[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY OF THE UNITED COLLEGE OF ST. SALVATOR AND ST. LEONARD, UNIVERSITY OF ST. ANDREWS]

HEPTAMETHYLSUCROSE: A CORRECTION

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According to published accounts,[1] when sucrose is methylated by methyl sulfate and alkali the product, after one treatment with the reagents, consists essentially of a heptamethylsucrose in which four methyl groups are present in the fructose half of the molecule and three in the glucose component. This reaction was, in fact, utilized by Haworth as the source of tetramethyl-γ-fructose in the constitutional study of the fructose residue in sucrose.

In the course of further work now in progress in this Laboratory on the structure of sucrose it has been necessary, as a side issue, to examine the methylated derivatives of the disaccharide in detail and to direct special attention to the "heptamethyl sucrose" described by Haworth. As a result of a prolonged research in which advantage was taken of improved methods for separating and identifying methylated sugars we find that the methylation of sucrose is far from being the simple reaction claimed by Haworth. Under the conditions described by him, the methylation of sucrose yields a complex mixture of compounds boiling at approximately a constant temperature and giving the correct analytical figures only as a result of numerous compensations. As this partially methylated sucrose is a sirup and, so far, has yielded no crystalline derivatives, vacuum distillation offers the only method of isolation; but the process is ineffective as a means of separating the mixture of compounds into its components and the lack of uniformity was revealed only when the material was hydrolyzed and the scission sugars carefully separated and identified. This we have done, keeping a record of the weight of each constituent, which is expressed in percentages of the weight of "heptamethyl sucrose" used. We find that, in addition to tetramethyl-γ-fructose (45%), the hydrolysis sugars contained 15% of tetramethylglucose and this result, making allowance for the amount of trimethyl-γ-fructose which was also present, shows that *octa*methylsucrose formed at least 27.5% of the original material. Partially methylated hexoses, belonging to both the glucose and fructose series, were also produced in the hydrolysis and amounted approximately to 40%, made up of 14% of mono- and di-methylglucoses together with 25% of various trimethylhexoses. The latter were not homogeneous in type, as at least 8% of trimethyl-γ-hexoses was present along with three, and possibly four, isomeric trimethylglucoses. In short, not more than

[1] Haworth, *J. Chem. Soc.*, **107**, 12 (1915); **117**, 199 (1920); Haworth and Mitchell, *ibid.*, **123**, 301 (1923).

one-third of the original methylated sucrose consisted of heptamethylsucrose and even this is not a chemical individual but a mixture of at least four isomerides.

The combined results give a clue to the order in which the methyl groups enter the sucrose molecule. Evidently the fructose component is substituted preferentially as the amount of tetramethyl-γ-fructose greatly exceeds that of tetramethylglucose and no ketoses with a lower methoxyl content than trimethylfructose were detected. Nevertheless, before the whole of the fructose residues are fully substituted, some of the glucose residues likewise undergo complete methylation while in the remainder methyl groups are scattered throughout the glucose chain. Positions 2,3,6-, 2,4,6-, and 2,3,4- in the glucose component definitely take part in the partial substitution and possibly also 2,3,5- as is shown by the isolation of the corresponding trimethylglucoses. Similarly 2,3-dimethylglucose was also identified with certainty in the lower methylated hexoses. The preference for positions 2 and 3 is interesting, but the possibility that the sucrose molecule may undergo methylation in position 5 of the glucose chain is disconcerting. The amount of 2,3,5-trimethylglucose detected was small, but nevertheless at least 5% of the total trimethylglucose consisted of this sugar[2] and its constitution is evident from the following properties. The compound was a reducing sugar and position 1 was therefore unmethylated. Position 4 was likewise unmethylated as the sugar gave an authentic γ-methylglucoside and not a semi-acetal when condensed with methyl alcohol under conditions favorable to γ-glucoside formation; position 6 was also unmethylated as the *p*-toluenesulfonyl derivative was convertible into the corresponding iodo derivative, a transformation which in the glucose series is restricted to the primary alcohol group.[3]

It is not inconceivable that this 2,3,5-trimethylglucose originated in partial hydrolysis of the sucrose molecule during methylation, although all experience is opposed to the idea that under such circumstances glucose reverts to the γ-form. There remains the more disquieting possibility that methylation is accompanied to some extent by a change in the position of the internal oxygen ring in a sugar. Although the validity of the methylation method as a means of determining structure has not escaped challenge, the evidence hitherto offered in criticism of the process has not been convincing, but it must be admitted that the case now presented opens up grave possibilities. If the oxygen ring alters in the glucose component

[2] The possibility that the sugar referred to may be 1,3,4-trimethylfructose has not been overlooked. The properties of the compound as displayed in the method of isolation are not strictly in accordance with either possibility, but as the sugar in the form of its γ-methylglucoside survived a treatment known to decompose the corresponding fructosides, the combined evidence favors the view that it is a derivative of glucose rather than of fructose.

[3] Oldham and Rutherford, THIS JOURNAL, **54**, 366 (1932).

of sucrose even to the extent of 5%, it may conceivably do so to a greater extent in the more reactive fructose half of the sucrose molecule, in which case the constitution of the disaccharide may require revision. The present formula accounts for the known properties of the disaccharide so far as these can be interpreted in terms of structure and its value is not necessarily impaired by the repeated failures to synthesize sucrose on the lines of the formula. But it is evident that an unsuspected element of uncertainty attends the methylation process and this may lead ultimately to further revision of the structures now assigned to many carbohydrates.

In order to economize description the general principles underlying the experimental methods we employed are summarized so as to indicate the reasons for the various operations.

1. Extraction of an aqueous solution of methylated hexoses with chloroform removes compounds containing four or five methyl groups in each chain of six carbon atoms (*e. g.*, tetramethyl reducing hexoses, the corresponding methylhexosides and also trimethylmethylhexosides).

2. Compounds containing three methyl groups in the hexose chain (*e. g.*, trimethylglucose or dimethylmethylglucoside) are extracted by chloroform from an aqueous solution saturated with potassium carbonate.

3. The above processes used in conjunction with the fact that an additional methyl group can be introduced into a sugar by conversion into the corresponding methylglucoside or, conversely, a methyl group can be eliminated from a methylglucoside by hydrolysis admits of a sharp separation of tetra-, tri-, di- and monomethylhexoses, when γ-derivatives are absent.

4. Condensation of mixed methylated sugars with methyl alcohol in the cold converts components which are capable of reacting in the γ-form into the corresponding γ-methylglucosides. When a sugar fails to form a γ-glucoside, position 4 must be methylated and when it is incapable of normal methylglucoside formation position 5 must be similarly substituted.

5. Mixtures of glucosides consisting partly of γ-forms and partly of normal forms can be separated into their respective classes by graded hydrolysis with $N/100$ hydrochloric acid which affects only the γ-isomerides.

6. When mixtures contain methylated reducing hexoses and also methylated glucosides, the former can be destroyed without damage to the latter by boiling with aqueous alkali.

7. When position 6 in the glucose series is unmethylated, the primary alcohol group can be substituted by the *p*-toluenesulfonyl residue which, in turn, is replaceable by iodine. The halogen can be removed quantitatively and estimated, thus giving the content, in a mixture, of any sugars unsubstituted in the terminal position.

Experimental

All evaporations and distillations were conducted under diminished pressure, all hydrolyses and condensations were followed by polarimetric observations, and the composition of each compound was verified by analysis or by comparison with standard specimens. The validity of each process was verified by means of control experiments, the account of which is omitted from the text.

Pure sucrose was methylated exactly as described by Haworth[1] and 100 g. of the product, which gave analytical figures agreeing for a heptamethylsucrose, was hydrolyzed by $N/100$ hydrochloric acid. On extraction from aqueous solution with chloroform the tetramethylhexoses were separated from lower methylated hexoses.

Tetramethylhexoses.—(Weight, 54 g.) The mixed sugars were condensed with cold methyl alcohol under conditions which give tetramethyl-γ-methylfructoside while tetramethylglucose remains unaltered. The latter was destroyed by boiling with alkali, the fructoside (44 g.) recovered, hydrolyzed as usual and the tetramethyl-γ-fructose (34.4 g.) isolated at 117–119° (0.05 mm.). Found: n_D 1.4533; $[\alpha]_D$ +30.7° in water for $c = 1.5155$. Control experiments showed that the above methods are effective in separating tetramethyl-γ-fructose from the stable form of tetramethylglucose.

Lower Methylated Hexoses.—(Weight, 38 g.) The mixed sugars, containing both partially methylated glucose and fructose, were condensed with acid methyl alcohol at the boiling point and the products extracted from water by chloroform. The extract (A) then contained trimethylmethylglucosides and trimethyl-γ-methylfructosides (24 g.) while the aqueous layer (B) retained di- and monomethylmethylglucosides (13.6 g.).

Treatment of A.—Regulated hydrolysis with $N/100$ hydrochloric acid converted the γ-fructosides into trimethyl-γ-fructose, which was isolated as usual and identified by analysis and specific rotation. The trimethylmethylglucosides which remained unaffected were then boiled with alkali to destroy reducing sugars and ultimately isolated by vacuum distillation (8.4 g.). Hydrolysis of the distillate with 8% hydrochloric acid gave a mixture of trimethylglucoses (6.2 g.) the separation of which is described later.

Treatment of B.—The mixture of di- and monomethylmethylglucosides and fructosides was recovered, dissolved in water, saturated with potassium carbonate and extracted with chloroform. Monomethylated methylglucosides remained in the aqueous layer from which 1.8 g. of monomethylmethylglucoside was isolated analytically pure. The chloroform extract contained 10.8 g. of dimethylmethylglucosides which were examined as afterward described.

Examination of Trimethylglucoses.—The sirupy product was allowed partially to crystallize. On treatment with dry ether the crystalline trimethylglucose (m. p. 121–123°) noted by Haworth remained. As this sugar was not identical with 2,3,6-, 2,3,4- or 3,4,6-trimethylglucose, specimens of which were available for comparison, it is probably the 2,4,6-isomeride. The sugar dissolved by the ether (14 g.) was condensed with methyl alcohol in the cold and, following the usual procedure, trimethyl-γ-methylglucoside (5.7 g.) was separated from normal trimethylglucose (8.1 g.) The former on hydrolysis gave, as anticipated, 2,3,6-trimethylglucose on hydrolysis of a sample but a more important result was obtained by conversion of the γ-glucoside into the corresponding *p*-toluenesulfonate. In this way trimethyl-5-*p*-toluenesulfonyl-γ-methylglucoside was obtained (5 g.) but the product did not consist exclusively of the 2,3,6-isomeride as when heated in acetone solution with sodium iodide an iodo derivative was formed to the extent of 14%. This was verified by heating in acetonitrile solution with silver nitrate and weighing the silver iodide formed. In view of its origin the parent sugar is unlikely to have been a fructose derivative and this was supported by control experiments, yet it forms a γ-glucoside and has no methyl group in position 6; it is probably 2,3,5-trimethylglucose.

The trimethylglucose (8.1 g.) left uncombined in the condensation with methyl alcohol were acetylated, treated with hydrogen bromide in glacial acetic acid solution and the product on isolation shaken with methyl alcohol in the presence of silver carbonate. In this way 7.8 g. of a mixture of mono-acetyltrimethyl-β-methylglucosides were obtained and this, after de-acetylation, was distilled in a high vacuum. The trimethyl-β-methylglucosides thus produced (4.6 g.) were treated in the customary manner with *p*-toluenesulfonyl chloride and the product heated at 100° for two hours in acetone solution with sodium iodide; 65% of the material remained as unchanged trimethyl-*p*-toluenesulfonyl-β-methylglucosides, indicating that to this extent position 6 was methylated so that the parent sugar must have been either 2,4,6- or 3,4,6-trimethylglucose or a mixture of both forms. The remainder of the glucoside sulfonate transformed smoothly into 6-iodo-trimethyl-β-methylglucoside, evidence that group 6 was unmethylated and was therefore derived from 2,3,4-trimethylglucose. This was confirmed by heating in acetonitrile solution with silver nitrate, thereby converting the iodo derivative into the corresponding nitrate and thereafter reducing with iron and acetic acid. Extraction of an aqueous solution of the reduced product with chloroform yielded 2,3,4-trimethyl-β-methylglucoside melting after recrystallization from light petroleum at 92–93° and identical with an authentic specimen. The aqueous layer yielded in equal amounts (a) a sirup which was not identified and (b) 1.4 g. of a product crystallizing from alcohol (m. p. 104–105°) which analysis proved to be 3,4,6- or 2,4,6-trimethyl-*p*-toluenesulfonyl-β-methylglucoside.

Examination of Dimethylglucoses.—This series of products, obtained in the form of the methylglucosides, was dissolved in water and extracted with chloroform to remove trimethylmethylglucoside (3 g.). The purified dimethylmethylglucoside (8.4 g.) was treated with *p*-toluenesulfonyl chloride giving 10 g. of sulfonated product, the composition of which was established by analysis. After heating in acetone solution with sodium iodide 6-iodo-2,3-dimethylmethylglucoside was isolated and identified but 46% of the dimethyl-di-*p*-toluene-sulfonyl methylglucoside remained unaltered, showing that to this extent position 6 was methylated. The parent sugar was in all probability 2,6-dimethylglucose.

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Summary

1. The "heptamethyl sucrose" described by Haworth in his constitutional study of sucrose is not a homogeneous compound but a complex mixture containing 30% of octamethylsucrose.

2. The nature of the mixture is revealed when the material is hydrolyzed and the scission sugars separated and identified individually.

3. The hydrolysis sugars consist as a minimum of ten compounds, *viz.*: (a) tetramethyl-γ-fructose, 45%, (b) tetramethylglucose, 15%, (c) trimethyl-γ-fructoses, 8%, (d) four isomeric forms of trimethylglucose, 17%, (e) 2,3-dimethylglucose, one other isomeride and monomethylglucoses amounting to 14% of the weight of methylated sucrose used.

4. Evidence has been obtained showing the order in which methyl groups enter the sucrose molecule and that, so far as the glucose component is concerned, this is similar to that in starch.

5. Evidence has also been obtained that the methylation of sucrose may

be accompanied to some extent by alteration in the position of the oxygen ring in the glucose component.

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SYNTHESIS OF 2,3,6-TRIMETHYLGLUCOSE

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2,3,6-Trimethylglucose was first prepared by Denham and Woodhouse[1] who, working in this Laboratory, obtained the sugar by hydrolyzing methylated cellulose; in justice to these workers whose pioneer efforts have been ignored by others, it is advisable to recall that we also owe to them the use of methyl sulfate as a methylating agent applicable to carbohydrates.

Obviously the constitution of 2,3,6-trimethylglucose is of unusual importance as the structural formulas meanwhile assigned to many disaccharides and to all the polysaccharides derived from glucose are based ultimately on the capacity of these particular compounds to be converted into this variety of trimethylglucose. The point is elaborated in a previous paper[2] where it is, however, emphasized that the formation of 2,3,6-trimethylglucose by the hydrolysis of a methylated saccharide is not conclusive evidence of structure as it fails to discriminate between the normal and γ-types of glucose residues. This much is nevertheless certain—when 2,3,6-trimethylglucose is obtained in a structural study conducted by the methylation method the parent compound must have contained unsubstituted hydroxyl groups in positions 2, 3 and 6; no further conclusions are justifiable.

The evidence upon which the constitution of this methylated sugar depends has been accumulated gradually. In the first place Denham and Woodhouse[1] showed that the compound failed to form an osazone and that when converted into the corresponding heptonic lactone a methoxyl group was eliminated. In this way, methyl groups are definitely assigned to positions 2 and 3 in the glucose chain and the proof was completed by Irvine and Hirst,[3] who found that when the sugar was oxidized to a dibasic acid one of the methyl groups was removed and this group must have occupied position 6. Confirmation of the formula was supplied by Irvine and McGlynn,[2] who showed that this particular trimethylglucose could react equally as a normal or as a γ-sugar, from which it follows that groups 1, 4 and 5 must be unmethylated.

[1] Denham and Woodhouse, *J. Chem. Soc.*, **105**, 2357 (1914).

[2] Irvine and McGlynn, THIS JOURNAL, **54**, 356 (1932).

[3] Irvine and Hirst, *J. Chem. Soc.*, **121**, 1213 (1922).