NOTES.

279. *Hydrogen–Deuterium Exchange in Aqueous Solutions of Glycollic Acid and of Sodium Formate.*

By L. D. C. Bok and L. B. Petters.

The rate of exchange of hydrogen attached to a carbon atom, rendered measurable by the presence of a carboxyl group as an immediate neighbour, is further influenced by the nature of other atoms or radicals attached to the same carbon atom. Thus measurement of the rate of this exchange in a series of saturated fatty acids gives information on the relative influence of various substituents.

By carrying out a number of exchange experiments with aqueous solutions of glycollic acid at 210°, 200°, and 190°, averaging the calculated first-order velocity constants, and extrapolating the plot of $\log_{10} k$ against $1/T$, 330 hours has been obtained as the calculated approximate time of half-change for the forward (protium-rich substance + deuterium-rich solvent) exchange of the $C_{(\alpha)}$-hydrogen, and 470 hours for the reverse reaction at 160°.

The result for the forward exchange may be compared with the values obtained under the same conditions for other acids—800 hours for sodium formate (Bok and Cohen, *J. South African Chem. Inst.*, in the press), 40 hours for potassium acetate (Bok and Geib, *Z. phys. Chem.*, 1939, *A*, **183**, 353), and 1·4 hours for glycine (Bok and Mitchell, *J. South African Chem. Inst.*, in the press). The electromeric effect of the hydroxyl oxygen would result in the α-carbon atom being more electronegative; on the other hand the inductive effect of the positive ammonium ion results in a depletion of the electrons from the α-carbon atom. Thus one would expect the order glycollic acid < acetic acid < glycine for a reaction involving a proton transfer from the α-carbon atom.

An approximate activation energy calculated for the hydrogen exchange in glycollic acid is 30 kcals.

Sodium formate partly converted into formic acid by addition of deuterium chloride exchanged the $C_{(\alpha)}$-hydrogen at about the same rate as the formate ion. The exchange was more rapid when excess of deuterium chloride was present.

Experimental.—Weighed quantities of pure glycollic acid (about 0·1 g.) and deuterium oxide (about 0·5 g.) were heated in sealed Pyrex-glass tubes (15 cm. × 0·75 internal diam.) in an oil-bath whose temperature was thermostatically controlled to within 0·1°. After the required time the solvent was distilled off under vacuum, and the substance was redissolved in water at room temp. The solvent was again distilled off, the substance dried under high vacuum, and then burned in a stream of dry oxygen, and the isotopic content of the water formed determined by the method described by Harteck (*Z. Electrochem.*, 1938, **44**, 3).

For study of the reverse exchange, a forward-exchange experiment was carried out using four times the quantities stated above, and considerable exchange allowed to take place. Part of the material was used for combustion and the measurement of isotopic content, and the remainder for reverse-exchange experiments, about 0·1 g. of deuterated substance and 0·5 ml. of water being used.

A similar procedure was used with sodium formate. Deuterium chloride was prepared by distilling deuterium oxide on to barium chloride–phosphorus pentachloride, and distilling off the deuterium chloride formed, together with excess of deuterium oxide. Deuterium chloride solutions, about 5N, were obtained, the normality being determined by titration of about 0·1 ml. of the solution with standard sodium hydroxide. The deuterium chloride solution could be diluted with deuterium oxide to the required normality. With sodium formate, the acid was neutralised before distillation of the solvent, with standard sodium hydroxide solution.

We are indebted to the South African Council for Scientific and Industrial Research for Grants for equipment and expenses to one of us (L. D. C. B.).

UNIVERSITY OF NATAL, DURBAN. [*Received, October 30th*, 1951.]

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280. *Methylene Derivatives of* D-*Galactose and* D-*Glucose.*

By L. HOUGH, J. K. N. JONES, and M. S. MAGSON.

THE condensation of formaldehyde with sugars was first recorded by Schulz and Tollens (*Annalen*, 1896, **289**, 20; *Ber.*, 1899, **32**, 2585) who, by treatment of grape sugar with formaldehyde in the presence of concentrated hydrochloric acid, obtained a crystalline product, containing a free reducing group; analysis indicated a monomethylene glucose. Later work by De Bruyn and Van Eckenstein (*Rec. Trav. chim.*, 1903, **22**, 159) led to the isolation of syrupy bismethylene derivatives of glucose and galactose from a melt of the sugars with paraformaldehyde in the presence of an acid catalyst. Further investigation by Schmidt, Distelmaier, and Heiss ("F.I.A.T. Review of German Science, Preparative Organic Chemistry," Part II, p. 90) has provided evidence for the presence of a free hydroxyl group on $C_{(6)}$ of bismethylene glucose. This conclusion follows from the formation of 6-methyl glucose from bismethylene glucose.

Following De Bruyn and Van Eckenstein's procedure (*loc. cit.*) we attempted to condense D-glucose and also D-galactose with formaldehyde by melting the sugar and paraformaldehyde with a little acid catalyst; very little of the desired products was obtained. In an attempt to obtain a homogeneous reaction D-glucose was heated with paraformaldehyde in glacial acetic acid with concentrated sulphuric acid as catalyst, and a crystalline acetyl bismethylene derivative was obtained. The syrupy bismethylene glucose, obtained from the acetyl derivative, yielded, on methylation, a crystalline methyl bismethylene derivative which, on hydrolysis, gave 6-methyl D-glucose. It is established, therefore, that bismethylene glucose has a free primary alcohol grouping on $C_{(6)}$, and it follows that the acetyl and methyl derivatives must be 6-acetyl and 6-methyl bismethylene D-glucose.

A similar series of derivatives has been prepared from D-galactose. In this case, how-

ever, the acetyl bismethylene derivative was not obtained crystalline, but deacetylation followed by methylation with Purdie's reagents gave a crystalline methyl bismethylene D-galactose. Hydrolysis of this gave crystalline 6-methyl D-galactose, thus providing conclusive evidence that, in bismethylene galactose, formaldehyde is not engaged in acetal formation with the hydroxyl group on $C_{(6)}$.

Whilst the structures of the methylene derivatives of the hexose sugars await solution, those of many of the polyhydric alcohols are known. Thus, for example, Ness, Hann, and Hudson (*J. Amer. Chem. Soc.*, 1944, **66**, 665, 670) have proved that 1 : 3-2 : 4-5 : 6-trismethylene sorbitol is obtained when formaldehyde reacts with sorbitol.

The bismethylene derivatives of galactose and glucose which we have prepared are non-reducing to Fehling's solution and show no mutarotation. It is clear that the formaldehyde residues may be attached in acetal formation with hydroxyl groups, other than those on $C_{(6)}$, on adjacent carbon atoms, or other carbon atoms, but that one residue must involve the $C_{(1)}$-glycosidic hydroxyl group. Thus the methylene residues on glucose or galactose could be represented as 1 : 2-3 : 4-, 1 : 3-2 : 4-, or even 1 : 4-2 : 3-, assuming that the sugars exist in the pyranose form, which is by no means certain. Furthermore, isomerism may arise through the α- and β-glycosidic forms.

Experimental.—Carbon and hydrogen determinations are by Drs. Weiler and Strauss, Oxford, and methoxyl by Mr. W. M. Eno, Bristol. M. p.s are uncorrected. Optical rotations were determined in a 10-cm. tube, aqueous solutions being used.

6-*Acetyl bismethylene* D-*glucose*. A solution of D-glucose (50 g.) in water (20 c.c.) was mixed with glacial acetic acid (200 c.c.); paraformaldehyde (55 g.) was then added, followed by cautious addition, with shaking, of sulphuric acid (*d* 1·84; 25 c.c.). The mixture was heated on the steam-bath for 1 hour, the clear yellow solution was then cooled, water (200 c.c.) was added, and the liquor was extracted thrice with chloroform (200-c.c., 100-c.c., and 100-c.c. portions). The combined chloroform extracts were repeatedly washed with water until the washings were neutral. The chloroform solution was then dried ($CaCl_2$), filtered, and evaporated under reduced pressure to a syrup (37·6 g.) which partially crystallised. The crystalline material was isolated on a tile and recrystallised from methylated spirits, giving 6-*acetyl bismethylene* D-*glucose* (9 g.), m. p. 104°, $[\alpha]_D$ +46·5° (*c*, 0·67) (Found: C, 48·8; H, 5·6; Ac, 16·7. $C_{10}H_{14}O_7$ requires C, 48·75; H, 5·7; Ac, 17·5%).

6-*Methyl bismethylene* D-*glucose*. 6-Acetyl bismethylene D-glucose (4·7 g.) was dissolved in methanol (10 c.c.), and a solution of sodium methoxide (0·5 g.) in methanol (10 c.c.) added. The methanol was evaporated under reduced pressure and the syrupy deacetylated product methylated by heating it under reflux with methyl iodide (20 c.c.) and silver oxide (20 g.; added in four portions during 6 hours). The reaction mixture was extracted with chloroform, filtered, and the clear solution evaporated under reduced pressure to yield crystalline 6-*methyl bismethylene* D-*glucose* (2·2 g.) which was recrystallised from absolute alcohol. It had m. p. 46°, $[\alpha]_D$ +45° (*c*, 1·14) (Found: C, 49·3; H, 6·2; OMe, 14·5. $C_9H_{14}O_6$ requires C, 49·5; H, 6·4; OMe, 14·2%).

6-*Methyl* D-*glucose*. A solution of 6-methyl bismethylene D-glucose (0·5 g.) in 2N-hydrochloric acid (2 c.c.) was heated at 100° for 3 hours. The cooled solution was neutralised by treatment with Amberlite resin IR-4B, and unchanged material extracted with chloroform. Evaporation of the neutral aqueous solution in a vacuum-desiccator afforded crystalline 6-methyl D-glucose (0·1 g.) which was recrystallised from ethyl alcohol and then had m. p. 140° (mixed m. p. with an authentic specimen 137—138°), $[\alpha]_D$ +89° (initial value) ⟶ +61° ± 3° (equilibrium; *c*, 0·29). An *X*-ray powder photograph of the crystals was identical with that of 6-methyl D-glucose. 6-Methyl glucose is readily separable from the 2-, 3-, or 4-methyl derivatives on the paper chromatogram by using the butanol–pyridine–water solvent (Hough, Jones, and Wadman, *J.*, 1950, 1702). The chromatogram was run for 30 hours to achieve good separation of the sugars, and on development with *p*-anisidine hydrochloride it was observed that 3- and 4-methyl glucose gave spots of higher R_F value, which were yellower than that of 6-methyl glucose. 2-Methyl glucose also moved at a faster rate than 6-methyl glucose, but gave a redder spot. The rates of movement of the methyl derivatives relative to rhamnose of assumed R_F 0·30 were 2- (0·27), 3- (0·29), 4- (0·27) and 6- (0·25), respectively. The corresponding figures in the butanol–ethanol–water solvent (40 : 11 : 19) were 0·28, 0·29, 0·25, and 0·24. After separation in this solvent, 6-methyl glucose gave a redder derivative with the spray reagent than did the 2-methyl glucose. The 6-methyl bismethylene D-glucose may be hydrolysed at 100°

by agitating it with a suspension of Amberlite resin IR 120 (Wadman, unpublished results; cf. Glen, Myers, and Grant, *J.*, 1951, 2568) in water containing a weight of resin equivalent to a 0·25N-solution for 3 hours. To isolate the sugar it is necessary only to filter and evaporate the solution; the product is free from coloured decomposition products which are usually present when mineral acids are employed for the hydrolysis.

6-*Acetyl bismethylene* D-*galactose*. The condensation of formaldehyde with D-galactose (50 g.) in glacial acetic acid with sulphuric acid catalyst followed the technique already described for glucose and gave syrupy 6-methyl bismethylene D-glactose (14 g.). Distillation of the product under reduced pressure gave a syrup which failed to crystallise even after several months.

6-*Methyl bismethylene* D-*galactose*. The above syrup (14 g.) was converted into the methyl derivative by dissolving it in a little methanol and proceeding as described for the corresponding glucose derivative. A crystalline solid (10 g.) was obtained which, on recrystallisation from light petroleum (b. p. 60—80°) gave 6-*methyl bismethylene* D-*galactose* (5·5 g.), m. p. 82—82·5°, $[\alpha]_D$ −55·5° (*c*, 1·37) (Found: C, 49·6; H, 6·3; OMe, 14·4. $C_9H_{14}O_6$ requires C, 49·5; H, 6·4; OMe, 14·2%).

6-*Methyl* D-*galactose*. Hydrolysis of the bismethylene compound (0·5 g.), as described for the glucose compound, gave a crystalline product which after recrystallisation from *n*-butanol had m. p. 119·5—120·5° (mixed m. p. 120—121°), $[\alpha]_D$ +117·5° (initial value ⟶ constant value). An *X*-ray powder photograph of the crystals was identical with that of an authentic secimen of 6-methyl D-glactose. 6-Methyl galactose is distinguished from the 2-, 3-, or 4-methyl derivatives by paper chromatography, the butanol–pyridine–water (*idem, ibid.*) mixture being used as mobile phase. 3- and 4-Methyl galactose gave lower R_F values (0·21 and 0·20, respectively) and were yellower than the synthetic material which moved at the same rate as 6-methyl galactose (R_F 0·22). 2-Methyl galactose (R_F 0·25) gave a redder spot than 6-methyl galactose (cf. the colour differentiation in the glucose derivatives). The rates of movement of the methyl glucoses in the butanol–ethanol–water solvent were 2- (0·24), 3- (0·20), 4- (0·19), and 6- (0·20), respectively.

We thank Dr. T. Malkin for *X*-ray analyses and one of us (M. S. M.) acknowledges receipt of an F.E.T.S. grant from the Ministry of Education.

THE UNIVERSITY, BRISTOL 8. [*Received, December 18th*, 1951.]

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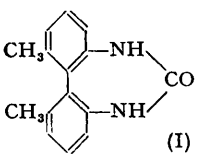
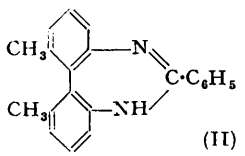
281. *The Stereochemistry of 2 : 2′-Bridged Derivatives of Diphenyl.*

By F. BELL.

KUHN AND GOLDFINGER (*Annalen*, 1929, **470**, 183) obtained (III) in an optically active condition by the interaction of (+)-2 : 2′-diamino-1 : 1′-dinaphthyl with benzil, and Sako (*Mem. Coll. Eng. Kyushu*, 1932, **6**, 283) obtained compounds (I) and (II) in active forms starting from (−)-2 : 2′-diamino-6 : 6′-dimethyldiphenyl. It was recognised that


(I)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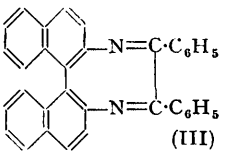
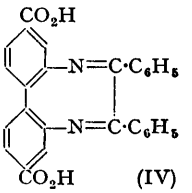
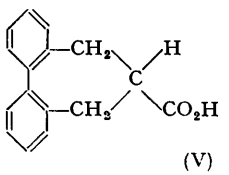

(III)

the ring system based on the 2 : 2′-positions of the diphenyl must exist in a non-planar form but the presence of obstacle groups in the 6 : 6′-positions was regarded as essential to preserve the non-planarity. This is now found to be unnecessary.


(IV)


(V)

Compound (IV), obtained by the interaction of 2 : 2′-diaminodiphenyl-4 : 4′-dicarboxylic acid and benzil, is readily resolved by crystallisation of the brucine salt to give

optically stable enantiomorphs. This result suggests that the compound (V) might be resolvable, but attempted resolution has so far failed.

Experimental.—2 : 2′-Diaminodiphenyl-4 : 4′-dicarboxylic acid (Jakubowski and Niementowski, *Ber.*, 1909, **42**, 650) was heated in a bath at 260° with the calculated quantity of benzil. The product was dissolved in sodium carbonate solution and reprecipitated with acetic acid. It was very sparingly soluble in acetone, chloroform, and *o*-dichlorobenzene but more soluble in boiling alcohol, acetic acid, cellosolve, and formamide. 6 : 7-*Diphenyl*-5 : 8-*diaza*-1 : 2-3 : 4-*dibenzo*cyclo*octatetraene*-2′ : 2″-*dicarboxylic acid* (IV) crystallised from these solvents on addition of water in pale yellow prisms, m. p. 348° (Found : C, 75·0; H, 4·1; N, 6·5. $C_{28}H_{18}O_4N_2$ requires C, 75·3; H, 4·0; N, 6·3%). On addition of (IV) (1·4 g.) to brucine (2·8 g.) in hot ethanol (50 c.c.) there was obtained a clear solution which almost immediately began to deposit rosettes of needles. The crop ($[\alpha]_D$ −540°; c = 1, in chloroform) was extracted with alcohol until constant rotatory power was obtained ($[\alpha]_D$ −566°). On decomposition with hydrochloric acid this gave the free acid, $[\alpha]_D$ −740° (c = 1, in N/2-sodium hydroxide) raised by crystallisation from aqueous alcohol to $[\alpha]_D$ −955°. The mother liquor from the brucine salt was concentrated, a small crystalline crop removed, and the residual liquor poured into dilute hydrochloric acid. The product had $[\alpha]_D$ +925° (c = 0·91, in N/2-sodium hydroxide).

The compound formed from 2 : 2′-diaminodiphenyl and benzil had m. p. 238° in agreement with Tauber (*Ber.*, 1892, **25**, 3287). It had negligible solubility in cold acetone and cold ethanol, low solubility in cold acetone, and moderate solubility in cold benzene and cold pyridine. Attempted resolution by adsorption from benzene solution on powdered cellulose or lactose was unsuccessful. The compound could be readily recrystallised from amyl alcohol or limonene.

1 : 2-3 : 4-Diben*zocyclo*hepta-1 : 3-diene-6-carboxylic acid (V) (Kenner, *J.*, 1913, **103**, 621) yielded non-crystalline brucine, morphine, quinidine, strychnine, and cinchonine salts. The quinine and cinchonidine salts could be readily recrystallised from ethanol, but the acid recovered from all crops was optically inactive.

HERIOT-WATT COLLEGE, EDINBURGH. [*Received, November 29th*, 1951.]

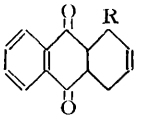
282. 1-o-*Methoxyphenylanthraquinone.*

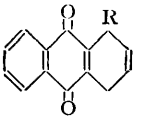
By E. A. BRAUDE and J. S. FAWCETT.

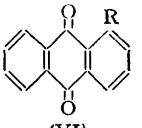
1-*o*-METHOXYPHENYLANTHRAQUINONE has been synthesised in the following manner. Reaction of *o*-methoxyphenylmagnesium bromide with crotonaldehyde gave *o*-methoxyphenylpropenylcarbinol (I), which undergoes oxotropic rearrangement to *o*-methoxystyryl-

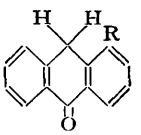
o-MeO·C_6H_4·CH(OH)·CH:CHMe (I) *o*-MeO·C_6H_4·CH:CH·CHMe·OH (II) *o*-MeO·C_6H_4·CH:CH·CH:CH_2 (III)

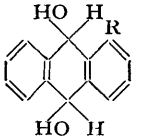
methylcarbinol (II) on treatment with dilute mineral acid and dehydration to 1-*o*-methoxyphenylbuta-1 : 3-diene (III) on distillation from potassium hydrogen sulphate (cf. Braude, Jones, and Stern, *J.*, 1947, 1087). Reaction of the diene with 1 : 4-naphthaquinone in ethanol at room temperature afforded the Diels–Alder adduct (IV), which, on treatment


(IV)


(V)


(VI)


(VII)


(VIII)

R = *o*-C_6H_4·OMe

in hot alkaline solution with a current of oxygen, was oxidised to a mixture of the dihydroanthraquinone (V) and the anthraquinone (VI).

On treatment with hot sodium dithionite (hydrosulphite) solution, 1-*o*-methoxyphenylanthraquinone is not reduced to the corresponding anthraquinol, but to a derivative which contains one less oxygen atom and is formulated as 1-*o*-methoxyphenyl-9-anthrone (VII).* The anthrone is readily oxidised back to the anthraquinone in alkaline solution. Treatment of 1-*o*-methoxyphenylanthraquinone with lithium aluminium hydride at 0° gave a tetrahydro-derivative, formulated as 9 : 10-dihydro-1-*o*-methoxyphenylanthraquinol (VIII) on the basis of its ultra-violet light absorption properties. Under similar conditions, anthraquinone itself is stated to give anthraquinol (Nystrom and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 3738) whereas anthracene does not react (Sampey and Cox, *J. Amer. Chem. Soc.*, 1949, **71**, 1507; Goodman, *J.*, 1951, 2209). On the other hand, the action of lithium aluminium hydride on 1 : 2-anthraquinone has been shown to give 1 : 2-dihydroanthraquinol (Booth, Boyland, and Turner, *J.*, 1950, 1188).

Experimental.—o-*Methoxyphenylpropenylcarbinol.* Freshly distilled crotonaldehyde (38 g.) in ether (100 ml.) was added to *o*-methoxyphenylmagnesium bromide (from magnesium, 13 g.; ethyl bromide, 0·5 g.; and *o*-bromoanisole, 100 g.) in ether (300 ml.) during 1 hour at 0° in an atmosphere of nitrogen. Stirring was continued for 2 hours, and excess of saturated ammonium chloride solution added at 0°. The ethereal layer was separated and dried (Na_2SO_4). Distillation from a trace of anhydrous potassium carbonate afforded o-*methoxyphenylpropenylcarbinol* (58 g., 67%) as a colourless liquid, b. p. 88°/0·05 mm., n_D^{22} 1·5431 (Found: C, 73·8; H, 7·9. $C_{11}H_{14}O_2$ requires C, 74·15; H, 7·9%). Light absorption in ethanol: $\lambda_{max.}$ 2230, 2730, and 2780 Å, ε = 8900, 2500, and 2300, respectively.

o-*Methoxystyrylmethylcarbinol.* The above carbinol (6 g.) was dissolved in a 0·01M-solution of hydrochloric acid in 60% aqueous acetone (100 ml.), and the solution kept for 24 hours at room temperature. On dilution with water, o-*methoxystyrylmethylcarbinol* (5 g.) separated as a viscous oil, which was distilled and had b. p. 100°/0·03 mm., n_D^{20} 1·5691 (Found: C, 74·3; H, 8·1. $C_{11}H_{14}O_2$ requires C, 74·15; H, 7·9%). Light absorption in ethanol: $\lambda_{max.}$ 2510 and 2990 Å, $\lambda_{infl.}$ 2560 Å, ε = 15 800, 5200, and 15 300, respectively. The p-*nitrobenzoate* crystallised from benzene–light petroleum (b. p. 80—100°) in colourless clusters of needles, m. p. 109° (Found: C, 66·6; H, 5·4; N, 4·4. $C_{18}H_{17}O_5N$ requires C, 66·1; H, 5·2; N, 4·3%). Light absorption in ethanol: $\lambda_{max.}$ 2560 and 2950 Å, $\lambda_{infl.}$ 2640 Å, ε = 36 000, 8500, and 29 800, respectively.

1-o-*Methoxyphenylbutadiene.* *o*-Methoxyphenylpropenylcarbinol (50 g.) was heated with anhydrous potassium hydrogen sulphate (5 g.) to 60° for 10 minutes and then rapidly distilled to give 1-o-*methoxyphenylbutadiene* (27 g., 60%), b. p. 68°/0·05 mm., n_D^{22} 1·6169 (Found: C, 82·9; H, 7·7. $C_{11}H_{12}O$ requires C, 82·5; H, 7·6%). Light absorption in ethanol: $\lambda_{max.}$ 2260, 2730, 2770, 3100, and 3160, $\lambda_{infl.}$ 2810 Å, ε = 14 400, 24 500, 24 000, 12 800, 12 800, and 23 200, respectively.

1-o-*Methoxyphenylanthraquinone.* A solution of the above diene (1 g.) and naphthaquinone (1 g.) in ethanol (6 ml.) was kept at room temperature for 48 hours. Filtration gave 1 : 4 : 13 : 14-*tetrahydro*-1-*o*-*methoxyphenylanthraquinone* (1·3 g.), which crystallised from ethyl acetate in colourless prisms, m. p. 154—155° (Found: C, 79·2; H, 5·8. $C_{21}H_{18}O_3$ requires C, 79·2; H, 5·7%).

The tetrahydroanthraquinone (5 g.) was dissolved in ethanol (100 ml.) containing potassium hydroxide (1 g.) and oxygen was passed through the hot solution for 15 minutes. After cooling, the product (4·8 g.) was filtered off and separated into two fractions by treatment with hot methanol (300 ml.). From the methanol-soluble fraction, 1-o-*methoxyphenylanthraquinone* (3 g.) was obtained, which crystallised from methanol in yellow prisms and rods, m. p. 160° (Found: C, 80·1; H, 4·75. $C_{21}H_{14}O_3$ requires C, 80·3; H, 4·5%). Light absorption in chloroform: $\lambda_{max.}$ 2440, 2495, 2670, and 3350 Å, ε = 22 000, 24 000, 19 000, and 3300, respectively. The methanol-insoluble fraction gave 1 : 4-*dihydro*-1-o-*methoxyphenylanthraquinone* (1 g.), which crystallised from xylene in yellow needles, m. p. 208° (Found: C, 79·8; H, 5·3. $C_{21}H_{16}O_3$ requires C, 79·8; H, 5·1%). Light absorption in chloroform: $\lambda_{max.}$ 2590 and 4130 Å, $\lambda_{infl.}$ 2640 Å, ε = 19 000, 1500, and 17 500, respectively.

Reduction of 1-o-*methoxyphenylanthraquinone.* (*a*) The quinone (3 g.) was suspended in a solution of sodium dithionite (4 g.) in 5% sodium hydroxide solution (50 ml.), and the mixture refluxed for 1 hour in an atmosphere of nitrogen. The product, formulated as 1-o-*methoxyphenyl*-9-*anthrone*, crystallised from methanol in colourless rods, m. p. 158° (Found: C, 83·8; H, 5·6. $C_{21}H_{16}O_2$ requires C, 84·0; H, 5·3%). On treatment with 1% alcoholic potassium hydroxide solution the anthrone was readily re-oxidised to the quinone.

* The alternative 10-anthrone structure is not excluded.

(*b*) Lithium aluminium hydride (0·2 g.) in ether (20 ml.) was slowly added to a solution of the quinone (1 g.) in benzene (50 ml.) at 0°, and the whole stirred at room temperature for 30 minutes; excess of 2N-sulphuric acid was added, and the ether–benzene extract evaporated, to give 9 : 10-*dihydro*-1-o-*methoxyphenylanthraquinol* (0·8 g.), which crystallised from methanol in colourless rectangular prisms, m. p. 195—196° (Found : C, 79·1; H, 6·0. $C_{21}H_{18}O_3$ requires C, 79·25; H, 5·65%). Light absorption in ethanol : $\lambda_{max.}$ 2800 Å, $\varepsilon = 3200$.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
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283. *The Separation of α-Acetamido-acids by Partition Chromatography on Kieselguhr.*

By G. HOWARD SMITH.

THE technique recently devised by Stein and Moore (*Cold Spr. Harb. Sym. Quant. Biol.*, 1949, **14**, 179; *J. Biol. Chem.*, 1951, **192**, 663) for the chromatographic separation of amino-acids on ion-exchange resins is much superior to the original partition method described by Martin and Synge (*Biochem. J.*, 1941, **35**, 1358) and Gordon, Martin, and Synge (*ibid.*, 1943, **37**, 79; 1944, **38**, 65) for the quantitative microanalysis of amino-acid mixtures. The latter technique has, however, certain advantages for preparative work in that the bands are made visible on the column by the use of an indicator held in the stationary phase and the α-acetamido-acids so separated can, if necessary, be estimated easily, quickly, and without their destruction, by titration with baryta solution.

One drawback to the partition method is the extreme degree of unreproducibility in the preparation of the silica gel used to support the stationary (aqueous) phase. Even those batches of silica which were satisfactory for the other faster-moving acetamido-acids still adsorbed acetyltryptophan to a greater or less extent, rendering separation in good yield of small quantities of this substance difficult or impossible (G. H. Smith, Ph.D. Thesis, University of London, 1950). At Dr. A. J. P. Martin's suggestion, columns were therefore prepared from kieselguhr. In addition to other advantages over precipitated silica, kieselguhr is almost non-adsorptive (Martin, *Biochem. Soc. Sym.*, 1949, **3**, 4), and it was found to give very satisfactory columns, which would carry a high load of acetyltryptophan.

The performance of the columns was highly reproducible. As an example, 9·9 mg. of acetyltryptophan ($R = 0·5$) was just separated from 7·4 mg. of acetylphenylalanine ($R = 0·9$) and well separated from 7·0 mg. of acetylvaline ($R = 0·2$) on a column (0·9 cm. × 10·2 cm.) containing 4·0 g. of kieselguhr. In several runs, estimated recoveries of acetylphenylalanine of 98—102% and of acetyltryptophan of 98—104% were obtained. The recovery of acetylvaline was only about 90% but the reason for this was not determined. By collection and separate titration of 1-ml. fractions of the eluate, it was shown that the zones were only slightly tailed at this column-loading. The degree of separation was, of course, greater for smaller amounts of the acids. The immediate aim of this work, however, was not so much to obtain a complete separation for analytical purposes, as to obtain a convenient and rapid method of isolating 5—10 mg. of tryptophan from protein hydrolysates for isotope analysis. It is suggested that the use of kieselguhr partition columns containing indicator in the stationary phase could be of wide applicability in the separation of acids and bases on a micro-preparative scale.

Experimental.—Hyflo "Supercel" (Johns–Manville Co. Ltd.) (about 150 g.) was added to water (1 l.) without stirring, and allowed to settle, and the yellow supernatant liquid and some floating material were decanted off. The wet kieselguhr was made into a column 5·8 cm. in diameter which was washed with 2N-nitric acid (500 ml.) to remove interfering metals and then with water until the effluent was neutral (methyl-orange). The column was sucked as dry as possible and extruded. The kieselguhr was then successively dried at 110°, roasted in a muffle furnace for 1½ hours to remove residual organic matter, and sieved (90 mesh).

For use in columns, the washed kieselguhr was first thoroughly mixed with 0·015% methyl-orange solution (0·5 ml. per 1 g. of kieselguhr) and dried at 110°. It was then mixed with the amount of 0·1N-sulphuric acid previously determined to give the most satisfactory colour of

the indicator (usually about 0·6 ml. acid to 1 g. of kieselguhr), and then redried. The acidified solid was not kept in the oven for more than 3—4 hours or at room temperature for more than a day before use, as it tended to become more alkaline. Water was added to the kieselguhr (0·7 ml. per 1 g.), and the mixture suspended in *n*-butanol–chloroform (1% by volume; water-saturated) in the usual way. Although the kieselguhr would hold appreciably more than its own weight of water without appearing wet, columns containing the above amount were the easiest to pack in 1% butanol in chloroform solution and were therefore generally used. The columns were packed with the aid of a perforated metal disc on the end of a long wire (Martin, *loc. cit.*) and then run in the usual way without regulation of the rate of flow of the developing solvent. The separated acetamido-acids were titrated with 0·01N-barium hydroxide (bromothymol-blue) (Gordon *et al.*, 1943, *loc. cit.*).

As kieselguhr is less adsorbent than silica gel, the methyl-orange was held less firmly in the stationary phase if the columns were prepared as for silica; the drying of the indicator on to the kieselguhr appeared largely to fix it, however. Nevertheless, a small amount of indicator was sometimes displaced by the substances being separated, particularly from the top of the column, but this effect was overcome by placing small pads of acid-treated kieselguhr containing water but no indicator at the top and bottom of the column. Liddell and Rydon's indicator (*Biochem. J.*, 1944, **38**, 68) might perhaps have been held more firmly, being less soluble in organic solvents, but methyl-orange was used owing to its greater sensitivity. The indicator was, of course, rapidly eluted by chloroform containing 20% *n*-butanol.

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284. 2 : 4 : 5-*Trichlorophenetole.*

By F. E. Smith.

In another investigation, it became necessary to prepare pure 2 : 4 : 5-trichlorophenetole. The only references to this are in D.R.-P. 411,052 (Agfa, 1925) and U.S.P. 2,072,797 (Gen. Electric Co., 1937). Both processes describe preparations from 1 : 2 : 4 : 5-tetrachlorobenzene, alcohol, and sodium hydroxide, which are heated under pressure, in the first case alone, and in the second case in the presence of cuprous chloride. Only Agfa describe the product, which is stated to crystallise from alcohol with m. p. 95°. This is unexpectedly high, since substituted phenetoles in general melt at lower temperatures than the corresponding anisoles and parent phenols.

2 : 4 : 5-Trichlorophenetole, prepared by treatment of the pure phenol with ethyl sulphate in alkaline solution, followed by recrystallisation from ethanol, then methanol, had m. p. 40·4°, unchanged by boiling dilute sodium hydroxide or by recrystallisation from toluene, so, as it seemed certain that the product was that required, the Agfa process was repeated.

1 : 2 : 4 : 5-Tetrachlorobenzene (7·3 g.), sodium hydroxide pellets (2·5 g.), and anhydrous industrial alcohol (50 ml.) were heated at 170—180° for 7 hours in a sealed tube. When cool, the mixture was transferred to a flask with the aid of water (100 ml.) and steam-distilled, giving: (*a*) an alcoholic fraction which on evaporation to dryness gave 0·2 g., m. p. 37°; (*b*) the main ether fraction which was separated and dried, giving 3·3 g. of oil + crystals; and (*c*) the phenol fraction, distilled after acidification with sulphuric acid, which was filtered off and dried, giving 3·5 g., m. p. 54—55°, mixed m. p. with pure 2 : 4 : 5-trichlorophenol 59—62·5°. The material from (*b*) was recrystallised three times from methanol until short colourless needles, m. p. 40·3°, were obtained. As these were identical in appearance and m. p. with the material prepared by alkylation, and as the mixed m. p. was 40·3°, it is evident that the m. p. 95° recorded for 2 : 4 : 5-trichlorophenol is incorrect and that the true m. p. is 40·4°.

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285. 4 : 6-*Dichloro*-2-*dimethylaminopyrimidine*.

By W. R. Boon.

King and King (*J.*, 1947, 726) have described the isolation of two substances from the reaction of 2 : 4 : 6-trichloropyrimidine with dimethylamine. To the substance, m. p. 113°, they assigned the structure 2 : 4-dichloro-6-dimethylaminopyrimidine, and a second substance of m. p. 102—103° was believed to be 4 : 6-dichloro-2-dimethylaminopyrimidine. On repetition of this work no difficulty was encountered in obtaining the higher-melting isomer pure, but only solids of indefinite m. p. could be obtained when using the conditions prescribed by King and King for the isolation of the low-melting isomer. Fractional crystallisation of such a mixture first from aqueous methanol and then from light petroleum showed it to consist of 2 : 4-dichloro-6-dimethylamino- and 4 : 6-dichloro-2-dimethylamino-pyrimidine, m. p. 54°. The latter substance has also been prepared by the reaction of 2-dimethylamino-4 : 6-dihydroxypyrimidine with phosphorus oxychloride. Reaction of this substance with ammonia, methylamine, and dimethylamine gave respectively 4-amino-6-chloro-2-dimethylamino-, 4-chloro-2-dimethylamino-6-methylamino- and 4-chloro-2 : 6-bisdimethylamino-pyrimidine, the first two compounds being identical with the corresponding compounds prepared by reaction of dimethylamine with 4-amino-2 : 6-dichloropyrimidine (Büttner, *Ber.*, 1903, **36**, 2227) and 2 : 4-dichloro-6-methylaminopyrimidine (Winklemann, *J. pr. Chem.*, 1927, **115**, 292).

Experimental.—2-*Dimethylamino*-4 : 6-*dihydroxypyrimidine*. Dimethylguanidine sulphate (91 g.) was stirred for 30 minutes with a boiling solution of sodium methoxide, from sodium (15 g.) and methanol (300 c.c.). Ethyl malonate (116 g.) was then added cautiously and the whole was stirred under reflux for 6 hours. After cooling, water (450 c.c.) was added, the solution acidified with acetic acid, and the *pyrimidine* collected (85 g.). It did not melt below 350° and for analysis was crystallised from water (Found: C, 41·6; H, 6·4; N, 24·6. $C_6H_9O_2N,3H_2O$ requires C, 41·6; H, 6·4; N, 24·2%).

4 : 6-*Dichloro*-2-*dimethylaminopyrimidine*. (*a*) 2-Dimethylamino-4 : 6-dihydroxypyrimidine (15·5 g.; dried at 120°) and phosphorus oxychloride (60 c.c.) were heated under reflux for 35 minutes, cooled, and poured with stirring into cold 2N-sodium hydroxide (1 l.); after 15 minutes the *dichloro*-compound was collected; purified first by steam-distillation and then by crystallisation from light petroleum, it (16 g.) had m. p. 54° (Found: C, 37·6; H, 3·8; N, 21·8; Cl, 36·4. $C_6H_7N_3Cl_2$ requires C, 37·5; H, 3·7; N, 21·9; Cl, 36·9%). (*b*) This substance was also isolated by fractional crystallisation, alternately from light petroleum and aqueous methanol, of the residue remaining after removal of the bulk of the 2 : 4-dichloro-6-dimethylamino-pyrimidine from the reaction product of trichloropyrimidine and dimethylamine (King and King, *loc. cit.*).

4-*Amino*-6-*chloro*-2-*dimethylaminopyrimidine*. 2-Dimethylamino-4 : 6-dichloropyrimidine (3·8 g.) and 10% ethanolic ammonia (10 c.c.) were heated at 110—120° for 18 hours. After the mixture had cooled, water was added, the crude product collected, and unchanged starting material removed by steam-distillation. The *aminopyrimidine*, crystallised first from benzene–light petroleum and then from aqueous ethanol, had m. p. 151° (Found: C, 41·5; H, 5·5; N, 32·8; Cl, 19·7. $C_6H_9N_4Cl$ requires C, 41·7; H, 5·2; N, 32·5; Cl, 20·6%). The same product (identified by m. p. and mixed m. p.) was obtained by reaction of 4-amino-2 : 6-dichloropyrimidine (3·3 g.) with dimethylamine (3 g. in 15 c.c. of ethanol) at 80° for 10 hours.

4-*Chloro*-2-*dimethylamino*-6-*methylaminopyrimidine*, m. p. 78° (from aqueous ethanol), b. p. 122—125°/1 mm. (Found: C, 45·4; H, 6·1; N, 30·6; Cl, 19·0. $C_7H_{11}N_4Cl$ requires C, 45·1; H, 5·9; N, 30·0; Cl, 19·0%), was obtained similarly from 4 : 6-dichloro-2-dimethylamino-pyrimidine and alcoholic methylamine or 2 : 4-dichloro-6-methylaminopyrimidine and alcoholic dimethylamine.

4-*Chloro*-2 : 6-*bisdimethylaminopyrimidine*, m. p. 52·5° after sublimation at 56°/0·1 mm. (Found: C, 47·4; H, 6·1; N, 27·1; Cl, 17·5. $C_8H_{13}N_4Cl$ requires C, 47·8; H, 6·5; N, 27·9; Cl, 17·7%), was similarly obtained from 4 : 6-dichloro-2-dimethylaminopyrimidine and alcoholic dimethylamine.

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