391. *The Structure of Osazones and the Isolation of a New Hexosazone Anhydride.*

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In a previous paper (Percival and Percival, J., 1935, 1398) the suggestion was put forward on the basis of methylation experiments that glucosephenylosazone possessed a 2 : 6-oxide ring structure. Engel (*J. Amer. Chem. Soc.*, 1935, **57**, 2419) has since adduced evidence in support of the original acyclic formula of Emil Fischer. He rejects the results obtained in his own methylation experiments as inconclusive, but the highest methoxyl content for the methylated glucosazone he obtained (OMe, 20·7%) approaches the value for a trimethyl derivative, and this is in agreement with our results. His results dealing with the absorption spectra of osazones are of interest, but the conclusion that they disprove a cyclic structure for glucosazone must be accepted with reserve, for it must be pointed out that the effect on the absorption of the group $\cdot O\cdot\overset{\cdot}{\underset{|}{C}}\cdot NH\cdot NHPh$ may not differ greatly from that of $\cdot\overset{\cdot}{\underset{|}{C}}\cdot N\cdot NHPh$ owing to the presence of the aromatic nuclei.

Engel (*loc. cit.*), Maurer and Schiedt (*Ber.*, 1935, **68**, 2187), and Wolfrom, Konigsberg, and Soltzberg (*J. Amer. Chem. Soc.*, 1936, **58**, 490) describe a tetra-acetyl glucosephenylosazone, and the last authors a tetra-acetyl galactosephenylosazone. Wolfrom *et al.*

(*loc. cit.*) claim that by using the method of Freudenberg and Harder (*Annalen*, 1923, **433**, 230) for the estimation of the total number of acetyl groups and the method of Kunz and Hudson (*J. Amer. Chem. Soc.*, 1926, **48**, 1982) for the determination of *O*-acetyl residues, they can distinguish between *O*-acetyl and *N*-acetyl groups. Thus β-*d*-glucoseoxime hexa-acetate is shown to possess five *O*-acetyl groups and one *N*-acetyl group. This method on application to the above osazone tetra-acetates indicated that all the acetyl groups were bound through oxygen in agreement with an acyclic structure. These experiments have been repeated, the potentiometric method described by Wolfrom being used both under the conditions employed by Kunz and Hudson (*loc. cit.*) and under those of Wolfrom, Konigsberg, and Soltzberg (*loc. cit.*). The results indicate that only three of the four acetyl groups are removed during the deacetylation in the cold, even when the reagent is in contact with the acetylated osazones for periods much in excess (6 hours) of the 1 hour period used by the latter authors. On the other hand, deacetylation at room temperature indicated the presence of four acetyl groups, and this was confirmed by the method of Freudenberg and Harder (*loc. cit.*). Unless, therefore, one *O*-acetyl group is difficult to remove, the present results appear to indicate that both tetra-acetyl glucosephenylosazone and tetra-acetyl galactosephenylosazone have cyclic structures. Although the structure of tetra-acetyl glucosazone cannot yet be defined with certainty, it has been pointed out by Behrend and Reinsberg (*Annalen*, 1910, **377**, 187) that acetylation on the nitrogen of a true hydrazone in the cold is difficult, whereas phenylhydrazides are easily acetylated, and it is thought probable, therefore, that the *N*-acetyl group is to be found on the phenylhydrazide residue (I).

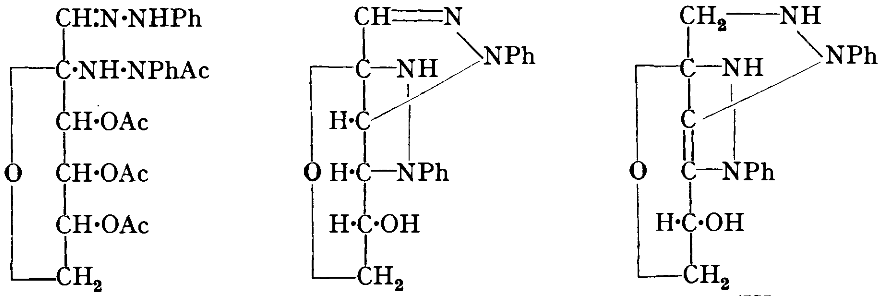
The description of the fully methylated glucosazone as trimethyl glucosazone (Percival and Percival, *loc. cit.*) must now be corrected to trimethyl glucosemethylphenylphenylosazone, since a subsequent estimation of methylimino-groups indicated the presence of one NMe residue per molecule introduced during the prolonged methylation process employed to secure complete methylation.

By deacetylating glucosazone tetra-acetate at room temperature a new derivative was isolated which by analysis and molecular weight determinations was shown to be glucosazone minus two molecules of water, $C_{18}H_{18}O_2N_4$. Diels and Meyer (*Annalen*, 1935, **519**, 157) had previously reported the isolation from glucosazone of a monoanhydroglucosazone, $C_{18}H_{20}O_3N_4$, which they considered to be **3 : 6**-anhydroglucosazone. The *dianhydro-hexosazone* described above contained one hydroxyl group only, since it gave rise to a monomethyl ether and a monoacetate. This demands the assumption of the presence of an oxide ring structure, a fact which is rendered the more probable by the existence of such a ring in the acetylated osazones themselves. By the addition of bromine in chloroform this dianhydride immediately gave rise to an insoluble dibromide, which appeared to indicate the presence of a double bond either C:C or C:N. The dianhydrohexosazone was very stable and resisted all attempts to remove the phenylhydrazine residues by means of benzaldehyde, *p*-nitrobenzaldehyde, or concentrated hydrochloric acid; it could in fact be recrystallised from the last reagent.

When galactosazone tetra-acetate was deacetylated in the same way, a compound of the same composition was obtained, identical in crystalline form, melting point, and specific rotation with that described above, and mixed melting point determinations appeared to prove their identity. Similarly the monoacetate of the "dianhydrogalactosazone" was identical in all respects with the corresponding compound prepared from glucosazone. It is suggested that on deacetylation of the osazone tetra-acetate (I) ring closure with the elimination of two molecules of water takes place between the hydroxyl groups on C_3 and C_4 and the hydrogen atoms of the imino-groups attached to C_1 and C_2, so that two five-membered rings are formed (II). To explain the formation of the same dianhydride from both glucose and galactose it is necessary to assume either a Walden inversion at C_4 on deacetylation and ring formation in one case (II), or a migration of hydrogen atoms from C_3 and C_4 so that the pyrazoline ring is converted into a pyrazolidine ring system with the production of a double bond between C_3 and C_4 (III). The latter structure appears from inspection of models to be impossible owing to the strained nature of the ring joining C_2 and C_4.

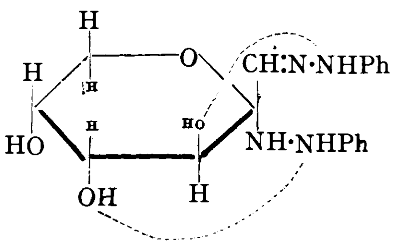
It is noteworthy that Karrer and Pfaeler (*Helv. Chim. Acta*, **1934**, **766**) by oxidation of glucosazone with periodic acid isolated 4-benzeneazo-1-phenylpyrazol-5-one by fission between C_3 and C_4.

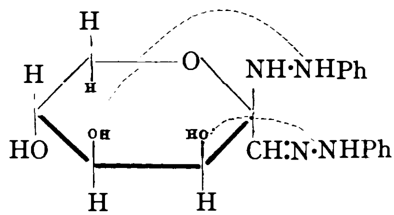
An examination of the possibilities of ring formation for derivatives of fructopyranose



(I.) (II.) (III.)

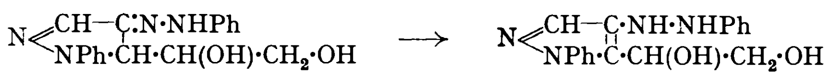
and tagatopyranose of the above type reveals the fact that only the α-fructopyranose derivative (IV) and the β-tagatopyranose derivative (V) can yield structures of the type (II) with any ease, and the Walden inversion may take place in the case of either derivative.

(IV.) 

 (V.)

From the specific rotations of the acetates of glucosazone and galactosazone it would appear that the former is present in the β- and the latter in the α-form. If the above mechanism is correct, however, unless Walden inversion also takes place on C_2, it must be assumed that the acetylated osazones are not pure α- and β-forms, but mixtures. This is probable both from the magnitude of the specific rotations observed and from the yields of the dianhydro-hexosazone isolated (30—40%).

Diels, Meyer, and Onnen (*Annalen*, 1936, **525**, 94) have now revised the structure proposed for the mono-anhydrides of the osazones and suggest their formulation as pyrazole derivatives by a process similar to (III) in which the 4-pyrazolone derivative (VI) isomerises into the pyrazole structure (VII).

(VI.)  (VII.)

The basis for this assumption rests on the observation that the monoanhydrides of *l*-arabinosazone and *d*-xylosazone are optical enantiomorphs, and since this must involve the abolition of the asymmetry on C_3, this would be accounted for by the formation of a double bond. Diels, Meyer, and Onnen (*loc. cit.*) have also prepared a dianhydro-maltosazone and have proposed a structure containing both pyrazole and pyridazine rings. So far it is not known whether the monoanhydro-hexazones of Diels possess an oxide ring structure. The fact that they form dibenzoates suggests this possibility, but triacetates have also been isolated. The possibility is not entirely excluded, however, as the authors point out that they have not attempted to discriminate between *O*-acetyl and *N*-acetyl residues.

Owing to the stability of the dianhydro-hexosazone it has not yet been found possible to confirm the structure by degradative experiments and further investigations are proceeding in order to establish the structure with certainty.

Experimental.

Acetylation of Glucosephenylosazone.—The method described by Maurer and Schiedt (*loc. cit.*) was employed, giving an amorphous yellow powder in almost quantitative yield; the method of Engel (*loc. cit.*) gave the same result. M. p. 70°, $[\alpha]_D^{20°}$ — 57° in alcohol (*c*, 0·7) [Found: C, 59·4; H, 5·6; CH_3·CO (Freudenberg), 33·5; N, 10·4. Calc. for $C_{26}H_{30}O_8N_4$: C, 59·3; H, 5·9; CH_3·CO, 32·7; N, 10·6%]. It was found difficult to crystallise the product, but eventually, following the technique of Maurer and Schiedt, the crystalline tetra-acetate was obtained in clumps of needles, m. p. 114—115°, $[\alpha]_D^{20°}$ — 58° in chloroform (*c*, 0·3) (Found: CH_3·CO, 33·1; N, 10·3%).

Acetylation of Galactosephenylosazone.—Galactosazone was acetylated as described by Wolfrom, Konigsberg, and Soltzberg (*loc. cit.*). The product, obtained in quantitative yield, was recrystallised from alcohol; m. p. 178—180°, $[\alpha]_D^{20°}$ + 90° in chloroform (*c*, 0·4) (Found: C, 59·4; H, 5·5; CH_3·CO, 32·5; N, 10·5. Calc. for $C_{26}H_{30}O_8N_4$: C, 59·3; H, 5·9; CH_3·CO, 32·7; N, 10·6%).

The Estimation of Acetyl Groups.—The amount of total acetyl was determined by the method of Freudenberg and Harder (*loc. cit.*) (Found: CH_3·CO, 33·5. Calc. for triacetate, 24·5; for tetra-acetate, 32·7%). Titrations were then carried out by the method of Kunz and Hudson (*loc. cit.*) using a potentiometer and the quinhydrone electrode. The substance (*ca.* 0·2 g.), dissolved in acetone (35 c.c.), was cooled to — 5°, and 0·1*N*-sodium hydroxide added drop by drop, the mixture being kept below 0° for the periods shown in the table. An excess of 0·1*N*-sulphuric acid was then added, and the solution back-titrated with 0·1*N*-sodium hydroxide. This method was used because of the coloured solutions produced but it was found that the use of phenol-red as indicator gave almost identical results. Deacetylations were also carried out at room temperature. The results are below:

Glucosazone Tetra-acetate.

	— 5° to — 0° (Kunz and Hudson).							18°.		
Time (hrs.)	1	1·25	1·75	2·5	2·75	3·25	6·25	2·25	4	18
CH_3·CO, %	24·5	25·0	24·9	25·4	25·4	25·8	27·9	33·7	33·2	34·9

Galactosazone Tetra-acetate.

	— 5° to — 0° (Kunz and Hudson).				18°.
Time (hrs.)	1	1·75	2	2·75	4
CH_3·CO, %	24·1	25·0	25·6	25·6	32·8

Analysis of "Trimethyl Glucosazone."—The "trimethyl glucosazone" described in the previous paper (Percival and Percival, *loc. cit.*) was purified by solution in chloroform and precipitation with a large quantity of light petroleum (b. p. 40—60°), this throwing out a small quantity of red tar, which was discarded. The syrup obtained on evaporation of the bulk of the solution was analysed (Found: C, 63·5; H, 6·9; N, 14·0; OMe, 21·6; NMe, 6·8. $C_{23}H_{30}O_4N_4$ requires C, 63·7; H, 7·3; N, 13·5; OMe, 22·5; NMe, 7·0%).

Preparation of the Dianhydro-hexosazone.—(a) Tetra-acetyl glucosazone (5 g.), dissolved in acetone (250 c.c.), was mixed with sodium hydroxide solution (320 c.c., 1·5%) at room temperature. After a few hours, pale yellow plates appeared; these (1·2 g.) were filtered off after 24 hours, and recrystallised from alcohol; m. p. 238°, $[\alpha]_D^{20°}$ — 88° in acetone (*c*, 0·3) with no mutarotation [Found: C, 67·0; H, 5·6; N, 17·4; *M* (Rast), 319. $C_{18}H_{18}O_2N_4$ requires C, 67·1; H, 5·6; N, 17·4%; *M*, 322].

(b) Tetra-acetyl galactosazone (30 g.) was deacetylated as described above to yield a product (6 g.) similar to that obtained from tetra-acetyl glucosazone, which after recrystallisation had m. p. 238°, not depressed on admixture with the *anhydro-compound* described above, and $[\alpha]_D^{20°}$ — 88° in acetone (*c*, 0·3) (Found: C, 67·1; H, 5·7; N, 17·4%). This result was twice confirmed.

Acetylation of the Dianhydro-hexosazone.—A solution of the above product (1 g.) in acetic anhydride (2 c.c.) and pyridine (5 c.c.) was kept overnight and then poured into water. The solid obtained, recrystallised from aqueous alcohol, formed shining crystals (0·7 g.), m. p. 135°, $[\alpha]_D$ + 108° in chloroform (*c*, 0·5) [Found: C, 65·8; H, 5·5; CH_3·CO, 12·0; N, 15·5; *M* (Rast), 339. $C_{20}H_{20}O_3N_4$ requires C, 65·9; H, 5·5; CH_3·CO, 11·8; N, 15·4%; *M*, 364]. Deacetylation of this *mono-acetyl* derivative yielded yellow needles of the original dianhydride, m. p. 235°, $[\alpha]_D^{20°}$ — 89° in acetone (*c*, 0·4). The same monoacetyl derivative was obtained from the di-

anhydride obtained from tetra-acetyl galactosazone and no differences could be detected by mixed m. p. determinations or in specific rotation.

Methylation of the Dianhydro-hexosazone.—The dianhydride (2 g.), dissolved in acetone (75 c.c.), was methylated at 60° with methyl sulphate (20 c.c.) and sodium hydroxide solution (50 c.c., 30%) in the usual way. The *dianhydro-hexosazone monomethyl ether* which separated was easily recrystallised (2·1 g.) and had m. p. 172°, $[\alpha]_D^{20°}$ — 170° in chloroform (*c*, 0·4) (Found: C, 68·0; H, 6·3; N, 16·3; OMe, 8·9; NMe, nil. $C_{19}H_{20}O_2N_4$ requires C, 67·8; H, 6·0; N, 16·7; OMe, 9·2%).

Isolation of a Dianhydro-hexosazone Dibromide.—The dianhydroglucosazone (0·2 g.) in chloroform (6 c.c.) was treated with a solution of bromine (0·6 g.) in chloroform (5 c.c.). An immediate precipitate (0·28 g.) was obtained, which was filtered off, washed with alcohol, and recrystallised from a large quantity of alcohol on account of its low solubility; m. p. 240° (decomp.) (Found: N, 11·2; Br, 34·2. $C_{18}H_{18}O_2N_4Br_2$ requires N, 11·6; Br, 33·2%).

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