# 274. *Studies on Cellobiosazone, Galactosazone, and Other Sugar Osazones.*

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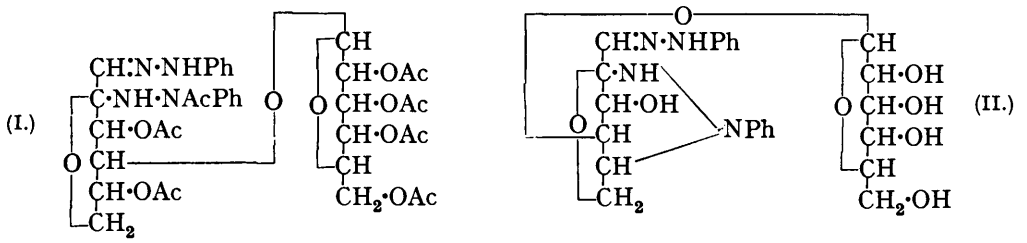
The deacetylation of *cellobiosazone hepta-acetate* produces a monoanhydrocellobiosazone hydrate which yields the same *penta-acetate* as the anhydrocellobiosazone prepared by Diels's method. The *hepta-acetates* of *gentiobiosazone* and *melibiosazone* yield no crystalline anhydro-osazones on deacetylation.

It is shown that galactosazone may be converted by methylation into a *trimethyl galactose methylphenylphenylosazone*, but that no more methoxyl residues can be introduced. This fact together with the failure to prepare a trityl derivative indicates the presence of a tagatopyranose ring in galactosazone.

The study of disaccharide osazones (Percival and Percival, J., 1937, 1320) has been extended to cellobiosazone, melibiosazone, and gentiobiosazone. The first of these yielded a crystalline *hepta-acetate* (I), which on deacetylation yielded a product (II), m. p. 218°, of the same analytical composition as cellobiosazone but having a markedly different specific rotation ($[\alpha]_D^{18°}$ — 142° in methyl alcohol). Acetylation yielded a crystalline *penta-acetate*, from which (II) could be recovered on deacetylation. The analytical figures for the penta-acetate were in agreement with those for a monoanhydrocellobiosazone penta-acetate but not for a dianhydrocellobiosazone penta-acetate, so the original deacetylation product is clearly a monoanhydrocellobiosazone hydrate. Diels, Meyer, and Onnen (*Annalen*, 1936, **525**, 94), by treating cellobiosazone with sulphuric acid in alcohol, have obtained an anhydrocellobiosazone hydrate, m. p. 225—245°, and by acetylation with acetic anhydride and sodium acetate claim to have isolated a hexa-acetate, but their analytical figures, in the absence of any acetyl determination, would seem to fit equally well for a penta-acetate. Using their method, we obtained a product, m. p. 218°, identical with that prepared by deacetylating cellobiosazone hepta-acetate, which yielded the same penta-acetate, so the case of cellobiosazone is parallel to that of lactosazone (Percival and Percival, *loc. cit.*).

Gentiobiosazone and melibiosazone yielded amorphous *hepta-acetates* and no crystalline products could be obtained on deacetylation, a result similar to that observed for the acetates of arabinosazone, xylosazone, and rhamnosazone (Percival and Percival, *loc. cit.*). It would seem, therefore, that, if a free primary alcohol residue is absent in the original sugar, crystalline anhydrides are not readily isolated from the osazone acetates by the deacetylation method. Previously (J., 1938, 1384) it had been shown by one of us that for glucosazone, galactosazone, and gulosazone, anhydride formation is concerned with the hydroxyl groups on $C_3$ and $C_4$, since the tetra-acetates of these osazones give the same dianhydrohexosazone on hydrolysis, which also has no free hydroxyl group on $C_6$. Since for steric reasons anhydride formation is unlikely to involve this position and a hydrogen atom attached to nitrogen on $C_2$, it appears that the 2 : 6-oxide ring known to be present in glucosazone and galactosazone (see p. 1480) is retained in the dianhydrohexosazone. By analogy the most probable formula for the monoanhydrocellobiosazone appears to be

(II), being formed from the hepta-acetate (I), although the possibilities of 1 : 3-anhydro (1 : 5-oxide), 1 : 3-anhydro (2 : 6-oxide) and 1 : 3-anhydro (2 : 5-oxide) cannot be rigidly




excluded. When $C_6$ is substituted (or absent), no ketopyranose structure is possible and, if this is a predisposing factor to the formation of crystalline anhydrides, this may explain the facts. There are, however, double the theoretical possibilities for anhydride formation when the hydroxyl group on position 6 is substituted rather than that on position 4, namely, three pyranose and five furanose monoanhydrides (apart from any stereochemical isomers), so it may well be that mixtures of anhydrides result in these cases.

Direct proof has not hitherto been furnished that galactosazone has an oxide ring structure. Diels, Cluss, Stephan, and König (*Ber.*, 1938, **71**, 1189) had shown, however, that glucosazone failed to react with triphenylchloromethane, indicating the absence of a primary alcohol group, and we have found that galactosazone behaves in the same way.

The results of methylation experiments are not so conclusive as those previously described for glucosazone (Percival and Percival, J., 1935, 1398), since, although what appeared to be a monomethyl galactosazone was obtained, it proved to be a mixture. Exhaustive methylation, however, failed to introduce more than three methoxyl residues into the molecule. It was found possible to isolate a crystalline *trimethyl galactosemethylphenylphenylosazone* by direct methylation with sodium hydroxide and methyl sulphate as well as a syrup of lower methoxyl content which on further methylation yielded a syrupy trimethyl galactosemethylphenylphenylosazone, but these compounds proved to be so difficult to convert into the corresponding osone that examination of the trimethyl ketose could not be carried out. The weight of evidence, however, justifies the formulation of galactosazone as tagatopyranosazone.

## Experimental.

*Cellobiosazone Hepta-acetate.*—A solution of cellobiosazone (10 g.) in a mixture of pyridine (50 c.c.) and acetic anhydride (20 c.c.) was kept overnight and poured into an excess of water. The yellow amorphous solid obtained (13 g.), recrystallised from alcohol, gave shining needles, m. p. 90°, $[\alpha]_D^{18°}$ — 37° in chloroform (*c*, 0·3) (Found: C, 55·8; H, 5·65; $CH_3$·CO, 36·4; N, 7·1. $C_{38}H_{46}O_{16}N_4$ requires C, 56·0; H, 5·7; $CH_3$·CO, 37·0; N, 6·9%).

*Anhydrocellobiosazone.*—Cellobiosazone hepta-acetate (4 g.), dissolved in acetone (180 c.c.) and water (100 c.c.), was mixed with sodium hydroxide solution (44 c.c., 8%) and kept overnight at room temperature. The solution was then neutralised with sulphuric acid and diluted with acetone until the precipitation of sodium sulphate was complete. After filtration the acetone was removed at 40°/20 mm. On standing, a yellow powder (1·7 g.) was obtained which crystallised from hot pyridine–alcohol–water in light yellow needles, m. p. 218°, $[\alpha]_D^{18°}$ — 142° in methyl alcohol (*c*, 0·2) (Found: C, 55·4; H, 6·6; N, 10·4. Calc. for $C_{26}H_{32}O_8N_4$: C, 55·4; H, 6·2; N, 10·7%).

This compound was acetylated as described above to yield light yellow needles (1·3 g.), which after recrystallisation from alcohol had m. p. 193°, $[\alpha]_D^{18°}$ — 153° in (1 : 1) pyridine–alcohol (*c*, 0·2), $[\alpha]_D^{18°}$ — 142° in acetone (*c*, 0·2) (Found: C, 57·2; H, 5·75; N, 8·1; $CH_3$·CO, 29·3. $C_{34}H_{40}O_{13}N_4$ requires C, 57·2; H, 5·65; N, 7·9; $CH_3$·CO, 30·1%).

This *penta-acetyl anhydrocellobiosazone* on deacetylation yielded the anhydrocellobiosazone above, m. p. 218°, $[\alpha]_D^{18°}$ — 142° in methyl alcohol (*c*, 0·2).

The anhydrocellobiosazone of Diels, Meyer, and Onnen (*loc. cit.*) was prepared; it had m. p. 218° (not depressed by the above anhydrocellobiosazone) and $[\alpha]_D^{18°}$ — 143° in methyl alcohol (*c*, 0·2). Acetylation yielded the same penta-acetate as before, m. p. 193° (not depressed by

the above anhydrocellobiosazone penta-acetate) and $[\alpha]_D^{19°}$ — 153° in (1 : 1) pyridine–alcohol (*c*, 0·2).

*Melibiosazone Hepta-acetate.*—Melibiosazone (1 g.), m. p. 179°, $[\alpha]_D^{18°}$ + 43° in pyridine (*c*, 0·7), on acetylation as described above yielded an amorphous *acetate* (1·6 g.), m. p. 105°, $[\alpha]_D^{17°}$ + 32° in chloroform (*c*, 0·4) (Found: C, 55·7; H, 5·95; N, 7·0; $CH_3$·CO, 36·9. $C_{38}H_{46}O_{16}N_4$ requires C, 56·0; H, 5·7; N, 6·9; $CH_3$·CO, 37·0%).

*Gentiobiosazone Hepta-acetate.*—Gentiobiosazone (1 g.), m. p. 170°, was acetylated as above to yield an amorphous *acetate*, m. p. 98°, $[\alpha]_D^{17°}$ — 46° in chloroform (*c*, 0·4) (Found: C, 56·0; H, 6·05; N, 7·2; $CH_3$·CO, 36·6. $C_{38}H_{46}O_{16}N_4$ requires C, 56·0; H, 5·7; N, 6·9; $CH_3$·CO, 37·0%).

The deacetylation of melibiosazone and gentiobiosazone hepta-acetates yielded dark brown solids which could not be recrystallised or purified.

*Methylation of Galactosephenylosazone.*—Methyl sulphate (60 c.c.) and 30% sodium hydroxide solution (140 c.c.) were added to galactosazone (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 15 minutes, diluted with hot water (500 c.c.), neutralised with glacial acetic acid, and kept overnight. The yellow precipitate and brown tarry matter (21·5 g.) were dissolved in boiling chloroform; on cooling, unchanged galactosazone (1·5 g.) separated. After filtration and precipitation from light petroleum (b. p. 60—80%) a yellow solid (A) (18 g., OMe 7·4%) was obtained together with a red syrup (B) (OMe, 12—14%). The solution deposited a crop (C) (0·4 g.) of pale yellow needles (OMe, 21·5%). Repeated precipitation of (A) gave a *product*, m. p. 78°, which could not be crystallised, $[\alpha]_D^{18°}$ + 27° (10 mins. in chloroform; *c*, 0·25); + 12° (72 hours); $[\alpha]_D^{18°}$ + 36·5° (10 mins. in alcohol; *c*, 0·3); + 16·5° (48 hours) (Found: C, 61·3; H, 6·5; OMe, 8·1; N, 13·0. $C_{19}H_{24}O_4N_4$ requires C, 61·3; H, 6·45; OMe, 8·3; N, 15·0%).

A further small quantity of the needles (C) (0·2 g.) was obtained from the filtrates from these precipitations and analysis indicated it to be a *trimethyl galactose methylphenylphenylosazone*, m. p. 160°, $[\alpha]_D^{18°}$ + 86·5° (5 mins. in chloroform; *c*, 0·2); + 32·4° (48 hours); $[\alpha]_D^{18°}$ + 93·5° (5 mins. in alcohol; *c*, 0·2); + 31° (48 hours) (Found: C, 63·5; H, 7·1; OMe, 21·5; N, 13·8; NMe, 6·7. $C_{22}H_{30}O_4N_4$ requires C, 63·7; H, 7·3; OMe, 22·5; N, 13·5; NMe, 7·0%).

Subsequent methylations gave variable yields of this substance. The syrup (B) on further methylation failed to yield more (C). Repeated methylation of (B), first with methyl sulphate and sodium hydroxide and then with silver oxide and methyl iodide, yielded a product (D), $[\alpha]_D^{18°}$ + 121° (10 mins. in alcohol; *c*, 0·2); + 65·5° (24 hours) (Found: OMe, 21·5; NMe, 6·2. Calc. for $C_{22}H_{30}O_4N_4$: OMe, 22·5; NMe, 7·0%).

When galactosazone was treated with *p*-nitrobenzaldehyde in an atmosphere of nitrogen, the conditions otherwise being the same as those described for glucosazone (Percival and Percival, *loc. cit.*), a 30% conversion into galactosone was obtained. This method was therefore applied to the supposed monomethyl galactosazone and the osone was reduced to the ketose as previously described. Application of the modified method described in the above paper of following the course of glycoside formation with 1% methyl-alcoholic hydrogen chloride in the cold led to indefinite results, both furanosides and pyranosides being formed, the conclusion necessarily following that the monomethyl tagatose was either a mixture of more than one monomethyl tagatose or a mixture of an aldose and a ketose.

Attempts to convert the crystalline compound (C) and the syrup (B) into osones and thence into the trimethyl ketose were abortive.

*The Attempted Preparation of Triphenylmethylgalactosazone.*—Dry galactosazone (10 g.) and triphenylchloromethane (7·8 g.) were dissolved in dry pyridine and warmed at 80° for 2 hours. On pouring into ice-water and standing, a yellow solid was produced, which was extracted with carbon tetrachloride to remove triphenylcarbinol. The insoluble yellow residue on recrystallisation from alcohol–pyridine–water gave needles, m. p. 185°, not depressed on admixture with galactosazone. A similar result was obtained for glucosazone.

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