NOTES.

399. *The Preparation of* 1-p-*Diphenylylethyleneimine.*

By J. N. Baxter and J. Cymerman-Craig.

In view of the cytotoxic and tumour-inhibiting properties of certain ethyleneimines (Hendry, Homer, Rose, and Walpole, *Brit. J. Pharmacol.*, 1951, **6**, 357) 1-*p*-diphenylylethyleneimine was prepared; it will be examined for carcinogenic properties by Dr. A. L. Walpole (Dr. F. L. Rose, personal communication).

The only 1-arylethyleneimines reported in detail are 1 : 2 : 3-triphenyl- (Taylor, Owen, and Whittaker, *J.*, 1938, 206) and 1-*p*-(*p'*-aminophenylsulphonyl)phenyl-ethyleneimine (Jackson, *J. Org. Chem.*, 1951, **16**, 1899). Reaction of 4-aminodiphenyl with 2-bromoethanol gave 4-bis-2'-hydroxyethylaminodiphenyl (Ross, *J.*, 1949, 183), with small amounts of 2-*p*-diphenylylaminoethanol (unpublished observations). The required 2-*p*-diphenylylaminoethanol was obtained in 36% yield by the action of 2-bromoethanol on 4-benzylideneaminodiphenyl followed by hydrolysis of the intermediate quaternary salt (cf. Decker and Becker, *Annalen*, 1913, **395**, 362). Many attempts to improve the yield gave much unchanged Schiff's base, together with 4-aminodiphenyl. Attempted isolation of the intermediate quaternary salt resulted in quantitative transformation into 4-diphenylylammonium bromide; instability of the intermediate quaternary compounds in this reaction has been noted previously (Hantzsch and Schwab, *Ber.*, 1901, **34**, 837; Knoevenagel, *ibid.*, 1923, **55**, 1921).

With 48% hydrobromic acid the alcohol readily gave the desired 2-*p*-diphenylylaminoethyl bromide; this and its hydrobromide showed evidence of chemical change on repeated recrystallisation.

Treatment of the base with methanolic sodium methoxide (1 mol.) afforded 1-*p*-diphenylylethyleneimine in excellent yield. When heated or repeatedly recrystallised this was partly transformed into a high-melting solid; the ease of polymerisation of ethyl-

eneimines is well known. The ethyleneimine readily gave 2-*p*-diphenylylaminoethyl chloride hydrochloride, identical with a sample prepared by the action of thionyl chloride on 2-*p*-diphenylylaminoethanol. The dimer, 1 : 4-bis-*p*-diphenylylpiperazine, was prepared from 4-bis-2′-chloroethylaminodiphenyl and 4-aminodiphenyl by Davis and Ross' method (*J.*, 1949, 2831).

Experimental.—2-p-*Diphenylylaminoethanol.* 4-Benzylideneaminodiphenyl (12·8 g., 0·05 mol.), suspended in absolute alcohol (150 c.c.) containing 2-bromoethanol (5 c.c., 0·075 mol.), was heated for 2·5 hours under reflux. The solution was cooled to 0° and unchanged Schiff's base filtered off (7·2 g., 56%). The filtrate was boiled with hydrochloric acid (100 c.c.; 3N) for 0·5 hour, alcohol being allowed to distil off. The cooled solution was extracted with ether to remove benzaldehyde, and the amine was then liberated with sodium hydroxide and extracted with ether. The residue, after removal of solvent from the dried (Na_2SO_4) extracts, was recrystallised from light petroleum (b. p. 60—90°), giving 2-p-*diphenylylaminoethanol* as needles, m. p. 112° (1·55 g., 36% calc. on Schiff's base which reacted) (Found: C, 78·6; H, 6·95; N, 6·4. $C_{14}H_{15}ON$ requires C, 78·85; H, 7·1; N, 6·55%). The mother-liquors contained some 4-aminodiphenyl isolated as the hydrochloride (1·15 g.). 2-p-*Diphenylylaminoethanol hydrochloride* crystallised from ethyl acetate in needles, m. p. 152—153° (Found: N, 5·6. $C_{14}H_{15}ON,HCl$ requires N, 5·65%).

Attempted isolation of N-*benzylidene*-N-2′-*hydroxyethyl*-4-*diphenylylammonium bromide.* The quaternary salt (m. p. >310°), obtained by addition of ether to the filtrate after removal of Schiff's base from an experiment as above, liberated benzaldehyde on treatment with dilute acid; it was transformed by repeated crystallisation from methanol–ether into 4-*diphenylylammonium bromide*, m. p. 340° (decomp.) (Found: C, 57·7; H, 4·65: N, 5·4; Br, 31·5. $C_{12}H_{11}N,HBr$ requires C, 57·6; H, 4·85; N, 5·6; Br, 31·95%), identified as the benzenesulphonate, m. p. and mixed m. p. 284° (Bauer and Cymerman, *J.*, 1950, 1826).

2-p-*Diphenylylaminoethyl bromide.* A solution of 2-*p*-diphenylylaminoethanol (2·94 g.) in hydrobromic acid (22 c.c.; 48%) was refluxed for 6 hours; 10 c.c. of distillate were then removed and the residual solution refluxed for a further 4 hours. The cooled solution was poured into ice-water and filtered, and the product washed with ice-water, alcohol, and ether, giving plates (4·49 g., 89%), m. p. 192—195°. Crystallisation from *iso*propanol gave plates, m. p. 196—197°, of 2-p-*diphenylylaminoethyl bromide hydrobromide* (Found: C, 47·1; H, 4·45. $C_{14}H_{14}NBr,HBr$ requires C, 47·1; H, 4·25%). The substance appeared to undergo some solvolysis on recrystallisation.

The free base, liberated with dilute ammonia and crystallised from light petroleum (b. p. 60—90°), gave 2-p-*diphenylylaminoethyl bromide*, prisms, m. p. 75—76° (Found: N, 5·5. $C_{14}H_{14}NBr$ requires N, 5·1%). Repeated recrystallisation caused the formation of a high-melting impurity, insoluble in light petroleum.

1-p-*Diphenylylethyleneimine.* A solution of 2-*p*-diphenylylaminoethyl bromide (1·12 g.) in methanol (100 c.c.) was treated with methanolic sodium methoxide until just alkaline to brilliant-yellow. The mixture was refluxed for 2·5 hours, filtered from sodium bromide, and evaporated to dryness. The washed (water) and dried (P_2O_5) residue (0·74 g., 93%) had m. p. 91—92° and on crystallisation from light petroleum (b. p. 40—60°) gave 1-p-*diphenylylethyleneimine* as prisms, m. p. 98—99° (Found: C, 86·05; H, 6·85; N, 6·95. $C_{14}H_{13}N$ requires C, 86·15; H, 6·7; N, 7·15%). Repeated recrystallisation gave increasing amounts of a high-melting, petrol-insoluble impurity.

2-p-*Diphenylylaminoethyl chloride.* (*a*) A solution of 1-*p*-diphenylylethyleneimine (0·13 g.) in dry ether was saturated with hydrogen chloride gas. The precipitate (0·12 g., 67%; m. p. 173—175°) crystallised from methanol–ether in prisms, m. p. 176—178°, of 2-p-*diphenylylaminoethyl chloride hydrochloride* (Found: C, 62·8; H, 5·8; N, 5·5. $C_{14}H_{14}NCl,HCl$ requires C, 62·7; H, 5·65; N, 5·25%). The mother-liquors contained a high-melting by-product (0·02 g.).

(*b*) A solution of 2-*p*-diphenylylaminoethanol (0·13 g.) in dry chloroform (10 c.c.) was refluxed with thionyl chloride (0·5 c.c.) for 0·5 hour. Evaporation to dryness *in vacuo* and trituration of the residue with dry ether gave a hydrochloride (0·085 g., 52%), m. p. 175—177°, undepressed on admixture with the material prepared as in (*a*) above.

1 : 4-*Bis*-p-*diphenylylpiperazine.* A solution of 4-aminodiphenyl (5 g., 0·03 mol.) and 4-bis-2′-chloroethylaminodiphenyl (2·95 g., 0·01 mol.) in 50% aqueous acetone (1100 c.c.) was refluxed for 4 hours, during which crystals separated. The hot solution (A) was filtered, and the crude product (2·3 g.) extracted with ether (100 c.c.). The ether-insoluble portion (1·05 g., 16%) was the *piperazine*, m. p. 305·5—306°, crystallising from toluene in plates of unchanged

m. p. (Found : N, 7·15. $C_{28}H_{26}N_2$ requires N, 7·15%). Evaporation of the filtrate (A) gave a further 0·1 g. (2·5%) of the piperazine, m. p. 300°.

Thanks are offered to Mrs. E. Bielski and the C.S.I.R.O. microanalytical laboratory for microanalyses. One of us (J. N. B.) is indebted to the University of Sydney for the award of a Monsanto (Aust.) Pty. Ltd. Scholarship.

ORGANIC CHEMISTRY DEPARTMENT, UNIVERSITY OF SYDNEY. [*Received, December 10th*, 1952.]

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400. *The Determination of* C-*Methyl Groups in Some Unsaturated Straight-chain Compounds.*

By A. D. CAMPBELL and V. J. CHETTLEBURGH.

CAMPBELL and MORTON (*J.*, 1952, 1693) showed that the Kuhn–Roth procedure for the determination of *C*-methyl groups is a unreliable method for branched-chain fatty acids. In the terpene series, side-chains attached to *cyclo*alkanes give low yields of acetic acid but when attached to *cyclo*alkenes, cyclic alcohols, and ketones give high yields (Petru, Jurecek, and Kovar, *Chem. Listy*, 1951, **45**, 300; *Chem. Abs.*, 1952, **46**, 4506), and some compounds containing a polymethylene chain but initially no *C*-methyl group have been found to give a positive *C*-methyl value (W. Baker, personal communication).

The modified Kuhn–Roth procedure (Ginger, *J. Biol. Chem.*, 1944, **156**, 452) when applied to some unsaturated straight-chain compounds has given anomalous yields of acetic acid. Our results (on 10—12-mg. samples by Campbell and Morton's procedure) were : oleic acid, 1·37, 1·43, 1·28*; methyl oleate, 1·36, 1·33; oleyl alcohol, 1·53; linoleic acid, 1·29, 1·16 (yields expressed as moles of acetic acid per mole of compound) (* digested in a sealed tube at 100° for 2 hr.).

In these cases the Kuhn–Roth precedure, when applied to unsaturated straight-chain compounds, gives entirely misleading results. As the yield of acetic acid per *C*-methyl group in branched chain fatty acids may be as low as 62% the above results are those which would be expected from a compound containing two *C*-methyl groups. Saturated dibasic acids such as azelaic, glutaric, and malonic acid did not give a *C*-methyl value.

We thank Mr. R. P. Hansen, Fats Research Laboratory, Wellington, for supplying pure samples of methyl oleate and linoleic acid.

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401. *The Alkaloids of* Senecio ruwenzoriensis.

By M. L. SAPIRO.

EXTRACTION of the plant *Senecio ruwenzoriensis* from the Nanyuki district of Kenya, where it has been suspected of causing poisoning of cattle, has yielded two new alkaloids, which have been named ruzorine and ruwenine.

Preliminary small-scale extraction in the cold yielded 0·38% of alkaloids; but extraction of 3 kg. of dry plant by Koekemoer and Warren's method (*J.*, 1951, 66) gave only 0·11% of ruwenine, of which one-tenth was from the reduction stage (*loc. cit.*) and 0·034% of ruzorine. The low yields in this process were due to the relative instability of the alkaloids.

Experimental.—M. p. and decomposition temperatures are corrected, a standard rate of heating, 2° per min., being adopted.

Separation and purification. The crude mixture obtained in the extraction process was treated with very dilute sulphuric acid and the extract, after filtering, was rendered alkaline with ammonia and re-extracted with chloroform. After removal of the chloroform the residue was treated with cold acetone. Ruzorine remained undissolved and was crystallised from

pyridine and washed with acetone or peroxide-free ether. Ruwenine, extracted by acetone, was freed from decomposition products *via* the sulphate or hydrochloride and crystallised from methanol.

Ruzorine formed colourless prisms or needles, m. p. 161—163° (decomp. from 134°), very soluble in water, soluble in the usual organic solvents except ether, ethyl acetate, or acetone (Found: C, 56·2, 55·9; H, 7·4, 7·35; N, 3·9, 3·65. $C_{18}H_{27}O_8N$ requires C, 56·2; H, 7·0; N, 3·6%). It gave a very unstable hydrochloride (by evaporation from a cold aqueous solution), gradual decomp. from *ca.* 100°, complete decomp. with partial melting at *ca.* 148°, a nitrate (prepared similarly), gradual decomp. from *ca.* 110°, complete decomp. with partial melting at 143—145°, and a picrate (from aqueous solutions), decomp. from *ca.* 125° to 133—137°.

Ruwenine formed colourless plates, m. p. 175·5—179° (decomp. from *ca.* 164°), readily soluble in the usual organic solvents except ether, very slightly soluble in water [Found: C, 60·45, 60·75; H, 7·3, 7·5; N, 3·7, 4·0%; *M* (Rast; poor accuracy owing to instability), 371, 348, 360. $C_{18}H_{27}O_6N$ requires C, 61·3; H, 7·7; N, 4·0%; *M*, 353]. It gave a hydrochloride (by cold evaporation from aqueous solution and recrystallisation from absolute ethanol), decomp. at 222—224° with partial melting, a nitrate (prepared in a similar manner), decomp. from *ca.* 100° to *ca.* 140—160°, and a picrate (from ethanolic solution with aqueous picric acid; recrystn. from boiling ethanol), m. p. 186—188° (decomp. from *ca.* 160°).

Physiological effects. Retrorsine was used as standard in toxicological tests in which the hydrochlorides were injected subcutaneously, in small doses during some days, into 300—350-g. rats. Results are tabulated.

Alkaloid	Quantity (mg.)	Period (days)	Effects
Ruwenine .	2 × 25	1	Death after further 3 days. Early liver cirrhosis, periportal (unlike characteristic *Senecio* poisoning).
Retrorsine .	4 × 25	10	Death after further 2½ days. Liver damage acute and centrilobular.
Ruzorine .	2 × 25 1 × 50	10	No outward symptoms. Mild degenerative changes in the liver.

Acknowledgment is made to the Director of Veterinary Services, Kenya, for permission to publish this work.

DEPARTMENT OF VETERINARY SERVICES,
KABETE, KENYA.

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402. *Kinetics of the Association of Maleic Anhydride and Butadiene.*

BY B. EISLER and A. WASSERMANN.

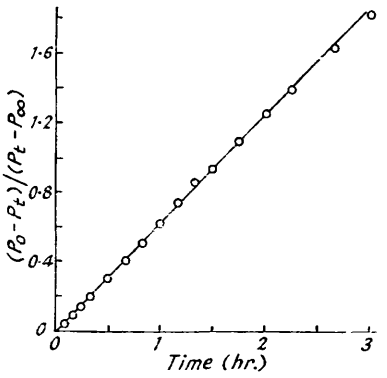
IN previous papers (*J.*, 1936, 1028; 1939, 362, 381; 1950, 2205) the kinetics of Diels–Alder associations were reported for benzoquinone, tetrachlorobenzoquinone, 1 : 4-naphthaquinone, acraldehyde, 5 : 8 : 9 : 10-tetrahydro-5 : 8-*endo*methylene-1 : 4-naphthaquinone, and *cyclo*pentadiene as dienophiles. We have now measured the kinetics of the reaction of maleic anhydride with butadiene to give Δ^4-tetrahydro-*o*-phthalic anhydride. Second-order velocity coefficients, *k*, were determined by a vapour-pressure technique (Eisler and

Kinetics of maleic anhydride–butadiene reaction in benzene.

Temp.	Equimol. initial conc. of reactants (mole/l.)	*k* (l. mole^{-1} hr.$^{-1}$)	%, final conversion	Temp.	Equimol. initial conc. of reactants (mole/l.)	*k* (l. mole^{-1} hr.$^{-1}$)	%, final conversion
14·4°	0·833	0·0647 ± 0·007	62	25·0°	1·200	0·133 ± 0·005 ‡	58
14·4	0·903	0·0465 ± 0·011	69	25·0	2·000	0·137 ± 0·005	65
14·4	0·914	0·0534 ± 0·008	57	35·0	1·125	0·216 ± 0·010	39
25·0	0·311	0·106 ± 0·020	47	35·0	1·450	0·244 ± 0·010	54
25·0	0·601	0·117 ± 0·008	41	35·0	1·500	0·247 ± 0·010	54
25·0	0·980	0·128 ± 0·005 *	74	45·0	1·335	0·480 ± 0·020	64
25·0	1·200	0·138 ± 0·005	52	55·0	1·063	0·8175 ± 0·020	65

* Reaction mixture 0·5M with respect to tri*iso*amylamine picrate.
‡ Reaction mixture saturated with maleic acid.

Wassermann, *J.*, 1953, 979), dry maleic anhydride and the same butadiene and solvent as in the previous work (*loc. cit.*) being used. The results of a typical experiment, and the values of k are given in the accompanying Figure and Table. The Arrhenius rate equation, $k = Ae^{-E/RT}$ is obeyed with $E_1 = 11{\cdot}7 \pm 0{\cdot}8$ kcals. and $\log_{10} A = 4{\cdot}2 \pm 0{\cdot}7$ (A in l. mole^{-1} sec.$^{-1}$). A previously investigated Diels–Alder reaction, involving benzoquinone and butadiene (Eisler and Wassermann, *loc. cit.*), is characterised by an activation energy $E_2 = 14{\cdot}6 \pm 0{\cdot}6$ kcals. The difference, $E_2 - E_1$ ($2{\cdot}9 \pm 1{\cdot}4$ kcals.) can be explained,



Maleic anhydride–butadiene reaction in benzene at 45·0°. Equivalent initial concentration of reactants, 1·335 mole/l. P_0, P_t, and P_∞ are the vapour pressures at zero time, time t, and after completion of reaction. The slope of the straight line gives $k = 0{\cdot}480 \pm 0{\cdot}020$ l. mole^{-1} hr.$^{-1}$.

inter al., by the influence of the C–O dipoles in maleic anhydride, which are liable to reinforce the dipole induction energy arising from the C=O groups (*J.*, 1935, 828, 1511).

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403. *The Polysaccharide of* Penicillium islandicum *Sopp.*

By J. BADDILEY, J. G. BUCHANAN, and E. M. THAIN.

DURING investigations on the colouring matters of *Penicillium islandicum* Sopp, Howard and Raistrick observed that addition of ferric chloride to the culture filtrate gave a heavy, amorphous precipitate with all six strains examined (*Biochem. J.*, 1949, **44**, 227). Although the nature of this material was not fully established it was thought to be a phosphorylated polysaccharide. Through the courtesy of Professor Raistrick we have re-examined this polysaccharide and the results of our studies are recorded here.

We confirmed that the material was insoluble in water but dissolved readily in dilute acids and alkalis, also that it reduced Fehling's solution only after acid hydrolysis at 100° (Howard and Raistrick, personal communication). Its solution in dilute hydrochloric acid gave a strong positive test for ferric ion (ferrocyanide), and phosphate determinations by the methods of Allen (*Biochem. J.*, 1940, **34**, 858) and Lowrey and Lopez (*J. Biol. Chem.*, 1946, **102**, 41) before and after acid hydrolysis indicated that the whole of the phosphate present (3·3%) was inorganic. It was concluded that the material was a mixture or complex of non-reducing carbohydrate and ferric phosphate. When a few drops of dilute aqueous ammonia were added to a suspension of this complex in water a rigid gel was produced. This was transformed into a relatively mobile solution on further addition of ammonia. It seems that the carbohydrate has a powerful solubilising effect on ferric phosphate.

After removal of ferric and phosphate ions by precipitation and ion-exchange methods, a solution of the carbohydrate was strongly acidic. This was a most unexpected observation on an eluate from a basic resin. The solution also passed unchanged through a column of the strongly basic Amberlite IRA–400 resin. A possible explanation of this phenomenon

is that the large molecules of an acidic polysaccharide are unable to penetrate the resin grains, thereby considerably reducing the acid-binding capacity of the column. The substance was recovered from solution by freeze-drying. Its ready and complete precipitation from aqueous solution by addition of alcohol was further evidence for its polymeric nature.

Acid hydrolysis of the polysaccharide liberated glucose, identified by paper chromatography in three solvent systems and by its characteristic pink colour in the carbazole reaction (Gurin and Hood, *J. Biol. Chem.*, 1939, **131**, 211). No other carbohydrate components were thus detected. Alkaline hydrolysis liberated a neutral polyglucoside and malonic acid, identified by paper chromatography and by isolation and comparison with an authentic sample. It appears then that the acidity of the polysaccharide arises from the presence of malonyl hemi-ester groups.

A 1 : 6-β-polyglucose bearing malonyl hemi-ester residues was isolated by Raistrick and Rintoul from *P. luteum* (*Phil. Trans.*, *B.*, 1931, **220**, 255; Anderson, Howarth, Raistrick, and Stacey, *Biochem. J.*, 1939, **33**, 272) and was called luteic acid. In absence of a more complete investigation of the physical and chemical properties of the polysaccharide from *P. islandicum* it is not possible to assert its identity with luteic acid. However, certain marked similarities are indicated. After acid hydrolysis it gave a reducing value equivalent to 81—85 g. of glucose per 100 g. of polysaccharide, in fairly close agreement with the value for luteic acid (Raistrick and Rintoul, *loc. cit.*). Titration of the polysaccharide with sodium hydroxide indicated an equivalent of 575. Hydrolysis with excess of alkali liberated acidic groups equivalent to those present in the original polysaccharide. The amount of malonic acid set free by hydrolysis corresponded to 19·3 g. per 100 g. of the polysaccharide. These results agree approximately with a structure containing one malonyl hemi-ester per two glucose units, as is found in luteic acid.

Experimental

Purification of P. islandicum *Polysaccharide.*—The ferric phosphate-containing sample (400 mg.) was suspended in water (50 c.c.) and sufficient dilute aqueous ammonia added to produce a mobile solution. Hydrogen sulphide was passed in and iron sulphide removed by centrifugation. The precipitate was washed twice with water, and the combined solutions were passed through a column of IR-120 and then IR-4B resin. The acidic eluate was freeze-dried, yielding an almost colourless, porous solid (240 mg.), $[\alpha]_D^{22}$ −27°. To an aqueous solution of the polysaccharide was added ferric chloride, then dilute sodium hydroxide : a clear brown solution was obtained. Addition of Fehling's solution yielded a gel.

Hydrolysis.—(*a*) A sample (2 mg.) in 0·5N-hydrochloric acid (2 c.c.) was hydrolysed at 100° for 10 hr. Solvent was removed by evaporation in a desiccator, and the residue dissolved in a little water. Samples were examined by paper chromatography, using an aniline phthalate spray. A single spot corresponding to glucose was observed with the following solvent systems : phenol–water (R_F 0·37), butyl alcohol–acetic acid–water (R_F 0·31), collidine–water (R_F 0·38). The colour of the spot was the same as that given by glucose.

(*b*) The acidic polysaccharide (0·15 g.) was dissolved in 0·1N-sodium hydrogen carbonate (5 c.c.) and dialysed in a Cellophane sac against 0·1N-sodium hydrogen carbonate solution for 8 days at 4°. The sac contents were made 0·1N with respect to sodium hydroxide and left at room temperature for 18 hr. Alkali was removed by passing the solution through a column (20 × 1 cm.) of IR-120 resin, and acid in the eluate adsorbed on a column of IR-4B (OH^- form), from which it was eluted with 2N-ammonia (20 c.c.). The ammoniacal eluate was evaporated to dryness under reduced pressure, and a fraction of the residue run on a paper chromatogram in propanol–water–ammonia (6 : 3 : 1) and sprayed with Universal indicator which had been adjusted to pH 10 (Long, Quayle, and Stedman, *J.*, 1951, 2198). Only one spot was observed; this had R_F 0·24, which corresponded with malonic acid.

The residual ammonium salt was dissolved in water (0·5 c.c.), and silver nitrate solution added until no further precipitate was produced. The insoluble silver salt was collected by centrifugation, washed thrice with water, and decomposed with hydrogen sulphide. The silver sulphide was removed by centrifugation, the aqueous solution evaporated to dryness, and the residue sublimed (0·1 mm., bath-temp. 100°). The colourless sublimate, m. p. 130—132°, was undepressed in m. p. on admixture with an authentic specimen of malonic acid.

(*c*) The polysaccharide (14·5 mg.) was dissolved in hydrochloric acid (40 c.c., N) and heated at 100°. Samples were withdrawn periodically for determination of reducing value (Somogyi, *J. Biol. Chem.*, 1937, **117**, 771).

Time (hr.)	1	2	4	8
Reducing value (as g. of glucose liberated per 100 g. of polysaccharide)	66·3	81·2	81·2	85·1

Titration.—The acidic polysaccharide was further purified for titration by passing its aqueous solution through a column of Amberlite IR–4B (OH^- form) and one of Amberlite IR–120 (H^+ form). This effected almost complete removal of metallic and ammonium ions. The aqueous solution was freeze-dried as before. The polysaccharide (325 mg., dried at 110°/0·1 mm. for 1 hr.) required 10·59 c.c. of 0·0534N-sodium hydroxide for neutralisation to phenolphthalein. Treatment with excess of sodium hydroxide for 12 hr. at room temperature liberated acid groups equiv. to 11·28 c.c. of 0·0534N-sodium hydroxide.

The equiv. of the polysaccharide (from the first titre) is 575. 100 g. of the polysaccharide gave, on hydrolysis, 19·3 g. of malonic acid (from the second titre). Raistrick and Rintoul (*loc. cit.*) found that luteic acid had an equiv. of 434·7 and liberated 23·4 g. of malonic acid per 100 g. on hydrolysis.

Thanks are offered to Professor H. Raistrick, F.R.S., and Dr. B. H. Howard for generous gifts of the partly purified polysaccharide. We acknowledge financial support from the Department of Scientific and Industrial Research. This work was carried out during the tenure by one of us (E. M. T.) of an I.C.I. Fellowship.

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