

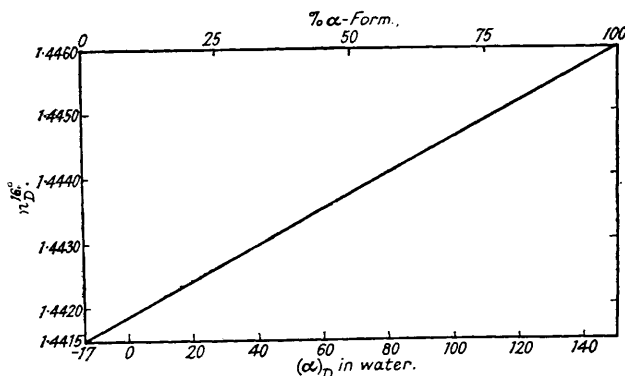
**236.** *Polysaccharides. Part XXVIII. The "End-group" Method as applied to Starch. An Improved Method for the Estimation of Tetramethyl Glucose in Admixture with Trimethyl Glucose.*

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A detailed study has been made of the accuracy of the method developed by Haworth and Machemer for the evaluation of tetramethyl glucose in the presence of trimethyl glucose. It is shown that, under the conditions adopted for chain-length determinations of methylated starch by the end-group method, accurate estimates of the proportions of tetramethyl methylglucoside can be made by fractional distillation of the mixed glucosides. A modification of the procedure is now proposed which renders the fractional distillation method still more accurate and increases its range of application.

In a recent paper Hess and Lung (*Ber.*, 1938, **71**, 815) have criticised the value given by Hirst, Plant, and Wilkinson (*J.*, 1932, 2375) for the yield of tetramethyl glucose obtained on hydrolysis of methylated amylose and methylated amylopectin from potato starch. In carrying out the estimation we used the simple and direct method developed by Haworth and Machemer (*J.*, 1932, 2270). This depends on the fractional distillation of the mixture of tetramethyl methylglucoside and trimethyl methylglucoside obtained after the hydrolysis of methylated starch. Control experiments with artificial mixtures of the pure sugars have proved on many occasions (see, for example, Haworth and Machemer, *loc. cit.*; Haworth, Hirst, and Isherwood, *J.*, 1937, 577) that when carried out in the prescribed manner on mixtures containing between 1% and 10% of tetramethyl glucose (the range of special interest for the present paper) the method is sufficiently accurate to account for 90–95% of the total amount of tetramethyl glucose present. These control experiments furnish a stringent test of the utility and accuracy of the analytical methods used and in consequence we regard Hess's criticism of our manner of estimating tetramethyl glucose as unjustified.

FIG. 1.  
*Mixtures of tetramethyl  $\alpha$ -methylglucoside and tetramethyl  $\beta$ -methylglucoside.*



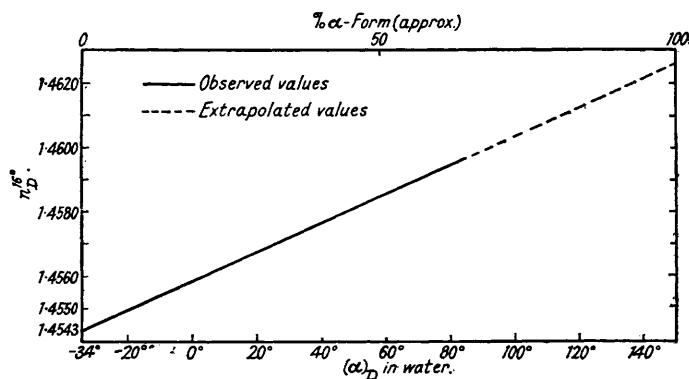
We have recently been engaged on a further study of the fractional distillation method with the view of rendering it still more widely applicable in chain-length determinations. Under the conditions normally adopted, the mixed glucosides of the two sugars have been allowed to reach equilibrium with respect to their  $\alpha$ - and  $\beta$ -forms during the formation of the glucosides. In these circumstances there is no difficulty in achieving, by refractive index measurements alone, the degree of accuracy obtained in the control experiments mentioned above, and the estimated experimental error in terms of chain length is  $\pm 3$  units in a chain length of 25 units. Additional evidence in support of this view is derived from the work now described.

If, however, glucoside formation is arrested before equilibrium is reached, there may be present an excess of the  $\beta$ -forms of the mixed glucosides, which have lower refractive indices and lower boiling points than those of the corresponding  $\alpha$ -isomerides, and in such a case the use of refractive index measurements pertaining to the equilibrium mixtures would lead to less accurate estimates of the proportion of tetramethyl glucose present, the estimated values tending to be higher than the real ones. We have now found that the difficulty can be overcome by measuring both the specific rotation and the refractive index at frequent intervals during the fractional distillation. When pure tetramethyl methylglucoside is being collected, the successive portions of the distillate will have  $n_D^{16}/[\alpha]_D$  values which fall on the line in Fig. 1. Again, when the distillate is pure trimethyl methylglucoside, the  $n_D^{16}/[\alpha]_D$  values must fall on the line of Fig. 2, but when both substances are present in the distillate its  $n_D^{16}/[\alpha]_D$  values will not fall on either line. It is possible, therefore, to detect with accuracy the precise points during the distillation: (A) at which the collection of pure tetramethyl methylglucoside ceases and (B) at which collection of pure trimethyl methylglucoside commences. The identity and purity of the fractions containing only tetramethyl methylglucoside can be established by reference to Fig. 1, with further

checks provided by the methoxyl content and the yield of crystalline tetramethyl glucose obtained on hydrolysis. Control experiments have demonstrated that the point (B) can be determined with ease and that there is no difficulty in evaluating the composition of the intermediate fractions containing both sugars, when the  $\alpha$ - and  $\beta$ -forms of the glucosides are present in such proportions that all the fractions are strongly dextrorotatory (equilibrium mixtures). In these circumstances, which are the usual ones met with in chain-length determinations, the  $n_D^{16}$  value for the  $\alpha\beta$ -mixture of tetramethyl methylglucosides present in the intermediate fraction does not differ appreciably from 1.4445. The refractive index of the mixed trimethyl methylglucosides distilling at point (B) is very close to 1.4580 under these conditions and it has been shown that with equilibrium mixtures there can be little variation in the latter figure throughout the period when the tetramethyl- and the trimethyl-methylglucosides are distilling together. These observations provide strong confirmation of the accuracy of the method hitherto adopted.

FIG. 2.

Mixtures of 2 : 3 : 6-trimethyl  $\alpha$ -methylglucoside and 2 : 3 : 6-trimethyl  $\beta$ -methylglucoside.



Even in the above favourable circumstances the measurement of rotation values is desirable in that a further check is thereby provided, but when the conditions of glucoside formation give rise to mixtures containing excess of the  $\beta$ -glucosides the use of rotations to supplement refractive indices becomes essential. A particularly difficult case—the most extreme we have been able to devise—is described in the experimental section in order to present a comparison between the scope of the old method and that of the revised procedure now suggested, but it is to be remembered that in practice a mixture so rich in  $\beta$ -forms is not likely to be encountered. The method adopted in calculating the composition of the fractions is best described by reference to the actual figures and is given fully in the experimental section. The results show that accurate estimates of the amount of tetramethyl glucose can be made with such  $\beta$ -rich mixtures even when only small quantities of material are used for the fractional distillation.

By comparison with our method that of Hess and Neumann (*Ber.*, 1937, 70, 710) is lengthy and complicated and is subject to deficiencies recognised by Hess and his collaborators, such as the incompleteness of the phosphorylation of the trimethyl methylglucoside and the loss of tetramethyl methylglucoside during the evaporation of its solution in light petroleum (Leckzyck, *Ber.*, 1938, 71, 829). But the main potential source of loss has escaped the notice of these authors. This occurs during the removal of the water in which the tetramethyl methylglucoside and the barium salt of the phosphorylated trimethyl methylglucoside are dissolved at one stage of the estimation. In typical experiments, following exactly the procedure described by these authors, we have found that half the total quantity of tetramethyl glucose recoverable was present in this aqueous distillate, which must contain none at all if Hess and Neumann's method is to be capable of giving accurate results. Furthermore their method appears to be uncertain in operation and on other occasions we have found, in agreement with Averill and Peat's observations

(preceding paper) that the final product, which according to Hess and Neumann consists of pure tetramethyl methylglucoside, was contaminated with trimethyl methylglucoside.

## EXPERIMENTAL.

In order to ascertain the degree of separation of the  $\alpha$ - and the  $\beta$ -form of tetramethyl methylglucoside which can be effected by fractional distillation under the conditions used in chain-length determinations, 2:3:4:6-tetramethyl glucose was boiled with methyl-alcoholic hydrogen chloride to convert it into the mixed glucosides. The composition of the mixture of glucosides (isolated in the usual way) was altered from experiment to experiment by varying the time of heating and the concentration of the hydrogen chloride, excess of the  $\beta$ -form being favoured by shorter times and lower concentrations. For instance, after 1 g. of the sugar in 25 c.c. of 1% methyl-alcoholic hydrogen chloride had been boiled for  $4\frac{1}{2}$  hours, distillation of the glucosides gave fractions with the following properties: (a)  $n_D^{16}$  1.4434,  $[\alpha]_D^{16} + 55^\circ$  in water; (b)  $n_D^{16}$  1.4440,  $[\alpha]_D + 76^\circ$ . After use of 3% methyl-alcoholic hydrogen chloride for 7 hours, distillation of the mixed glucosides gave fractions (a)  $n_D^{16}$  1.4441,  $[\alpha]_D + 79^\circ$ ; (b)  $n_D^{16}$  1.4444,  $[\alpha]_D + 94^\circ$ ; (c)  $n_D^{16}$  1.4448,  $[\alpha]_D + 108^\circ$  (total distillate, 90% of the theoretical amount). In this way it was found that fractions varying in  $n_D^{16}$  from 1.4428 to 1.4448 could be obtained, but when the glucosides are made under conditions which give the equilibrium mixture, the  $n_D^{16}$  of the various fractions differ only slightly from 1.4445 (see second experiment recorded above). The properties of typical fractions from the series of experiments are collected in Table I, in which the percentage of  $\alpha$ -form is calculated from the known rotations of the  $\alpha$ - and the  $\beta$ -form. The observed points lie on the straight line of Fig. 1 and it will be seen that rotations are more sensitive than  $n_D$  values as a guide to composition. It was found that the refractive index values diminish by 0.0004 for  $1^\circ$  rise in temperature, and this figure can be used in reducing observed  $n_D$  values for comparison with the figure for  $16^\circ$ .

TABLE I.

*Mixtures of the  $\alpha$ - and the  $\beta$ -Form of Tetramethyl Methylglucoside obtained by Fractional Distillation.*

|                                  |        |        |        |        |        |        |        |
|----------------------------------|--------|--------|--------|--------|--------|--------|--------|
| % $\alpha$ -Form .....           | 29     | 38     | 43     | 50     | 57     | 60     | 74     |
| $n_D^{16}$ .....                 | 1.4428 | 1.4432 | 1.4434 | 1.4437 | 1.4441 | 1.4444 | 1.4448 |
| $[\alpha]_D^{16}$ in water ..... | +32°   | +46°   | +55°   | +67°   | +79°   | +94°   | +108°  |

A similar series of experiments was carried out with 2:3:6-trimethyl glucose. In this case there is a much greater tendency for the mixture of glucosides to contain an excess of the  $\beta$ -form and there is greater separation of the isomerides during fractional distillation. For instance, after glucoside formation by boiling with 0.6% methyl-alcoholic hydrogen chloride for 4 hours, the first three fractions of the distilled glucosides had (a)  $n_D^{16}$  1.4548,  $[\alpha]_D^{16} - 24^\circ$  in water; (b)  $n_D^{16}$  1.4549,  $[\alpha]_D - 20^\circ$ ; (c)  $n_D^{16}$  1.4556,  $[\alpha]_D - 5^\circ$ . The equilibrium mixture of glucosides (3% boiling methyl-alcoholic hydrogen chloride for 5 hours) gave (a)  $n_D^{16}$  1.4579,  $[\alpha]_D + 45^\circ$ ; (b)  $n_D^{16}$  1.4586,  $[\alpha]_D + 65^\circ$ ; (c)  $n_D^{16}$  1.4596,  $[\alpha]_D + 85^\circ$ . The properties of typical fractions are given in Table II, in which the figures for the proportion of  $\alpha$ -form have been calculated for the known value ( $-35^\circ$ ) of  $[\alpha]_D$  for the  $\beta$ -form and an approximate value ( $+150^\circ$ ) for the  $\alpha$ -form. These are given for comparison, but are not required when Fig. 2 is used for the revised method of estimating tetramethyl glucose. The observed points lie on the line of Fig. 2 and by extrapolation the value of  $n_D^{16}$  for the  $\alpha$ -form is *ca.* 1.4625. The decrease of  $n_D$  per  $1^\circ$  rise in temperature was found to be 0.00035 in this series.

TABLE II.

*Mixtures of the  $\alpha$ - and the  $\beta$ -Form of Trimethyl Methylglucoside obtained by Fractional Distillation.*

|                                |        |        |        |        |        |        |        |        |        |
|--------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| % $\alpha$ -Form .....         | 6      | 16     | 24     | 31     | 35     | 38     | 43     | 54     | 65     |
| $n_D^{16}$ .....               | 1.4548 | 1.4556 | 1.4561 | 1.4568 | 1.4571 | 1.4573 | 1.4579 | 1.4586 | 1.4596 |
| $[\alpha]_D^{16}$ in water ... | -24°   | -5°    | +10°   | +23°   | +31°   | +35°   | +45°   | +65°   | +85°   |

*Elimination of Possible Error due to Presence of Furfural.*—The high  $n_D^{16}$  value of furfural (1.52) causes serious error in the estimation if it is present in concentrations greater than 0.5% in the early fractions, where it accumulates. It can be removed by treatment of the mixed glucosides with cold  $N/25$ -aqueous potassium permanganate, added drop by drop until the solution gives

no colour with aniline acetate. The aqueous solution is then evaporated, and the glucosides dissolved in ether. Slight loss of trimethyl methylglucoside occurs and it is very desirable to adopt conditions of glucoside formation which ensure absence of furfural, *e.g.*, boiling the sugars for 10 hours with 1% methyl-alcoholic hydrogen chloride. If the acid is completely neutralised before removal of the methyl alcohol and the mixed glucosides are dried at 90°/12 mm. before distillation, the amount of furfural in the distillate should be negligible. It is desirable, however, to apply a routine test for furfural in all chain-length determinations, using for detection and quantitative estimation the band at  $\lambda$  2750 A.,  $\epsilon$  20,000.

Occasionally (see Averill and Peat, preceding paper) small amounts of methyl lævulate appear as impurity. This is revealed by low *b. p.*, low refractive index, and the absorption band at 2630 A. Its presence in the early fractions of the distillation is marked by irregularities in the  $n_D/[\alpha]_D$  values. It can be eliminated by careful fractionation (compare Averill and Peat, *loc. cit.*).

*Revised Method for Estimation of Tetramethyl Glucose in Admixture with 2 : 3 : 6-Trimethyl Glucose.*—A mixture of 0.180 g. of tetramethyl glucose and 1.75 g. of 2 : 3 : 6-trimethyl glucose was boiled with 1% methyl-alcoholic hydrogen chloride for 5 hours, these conditions being selected in order to obtain an excess of the  $\beta$ -forms of the glucosides. The quantities used are much smaller than those normally handled in starch chain-length determinations and for both these reasons the test is rendered all the more stringent. [The proportion of tetramethyl glucose (1 : 10) is approximately that present in the mixtures used by Hess and Lung (*loc. cit.*) in their phosphorylation experiments.] The glucosides were isolated in the usual manner, no furfural was found, and on distillation the following observations were made :

| Fraction. | Wt., g. | $n_D^{16^\circ}$ . | $[\alpha]_D$ in water. | OMe, %. | Revised method. |                  | Old method. |                  |
|-----------|---------|--------------------|------------------------|---------|-----------------|------------------|-------------|------------------|
|           |         |                    |                        |         | % "tetra."      | Wt. "tetra.," g. | % "tetra."  | Wt. "tetra.," g. |
| I.        | 0.101   | 1.4456             | +56°                   | 58.2    | 85              | 0.086            | 88          | 0.089            |
| II.       | 0.463   | 1.4533             | -11                    | 52      | 14              | 0.065            | 30          | 0.139            |
| III.      | 0.252   | 1.4553             | +7                     | 53      | 6               | 0.015            | 15          | 0.038            |
| IV.       | 0.198   | 1.4565             | +16                    | 51      | Nil             | —                | 5           | 0.010            |
| V.        | 0.165   | 1.4571             | +27                    | —       | Nil             | —                | —           | —                |

It will be seen by reference to Fig. 2 that fractions IV and V have  $n_D/[\alpha]_D$  values falling on the line for 2 : 3 : 6-trimethyl methylglucoside; the material which was collected thereafter was pure trimethyl methylglucoside. Reference to Figs. 1 and 2 reveals that the  $n_D/[\alpha]_D$  values for fractions I—III lie on neither of the two lines, and therefore these fractions contain mixtures of "tetra" and "tri."

*Fraction I.* This is obviously mainly tetramethyl methylglucoside. Its positive rotation, regarded in conjunction with the negative rotation of fraction II, indicates that the  $n_D$  of the tetramethyl portion is approximately 1.4440. That of the trimethyl portion, in view of the rotation of fraction II, is approximately 1.4548 (see Figs. 1 and 2). Fraction I therefore contains 85% of tetramethyl methylglucoside.

*Fraction II* contains mainly trimethyl methylglucoside, and in view of the rotation, the  $n_D^{16^\circ}$  of this portion can be taken as 1.4548 (see Fig. 2). The percentage of tetramethyl methylglucoside ( $n_D^{16^\circ}$  1.4440) is therefore 14. A direct estimation by the older method involving no corrections for rotations would indicate about 30% for the "tetra" content and would involve serious error.

*Fraction III* contains a little tetramethyl methylglucoside, since the  $n_D^{16^\circ}/[\alpha]_D$  values do not fall on the line of Fig. 2. Taking for the trimethyl portion  $n_D^{16^\circ}$  1.4560 (the next fraction of pure "tri" having  $n_D$  1.4565) and  $n_D$  1.4445 for the tetramethyl portion, the percentage of the latter present is 6 (by the older method the estimate would be 15%). The total tetramethyl methylglucoside estimated by the revised method is therefore 0.166 g. and by the older method 0.276 g., corresponding respectively to 0.156 g. and 0.260 g. of tetramethyl glucose. Since 0.18 g. of tetramethyl glucose was used at the start of the experiments, the revised method has accounted for 87% of the tetramethyl glucose, whilst the older method would give 145%. It must be emphasised that the case chosen is a particularly difficult one, never likely to be met with in the routine work of chain-length determinations, and was chosen specially to reveal the advantages of the proposed procedure.

By continuing the fractional distillation it is possible to detect by reference to Fig. 2 the point at which collection of trimethyl methylglucoside ceases and a mixture of the latter with dimethyl methylglucoside begins to come over.

*Examination of Hess's Method for End-group Assay.*—(a) 5.47 G. of a mixture of 2 : 3 : 4 : 6-tetramethyl methylglucoside (0.56 g.) and 2 : 3 : 6-trimethyl methylglucoside were separated under the conditions recommended by Hess and Neumann (*loc. cit.*). [During the removal of water in a vacuum from the barium salts obtained after phosphorylation, the distillate (C) was retained.] After distillation over sodium, 0.16 g. of tetramethyl methylglucoside was obtained,  $n_D^{18}$  1.4435, OMe 60.4%. 0.19 G. of 2 : 3 : 4 : 6-tetramethyl methylglucoside was isolated from the distillate (C) by continued extraction with benzene in all-glass apparatus. After the solution had been dried over anhydrous sodium sulphate, the benzene was removed at 90°/760 mm. The residual syrup was transferred to a flask fitted with a fractionating column. The syrup was heated at 80°/12 mm. for 8 hours to remove the last traces of solvent, the use of a column being essential to prevent loss of tetramethyl methylglucoside by volatilisation. The remaining syrup had  $n_D^{18}$  1.4440, OMe 61.8%, and was pure tetramethyl methylglucoside (yield, 0.19 g.).

(b) In a second experiment, 3.5 g. of 2 : 3 : 6-trimethyl methylglucoside and 0.45 g. of 2 : 3 : 4 : 6-tetramethyl methylglucoside were used (obtained from recrystallised 2 : 3 : 6-trimethyl glucose and 2 : 3 : 4 : 6-tetramethyl glucose respectively). By Hess's method 0.11 g. of syrup,  $n_D^{19}$  1.4520, OMe 54.0%, was obtained. From these figures, the mixture contained 35% (*i.e.*, 0.04 g.) of tetramethyl methylglucoside. From the aqueous distillate obtained by evaporation of the solution containing the barium salts (compare C, above), 0.18 g. of syrup,  $n_D^{19}$  1.4505, OMe 55.7%, was obtained. This contained 50% (*i.e.*, 0.09 g.) of tetramethyl methylglucoside.

The above results are summarised in the following Table, from which it is apparent that in

| Expt. | Wt. of "tetra" used, g. | Wt. of "tetra" recovered      |                    | Total recovery. |
|-------|-------------------------|-------------------------------|--------------------|-----------------|
|       |                         | by Hess and Neumann's method. | from distillate A. |                 |
| a     | 0.56                    | 0.16 g. (29%)                 | 0.19 g. (34%)      | 0.35 g. (63%)   |
| b     | 0.45                    | 0.04 g. (10%)                 | 0.09 g. (20%)      | 0.13 g. (31%)   |

both experiments, more tetramethyl methylglucoside was recovered from the aqueous distillate (C) than was obtained by Hess and Neumann's procedure. Hess and Neumann disregard this aqueous distillate, and for this and other reasons the inaccuracy of the results obtained by their method is evident.

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