335. *The Methylation of Glucosephenylosazone and its Formulation as a Derivative of Fructopyranose.*

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According to many workers, osazones such as glucosazone, galactosazone (Levene and Laforge, *J. Biol. Chem.*, 1915, **20**, 429), and 3-methyl glucosazone (Anderson, Charlton, and Haworth, J., 1929, 1329) in solution in alcohol or pyridine exhibit mutarotation which, unless the rotational changes are due to decomposition, may indicate the existence of some type of ring structure.

After a single methylation of glucosephenylosazone with methyl sulphate and sodium hydroxide, excess of alkali being avoided, a new crystalline *monomethyl glucosazone* was isolated, which did not agree in physical properties with any of the known monomethyl glucosazones (see Table I). It was a true osazone, since treatment with *p*-nitrobenzaldehyde gave an osone, from which the original osazone was regenerated in five minutes at room temperature by treatment with phenylhydrazine acetate.

Table I.

Glucosazone.	M. p.	$[\alpha]_D$ in alcohol.	Form.	Reference.
3-Methyl	178—179°	− 109° → − 9°	Needles	Anderson, Charlton, and Haworth, J., 1929, 1329.
4-Methyl	158—159	− 33 → − 15	Needles	Pacsu, *Ber.*, 1925, **58**, 1463; Schinle, *Ber.*, 1932, **65**, 315; Munro and Percival, this vol., p. 873.
6-Methyl	184—187	− 69; no mutarotation	Needles	Helferich and Günther, *Ber.*, 1931, **64**, 1276.
New methyl	116—117	− 50 → − 12	Plates (aqueous alcohol) Needles (equilibrium solution in alcohol)	

The evidence presented below gives no reason to doubt that the new compound is the missing 5-methyl glucosazone.

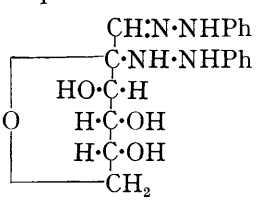
p-Nitrobenzaldehyde, having given a better yield of glucosone from glucosazone than benzaldehyde (Fischer and Armstrong, *Ber.*, 1902, **35**, 3143), was used to prepare the methyl glucosone. The yield was poor (10%) and attempts to improve it by using hydrochloric acid (Fischer, *Ber.*, 1889, **22**, 87) failed. By reduction with zinc dust and acetic acid (Fischer, *loc. cit.*) the corresponding ketose was obtained as a syrup of negative rotation, from which the original osazone could be regenerated in the usual manner. Its properties agreed with those of a monomethyl fructose and the negative rotation indicated its relationship to fructopyranose, so substitution in position 6 was unlikely.

The course of glycoside formation in the cold was followed as described by Levene, Raymond, and Dillon (*J. Biol. Chem.*, 1932, **95**, 699). A comparison of the results (Table III) with those given by fructose (Table II) under parallel conditions showed that, whereas the sugar under review gave 62% of a pyranoside in 24 hours, fructose was exclusively transformed into furanoside. A small constant amount of furanoside appeared to be formed, but this was ascribed to the presence of a 5-methyl aldose produced by a Lobry de Bruyn transformation during the removal of zinc with barium hydroxide. These observations are in harmony with the substitution of methyl in the penultimate hydroxyl group in fructose, such a 5-methyl fructose being capable only of a pyranose formulation. The sugar was accordingly transformed into the pyranoside by heating with methyl-alcoholic hydrogen chloride, and methylation, followed by distillation and hydrolysis, yielded crystalline tetramethyl fructopyranose, indicating that we were dealing with a genuine derivative of fructopyranose.

That 5-methyl glucosazone can be so readily obtained from glucosazone is to be ascribed to the fact that the particular hydroxyl group in question is the most vulnerable to attack by methyl sulphate and alkali. Obviously, whatever rings, if any, exist in the original glucosazone, neither a 1 : 5- nor a 2 : 5-oxide ring is possible.

In order to obtain more evidence on this point glucosazone was methylated by three treatments with methyl sulphate and sodium hydroxide, followed by three with methyl iodide and silver oxide. A red syrup, the methoxyl content of which could not be increased by further methylation, was obtained which gave the analytical figures required for trimethyl glucosazone. Evidently, therefore, there is only one ring in glucosazone, provided that one position is not made unavailable for methylation by steric effects. Repeated attempts to crystallise the syrup failed and it was apparently not identical with the crystalline 3 : 5 : 6-trimethyl glucosazone of Anderson, Charlton, and Haworth (*loc. cit.*) or the 3 : 4 : 6-trimethyl fructosazone of Haworth and Learner (J., 1928, 619). 3 : 4 : 5-Trimethyl fructopyranose has been prepared (Irvine and Patterson, J., 1922, **121**, 2159; for structure see Haworth, Hirst, and Learner, J. 1927, 1040), but there is no record of its phenylosazone.

The syrupy trimethyl glucosazone was converted into a trimethyl fructose by way of the osone as before. Methyl-alcoholic hydrogen chloride reacted slowly at room temperature to form almost exclusively a pyranoside (Table IV), again indicating substitution in position 5 and a free hydroxyl group in position 6. Further methylation of this pyranoside, followed by hydrolysis, again yielded tetramethyl fructopyranose, proving that the sugar was essentially 3 : 4 : 5-trimethyl fructose. Position 6 in the glucosazone is therefore either prevented from undergoing methylation by steric effects, which is improbable, or is concerned with ring formation, and it is considered probable that the osazone contains a pyranose ring and has the annexed structure. Examination of a model of this substance shows that the hydroxyl group on carbon atom 5 is the one most remote from the phenylhydrazine residues, and this may be the reason for the preferential formation of 5-methyl glucosazone.



Although 3-, 4-, and 5-methyl glucosazones may all have the fructopyranose structure, this cannot be the case for 6-methyl glucosazone. The observation of Helferich and Günther (*loc. cit.*) that the purest 6-methyl glucosazone so far obtained exhibits no muta-

rotation in alcohol is in agreement with this, though earlier workers (Kuhn and Ziese, *Ber.*, 1926, **59**, 2314; Ohle and v. Vargha, *Ber.*, 1929, **62**, 2434; Levene and Raymond, *J. Biol. Chem.*, 1932, **97**, 751) had apparently observed mutarotation in this case. The fact that 6-methyl glucosazone is only slowly precipitated (6 hours) during its formation from 6-methyl glucose in the usual way may also have some significance in connexion with its structure.

Experimental.

5-Methyl Glucosazone.—Methyl sulphate (60 c.c.) and 30% sodium hydroxide solution (140 c.c.) were added to glucosazone (20 g.), dissolved in acetone (50 c.c.) and alcohol (125 c.c.), during 2 hours with constant stirring at 50°. The mixture was then maintained at 70° for 10 minutes, diluted with hot water (500 c.c.), neutralised with glacial acetic acid, and kept overnight. The yellow precipitate and brown tarry matter were filtered off and dissolved in boiling alcohol, and water added until precipitation was just maintained on further heating. The tar that separated was removed; the filtrate on cooling deposited a yellow precipitate, which was collected and subjected to further treatment as above. After ten recrystallisations a pale yellow solid was obtained (Found: OMe, 5·0%). This was dissolved in boiling chloroform; unchanged glucosazone crystallised from the cold solution, and the mother-liquor on removal of the solvent under diminished pressure left a product, which was crystallised from aqueous alcohol (Found: OMe, 9·3%). Fractional crystallisation from aqueous alcohol then gave *5-methyl glucosazone* (5 g.) in shining rectangular plates with saw-like edges, m. p. 116—117°, $[\alpha]_D^{20°}$ − 44° in chloroform (*c*, 0·7), − 49° in alcohol (10 mins. after dissolution; *c*, 0·7), − 12° (36 hrs., constant value). This equilibrium solution crystallised in fine needles, m. p. 117° (Found: C, 61·3; H, 6·5; OMe, 7·9; N, 14·8. $C_{19}H_{24}O_4N_4$ requires C, 61·3; H, 6·45; OMe, 8·3; N, 15·0%).

Conversion of Glucosazone into Glucosone.—(1) Glucosazone (1 g.) was dissolved in alcohol (30 c.c.), and water added to produce a turbidity. The vigorously stirred mixture was heated with benzoic acid (0·5 g.) and benzaldehyde (10 c.c.) on a boiling water-bath for 1 hour. After 30 minutes water (50 c.c.) was added. The cooled filtered solution was extracted with ether and evaporated to 20 c.c. at 35° under diminished pressure; on treatment with phenylhydrazine acetate a yellow precipitate of glucosazone appeared after 3 minutes at room temperature (yield, 7%).

(2) Glucosazone (1 g.), in alcohol (100 c.c.), was stirred with benzoic acid (1 g.) and *p*-nitrobenzaldehyde (5 g.) at 90—100° until all the solid had dissolved. Water (150 c.c.) was then added, and the heating continued for 65 minutes, alcohol (50 c.c.) being added after 30 minutes to replace the loss by evaporation. The solution was cooled, filtered (residue A), extracted three times with ether, and evaporated to 20 c.c. at 35° under diminished pressure; treatment with phenylhydrazine acetate gave glucosazone (0·3 g.). The residue (A), similarly treated with *p*-nitrobenzaldehyde (3 g.), yielded glucosazone (0·1 g.), so the conversion was complete to the extent of 40% (cf. Fischer, *loc. cit.*).

Conversion of 5-Methyl Glucosazone into 5-Methyl Glucosone.—By method (2) above, a light yellow syrup of the osone was obtained, which strongly reduced Fehling's solution and on treatment with phenylhydrazine acetate gave a yellow precipitate after 5 minutes in the cold (yield, 10%). On recrystallisation this gave the characteristic shining plates of 5-methyl glucosazone, m. p. 116—117° (Found: OMe, 7·8%).

Reduction of 5-Methyl Glucosone to 5-Methyl Fructose.—5-Methyl glucosazone (5 g.) was converted into 5-methyl glucosone in the above manner. After extraction with ether, in order to avoid decomposition, zinc dust (1 g.) and glacial acetic acid (0·5 c.c.) were added and the solution was evaporated to 80 c.c. This was heated with zinc dust (20 g.) and a few drops of platinic chloride solution on a boiling water-bath with vigorous stirring for 90 minutes during the addition of glacial acetic acid (8 c.c.). A portion (1 c.c.) of the cooled filtered solution failed to give an osazone on treatment with phenylhydrazine acetate in the cold or on heating for 10 minutes, but after 1 hour's heating the osazone came down in the characteristic plates. 2*N*-Barium hydroxide was added to the main bulk until all the zinc was precipitated as zinc hydroxide; the filtrate gave no precipitate with ammonium sulphide. Barium was removed by means of 2*N*-sulphuric acid, and the filtered solution evaporated to dryness at 40°/20 mm. The resulting solid was extracted three times with absolute alcohol (200 c.c.), and the extracts evaporated to dryness, leaving a pale yellow, reducing glass (0·3 g.) consisting of barium acetate mixed with monomethyl fructose. It was considered inadvisable to remove the whole of the

barium because of the danger of decomposition in the presence of a trace of sulphuric acid, but solution in water and addition of more sulphuric acid removed a large part of the inorganic material. The liquid was filtered and evaporated at 40°/20 mm. $[\alpha]_D^{20°}$ — 40° in water (*c*, 0·5) (Found: OMe, 11·8; Ba, 3·8. The assumption that all the barium was present as barium acetate gives $[\alpha]_D^{20°}$ — 50°; OMe, 14·8. Calc. for $C_7H_{14}O_6$: OMe, 16·1%).

Attempted Furanoside Formation.—The method of Levene, Raymond, and Dillon (*loc. cit.*) is by no means quantitative as regards the estimation of fructofuranoside by hydrolysis with 0·1*N*-hydrochloric acid at 100°, since it was found that the monomethyl methylfructopyranoside was hydrolysed to the extent of 15% under the experimental conditions. This is not surprising, since 1 : 3 : 4 : 5-tetramethyl methylfructopyranoside is completely hydrolysed by 0·7*N*-hydrochloric acid during 30 minutes. Further, the effect of acid treatment on the free sugar itself under the conditions obtaining during the hydrolysis is such that a higher reducing value is obtained by the Hagedorn–Jensen ferricyanide method, modified by Hanes (*Biochem. J.*, 1929, **23**, 99), notably to the extent of 25%. The figures recorded for the percentages of pyranoside and furanoside are corrected according to these factors. Table III shows the effect at 20° of 0·5% methyl-alcoholic hydrogen chloride on monomethyl fructose (*ca.* 0·3%). The method employed was to withdraw two samples of 1 c.c. at a time, one being treated with a 20% excess of 0·4*N*-sodium carbonate solution, the volume made up to 5 c.c., 5 c.c. of the standard potassium ferricyanide–sodium carbonate mixture [8·25 g. $K_3Fe(CN)_6$, 10·6 g. Na_2CO_3/litre] added, and the solution heated for 15 minutes at 100°. After cooling for 3 minutes, 5 c.c. of a solution (potassium iodide, 12·5 g., zinc sulphate, 25·0 g., and sodium chloride, 125·0 g./litre) were added, followed by 3 c.c. of 1% acetic acid, the liberated iodine being titrated with 0·015*N*-sodium thiosulphate. The difference between this titre and a blank carried out under the same conditions gave the figure for the reducing value. To the second 1 c.c. portion, 0·4*N*-hydrochloric acid and water were added so that the solution was 0·1*N* with respect to hydrochloric acid, and the solution was heated at 100° for 10 minutes. Sodium carbonate (20% excess) was then added as before, the amounts being adjusted to bring the final volume to 5 c.c. The reducing power was then determined as above. Table II shows parallel experiments with fructose.

TABLE II.

Time.	0·015*N*-Thiosulphate, c.c. Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	4·2	4·5	100	—	—
40 mins.	0·1	4·5	2	100	—
3 hrs.	—	4·4	—	100	—
5 ,,	0·1	4·6	2	100	—
24 ,,	—	4·5	—	100	—

TABLE III.

Time.	0·015*N*-Thiosulphate, c.c. Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	5·2	6·5	100	—	—
30 mins.	3·4	5·1	65	14	21
2 hrs.	3·0	4·6	58	14	28
10 ,,	1·8	3·2	35	15	50
24 ,,	1·2	2·5	23	15	62

At the end of 24 hours the solution was still reducing to Fehling's reagent. The table shows that, whereas furanoside formation from fructose is complete after 1 hour, in the case of the sugar under review about 14% of furanoside appears to be formed at once but this figure remains constant while the amount of pyranoside gradually increases. The method is only regarded as semi-quantitative, indicating that in contrast with fructose the sugar forms a pyranoside in preference to a furanoside, an observation which agrees with the structure assigned to the monomethyl osazone.

Preparation of 5-Methyl Methylfructopyranoside and Methylation of the Product.—The sugar (0·25 g.) was dissolved in 3% methyl-alcoholic hydrogen chloride (20 c.c.) and heated at 75° under reflux for 5 hours; reducing action had then ceased. After neutralisation with barium carbonate the methyl-alcoholic solution, to which acetone (20 c.c.) had been added, was methylated twice with methyl sulphate (25 c.c.) and 30% sodium hydroxide solution (70 c.c.). The product was extracted with chloroform, the solvent removed, and the mobile syrup remethylated by two treatments with silver oxide (10 g.) and methyl iodide (30 c.c.). This resulted in the isolation of a syrup, which was distilled at 110° (bath temp.)/0·03 mm. to yield a mobile liquid (0·10 g.), $n_D^{20°}$ 1·4540; this was evidently a fully methylated fructoside.

Isolation of 1 : 3 : 4 : 5-*Tetramethyl Fructose.*—The tetramethyl methylfructoside was hydrolysed during 30 minutes with 3% hydrochloric acid, the solution neutralised with barium carbonate and evaporated to dryness under diminished pressure, and the residue extracted with ether. Removal of the solvent yielded a syrup, which partly crystallised in the square

plates of tetramethyl fructopyranose, m. p. 94—96° after recrystallisation from light petroleum, alone or in admixture with a specimen prepared directly from fructose, $[\alpha]_D^{20°}$ — 109° in water (*c*, 0·5).

Preparation of Trimethyl Glucosazone and Conversion into Trimethyl Fructose.—The tar (4 g.) obtained from the original methylation was found to contain OMe, 14·1%, and was subjected to two further methylations with methyl sulphate (20 c.c.) and 30% sodium hydroxide solution (50 c.c.) at 50° during 2 hours. Water (300 c.c.) was added, and the mixture cooled and filtered. The tarry residue was extracted with chloroform, the chloroform solution washed with water till neutral and dried with sodium sulphate, and the solvent removed under diminished pressure. The red syrup obtained (Found: OMe, 18·2%) was subjected to three methylations with methyl iodide (30 c.c.) and silver oxide (10 g.), which was added in 1 g. portions every 20 minutes. The product was isolated as a red syrup in the usual way (Found: C, 63·3; H, 6·9; OMe, 22·6; N, 14·1. $C_{21}H_{28}O_4N_4$ requires C, 63·0; H, 7·0; OMe, 23·2; N, 14·0%).

The conversion of this *trimethyl glucosazone* (12 g.) into trimethyl glucosone and the reduction to the trimethyl fructose were carried out precisely as described for the monomethyl derivative, with the exception that the sugar was extracted from the mixture with barium acetate by means of boiling chloroform. This yielded a reducing syrup (0·4 g.), $[\alpha]_D^{20°}$ — 43° in methyl alcohol (*c*, 0·4), — 39° in water (*c*, 0·3) (Found: OMe, 42·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Attempted Fructofuranoside Formation.—This was carried out as described for the monomethyl derivative, and the results are in Table IV. Blank experiments showed that the trimethyl methylfructopyranoside was 20% hydrolysed and the reducing value of free sugar increased by 20% under the experimental conditions. The figures recorded are for the relative amounts of the glycoside corrected by these factors.

TABLE IV.

	0·015*N*-Thiosulphate, c.c.				
Time.	Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	5·3	6·4	100	—	—
1 hr.	4·1	5·5	78	9	13
4 hrs.	3·8	4·9	72	6	23
19 ,,	3·2	4·4	62	8	30
24 ,,	3·1	4·2	58	8	34
48 ,,	2·4	3·4	45	8	47

At the end of 48 hours the solution was still reducing to Fehling's reagent. The results show in a roughly quantitative manner the gradual formation, as the available sugar disappears, of a glycoside which is hydrolysed only with difficulty. The values calculated as furanoside are uniformly low and constant, as in Table III, and are ascribed to impurity.

Preparation of 3 : 4 : 5-*Trimethyl Methylfructopyranoside and Methylation of the Product.*—The sugar (0·3 g.) was dissolved in 3% methyl-alcoholic hydrogen chloride (20 c.c.) and heated at 75° under reflux for 5 hours; reducing action had then ceased. After neutralisation with barium carbonate the methyl-alcoholic solution, to which acetone (20 c.c.) had been added, was methylated once with methyl sulphate and sodium hydroxide and once with silver oxide and methyl iodide. This resulted in the isolation of a syrup, which distilled at 110° (bath temp.)/0·03 mm. to yield a mobile liquid (0·15 g.), $n_D^{20°}$ 1·4520; this was evidently a fully methylated fructoside.

Isolation of 1 : 3 : 4 : 5-*Tetramethyl Fructose.*—This tetramethyl methylfructoside was hydrolysed as in the previous case to yield a syrup, which partly crystallised in the square plates of tetramethyl fructopyranose, m. p. 94—96° alone or in admixture with a specimen prepared directly from fructose.

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