235. *Polysaccharides. Part XXVII. The "End-group" Method as applied to Cellulose.*

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A study of the "end-group" method for determining the molecular size of polysaccharides shows that the accuracy claimed for it by Haworth and Machemer has not been over-estimated and that the criticism of Hess and Neumann is without foundation. On the other hand, it has been found impossible to substantiate the claims made by the latter authors for an alternative method of separating tetramethyl methylglucoside from trimethyl methylglucoside which involves the conversion of the latter substance into an ether-insoluble barium phosphate derivative.

By the estimation of the proportion of tetramethyl glucose liberated when a methylated cellulose was hydrolysed, Haworth and Machemer (J., 1932, 2270) were able to determine a lower limit for the molecular size of the cellulose. This "end-group" method has been employed subsequently in the elucidation of the structures of other polysaccharides, including starch and glycogen of various origins, xylan, mannan, inulin and levan, and in no case has occasion arisen to doubt the validity of the method. In the original work of Haworth and Machemer, on the basis of an analysis of artificial mixtures of tetramethyl and trimethyl glucoses, the correction to be applied for experimental loss was estimated as 10%.

Obviously, this correction factor will vary with the proportion of tetramethyl glucose present and the work described here was undertaken in the first place to determine the experimental loss at various concentrations (ranging from 1% to 0·1%) of tetramethyl glucose in trimethyl glucose when artificial mixtures of these compositions were submitted to the procedure of Haworth and Machemer.

Meanwhile, Hess and Neumann (*Ber.*, 1937, **70**, 710) have criticised the method of fractional distillation employed by Haworth and Machemer for the separation of tetramethyl methylglucoside on the basis of their own failure to repeat the estimations made by the latter authors.

The experiments described here show that the separation of tetramethyl methylglucoside can be adequately effected by fractional distillation. Mixtures of tetramethyl glucose and 2 : 3 : 6-trimethyl glucose were prepared in the specified proportions from pure recrystallised specimens of each sugar. Preliminary separation was achieved by chloroform extraction from an aqueous solution of the mixture and the extracted material, after conversion into the glucosides, was fractionally distilled from a Widmer flask. In each case, the first fraction contained the bulk of the tetramethyl methylglucoside. The recovery

of the tetramethyl glucose effected by the Haworth and Machemer method at each concentration is summarised as follows :

Mixtures of Tri- and Tetra-methyl Glucoses.

% Tetramethyl glucose in mixture	1·05	0·216	0·132	0·106
% Tetramethyl glucose found	0·93	0·142	0·082	0·056
% Recovery	89	66	62	53

A curve drawn from these figures indicates that at the concentration of tetramethyl glucose found by Haworth and Machemer (0·5%) the correction to be applied for experimental loss is in fact very nearly 10%. It is further to be observed that the applicability of the method extends to much lower concentrations of tetramethyl glucose than are usually obtained in the hydrolysates of methylated cellulose. For instance, tetramethyl glucose was detected with certainty in admixture with one thousand times its weight of trimethyl glucose.

One of the mixtures, that containing 1 part of tetramethyl glucose to 750 parts of trimethyl glucose, was treated first with glacial acetic acid and hydrochloric acid under the conditions employed for the hydrolysis of methylated cellulose. From this mixture a small amount of methyl lævulate was isolated as a first fraction in the subsequent distillation of the glycosides.

Neumann and Hess, rejecting the method of fractional distillation, have sought to separate mixtures of tri- and tetra-methyl glucose by an alternative procedure. This method (*Ber.*, **1937**, **70**, 721) exploits the possibility of introducing a phosphate group into the molecule of trimethyl methylglucoside and of extracting the tetramethyl methylglucoside with ether from the barium salt of trimethyl methylglucoside phosphate. These authors claimed to be able by these means to separate a mixture containing only 0·05% of tetramethyl methylglucoside. The method requires that the conversion of the trimethyl methylglucoside into a non-volatile derivative should be quantitative and it would be a matter of some surprise if this should be so. We had previously attempted without success to effect a quantitative conversion of trimethyl methylglucoside into the phosphate and when the details of the method of Neumann and Hess became available we repeated their work, using a mixture of glucosides containing 0·1% of tetramethyl methylglucoside.

We have been unable to substantiate the claim of Neumann and Hess as to the accuracy of this method. The figures given in the experimental section show that the tetramethyl methylglucoside even after distillation over metallic sodium contains a relatively large proportion of trimethyl methylglucoside.

Experimental.

The Estimation of Tetramethyl Glucose in the Presence of Trimethyl Glucose.—The procedures adopted with three of the four artificial mixtures were identical and will be described in full for one only. The materials used were 2 : 3 : 6-trimethyl glucose, purified by four recrystallisations from ether–methyl alcohol mixtures, and pure recrystallised tetramethyl glucose. The fourth mixture (1 : 750) was submitted to a modified treatment (see below).

Concentration 1 in 500. Tetramethyl glucose (0·2025 g.) was mixed with trimethyl glucose (100 g.), and the mixture dissolved in water (700 c.c.). This solution was extracted 15 times with chloroform (total volume, 1 l.), and the combined extracts concentrated to a syrup (2·55 g.) (A). The latter was boiled for 7 hours with 2% methyl-alcoholic hydrogen chloride (200 c.c.), and the solution neutralised with silver carbonate, filtered, and concentrated to a syrup, which was then distilled from a Widmer flask at a pressure of 0·008 mm. The following fractions were collected :

Fraction.	Bath temp.	Weight (g.).	n_D (temp.).	OMe, %.
1a	115°	0·054	1·4440 (21°)	56·6
2a	118	0·170	1·4522 (21°)	53·4
3a	118	0·188	1·4550 (20°)	—
4a	118	0·476	1·4560 (20°)	52·1

From these figures it was estimated that fractions 1a, 2a, and 3a contained together 0·108 g. of tetramethyl methylglucoside.

The aqueous solution left after the chloroform extraction was concentrated to 200 c.c. and again extracted with chloroform (6 times, total volume 270 c.c.). The extracted syrup (B) was converted into glucosides (9·5 g.) in the manner described, and the latter fractionally distilled at 0·02 mm. pressure:

Fraction.	Bath temp.	Weight (g.).	$n_D^{20°}$.
1b	112°	0·130	1·4540
2b	112	0·690	1·4558

It was estimated that fractions 1b and 2b contained together 0·034 g., making the total recovery of tetramethyl methylglucoside to be 0·142 g. This corresponds to a 66% recovery of the tetramethyl glucose added.

Concentration 1 *in* 100. Tetramethyl glucose (1·000 g.) was mixed with trimethyl glucose (100 g.). The extracts A and B were combined, converted into the glycosides, and fractionated at 0·04 mm. pressure:

Fraction.	Bath temp.	Weight (g.).	$n_D^{20°}$.	OMe, %.
1	140°	0·458	1·4425	61·1
2	148	0·585	1·4490	—
3	150	0·948	1·4540	—
4	150	0·865	1·4565	51·9

From these figures the yield of tetramethyl methylglucoside was calculated to be 0·941 g., corresponding to a recovery of 89%.

Concentration 1 *in* 1000. Tetramethyl glucose (0·1650 g.) was mixed with 165 g. of trimethyl glucose, and the mixture submitted to the above process. Again extracts A and B were united:

Fraction.	Bath temp.	Weight (g.).	n_D (temp.).	OMe, %.
1	120—125°	0·128	1·4465 (20°)	57·5
2	,,	0·404	1·4560 (19°)	—
3	,,	0·521	1·4560 (19°)	51·6

It was estimated that fraction 1 contained 0·0931 g. of tetramethyl methylglucoside, corresponding to a recovery of 53%.

Concentration 1 *in* 750. The procedure was varied here in that tetramethyl methylglucoside (0·22 g.) was used in admixture with the crystalline trimethyl glucose (167 g.) and the mixture was submitted to a preliminary treatment with glacial acetic acid (1500 c.c.) and 10% hydrochloric acid (1500 c.c.) under the conditions adopted for the hydrolysis of methylated cellulose. The product was then isolated in the manner described for other artificial mixtures. The extracts A and B after conversion into the glucosides were separately distilled at 0·005 mm.:

Fraction.	Weight (g.).	$n_D^{20°}$.	OMe, %.
1a	0·142	1·4275	26·3
2a	0·255	1·4515	54·0
3a	0·242	1·4552	51·5
4a	0·650	1·4565	51·2
1b	0·394	1·4555	51·3
2b	0·583	1·4560	51·1

Fraction 1a consisted of methyl lævulate. It was estimated that fractions 2a, 3a, and 1b contained respectively 0·098, 0·024, and 0·015 g. of tetramethyl methylglucoside. Total, 0·137 g., corresponding to a recovery of 62%.

Attempted Separation of Tri- and Tetra-methyl Methylglucosides by the Action of Phosphorus Oxychloride.—I. A mixture of trimethyl methylglucoside (14 g.) and tetramethyl methylglucoside (2·6 g.) was dissolved in dry pyridine (65 c.c.) and to the solution was added slowly phosphorus oxychloride (13 c.c. ≡ 1·2 mols.) dissolved in dry pyridine (100 c.c.). The mixture was kept at room temperature for 12 hours, cooled in an ice–salt mixture, and diluted with water (20 c.c.). After neutralisation with barium hydroxide solution, the mixture was concentrated to dryness, and the solid residue extracted three times with ether (total volume, 750 c.c.). Concentration of the extract left a syrup (3·8 g.), which was distilled at 0·01 mm. pressure. Two fractions were obtained, the first containing tetramethyl methylglucoside (1·41 g.) and the second consisting of unchanged trimethyl methylglucoside.

II. The separation was repeated, trimethyl methylglucoside (17·5 g.) and tetramethyl

methylglucoside (1·4 g.) being used with 2·5 mols. of phosphorus oxychloride (34 c.c.). Again a considerable amount of unchanged trimethyl methylglucoside was obtained.

III. The experiment was repeated as under II and rigorous precautions were observed to keep the temperature throughout at — 10°. Again quantitative separation was not achieved.

IV. *The method of Neumann and Hess.* An attempt was made to separate a mixture of trimethyl methylglucoside (5·1 g.) and tetramethyl methylglucoside (48·2 mg.; $n_D^{20°}$ 1·4430) by a strict adherence to the conditions prescribed by Neumann and Hess (*loc. cit.*). The mixture was dissolved in pyridine (15 c.c.) which had been dried by distillation from barium oxide. The solution was cooled to — 20°, treated with a mixture, similarly cooled, of phosphoryl chloride (2·5 c.c., distilled over calcium carbonate) and dry pyridine (20 c.c.), and kept in the cooling bath for 30 minutes and then at room temperature for 12 hours. Thereafter 5 c.c. of aqueous pyridine (20% water) were slowly added, and the acid solution shaken with finely powdered barium hydroxide (30 g.). The excess of barium hydroxide was removed by precipitation with carbon dioxide, the filtered solution evaporated to dryness, and the dry residue extracted three times with cold acetone (300 c.c.). The addition of ether (1000 c.c.) and light petroleum (1000 c.c.) to the acetone solution precipitated the barium salt, which was removed by filtration, and the filtrate was concentrated to dryness in the flask of the distillation apparatus. The residue in the flask was a syrup (1·46 g.), which was dissolved in benzene and heated under reflux for 1 hour with freshly-cut sodium. The benzene was then distilled off at atmospheric pressure, and the residual mixture of syrup and metallic sodium submitted to distillation at a pressure of 0·02 mm. and a bath temperature of 60—70°. The distillation apparatus was of the same design as the larger of the two types used by Neumann and Hess (*loc. cit.*, Fig. 2, p. 723). The relative dimensions of the parts of this apparatus were observed in the design used by us, although we reduced the absolute scale, since the scale given by Hess and Neumann in Fig. 2 is such that the reflux condenser (and the attached collecting boat) is too heavy and too unwieldy for accurate weighing.

The water-cooled receiver was weighed at intervals of 6 hours, the condenser being dried before each weighing by running acetone through it, followed by pre-heated air. The weight of distillate collected in the boat was as follows:

Time (hrs.)	6	12	18	24	30
Weight of distillate (mg.)	14·50	32·31	49·17	68·86	79·71

It is seen that after 18 hours, the distillate weighs more than the whole of the tetramethyl methylglucoside (48·2 mg.) originally present. Analysis of the distillate collected after 30 hours showed the undoubted presence in it of trimethyl methylglucoside (Found: C, 51·9; H, 8·2; OMe, 55·2. Calc. for $C_{11}H_{22}O_6$: C, 52·8; H, 8·8; OMe, 62·0%. Calc. for $C_{10}H_{20}O_6$: C, 50·9; H, 8·5; OMe, 52·6%).

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