CXLI.—*The Behaviour of Mannose Diacetone on Methylation.*

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In the course of a discussion on the application of the methylation process to the structural problems of carbohydrates, it was stated (Irvine and Oldham, J., 1925, **127**, 2910) that the method is valid only if certain conditions are fulfilled. These need not be enumerated again, but obviously the fundamental requirement is that during alkylation methyl groups are introduced into the vacant hydroxyl positions without disturbing any substituting groups already present. Equally important is the condition that, once methyl groups have entered the non-glucosidic positions of a sugar chain, they should remain unaffected during the standard processes employed to hydrolyse glucosides, disaccharides or polysaccharides. So far, no authentic case has been encountered which indicates that the above primary conditions are not fulfilled. One apparent exception was, however, recorded by Freudenberg and Hixon (*Ber.*, 1923, **56**, 2119), who converted mannose diacetone into a monomethyl derivative and subjected the product to mild hydrolysis in the hope that monomethyl mannose would be formed. Contrary to expectation, not only the unstable *iso*propylidene residues were removed, but the methyl group also was eliminated so that the final product consisted of mannose. The subject was reinvestigated by Levene and Meyer (*J. Biol. Chem.*, 1924, **59**, 145), who varied the procedure by applying the silver oxide method of alkylation to mannose diacetone, but the monomethyl derivative obtained, whilst differing in optical activity from Freudenberg's product, displayed precisely the same instability towards acid hydrolysts.

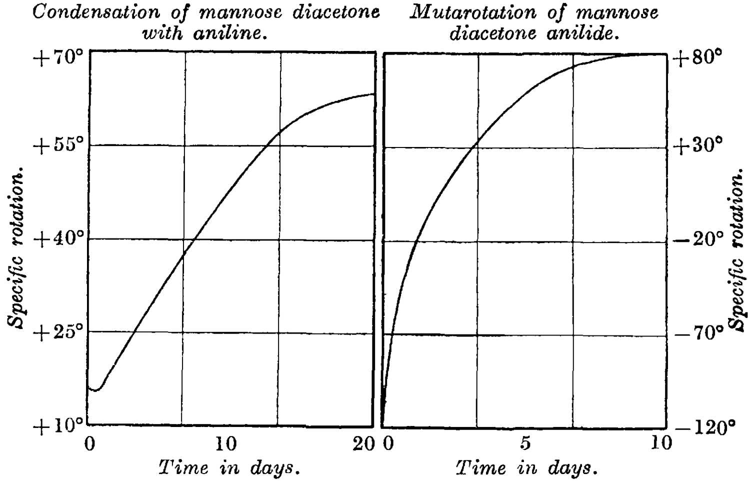
These observations, although capable of simple explanation, have elicited some criticism of the principles underlying the researches associated with this laboratory, and it is advisable to clear up the situation. It was assumed, prematurely, that, as mannose diacetone has no effect on Fehling's solution, the reducing position of the parent sugar had been substituted by a ketonic residue. In such an event, one hydroxyl group should persist in the sugar chain and undergo methylation, giving, irrespective of the method employed, the same monomethyl derivative. This product, in turn, when treated with aqueous acid should give a reducing sugar, *viz.*, monomethyl mannose. Two views have been put forward to account for the fact that the methyl group is removed on hydrolysis, the first being that the stability of methylated sugars is not a general feature of these compound and is considerably less than our published papers indicate. Alternatively, it has been suggested that, in the particular case under consideration, the entry of a methyl group had dislodged a ketonic residue from its initial attachment to the reducing position and, through subsequent rearrangement, had formed a derivative differing in type from the parent compound.

Either of the above explanations, if supported by convincing experimental evidence, would be damaging to the fundamental ideas underlying much of our work, but the suggestions are both highly improbable and opposed to experience. To the worker familiar with the properties of methylated sugars and glucosides, the apparently irregular behaviour of monomethyl mannose diacetone presents no difficulties whatsoever. The properties of the compound clearly show that it is *γ-methylmannoside diacetone*, and this could be formed only if mannose diacetone contained a labile hydrogen atom in the 1-position. That such is the case is shown by the fact that the compound forms a sodium derivative convertible into a monomethyl ether showing $[\alpha]_{Hg}$ — 41° in acetylene tetrachloride, whereas when methylation is effected directly by the silver oxide reaction the specific rotation of the product is widely different ($[\alpha]_D + 23°$). This is but an example of the general rule that when a reducing sugar is acted on by silver oxide and methyl iodide two stereoisomeric glucosides are produced, the β-variety being formed in excess. It follows that the monomethyl mannose diacetone obtained respectively by Freudenberg and by Levene differed merely in the relative proportions of two stereoisomeric substituted methylmannosides. From such compounds, again following a general rule, the alkyl group should be removed, as was the case, by hydrolysis. The only apparent irregularity is the extreme ease with which this group is lost, but even this feature is diagnostic and the explanation was already available. It has been known for a considerable time that γ-glucosides undergo hydrolysis with a facility comparable with that displayed by sucrose, and more recently (Irvine and Burt, J., 1924, **125**, 1343) γ-methylmannoside has been shown to posses the same instability. Any confusion which may have arisen through the behaviour of mannose diacetone on methylation is due to attaching more importance to a single test, *viz.*, the action of Fehling's solution, than to the numerous and more regular reactions of methylated sugars generally. Without additional experimental work it was possible to predict that mannose diacetone would (1) display mutarotation, (2) form the corresponding methylmannoside by the action of acid methyl alcohol, (3) undergo oxidation and reduction without removal of the acetone residues, and (4) condense with amino-bases.

In the interests of our general research programme we decided to explore the case, but meanwhile Freudenberg has extended his work and has shown that mannose diacetone exhibits mutarotation and enters into condensation with ammonia (Freudenberg and Wolf, *Ber.*, 1925, **58**, 300). More recently, Ohle and Berend (*ibid.*, p. 2950) have succeeded in oxidising mannose diacetone to a substituted mannonic acid, in which the ketonic residues persist, thus adding to the evidence that in the parent compound the reducing group is free. As our additional results are conclusive, and incidentally, have a bearing on the constitution of mannose diacetone, they are now submitted.

We have subjected mannose diacetone to the action of methyl alcohol containing 0·1% of hydrogen chloride, a methylating reagent

FIG. 1.



which is selective in the case of sugars, as it affects only the reducing position. Condensation proceeded readily and the optical changes involved, which are quoted in the experimental part, reveal that the substituted methylmannoside formed belongs to the γ-series. As anticipated, methylation proceeded without removing the acetone residues and the product was completely hydrolysed with the same facility as a γ-mannoside. Evidence was also obtained that mannose diacetone forms a phenylhydrazone, but as the product was a syrup and as, in the mannose series, aniline is a superior reagent to phenylhydrazine, the anilide was studied in greater detail. Mannose diacetone reacts in the cold with an alcoholic solution of aniline to give a well-defined crystalline *mannose diacetone anilide.* The polarimetric curve illustrating the change shows that two simultaneous reactions are involved and, following the general rule, the anilide displayed extensive mutarotation in alcoholic solution.

It will be observed that whereas during anilide formation the activity increases in the dextro-sense the compound when isolated is lævorotatory. In the preparation of mannoseanilide a similar anomaly is encountered.

The mutarotation of mannose diacetone in various solvents has also been studied, the observations being made on material which had been repeatedly crystallised, or preferably, sublimed at 120°/0·2 mm. A number of interesting features were encountered, particularly in the case of aqueous solutions where the phenomenon is abnormal and is probably more correctly described as "pseudo-mutarotation." Using dry acetone as the solvent, the mutarotation remained suspended and consequently the initial value ($[\alpha]_D + 38{\cdot}4°$) may be taken as a standard for the compound. The reaction was, however, catalysed by the addition of a trace * of *N*/50-sodium hydroxide, the constant value + 16·9° being reached in 6 hours. A solution in acetylene tetrachloride showed practically the same range, but gave the initial specific rotation $[\alpha]_D + 31{\cdot}5°$, which diminished regularly to 9·8°. Incidentally, the value quoted by Freudenberg for the same solvent (+ 14·3°) is intermediate between these extremes and corresponds to the readings obtained 40 minutes after the solution was prepared. The change, as shown by the diagram reproduced below, was complete in 100 minutes and this comparatively rapid mutarotation in an anhydrous solvent suggests that in addition to the interchange of the hydrogen and hydroxyl of the reducing position other factors may be operative.

On the whole, the results quoted above are regular, but the use of absolute alcohol as the solvent gave a negative result, the initial value ($[\alpha]_D + 15{\cdot}9°$) remaining constant and showing very little change on the addition of a trace of alkali. Reasons exist for the opinion that in this solvent the mutarotation is so rapid as to be undetectable and, in the circumstances, little success was anticipated in the case of aqueous solutions, particularly as only low concentrations are then possible. The result was, however, positive and the specific rotation diminished from + 11·9°, became lævo, and finally attained equilibrium when a small dextrorotation was just detectable. This consecutive fall and rise in activity shows that the mutarotation involves at least two reactions. If water plays a

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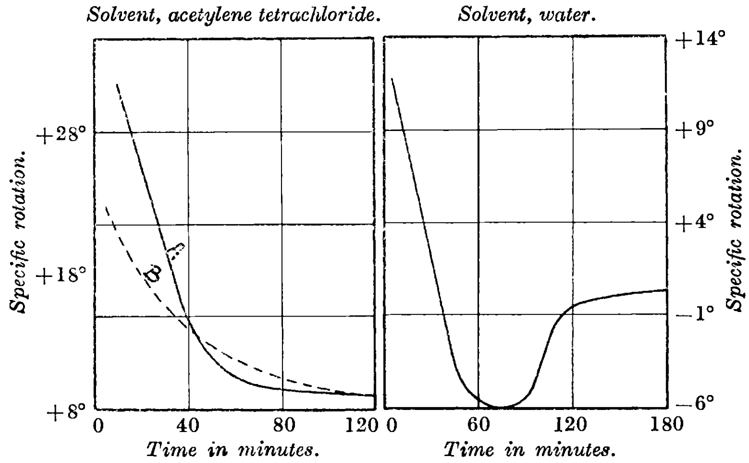
* Judging from published accounts, it would appear that experiments on mutarotation are frequently vitiated by the excessive use of alkali. In the sugar group particularly, this practice is regrettable, as prolonged changes in activity may thus be promoted which have no relationship to mutarotation in the accepted sense of the expression.

specific as well as a catalytic part in the changes, any compound thus produced must be unstable, as the original mannose diacetone was isolated from the solution by extraction with chloroform after mutarotation was complete. Additional information bearing on this point was obtained from the examination of a solution in 50% alcohol which had attained the permanent specific rotation of + 8·5°. On increasing the aqueous concentration to 75%, the activity was much reduced and ultimately vanished.

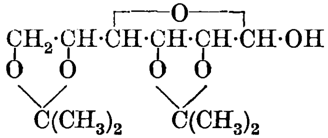
FIG. 2.

Mutarotation of mannose diacetone.



A. *Sublimed material.* B. *Crystallised material.*

The combined results, when plotted on curves, suggest that the constitution of mannose diacetone is more complex than is generally supposed. As already stated, our opinion is that the compound is derived from γ-mannose, and this is supported by other results, obtained in this laboratory but still unpublished, which prove definitely that glucose diacetone is a derivative of γ-glucose. There remains, however, the problem as to why γ-glucose condenses with acetone to give a compound in which the reducing group is substituted while the same group, usually the most reactive in the molecule, remains free in the case of γ-mannose. For the time being, the formula for mannose diacetone which finds most favour represents the compound as :



$$\mathrm{CH_2{\cdot}CH{\cdot}CH{\cdot}CH{\cdot}CH{\cdot}CH{\cdot}OH \quad (O\ bridge\ CH{-}CH;\ O{-}C(CH_3)_2{-}O \ \text{on}\ CH_2, CH;\ O{-}C(CH_3)_2{-}O\ \text{on}\ CH, CH)}$$

Considering recent views as to the structure of the stable and unstable varieties of mannose, it is possible that the above constitution is approximately correct, and it is our intention to investigate the subject further. Meanwhile it may be said that Ohle's criticism of Haworth's conclusions as to the ring structure of mannose is not justified, as evidently the acetone derivatives of the sugar have no relationship with the amylene-oxidic variety of the hexose.

EXPERIMENTAL.

The mannose diacetone was prepared by the standard method and purified by recrystallisation from light petroleum (b. p. 40—70°) diluted with 20% of dry ether. After the second crystallisation the m. p. was 122°, and this remained unaltered on further crystallisation or on subliming the compound in a vacuum. A Zeisel estimation, carried out as a check on the purity of the reagents used in the preparation, gave a negative result and the absence of any action upon Fehling's solution was also confirmed.

Rotation and Mutarotation of Mannose Diacetone.

Solvent, acetone. As the compound displays suspended mutarotation in acetone, this solvent was employed in determining the standard specific rotation.

I. The mannose diacetone was recrystallised three times and thereafter sublimed by heating for 5 hours at 120°/0·2 mm. $c = 1{\cdot}016$; $[\alpha]_D^{14^\circ} = +38{\cdot}4^\circ$. This value remained unchanged for 80 minutes.

II. In this experiment the sublimation process was omitted. $c = 1{\cdot}559$.

Time from start.	$[\alpha]_D^{14^\circ}$.	Time from start.	$[\alpha]_D^{14^\circ}$.
8 minutes	+27·9°	4½ hours	+27·1°
25 ,,	27·9	8 ,,	27·2
75 ,,	27·5	24 ,,	26·9
150 ,,	27·1	30 ,,	26·8

On adding a trace of sodium hydroxide the specific rotation diminished steadily to the constant value + 16·9°, which was reached in 6 hours. The material recovered from this experiment melted at 123°.

Solvent, acetylene tetrachloride. This solvent was selected as it was employed by Freudenberg when he obtained the value $[\alpha]_{\text{Hg yellow}} = +14{\cdot}3^\circ$. We find that when the mannose diacetone was recrystallised three times and dried at 80°/15 mm. before use, the maximum initial specific rotation observed was $[\alpha]_{\text{Hg yellow}}^{15^\circ} =$

+ 26·2° and regular mutarotation ensued. Typical figures are given. $c = 1{\cdot}980$.

Time from start.	$[\alpha]_D^{15^\circ}$.	Time from start.	$[\alpha]_D^{15^\circ}$.
5 minutes	+22·6°	60 minutes	+11·6°
15 ,,	18·6	75 ,,	10·0
20 ,,	17·1	90 ,,	9·6
40 ,,	14·1	100 ,,	9·1
50 ,,	12·6	24 hours	9·1

The above values were verified on four occasions and the figure quoted by Freudenberg corresponds with observations made 40 minutes after the first contact of solvent and solute.

A more striking result was obtained with mannose diacetone which had been sublimed by heating for 5 hours at 120°/0·2 mm. $c = 0{\cdot}509$.

Time from start (mins.) .	10	15	25
$[\alpha]_D^{14^\circ}$	+31·5°	+27·5°	+21·6°
Time from start (mins.) .	35	45	55
$[\alpha]_D^{14^\circ}$	+15·7°	+11·8°	+9·8° (constant)

Solvent, absolute alcohol. Recrystallised material was used. $c = 2{\cdot}574$. The initial specific rotation was + 15·9° and this remained unaffected for 150 minutes. After adding a trace of sodium hydroxide the optical value diminished very slowly.

Time (mins.)	5	30	150	300	48 (hours)
$[\alpha]_D^{16^\circ}$....................	+15·8°	+15·7°	+14·5°	+14·0°	+13·6° (constant)

The dissolved material was recovered and, after recrystallisation, proved to be unaltered mannose diacetone.

Solvent, water. Recrystallised material was used and, owing to the limited solubility, only dilute solutions were available. Mutarotation was exceedingly rapid and consequently the initial values varied considerably, but the most striking feature of the optical changes was the inversion of sign and the subsequent return to a small dextrorotation. This is illustrated in the following typical case. $c = 0{\cdot}4455$.

Time from start.	$[\alpha]_D^{16^\circ}$.	Time from start.	$[\alpha]_D^{16^\circ}$.
5 minutes	+11·9°	1 hour	−5·6°
15 ,,	5·6	2 hours	−0·6
25 ,,	3·6	3½ ,,	Inactive
35 ,,	2·8	45 ,,	+1·1°

The end value remained constant for 8 hours after the addition of a trace of alkali.

Although the development of a lævorotation was not observed on every occasion, the initial dextro-value always diminished and thereafter increased. By extraction with chloroform, the dissolved material was recovered and, after recrystallisation, was proved to be unchanged mannose diacetone.

Solvent, 50% aqueous alcohol. Freudenberg has already recorded that mannose diacetone preserves a constant specific rotation in this solvent for the first 24 hours and that thereafter the value falls to + 9·2°. For $c = 1{\cdot}2525$ and $T = 13°$ we found that in 3 days the end-point was $[\alpha]_D$ + 8·5° and that very little further alteration took place when a trace of dilute alkali was added. On halving the concentration by the addition of water, the liquid showed $[\alpha]_D$ + 0·5° and became inactive in the course of 2 hours.

Condensation of Mannose Diacetone with Phenylhydrazine.—A 3·5% solution of mannose diacetone was prepared in absolute alcohol containing recrystallised redistilled phenylhydrazine (1 mol. plus 10% excess). The solution was preserved at 12° in the dark, polarimetric readings being taken at intervals.

Time from start.	$[\alpha]_D^{12°}$.	Time from start.	$[\alpha]_D^{12°}$.
0 minute	+18·0°	10 days	+8·4°
2 days	13·3	14 ,,	9·8
4 ,,	8·7	19 ,,	12·7
6 ,,	4·2	28 ,,	15·5
7 ,,	7·0		

The results are typical of the condensation of a reducing sugar with the base, and indicate the superposition of an initial rapid reaction on a subsequent slower change. On isolation in the usual manner, the phenylhydrazone proved to be a syrup, which was not further examined.

Condensation of Mannose Diacetone with Aniline.—A 3·6% solution of mannose diacetone in absolute alcohol containing five times the theoretical amount of pure aniline was preserved in the dark at the temperature of the room. Polarimetric readings showed that two consecutive reactions then ensued.

Time from start.	$[\alpha]_D$.	Time from start.	$[\alpha]_D$.
0 minute	+16·5°	10 days	+50·5°
1 day	16·6	12 ,,	56·4
3 days	27·6	14 ,,	58·6
6 ,,	37·9	17 ,,	63·1

At this stage the crystalline product began to separate and the liquid after filtration was concentrated to a small bulk at 15°/15 mm. Clusters of needles were thus obtained in excellent yield and, after purification from light petroleum, the product melted at 114° (Found: C, 64·7; H, 7·6; N, 4·2. Calc. for mannose diacetone anilide, C, 64·5; H, 7·5; N, 4·2%). Mannose diacetone anilide is lævorotatory, but, in common with sugar anilides generally, displayed extensive mutarotation in absolute alcohol.

Time from start.	$[\alpha]_D$.	Time from start.	$[\alpha]_D$.
5 minutes	−118·3°	50 hours	Inactive
15 ,,	118·3	4 days	+39·8°
50 ,,	106·9	5 ,,	50·2
180 ,,	88·7	6 ,,	72·8
5 hours	69·4	8 ,,	79·6
20 ,,	22·7	12 ,,	83·0
30 ,,	12·5		(constant)

The results when plotted on a curve showed a marked resemblance to those given by mannose anilide, although the whole of the rotations are shifted markedly in the dextro-direction. The anilide was recovered unchanged from the above solution.

Condensation of Methyl Alcohol with Mannose Diacetone.—A 3% solution of mannose diacetone in dry methyl alcohol containing 0·1% of hydrogen chloride was maintained at 17°, polarimetric readings being taken at intervals.

Time from start.	$[\alpha]_D^{17°}$.	Time from start.	$[\alpha]_D^{17°}$.
0 minute	+13·6°	8 hours	+18·9°
2 hours	16·0	24 ,,	21·1
3 ,,	17·1	48 ,,	21·7
4 ,,	17·9	72 ,,	21·7

The acid was removed with silver carbonate, and the filtrate taken to dryness in a high vacuum, the residue then remaining being extracted repeatedly with dry ethyl acetate in the cold. On removal of the solvent under diminished pressure a syrup was obtained which, as it reduced Fehling's solution slightly, was taken up in cold ether and filtered from a small quantity of reducing material. The ether was then evaporated and the syrup isolated by redistillation, a fraction, b. p. 115—125°/0·3—0·7 mm., being collected. The distillate consisted of a mixture of methylmannoside diacetone (64%) and unchanged mannose diacetone (36%). The conditions of the condensation with acid methyl alcohol were varied, and it was ascertained that when the reaction was continued until all the mannose diacetone had disappeared, the bulk of the product then consisted of γ-methylmannoside. This was isolated by solution in cold ethyl acetate, the syrup thus extracted being afterwards treated with ether to remove extraneous products. The mannoside was obtained as a colourless syrup which slowly crystallised owing to its spontaneous conversion into the α-form.

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