hydroxide), (2) sodium salicylate, (3) thiocyanates (Na, K and NH_4 salts), (4) iodides (Na and K salts), (5) bromides (Na and K salts), (6) alkaline earth chlorides, and (7) urea. Viscosity, determined by means of an Ostwald pipet, was used as a measure of swelling or gelatinization of the starch.

2. Viscosity or swelling power of the reagents, in general, increased with concentration. Considerable variation in swelling or gelatinizing power of different reagents was noted. A Hofmeister series or lyotropic effect of anions was noted in the concentration-viscosity studies as follows: Cl < B < I < CNS < salicylate < OH. The relations of different cations indicated some effect other than a lyotropic series.

3. Microscopical examination indicates that viscosity at lower concentrations of reagents is due to jostling of swollen granules. As concentrations are increased, the granules burst, and the amylopectin is dispersed, forming a true colloidal dispersion.

MINNEAPOLIS, MINNESOTA

RECEIVED SEPTEMBER 29, 1932
PUBLISHED MAY 6, 1933

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

The Methylation and Constitution of Inulin

BY JAMES COLQUHOUN IRVINE AND TERENCE NEIL MONTGOMERY

The application of the methylation method to the elucidation of the constitution of inulin is due to Irvine and Steele,[1] who showed that the polysaccharide could be converted into a trimethyl derivative which on hydrolysis yielded a trimethyl-γ-fructose as the essential product. This result, which was confirmed in various ways[2] and characterized inulin as the only known natural compound composed exclusively of γ-fructose residues, was followed by the observation that the hydrolysis sugar referred to above is 3,4,6-trimethylfructose.[3] It then appeared that the constitution of inulin was in large measure solved, but subsequent developments have shown that such is not the case. It is known, for example, that under certain conditions small quantities of glucose are formed when inulin is hydrolyzed and the isolation from inulin of one or more anhydro-difructoses showing greater resistance to hydrolysis than the major portion of the polysaccharide has added further complications to the problem.[4] Obviously, if these products are not adventitious but represent fractions of the inulin molecule, the constitution must be adjusted to accommodate

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[1] Irvine and Steele, *J. Chem. Soc.*, **117**, 1474 (1920).

[2] Irvine, Steele and Shannon, *ibid.*, **121**, 1060 (1922).

[3] Haworth and Learner, *ibid.*, 619 (1928).

[4] Jackson, *Bur. Standards J. Research*, **3**, 27 (1929); **6**, 709 (1931); Irvine and Stevenson, THIS JOURNAL, **51**, 2197 (1929); Pringsheim and Hensel, *Ber.*, **64**, 1431 (1931).

them. In addition, small quantities of tetramethyl-γ-fructose are detectable among the hydrolysis products of trimethylinulin. This is not surprising in view of the extreme ease with which inulin undergoes partial hydrolysis but there remains the possibility that tetramethylfructose does not originate in this accidental manner but is a genuine hydrolysis product derived from an undegraded unhydrolyzed polysaccharide. Should this be the case inulin can no longer be represented as a non-reducing closed-ring system but must be regarded as a chain of anhydro-γ-fructose residues terminating in two fructose residues, one of which is reducing and the other non-reducing. The point is important. Our experience with cellulose and starch has been that under similar conditions tetramethylglucose is formed in small yields which, in place of being constant, vary according to the experimental procedure. We have therefore exercised much caution and have repeated our work on several occasions before submitting the results now contributed.

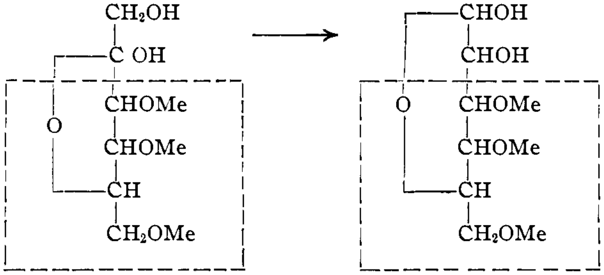
We have prepared 300 g. of trimethylinulin by an improved method designed to remove any fully methylated fructose derivatives which may have been formed during the methylation. When hydrolyzed by means of aqueous alcoholic oxalic acid the following products were isolated, the yields being expressed as percentages of the weight of trimethylinulin used.

		Minimum yield, %	Maximum yield, %
A	Trimethyl-γ-fructose	87.0	88.5
B	Tetramethyl-γ-fructose	1.7	2.7
C	Trimethyl anhydro-γ-fructose	4.5	4.6
D	Trimethylglucose	1.0	3.3
E	ω-Methoxy-5-methylfurfural	1.5	2.5

The significance of these products is now discussed.

Product A.—The constitution assigned to the trimethyl-γ-fructose as the 3,4,6-derivative was confirmed by proving the identity of the phenylosazone with that prepared from 3,4,6-trimethylglucose recently synthesized in this Laboratory. As the trimethylfructose is a sirup and might be a mixture of closely-related isomerides, it was carefully examined in order to ascertain if it is actually a homogeneous chemical individual. For this purpose the sensitive reaction of condensation with acetone was utilized and successive specimens of trimethylfructosemonoacetone were isolated at regular intervals as the condensation proceeded. These specimens were chemically identical (save in respect of the varying proportions of α- and β-forms produced) and were convertible into specimens of trimethyl-γ-fructose showing the same physical constants throughout. From this and other evidence quoted in the experimental part we conclude that the sugar consists uniformly of 3,4,6-trimethyl-γ-fructose, a result which has an important bearing on the structure of inulin.

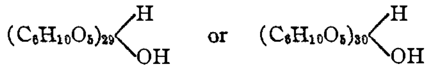
Products B, C, D and E are invariably formed although in small and fluctuating amount, and the question at once arises as to how far they must be taken into account in formulating the constitution of trimethylinulin and therefore of inulin itself. The furfural derivative (E) can be readily disposed of as its formation has been traced to secondary reactions of which the most productive is the decomposition of the trimethyl-γ-fructose by the oxalic acid used in hydrolysis. This was to be expected but it is remarkable that the formation of trimethylglucose (D) must also be attributed to the same cause. Under conditions parallel with those involved in the hydrolysis of trimethylinulin an equilibrium is established between trimethyl-γ-fructose and normal trimethylglucose, the reaction involving a unique example of the fructose ⟶ glucose conversion.



This accounts for the detection of glucose among the hydrolysis products of inulin when the reaction is carried out by acids and the absence of this sugar when enzymes are employed.

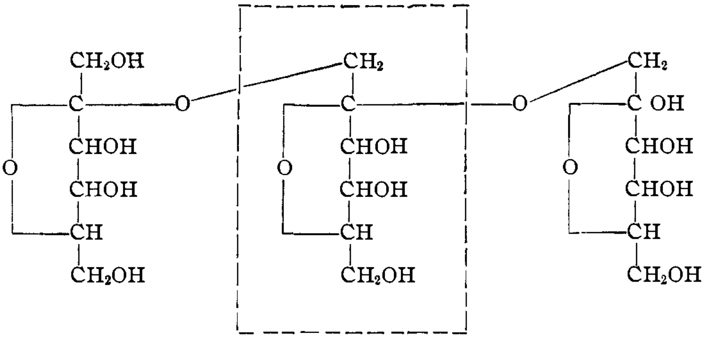
Discussion can now be limited to products B and C. With regard to C we have shown that the trimethylanhydro-γ-fructose although remarkably stable to hydrolysis is convertible completely into 3,4,6-trimethyl-γ-fructose and in this respect resembles the inulin molecule as a whole. Further, compounds of similar type were produced in control experiments in which the trimethylfructose was heated with oxalic acid solution and from this we conclude that the formation of these anhydro compounds from inulin is due to dehydration of the hydrolysis sugar after its liberation and is not valid evidence of constitution. Three fundamental factors therefore remain: (a) what is the maximum yield of 3,4,6-trimethyl-γ-fructose from inulin; (b) is tetramethylfructose a genuine product; (c) if so, in what amount is it formed? Our work has been conducted on a sufficiently large scale and all yields have been checked by control experiments so that answers can be provided to at least two of the above questions. As the products indexed as C and D are produced from A the yield of the latter must be augmented accordingly and must be of the order of 87% of the theoretical amount, calculating on the basis that trimethylinulin is completely convertible into trimethylfructose. But this does not take into account the fact that product E is likewise adventitious and originates in both A and B. It is impossible to allocate this factor accurately but a fair estimate, arrived at by controls, shows that the yield of trimethyl-γ-fructose from trimethylinulin is approximately 95%. This leaves little margin for tetramethyl-γ-fructose as an authentic product. That the compound is formed is certain and although the maximum yield actually obtained was only 2.7% of the total hydrolysis product this value must be supplemented in view of experimental losses and of partial conversion into the furfural derivative. A review of all our results shows that under the conditions employed by us the ideal yields of trimethyl and tetramethyl-γ-fructose derivable from "trimethylinulin" are, respectively, 95 and 4%, calculating on the basis: n mols. methylated inulin $\longrightarrow n - 1$ mol. trimethyl fructose + 1 mol. tetramethylfructose.

Applying this result, the molecular formula of inulin approximates to



$$(C_6H_{10}O_5)_{29}\begin{matrix}\diagup H\\ \diagdown OH\end{matrix} \quad \text{or} \quad (C_6H_{10}O_5)_{30}\begin{matrix}\diagup H\\ \diagdown OH\end{matrix}$$

and may be regarded as a chain of 1,2-anhydro-γ-fructose units terminated at each end by a fructosidic residue, one reducing and the other non-reducing as shown below:



One non-reducing fructose unit; gives tetramethylfructose

27 or 28 anhydrofructose units; give trimethylfructose

One reducing fructose unit; gives trimethylfructose

The formula displayed above does not exhaust the possibilities but is quoted chiefly for illustration and as the simplest constitution derivable from our results. A polymerized aggregate of 1,2-anhydrofructose residues which had undergone partial hydrolysis would lead to the same result and at present no purely chemical methods can discriminate between these possibilities. The actual multiple of $C_6H_{10}O_5$ arrived at by calculation from our results is not far removed from the factor 20–24 suggested by Haworth or the 23–30 found by Berner by physical methods and it is conceivable that inulin does react in molecular magnitudes of this order. But considering the ready tendency of inulin to undergo hydrolysis it is hazardous to conclude that the open-chain structure is characteristic of the polysaccharide. We have certainly taken every known precaution to avoid hydrolysis during methylation but it is impossible to guarantee the entire absence of such a side reaction. So far as present knowledge leads to a conclusion the constitutional formula now given is the simplest expression of our results.

Experimental

Trimethylinulin.—The inulin employed showed $[\alpha]_D$ —38.5° for $c = 1.95$. In each methylation 200 cc. of methyl sulfate was used for 25 g. of inulin, the temperature was restricted to 40° and the product was isolated by extracting the alkaline liquor with chloroform. Three subsequent methylations by the silver oxide method sufficed to raise the methoxyl content to a maximum, after which the powdered product was extracted in a Soxhlet apparatus for several days with dry ether. A snow-white solid remained (average yield 25.1 g.) and in six consecutive preparations $[\alpha]_D$ in chloroform varied between -50.4 and -53.7°. More than 300 g. was prepared in the manner described.

Hydrolysis of Trimethylinulin.—The following is an account of a typical experiment. Trimethylinulin in lots of 40 g. was dissolved in 400 cc. of 70% alcohol containing 1% of oxalic acid and the solution was boiled for six hours. After neutralization with calcium carbonate and distillation of the alcohol under reduced pressure the product was boiled under a reflux condenser for two hours with 0.5% aqueous oxalic acid. The solution was again neutralized and filtered, being then extracted with chloroform which removed a sirup amounting to 12% of the initial weight of trimethylinulin. From the aqueous layer crude trimethylfructose was isolated as a sirup in the usual way, the yield at this stage being about 85% of the theoretical amount. It should be noted that as some trimethylfructose passes into the chloroform layer it is necessary in exact working to extract the chloroform solutions twice with water to remove this sugar, the aqueous washings being again extracted with chloroform. This procedure was followed throughout.

Treatment of the Trimethyl-γ-fructose Fraction.—The sugar was condensed in the cold with methyl alcohol containing 1% of hydrogen chloride and the total product, when isolated in the usual manner, was dissolved in water and extracted with chloroform. Trimethyl-γ-methylfructoside passed into the chloroform layer while unchanged trimethylglucose remained in the water together with traces of lower methylated fructoses. The latter were removed by condensing the total product with acetone containing 0.5% of hydrogen chloride and extracting an aqueous solution of the resulting sirup with chloroform. As the trimethylglucose failed to condense with acetone it remained in the aqueous layer from which it was recovered as a reducing sirup.

Anal. Calcd. for $C_9H_{18}O_6$: OCH_3, 41.9. Found: 40.0; $[\alpha]_D$ in chloroform $+57.4$°; n_D 1.4722.

The identity of the compound was confirmed by (a) conversion into the corresponding methylglucoside ⟶ (b) methylation of this by the silver oxide method ⟶ (c) hydrolysis with aqueous hydrochloric acid. In this way pure crystalline 2,3,4,6-tetramethylglucose was obtained in good yield.

Isolation of Pure 3,4,6-Trimethyl-γ-fructose.—As described, this sugar was obtained free from trimethylglucose in the form of the corresponding methylfructoside which was distilled under diminished pressure and reconverted by hydrolysis into the parent sugar. Systematic fractionation of the sirup failed to disclose any difference in the physical constants of the successive fractions and the homogeneity was confirmed by condensing with acetone containing 0.5% of hydrogen chloride. It should be noted that this reaction attained equilibrium when only 85% of the sugar had reacted but the uncondensed sugar was identical with that which took part in the change and could be subjected to a second treatment. In other experiments the condensation was arrested at intermediate stages, the acid acetone solution being poured into excess of aqueous potassium bicarbonate solution. Extraction with chloroform removed trimethylfructosemonoacetone while unchanged sugar remained in the aqueous layer. The following synopsis of results shows the uniformity of the different specimens of trimethylfructose, the rotations being taken in chloroform. As the sugar shows slight mutarotation in this solvent permanent values are quoted.

Specific rotation of initial trimethyl-γ-fructose,	+21.4°
Rotation of the sugar recovered from the acetone derivative	
(a) when 85% of the sugar had reacted	21.8°
(b) when the remaining 15% had reacted	22.6°
(c) when 44% of the sugar had reacted	20.7°

Rotation of the uncombined sugar from (c), 22.0°. It should be stated that the rotations of the different specimens of trimethylfructosemonoacetone showed wide variation ($[\alpha]_D$ +37° $\longrightarrow$ 80°) but this we attribute to the existence of α- and β-forms.

Fractional hydrolysis of the acetone derivatives likewise failed to reveal any lack of uniformity in the parent sugar.

Ratio of hydrolysis of acetone derivative	Specific rotation of the trimethylfructose formed
60% complete	+21.9°
80% complete	21.2
100% complete	21.4

Chloroform Extract of Hydrolysis Products from Trimethylinulin.—The sirup was separated by means of fractional distillation into three components; the yields obtained in three consecutive experiments are given below, expressed as percentages of the weight of initial trimethylinulin.

(a) **ω-Methoxy-5-methylfurfural.**—Yield, 2.2, 1.5, 2.5%; bath temperature, 85–90° (0.06 mm.); phenylhydrazone m. p. 88.5–89.5°; semicarbazone m. p. 164–165°. These compounds showed no depression of melting point when mixed with the corresponding derivatives of ω-methoxy-5-methylfurfural prepared from tetramethyl-γ-fructose according to the method of Haworth, Hirst and Nicholson.[5]

(b) **Tetramethyl-γ-fructose.**—Yield, 1.7, 2.7, 2.5%; bath temperature, 100–105° (0.06 mm.). The substance reduced Fehling's solution and decolorized permanganate solution in the cold; showed $[\alpha]_D$ +32.0° in water (final value) for $c = 0.72$; n_D, 1.4540; and gave satisfactory analytical figures for tetramethylfructose.

On treatment of the sugar with acid methyl alcohol in the cold the polarimetric changes observed were similar to those given by tetramethyl-γ-fructose, and the product when distilled at 100° (0.1 mm.) proved to be tetramethyl-γ-methylfructoside.

(c) **Trimethylanhydrofructose.**—Yield, 4.5, 4.6%; bath temperature, 180–190° (0.06 mm.). The substance consisted of a viscous, pale yellow sirup which reduced Fehling's solution only slightly.

Anal. Calcd. for $C_9H_{16}O_5$: OCH_3, 45.6. Found: 42.4. $[\alpha]_D$ +45.1° in chloroform for $c = 2.98$; n_D 1.4738.

On treatment with methyl iodide and silver oxide no significant rise in methoxyl content took place. The compound was hydrolyzed by boiling for thirty hours with 1% aqueous oxalic acid, to yield a trimethylfructose which condensed readily with acetone and showed $[\alpha]_D$ +22.4° for $c = 3.86$.

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(5) Haworth, Hirst and Nicholson, *J. Chem. Soc.*, 1513 (1927).

Conversion of Trimethylfructose into Trimethylglucose.—Trimethyl-γ-fructose from which trimethylglucose and substances extractable from water with chloroform had been removed was subjected to the treatment used to hydrolyze trimethylinulin, the products being isolated in the usual way. In order to ensure the authenticity of the results, three consecutive cycles of experiments were carried out with the same specimen of trimethylfructose. In the third experiment, however, the conditions were modified and six hours of treatment with 1% aqueous oxalic acid alone was given. In each cycle successive quantities of trimethylglucose were formed and the identity of the sugar was confirmed by conversion of the material into crystalline tetramethylglucose. The ketose-aldose conversion was accompanied by a side reaction involving dehydration.

Yields shown below are expressed as percentages of the weight of trimethylfructose treated.

Yield of furfural derivative	Yield of trimethylglucose	Yield of anhydro sugar
1.5	0.7	1.9
...	1.3	...
4.4	1.1	3.4

Trimethylanhydrohexose.—This material consisted of a viscous pale yellow sirup boiling at 170–180° (0.04 mm.) which reduced Fehling's solution slightly and the methoxyl content did not increase beyond OCH_3, 41% on further methylation. Two specimens showed n_D 1.4762, 1.4730; $[\alpha]_D$ +62.7°, 61.6° in chloroform. When a small quantity was hydrolyzed with 2% oxalic acid, the specific rotation of the solution altered smoothly to that of 3,4,6-trimethylfructose.

The authors desire to express acknowledgment to the Carnegie Trust for the Universities of Scotland for a Research Scholarship awarded to one of them.

Summary

1. On hydrolysis of trimethylinulin, in addition to trimethyl-γ-fructose which is the chief product, small quantities of the following are also produced: (1) tetramethyl-γ-fructose, (2) trimethylanhydrofructose, (3) trimethylglucose, (4) ω-methoxy-5-methylfurfural.

2. The uniform nature of the trimethyl-γ-fructose has been definitely proved for the first time and its identity as the 3,4,6-derivative has been confirmed.

3. The formation of trimethylglucose from trimethylinulin has been traced to a secondary reaction which involves an unexpected transformation of methylated fructose into the corresponding glucose derivative.

4. With the exception of tetramethylfructose the remaining hydrolysis products are adventitious. The ideal yields of trimethylfructose and tetramethylfructose from inulin are, respectively, of the order 95 and 4%.

5. The combined evidence favors a formula for inulin consisting of twenty-seven or twenty-eight 1,2-anhydro-γ-fructose units terminated at each end by a fructosidic residue.

ST. ANDREWS, SCOTLAND

RECEIVED OCTOBER 3, 1932
PUBLISHED MAY 6, 1933