390. *Addition Compounds of the Carbohydrates. Part III. Potassium Hydroxide Derivatives of Cellobiose, Lactose, and Galactose.*

By EDMUND G. V. PERCIVAL and GEOFFREY G. RITCHIE.

IN Part I (J., 1934, 1160) it was pointed out that certain disaccharides appear to unite with more than one molecular proportion of potassium hydroxide. This has now been confirmed for cellobiose, which in the presence of excess of the reagent forms a *complex*, $C_{12}H_{22}O_{11}$,2KOH, as indicated by titration experiments. Controlled methylation resulted in the isolation of a monomethyl methylcellobioside, from which on hydrolysis and suitable treatment 6-methyl glucosephenylosazone has been isolated. These results can be explained if we consider the reducing group and one of the primary alcoholic residues to be concerned in the union. Since glucose itself only takes up one potassium hydroxide residue, it seems reasonable to suppose that in the cellobiose complex the second potassium hydroxide residue is to be found in the non-reducing glucopyranose unit (I).

Indirect support for this view was afforded by a study of the compounds which lactose forms with potassium hydroxide, since in this case the production or otherwise of methylated galactose derivatives would indicate whether the non-reducing unit were involved or not. Titration experiments showed that lactose combines with more potassium hydroxide than does cellobiose under the same conditions, the results indicating the presence of a mixture of $C_{12}H_{22}O_{11}$,2KOH and $C_{12}H_{22}O_{11}$,3KOH. Methylation under anhydrous conditions, followed by acetylation, gave a non-reducing syrup, from which, by hydrolysis, acetylation and distillation in a high vacuum, a triacetyl dimethyl hexose and a tetra-acetyl monomethyl hexose were isolated. Complete methylation of both these products and the isolation from each in good yield of crystalline tetramethyl galactopyranose anilide, together with the absence of any glucose derivatives, showed that, except for the normal reaction at the reducing group, substitution had taken place exclusively in the galactose portion of the lactose molecule.

The monomethyl galactose, unlike 6-methyl galactose, gave no crystalline phenylhydrazone. On heating with phenylhydrazine, however, a crystalline osazone (yield, 40% of the theoretical) was obtained which contained no methoxyl and was identical with galactosazone. No mucic acid could be isolated on oxidation of the original syrup with nitric acid; therefore it is necessary to assign the methoxyl residue to position 2. Robertson and Lamb (J., 1934, 1321) have recorded a case where the 2-methyl group in 2 : 3-dimethyl galactose is eliminated on osazone formation.

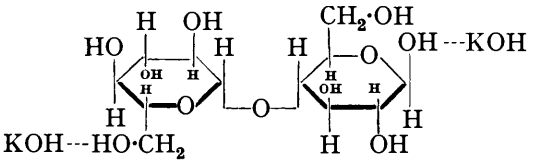
Similarly the dimethyl galactose gave a monomethyl galactosazone (m. p. 150°), and a rigorous search failed to reveal the presence of any dimethyl galactosazone. This monomethyl galactosazone was clearly not identical with 6-methyl galactosazone, m. p. 204° (Munro and Percival, this vol., p. 640), or 3-methyl galactosazone, m. p. 176—179° (Robertson and Lamb, *loc. cit.*), and 5-methyl galactosazone was excluded by the isolation of tetramethyl galactopyranose anilide. Furthermore the osazone was found to be identical with authentic 4-methyl galactosazone* kindly supplied by Professor W. N. Haworth, F.R.S., thus indicating that the dimethyl galactose was 2 : 4-dimethyl galactose, and further experiments confirmed this view. Glycoside formation with methyl-alcoholic hydrogen chloride in the cold followed a different course from that described by Robertson and Lamb (*loc. cit.*) for 2 : 3-dimethyl galactose, since the rotation remained strongly positive, indicating the absence of galactofuranosides, and this was confirmed by titration experiments after Levene, Raymond, and Dillon (*J. Biol. Chem.*, 1932, **95**, 699). Oxidation with nitric acid, followed by esterification and amide formation, yielded no *d*- or *i*-dimethoxysuccinamides, the presence of adjacent methyl groups (*e.g.*, in positions 2 : 3 or 3 : 4) thus being excluded.

From this examination of the products of the controlled methylation of the potassium hydroxide–lactose complexes, it appears that substitution has taken place at the reducing group of the glucopyranose unit and at the hydroxyl groups at positions 2 and 4 in the

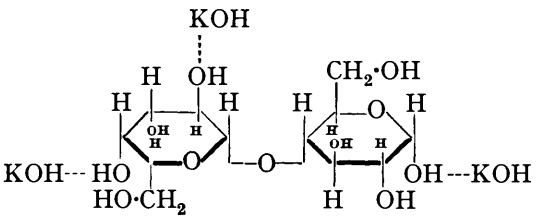
* Private communication.

galactopyranose residue. It is suggested, therefore, that lactose associates itself with potassium hydroxide residues at these points (II).

The difference in the results obtained for cellobiose and lactose is not due to the greater affinity of galactose than glucose for potassium hydroxide, since galactose forms a compound $C_6H_{12}O_6$,KOH, and this formulation is supported by methylation with dry methyl sulphate, no evidence for substitution beyond the methylgalactoside stage being found. While, therefore, it is reasonable to suppose that the tendency is for the reaction to take place in the sugar unit remote from the reducing group, there is no explanation yet avail-



(I.)



(II.)

able for the anomalous behaviour of lactose, since the other disaccharides so far examined, cellobiose and sucrose (Part II, J., 1935, 648), undergo substitution in the primary alcoholic residues.

In Part I (*loc. cit.*) it was suggested that the addition compounds were comparable with the hydrates of the alkali hydroxides and a structure involving co-ordination through hydrogen was proposed. In the light of modern views, however, this is doubtful and an alternative system is provided by the theory of resonance. At this stage, therefore, it does not seem desirable to define the union between the sugars and alkali hydroxides other than by some type of loose combination probably best represented by a dotted line in the manner of the residual valencies of Werner.

Experimental.

Titration Experiments with Cellobiose.—Cellobiose (0·2 g.), prepared by Zemplén's method (*Ber.*, 1923, **56**, 1705) from the octa-acetate, was dissolved in alcohol (6 c.c., 90%), standard alcoholic potassium hydroxide (20 c.c.) added, and the mixture kept for 10 minutes. The precipitate was removed by filtration, and an aliquot portion of the filtrate titrated with standard acid (method 1). The precipitate was drained, washed with the minimum quantity of alcohol, dissolved in water, and titrated (method 2). The results are below :

Total concn. of cellobiose, %		0·57	0·77	0·88
Concn. of KOH, N	initial	0·839	0·742	0·76
	final	0·807	0·697	0·69
KOH combined, %	method (1)	32·5	33·2	32·9
	,, (2)	25·7	25·1	23·0

For $C_{12}H_{22}O_{11}$,2KOH, 100 g. of cellobiose require 32·8 g. of potassium hydroxide.

Typical Preparation of Potassium Hydroxide–Cellobiose.—Cellobiose octa-acetate (20 g.), prepared by Haworth and Hirst's method (J., 1921, **119**, 193), was made into a paste with alcohol (40 c.c.), potassium hydroxide (40 g.) in alcohol (175 c.c.) stirred in, and stirring continued for 2 hours in a closed flask. The insoluble *product* (14 g.) was then filtered off, washed quickly with alcohol and ether, and dried in a vacuum over phosphoric oxide [Found : KOH (titration), 22·1. $C_{12}H_{22}O_{11}$,2KOH requires KOH, 24·7%].

Reaction with Methyl Sulphate.—The method described in Parts I and II (*loc. cit.*) was employed, the dry addition compound (8 g.) being stirred with dry, neutral methyl sulphate for 5 minutes at 35—40° and for 10 minutes at 70°. After removal of the potassium methyl sulphate, and unchanged cellobiose (4·9 g.) by precipitation with alcoholic potassium hydroxide,

the residue on acetylation yielded a syrup (4·7 g.), which partly crystallised on trituration with alcohol. The crystals (1·4 g.), m. p. 178°, $[\alpha]_D^{20°}$ — 22° in chloroform (*c*, 1·5), were hepta-acetyl β-methylcellobioside (Found: C, 50·0; H, 5·9; OMe, 4·5. Calc. for $C_{27}H_{38}O_{18}$: C, 49·9; H, 5·9; OMe, 4·9%).

Examination and Hydrolysis of the Residual Syrup.—The non-reducing syrup (3·2 g.), $[\alpha]_D^{20°}$ + 2° in chloroform (*c*, 5·0), appeared to be a hexa-acetyl monomethyl methylcellobioside (Found: OMe, 8·5; CH_3·CO, 41·4. Calc. for $C_{26}H_{38}O_{17}$: OMe, 9·9; CH_3·CO, 41·5%). It was accordingly deacetylated by Zemplén's method (*loc. cit.*) and hydrolysed in hydrochloric acid (80 c.c., 7%) at 90° until a constant rotation was attained:

Time (minutes)	0	65	125	220	300	380
$[\alpha]_D^{20°}$	+3°	13°	27°	37°	50°	50°

After neutralisation with silver carbonate and filtration, the solvent was removed at 50°/15 mm. to yield a reducing syrup (1·2 g.).

Removal of glucose. Potassium hydroxide (1 g.) in alcohol (15 c.c.) was added to the syrup dissolved in alcohol (20 c.c.). The precipitate (1·2 g.) was filtered off after 15 minutes, dissolved in water, acidified with acetic acid, and treated with phenylhydrazine to yield glucosephenylosazone (0·3 g.), m. p. 206° (Found: OMe, nil).

Isolation of 6-methyl glucosephenylosazone. The filtrate was acidified with acetic acid, alcohol removed under diminished pressure, and the residue dissolved in water (10 c.c.) and heated with phenylhydrazine (1 g.) and glacial acetic acid (1 c.c.) at 90° for 30 minutes. An orange osazone (0·5 g.) was obtained, which was recrystallised from aqueous pyridine; m. p. 180—184°, $[\alpha]_D^{20°}$ — 70° in 50% alcohol–pyridine (*c*, 0·3) (Found: C, 61·2; H, 6·6; OMe, 7·6; N, 14·75. Calc. for $C_{19}H_{24}O_4N_4$: C, 61·3; H, 6·45; OMe, 8·3; N, 15·0%). This osazone agreed in properties with 6-methyl glucosazone (see Part II, *loc. cit.*) and its m. p. was unaltered by authentic 6-methyl glucosazone, but was depressed to 164° by 3-methyl glucosazone (m. p. 179°).

Potassium Hydroxide–Lactose. Titration Experiments.—An approximate estimate of the alkali-combining capacity of lactose was obtained by the titration method previously used. Lactose monohydrate, dissolved in 75% alcohol, was employed and the results are below:

Total concn. of lactose, %		0·72	0·94	1·00	1·01
Concn. of KOH, *N*	initial	0·221	0·427	0·747	0·774
	final	0·181	0·373	0·685	0·704
KOH combined, %	method (1)...	30·9	32·1	35·1	39·2
	,, (2)...	28·1	26·0	34·2	41·6

$C_{12}H_{22}O_{11}$,3KOH requires 49 g. of potassium hydroxide for 100 g. of lactose. Thus it would appear that, in the higher concentrations of alkali examined, some of this higher compound was present; it was found impracticable to use very concentrated alkaline solutions, since brown solutions were thereby produced.

Preparation of Potassium Hydroxide–Lactose.—Lactose octa-acetate (35 g.), prepared by Hudson and Johnson's method (*J. Amer. Chem. Soc.*, 1915, **37**, 1270), was suspended in alcohol (200 c.c.), potassium hydroxide (66 g.) in alcohol (1 l.) added, and the mixture stirred for 2 hours, the insoluble product (20 g.) being isolated as before (Found: KOH, 26·0 $C_{12}H_{22}O_{11}$,3KOH requires KOH, 32·9%).

Reaction with Methyl Sulphate.—The powdered product thus obtained (20 g.) was stirred with methyl sulphate under the conditions obtaining above. Potassium methyl sulphate was removed, followed by the removal of lactose by the addition of potassium hydroxide (7 g.) in alcohol (100 c.c.). Ether was then added to bring about the complete removal of unchanged lactose until the precipitate obtained was no longer reducing. The residual solution was acidified with acetic acid and evaporated under diminished pressure. The product was acetylated at 95° for 1 hour with acetic anhydride (120 c.c.) and anhydrous sodium acetate (23 g.). After the usual treatment a non-reducing syrup (4·4 g.) was obtained (OMe, 12·1%). This was deacetylated by Zemplén's method (*loc. cit.*), and the product hydrolysed with sulphuric acid (1·5*N*) at 95° till a constant rotation was reached:

Time (minutes)	0	150	290	395	435	480
$[\alpha]_D^{20°}$	+18°	34°	41°	45·6°	47·4°	48° (const.)

The solution was neutralised with barium carbonate and evaporated to dryness at 50°/15 mm., yielding a syrup.

Separation of the Products of Hydrolysis.—The syrup was extracted with boiling alcohol (50 c.c.). To the extracts was added an alcoholic solution of potassium hydroxide (2 g. in 40 c.c.), and a precipitate (A) was filtered off rapidly, washed with alcohol, and dried. The addition of dry ether (250 c.c.) to the filtrate deposited another precipitate (B), which was collected and dried. The filtrate was neutralised with acetic acid and evaporated to dryness, and the residue (C) acetylated with acetic anhydride (35 c.c.) and anhydrous sodium acetate (7 g.) at 100° for 1 hour, a brown syrup being obtained by the usual method of isolation. The dry sugar–alkali compounds (A) and (B) were similarly acetylated. (A) Yield, 0·98 g.; OMe, 2·0%. (B) 0·49 g.; OMe 8·1%. (C) 0·83 g.; OMe, 15·5%. An approximate fractionation of the mixture had thus been achieved. Fraction (B) distilled almost completely at 182—183° (bath temp.)/0·04 mm. to yield a colourless reducing syrup (Found : OMe, 8·3. Calc. for $C_{15}H_{22}O_{10}$: OMe, 8·6%). Fraction (C) was distilled at 155—162° (bath temp.)/0·05 mm. (Found : OMe, 16·7. Calc. for $C_{14}H_{22}O_9$: OMe, 18·6%).

Examination of the Monomethyl Hexose Acetate.—Complete methylation. In order to determine whether this was derived from glucose or galactose a portion of the syrup (B) (0·5 g.; OMe, 8·3%), dissolved in acetone (10 c.c.) and water (10 c.c.), was methylated with methyl sulphate (10 c.c.) and aqueous sodium hydroxide (25 c.c., 30%) with the usual precautions for the methylation of a reducing sugar. After isolation of the product two further methylations with methyl iodide (10 c.c.) and silver oxide (2 g.) were conducted and the product was distilled at 113°/0·08 mm. to yield a clear, mobile, non-reducing syrup (0·26 g.). The glucosidic methoxyl residue was removed by heating for 2 hours at 100° in hydrochloric acid (10 c.c., 7%). The solution was neutralised with barium carbonate, alcohol was added to precipitate barium salts, which were filtered off, and the solution was evaporated to dryness under diminished pressure. The residue was extracted three times with ether, and from the filtered extracts on evaporation a syrup (0·2 g.) was obtained. Inoculation with tetramethyl glucopyranose failed to induce crystallisation.

Tetramethyl galactopyranose anilide. The anilide was prepared from the above sugar by boiling the syrup (0·19 g.) under reflux with aniline (0·6 g.) in alcohol (2 c.c.) for 3 hours. White needles (0·14 g.) separated on cooling, which after two recrystallisations from alcohol had m. p. 193°, not depressed in admixture with an authentic specimen. No product corresponding to tetramethyl glucopyranose anilide could be isolated from the mother-liquors.

Attempted phenylhydrazone formation. The method described by Munro and Percival (*loc. cit.*) for the isolation of 6-methyl galactosephenylhydrazone was applied to the deacetylated monomethyl galactose (B), but in spite of various modifications no crystalline phenylhydrazone could be isolated.

Osazone formation. A specimen of the syrupy monomethyl galactose tetra-acetate (0·58 g.) was deacetylated by Zemplén's method and treated with phenylhydrazine acetate. The first crop of osazone (0·17 g.) had m.p. 175°, which was raised to 186—188° by one recrystallisation (Found : OMe, nil). The m. p. was not depressed in admixture with authentic galactosazone. A second crop (0·08 g.), m. p. 186—188°, was obtained (OMe, nil). That this galactosazone could not have been derived from free galactose was shown by repeated failures to isolate mucic acid, from the deacetylated syrup used for osazone formation, by oxidation with nitric acid (*d* 1·15). It is therefore necessary to conclude that the galactosazone was derived from 2-methyl galactose.

Examination of the Dimethyl Hexose Acetate.—The colourless syrup was reducing to Fehling's solution and had $[\alpha]_D^{20°}$ + 59° in chloroform (*c*, 0·8); $n_D^{20°}$ 1·4525° (Found : C, 50·0; H, 6·4; OMe, 16·7. Calc. for $C_{14}H_{22}O_9$: C, 50·3; H, 6·6; OMe, 18·6%).

Complete methylation was carried out as previously described for 2-methyl galactose, and tetramethyl galactopyranose anilide (yield, 46%), m. p. 192°, unchanged in admixture with an authentic specimen, was obtained as before. No glucose derivatives could be detected and it was concluded that the specimen was triacetyl dimethyl galactopyranose.

Osazone formation.—Triacetyl dimethyl galactose (0·27 g.) was deacetylated by Zemplén's method. To the solution of the deacetylated sugar in water (20 c.c.) were added phenylhydrazine (0·5 g.), acetic acid (2 c.c.), sodium acetate (1 g.), and a trace of sodium bisulphite, the mixture being heated at 90—100°. Osazone formation was slow, but after 3 hours a yellow oil (0·07 g.) separated which solidified on standing (OMe, 7·5%). Purification by solution in chloroform, filtration, and precipitation by light petroleum gave a yellow solid (0·05 g.) (OMe, 8·0%), and the solution on evaporation gave a yellow glass (0·01 g.) (Found : OMe, 8·1. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3%). It thus appeared that from a dimethyl sugar a monomethyl osazone was obtained. As this would establish one of the methyl groups in the 2-position,

an exhaustive search for the presence of dimethyl osazone was carried out. A further crop of osazone (0·01 g.) had m. p. 150° (OMe, 7·2%). After six recrystallisations from aqueous alcohol a small amount of galactosazone, m. p. 186°, not depressed in admixture with an authentic specimen, was obtained (OMe, nil). This was undoubtedly derived from a small amount (*ca.* 10%) of 2-methyl galactose which could not be completely separated from the initial material. The search for a dimethyl osazone was continued by extraction of the original aqueous solution with chloroform, washing with acetic acid, drying, and evaporation (Found for the glass obtained : OMe, 4%).

Another series of experiments confirmed these results and five crops of osazone were isolated in a total yield of over 80% of the dimethyl galactose used. Four crops each had OMe, 7% and the other, OMe, 5%. The best crystalline fraction (20%) had m. p. 145—150° (OMe, 7·1%).

Identification of the osazone as 4-methyl galactosazone. The main portion of monomethyl osazone on recrystallisation from alcohol had m. p. 147—150° and showed no depression in admixture with an authentic specimen of 4-methyl galactosazone, m. p. 148—150° (Found : OMe, 7·1; N, 14·9. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3; N, 15·0%). No evidence was found for the presence of 6-methyl galactosazone (m. p. 204°).

The Course of Glycoside Formation.—A portion of the dimethyl galactose acetate was deacetylated by Zemplén's method and the aqueous extract of the free sugar was evaporated to dryness (diminished pressure). The product was dissolved in methyl alcohol (10 c.c.) containing 1·9% of hydrochloric acid; the concentration of sugar being 0·94% :

Time (hours)	0	19	43	67
$[\alpha]_D^{18°}$	+49°	34°	33°	33° (const.)

The equilibrium solution was non-reducing. These values differ from those given by Robertson and Lamb (*loc. cit.*) for 2 : 3-dimethyl galactose, the rotation of which under the same conditions fell during 7 days from $[\alpha]_D^{15°}$ + 38° to − 24°.

The semi-quantitative method of Levene, Raymond, and Dillon (*loc. cit.*; see also this vol., p. 643) was employed to follow the course of glycoside formation at room temperature. Methyl-alcoholic hydrogen chloride (0·5%) was employed, the concentration of sugar in the solution being 9 mg./c.c. The reducing values were corrected for the hydrolysis of the pyranoside, which was found to take place to the extent of 13%.

	0·01*N*-$Na_2S_2O_3$, c.c.		Free sugar, %.					
Time (hours).	Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.	Corrected.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	2·20	2·40	100	100	100	100	0	0
1	1·70	2·20	77·5	92	91	77·5	13·5	9
23·5	0	0·70	0	29	16	0	16	84
27·0	0	0·67	0	28	15	0	15	85

If the sugar in question was, as supposed, 2 : 4-dimethyl galactose, then no furanoside should have been formed. The small amount present is accounted for by the presence of 2-methyl galactose in the sample used. Under the experimental conditions which favour furanoside formation the main portion is obtained as pyranoside, and this is evidence for the conclusion that position 4 is occupied by a methyl group.

The Oxidation of Dimethyl Galactose.—Triacetyl dimethyl galactose (0·52 g.) was deacetylated by Zemplén's method, and the aqueous solution of the sugar evaporated under reduced pressure. The product was dissolved in nitric acid (5 c.c., *d* 1·44), and after standing at room temperature for 2 hours was heated at 85—90° for 6 hours. The excess of nitric acid was then removed by distillation at 70° under reduced pressure for 48 hours with the continuous addition of water and the product was subjected to esterification, distillation, and amide formation according to the method described by Herbert, Hirst, *et al.* (J., 1933, 1286). After 4 days, the syrup obtained on evaporation of the solution of the ester in ammoniacal methyl alcohol was examined for both *d*- and *i*-dimethoxysuccinamides with a negative result. This experiment was repeated. The two methyl groups present in the dimethyl galactose could not therefore occupy adjacent positions in the molecule.

Potassium Hydroxide–Galactose. Titration Experiments.—On account of the low solubility of galactose in alcohol and since the presence of water led to the formation of syrupy products it was necessary to use penta-acetyl galactose for the titration experiments. The method had to

be modified, since both deacetylation and compound formation were responsible for removing alkali from solution and furthermore the amount of potassium hydroxide required for deacetylation was not that theoretically required for 5 acetyl groups owing to the catalytic nature of the process of deacetylation by alkali in alcoholic solution (Zemplén, *loc. cit.*). To each of two similar suspensions of galactose penta-acetate (0·2 g.) in absolute alcohol (5 c.c.) a definite quantity of standard alcoholic potassium hydroxide was added. One sample was then filtered, the filtrate titrated, and from the decrease in alkalinity the total amount of potassium hydroxide removed from solution was determined. By titration of the second sample without filtration the quantity of alkali required for deacetylation was determined and by difference the potassium hydroxide engaged in compound formation was found. A second value was obtained by direct titration of the compound as before:

Concn. of KOH, *N* {initial	0·27	0·41	0·61
Concn. of KOH, *N* {final	0·07	0·18	0·41
KOH, g., required to deacetylate 100 g. of penta-acetate	68·5	72·0	69·0
KOH, g., combined with 100 g. of galactose {indirect method	18·0	32·2	27·0
KOH, g., combined with 100 g. of galactose {direct method	25·3	26·1	27·5

Potassium Hydroxide–Galactose.—Galactose penta-acetate (18 g.), suspended in absolute alcohol (200 c.c.), was stirred with potassium hydroxide (45 g.) in alcohol (1 l.) in a closed flask. The *product* (10 g.), isolated in the usual way, was a white deliquescent powder more sensitive to moisture than the compounds previously described (Found: KOH, 21·2. $C_6H_{12}O_6$,KOH requires KOH, 23·7%).

Methylation. The compound (10 g.) was mechanically stirred in a closed flask with neutral methyl sulphate (75 c.c.) for 10 minutes at 34—40° and for 10 minutes at 70°. After the removal of potassium methyl sulphate, potassium hydroxide (8 g.) in alcohol (100 c.c.) was added, and the precipitate removed. Ether (1200 c.c.), added to the filtrate, brought down a further precipitate, which was discarded. The solution was then acidified with acetic acid and evaporated to dryness, and the residue acetylated, finally yielding a non-reducing syrup (4·5 g.) consisting of a mixture of α- and β-tetra-acetyl methylgalactosides; $[\alpha]_D^{18°}$ + 21·7° in chloroform (*c*, 2·3) (Found: OMe, 8·1. Calc. for $C_{15}H_{22}O_{10}$: OMe, 8·6%).

The authors thank the Carnegie Trust for the award of a Teaching Fellowship (E. G. V. P.) and a Scholarship (G. G. R.), and the Earl of Moray Endowment and Imperial Chemical Industries, Ltd., for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, September 30th,* 1936.]