products will not react, it can be applied to stability studies. A method suitable for tablets and ampoules is described. No colour is produced when R″ = H or nicotoyl or when the terminal nitrogen is disubstituted. D. B. C.

Isoniazid, Photometric Determination of. B. Wesley-Hadžija and F. Abaffy. (*Acta Pharm. Jug.*, 1957, **7**, 137.) A simple and sensitive method for the determination of isoniazid in pharmaceutical preparations depends upon the development of a yellow colour with *p*-dimethylaminobenzaldehyde. A solution containing about 1 mg. of isoniazid in 50 ml. of water is used, and on the addition of 0·1 ml. of a 4 per cent solution of *p*-dimethylaminobenzaldehyde in 2N sulphuric acid, the maximum colour develops in 10 minutes. Measurements of the colour intensity are made at 425 mμ, the quantity of isoniazid being read from a calibration curve. Details are given for the assay of tablets and injections by this method. G. B.

Riboflavine in Pharmaceutical Specialities, Determination of. A. Maquinay and N. Brouhon. (*J. Pharm. Belg.*, 1957, **39**, 350.) For the determination of riboflavine in tablets by a polarographic method, a tablet is powdered, and sufficient sodium salicylate is added to make the polarographic solution contain 1 per cent. A phosphate buffer solution is added to make the final solution 0·1M, and the sample made up to volume. After shaking for 30 minutes (in the dark) the solution is placed in the cell and the polarographic curve recorded under suitable conditions. The height of the wave between −0·3 and −0·6 volt is read, and the result calculated by reference to a standard curve. The presence of salicylate aids solution of the riboflavine. The method has been applied successfully to a number of multivitamin preparations. Novalgin depresses the height of the wave, and should be removed by precipitation with cadmium sulphocyanide before making the polarogram. G. B.

Tyrothricin, Investigation of the Spectrophotometric Determination of. W. Oberzill. (*Sci. Pharm.*, 1957, **25**, 148.) The factors influencing the design of a direct spectrophotometric method for the determination of tyrothricin in solutions and pharmaceutical preparations are considered. Firstly it is shown that the extinction values for tyrothricin, which is a mixture of tyrocidin and gramicidin, are equal to the sum of those of the two components. Thus determinations can only be expressed in terms of a particular sample of tyrothricin since this is a mixture of at least two main components. The same applies to the colorimetric determination by means of the tryptophane reaction (treatment with *p*-dimethylaminobenzaldehyde in strong hydrochloric acid and sodium nitrite solution). Secondly it is shown that in preparations containing tyrothricin and cetyltrimethylammonium bromide, the extinction value is again equal to the sum of the individual components. In all cases the extinction is measured at 281 mμ where the curve for cetyltrimethylammonium bromide is almost horizontal. The concentrations used are of the order of 100 μg./ml. Measurements with known mixtures are quoted, and show that the results agree with those predicted to within a few per cent. D. B. C.

GLYCOSIDES

***Digitalis purpurea* Leaves, Presence of Gitoriside in.** D. Satoh, T. Wada, H. Ishii, Y. Oyama and T. Okumura. (*Pharm. Bull. Japan*, 1957, **5**, 253.) Gitoriside is the name given to a newly discovered glycoside present in very small quantity in dried digitalis leaves. This glycoside is gitoxigenin mono-(+)-digitoxoside. Some evidence is also produced to indicate that digitalonin (*Pharm. Bull. Japan*, 1956, **4**, 284) is diginigenin mono-(+)-digitaloside. Details for the production of crystalline gitoxin penta-acetate are also given.

J. W. F.

***Digitalis purpurea* Seeds, Presence of Glucodigifucoside in.** A. Okano. (*Pharm. Bull. Japan*, 1957, **5**, 272.) Further work on some of the 17 unknown glycosides of digitalis seeds, reported earlier (*ibid.*, 157) has led to the production of crystalline glucodigifucoside. This substance is a cardiotonic glycoside of empirical formula, $C_{35}H_{54}O_{13}.2H_2O$. On suitable hydrolysis it yields digitoxigenin, fucose and one molecule of glucose.

J. W. F.

***Digitalis purpurea* Seeds, Partition Chromatography of the Glycosides in.** K. Miyatake, A. Okano, K. Hoji and T. Mike. (*Pharm. Bull. Japan*, 1957, **5**, 157, 163, 167, 171.) A suitable extract of the seeds was subjected to adsorption chromatography on alumina using water saturated butanol as the developing solvent. Each fraction was subjected to ascending paper chromatography, using water saturated methyl ethyl ketone as the moving phase and water as the stationary phase. These paper chromatograms were done in triplicate and were treated separately by (*a*) $SbCl_3$-$CHCl_3$ solution, (*b*) 1 per cent HCl-MeOH solution and (*c*) 25 per cent *m*-dinitrobenzene-benzene solution. By this means, purpurea glycosides A and B, gitoxin, strospeside, digitalinum verum and 17 other substances (probably glycosides) were shown to be present. It was also shown that digitalinum verum was closely associated with a small quantity of one of these unknown glycosides. The presence of this impurity prevented crystallisation: when it was removed (by partition chromatography on Celite 535) crystals of pure digitalinum verum were readily obtained. This unknown glycoside is probably glucogitofucoside. Further work on one other of these unknown glycosides, which appeared to be present in comparatively large amount, was done. It was obtained in a crystalline form by using similar techniques employed previously for digitalinum verum. This new glycoside was named gitostin and further work showed that it was gitoxigenin-glucosido-glucosido-digitaloside. Partial decomposition by enzyme produces digitalinum verum and glucose.

J. W. F.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenaline and Noradrenaline, Biosynthesis of, *In Vitro*. McC. Goodall and N. Kirshner. (*J. biol. Chem.*, 1957, **226**, 213.) Evidence for the conversion of tyrosine to adrenaline via dopa, hydroxytyramine and noradrenaline in bovine adrenal slices is presented. The tissue slices were incubated in a Krebs phosphate buffer solution with labelled L-tyrosine-^{14}C. At the end of the period of incubation the slices were homogenised and extracts of the homogenates were prepared for ion exchange chromatography by precipitating

the protein with trichloroacetic acid. The resin used was Amberlite IRC–50. The amounts of adrenaline, noradrenaline and hydroxytyramine were determined by measuring the optical density of the eluate at 279 mμ. An aliquot of each fraction was assayed for radioactivity. It was found that adrenal slices formed hydroxytyramine, noradrenaline and adrenaline from tyrosine and from 3:4-dihydroxyphenylalanine. Noradrenaline and adrenaline were formed from hydroxytyramine. In the presence of unlabelled dopa and unlabelled hydroxytyramine the amount of noradrenaline formed from ^{14}C-labelled tyrosine was decreased. Tyramine, under identical conditions, did not decrease the formation of noradrenaline from tyrosine. The data do not exclude the possibility that dihydroxyphenylserine may be involved in the pathway. Whether the sequence of the reactions is dopa to hydroxytyramine to noradrenaline or is dopa to dihydroxyphenylserine to noradrenaline has not been unequivocally established but the evidence presented supports the former sequence. M. M.

Digitoxin, C(12)-Hydroxylation of. B. T. Brown, S. E. Wright and G. T. Okita. (*Nature, Lond.*, 1957, **180**, 607.) When digitoxin was administered to rats or to humans, the urine was shown to contain, besides unchanged digitoxin, two cardioactive metabolites. One of these metabolites was shown to be digoxin (12-hydroxydigitoxin) by careful comparison with pure digoxin on paper chromatograms and by hydrolysis, and by the use of carbon-14 labelled digitoxin. J. W. F.

Mast Cells, Mechanism of the Disruption of. B. Högberg and B. Uvnäs. (*Acta physiol. scand.*, 1957, **41**, 345.) This paper presents the experimental work upon which is based the theory postulated by Uvnäs in his Review Article in the *J. Pharm. Pharmacol.*, 1958, **10**, 1. Using mast cells from the rat mesentery the authors found that disruption of the cells by compound 48/80 was inhibited by metal salts and other enzyme inhibitors. Of the enzymes investigated, lecithinase A was the only one that disrupted the cells, and its action was inhibited by the same agents that affected 48/80 activity. The action of 48/80 was temperature-dependent and inhibited by incubation with acetic anhydride and 1:3-diphosphoimidazole. Dephosphorylation caused disruption without the addition of a liberator. The disruptive action of both enzyme and 48/80 was blocked by high doses of a polyvalent serum (against snake venom) and a specific serum (against lecithinase A). The observations supported the hypothesis that 48/80 acts by activating a lytic enzyme attached to the mast cell membrane. The enzyme normally blocked by an inhibitor becomes active when the inhibitor is removed by liberators such as 48/80. J. R. F.

Polymyxin B, Structure of. G. Biserte and M. Dautrevaux. (*Bull. Soc. Chim. Biol.*, 1957, **39**, 795.) In order to study the peptide sequence of polymyxin B, partial acid hydrolysates were prepared, and the components separated by chromatography on a strongly acidic cation exchange resin, followed by electrophoresis and chromatography on paper. The peptides containing phenylalanine were isolated by chromatography on charcoal, followed by electrophoresis and chromatography on paper. It is suggested that polymyxin B consists of an octacyclopeptide or heptacyclopeptide structure with a side chain consisting of 2 or 3 amino acid residues connected at a α:γ-diaminobutyric acid residue; the α-amino group of the α:γ-diaminobutyric acid terminating this side-chain is joined to the carboxyl group of *iso*pelargonic acid. G. B.

BIOCHEMICAL ANALYSIS

Morphine, New Method for Determination of, in Urine. P. Paerregaard. (*Acta pharm. tox. Kbh.*, 1957, **14**, 38.) A new method is described for determining morphine in human urine. It is based on extraction of the bicarbonate-saturated urine with chloroform-*iso*propanol, paper chromatographic isolation of the morphine and finally quantitative determination by polarography. Pipette 10 ml. of the urine into an ampoule, add 1 ml. of 10 N hydrochloric acid, seal and autoclave at 120° for 30 minutes. Cool, saturate with sodium bicarbonate (pH = 8–9, the isoelectric point of morphine) and extract three times with equal volumes of a 3:1 mixture of chloroform and *iso*propanol. From the total solvent phase withdraw 15 ml. (corresponding to 5 ml. of urine) and evaporate to dryness in a flat-bottomed tube. Dissolve the residue in 100 μl. of methanol and apply 10 μl. to a strip of Whatman No. 1 filter-paper using a Carlsberg constriction pipette. Carry out descending paper chromatography with one of the two solvent mixtures (1) *n*-butanol formic acid and water 12:1:7 or (2) amylene hydrate di-*n*-butylether and water 80:7:13. Elute the morphine localised on the paper with N hydrochloric acid and determine the morphine present polarographically after transforming into 2-nitrosomorphine by means of nitrite in acid solution. G. F. S.

Oxytocin in Preparations of Vasopressin, Assay of. A. T. Nielsen. (*Dansk Tidsskr. Farm.*, 1958, **32**, 1.) While theoretically the oxytocin content of preparations of vasopressin can be calculated from the difference between the total oxytocin activity and the activity due to vasopressin, the conditions are such that a statistically significant result is hardly to be expected. To obtain a more accurate result the author describes a method for the assay of oxytocin in injection of vasopressin in which the oxytocic activity is assayed before and after inactivating the vasopressin enzymatically by a tryptic digest (trypsin 10 μg./ml. of injection) at pH 8–9 and 22° for 30 minutes. The oxytocic activity is assayed as described by Holton on the isolated uterus of the rat in oestrus, using a modified de Jalon Ringer. The intrinsic oxytocic activity of the pressor hormone was found to average about 3·8 units per 100 pressor units.

J. R. F.

PHARMACY

Adrenaline, Decomposition of, in the Presence of Copper. P. Varène. (*Bull. Soc. Chim. Biol.*, 1957, **39**, 1099.) Sodium chloride solution (0·9 per cent), buffered with 0·05M phosphate buffer was mixed with 1 ml. of solution containing a known concentration of copper sulphate, the total volume being 99 ml. This solution was heated to 37°, and 1 ml. of adrenaline solution added. The reaction was carried out in darkness, using pure materials and a high degree of cleanliness to avoid spurious results. The destruction of adrenaline in the presence of copper was measured in two ways; (1) by determination of the hypertensive action of the solution in the pithed dog treated with atropine and chloralose, and (2) photometrically using a blue-green filter having its maximum transmission at 490 mμ. The activity determined by method (1) decayed to zero at the end of 13 minutes contact with the copper sulphate solution. The blue colour reached a maximum at about that time, and on prolonged contact with the copper sulphate solution, faded to pale brown. The rate of destruction of adrenaline increased with increase in pH, copper concentration and temperature. G. B.

Dressings, Sterilization of. V. G. Adler and W. A. Gillespie. (*J. clin. Path.*, 1957, **10**, 299). An experimental study has been made of the factors affecting the sterilisation of surgical dressings. The experiments were carried out in cylindrical jacketed autoclaves. Two methods of sterilisation were used, (*a*) the double vacuum method, and (*b*) the downward displacement method. The drums were loaded with folded "huckaback" towels and the temperature inside measured with a thermistor. Bacteriological tests were carried out with filter papers impregnated with a spore suspension of *B. stearothermophilus* and chemical tests for heat penetration with Browne steriliser control tubes. The results showed that the double vacuum method was slightly better than the downward displacement method for removing air from the drum and the dressings, but this was never complete. Delay in heat penetration was due to entrapped air. Exposure to steam at 20 lb./sq.in. for 15 minutes was sufficient to sterilise all spore papers, but exposure for 10 minutes allowed some spores to survive at the centre of closely packed drums. Packing of the drums and incorrect positioning delayed sterilisation, and sterilisation was easier when the towels were wrapped in cloth instead of in drums. Browne's chemical tests were found to be good indications of safe sterilisation. G. F. S.

Silicone and Petrolatum Ointment Bases, Comparison of *In Vivo* and *In Vitro* for Absorption, Penetration and Diffusion of Medicinals from. J. B. Plein and E. M. Plein. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 705.) Six ointment bases were prepared, all of about the same consistency, including a simple paraffin ointment, an absorption base (containing about 10 per cent of wool fat), an emulsion base, and similar bases prepared with silicone fluids in place of paraffins. Sulphanilamide, iodine, sodium radio-iodide (^{131}I), salicylic acid and chlortetracycline hydrochloride were incorporated in the bases, which were subjected to *in vitro* diffusion tests. The ointments were also applied to the intact and abraded skin of white rats, the penetration into the inuncted skin being determined by analysis of a skin sample, and the absorption into the systemic circulation by analysis of the blood or a storage organ. Results of the skin penetration and intact skin absorption tests showed no correlation with the *in vitro* diffusion of the ointments. For 3 of the 5 drugs investigated, the absorption through the abraded skin was in accordance with the *in vitro* diffusion data. The significance of these results is discussed. G. B.

Sterile Ophthalmic Solutions, Preparation of. J. Schmid. (*Die Pharmazie*, 1957, **12**, 748.) Owing to the large number of cases of optical infection in recent years following cataract operations resulting in loss of sight traceable to infected opthalmic solutions, the use of a 1 : 50,000 solution of alkyldimethylbenzylammonium chloride was employed as solvent and the solutions prepared from previously sterilised materials in sterile containers with aseptic precautions. In no case during an 18-month trial were bacteria detected in the residues in bottles after one to two months use, and no case of irritation was reported. D. B. C.

Syringes, Sterilization of, by Infra-red Radiation. E. M. Darmady, K. E. A. Hughes and W. Tuke. (*J. clin. Path.*, 1957, **10**, 291.) An infra-red steriliser for syringes is described. It consists of a metal moving belt on which the assembled syringes are loaded on trays in their pre-sealed containers. They pass through an insulated tunnel over which infra-red projectors are placed at predetermined intervals. Experimental studies showed that the heating up time was rapid and a sterilisation time of 11 minutes at 180° ensured sterility. The apparatus can be used for the sterilisation of other glassware articles. G. F. S.

PHARMACOLOGY AND THERAPEUTICS

Anthelmintics, New Series of. F. C. Copp, O. D. Standen, J. Scarnell, D. A. Rawes and R. B. Burrows. (*Nature, Lond.*, 1958, **181**, 183.) A series of compounds of the general formula $R{\cdot}C_6H_4{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2{\cdot}CH_2{\cdot}C_6H_4{\cdot}R'$ were examined for their activity against nematodes parasitic in the gastro-intestinal tract of animals. In all compounds R and R′ were in the *ortho* position, and those in which R = H, Me or Cl, R′ = H, Me, Cl or F were shown to be more active than others in which R = NO_2 or Br and R′ = Br. Some of the compounds examined were active against a wide range of species, particularly those nematodes which live in the mucosa rather than the lumen of the gut.

G. B.

Chlorothiazide, an Oral Diuretic. R. I. S. Bayliss, D. Marrack, J. Pirkis, J. R. Rees and J. F. Zilva. (*Lancet*, 1958, **1**, 120.) Chlorothiazide (6-chloro-7-sulphamyl-1 : 2 : 4-benzothiadiazine-1 : 1-dioxide) is a non-mercurial oral diuretic, with an action resembling that of mercurial diuretics. The value of chlorothiazide was assessed in 24 oedematous patients, of whom 17 had congestive heart failure and 11 had responded poorly to mersalyl. A daily dose of 2 g. (1 g. at 8.30 a.m. and 1 g. at 4.30 p.m.) produced good results, with clearing of the oedema, in 14 patients: in 7, the results were less satisfactory, and in 3 they were poor. Chlorothiazide may be effective in patients who do not respond to mersalyl, and it enhances the response to mersalyl even in patients who have become mersalyl-resistant. No toxic effects were observed except in 1 patient who developed malaise and anorexia. Chlorothiazide caused an approximately equimolar loss of chloride and sodium. It may cause potassium depletion, particularly if continuous treatment with 2 g. daily is given over a long period: in cases where this dosage is necessary the treatment should be supplemented with 2 to 6 g. of potassium chloride. In less severe cases, which respond quickly, a dose of 1·0 to 1·5 g. daily of chlorothiazide may suffice; for maintenance therapy the drug may be given intermittently on 3 or 4 days each week.

S. L. W.

Chlorothiazide, Clinical Experience with. J. D. H. Slater and J. D. N. Nabarro. (*Lancet*, 1958, **1**, 124.) Chlorothiazide was used successfully in 3 patients with the nephrotic syndrome, in 1 patient with ascites due to portal hypertension, and 1 patient with congestive heart failure. Two of the patients with nephrotic syndrome were satisfactorily controlled for 5 to 6 months. The chief action of chlorothiazide appears to be to cause a rapid increase in the rate of excretion of chloride by the kidneys, resembling closely the diuretic action of organic mercurial compounds. A subsidiary effect, suggesting inhibition of carbonic anhydrase, was observed in a short-term study. The drug is very well tolerated and seems to be free from side-effects, apart from the development of a hypokalaemia which is difficult to control with oral potassium supplements. If a patient has oedema persistent enough to need both severe restriction of sodium and chlorothiazide therapy (either continuous or intermittent), it is essential to have regular estimations of plasma-potassium concentrations. Initially, these should be made at weekly intervals and suitable supplements of potassium chloride given.

S. L. W.