# 973. *The Structure of Fischer's " Anhydro-lactosazone " and Other Anhydro-osazones.*

By S. BAYNE.

Fischer's " anhydro-lactosazone," which is readily formed from lactose phenylosazone in hot alcohol, is identified as 3 : 6-anhydro-4-β-D-galactosido-D-allose phenylosazone by formation and hydrolysis of its phenylosotriazole.* " Anhydro-cellobiosazone " is similarly identified.

L-Gulose phenylosazone, when heated with acid methanol, undergoes anhydride formation with Walden inversion on $C_{(3)}$ to form 3 : 6-anhydro-L-galactose phenylosazone. The phenylosotriazoles from this compound and from the D-isomer are described.

MONTGOMERY and HUDSON (*J. Amer. Chem. Soc.*, 1930, **52**, 2105) noted that lactosazone did not exhibit a constant final rotation on repeated recrystallisation. In the purification of lactosazone for the preparation of lactosone (Bayne, *Biochem. J.*, 1952, **50**, xxvii) it was observed that on recrystallisation from hot ethanol and treatment with decolorising charcoal the osazone was partly converted into Fischer's " anhydro-lactosazone " (*Ber.*, 1887, **20**, 829). A sample which had been recrystallised four times contained about 25% of lactosazone and 75% of anhydro-osazone and was moderately lævorotatory. The components were separated by extraction with hot water in which the anhydro-osazone is practically insoluble, and the lactose phenylosazone prepared in this way had a fairly sharp m. p. (cf. Table) and a reproducible optical rotation. The initial dextrorotation of lactose phenylosazone in pyridine–ethanol has not previously been observed. The initial lævorotation in the same solvent observed by Klages and Maurenbrecher (*Annalen*, 1938, **535**, 175) may be attributed to the presence of a small amount of anhydro-osazone; they remarked, however, that polarimetric readings were made difficult by darkening. This was presumably due to impurity and has not been encountered in the present investigation even after a week. The changes of rotation in methanol are in good agreement with the measurements recorded by Votocek and Valentin (*Coll. Trav. chim. Tchécosl.*, 1932, **3**, 435) (see Table).

The " anhydro-lactosazone " and benzaldehyde under reflux gave an osone which was reconverted into the anhydro-osazone by phenylhydrazine. The osone was surprisingly

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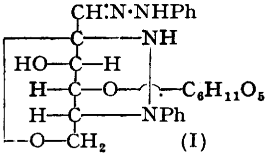
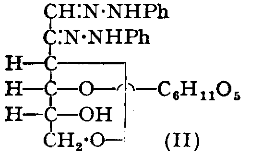
* The name " phenylosotriazole " is used (cf. Haskins, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1946, **68**, 1766) to describe the derivatives of 2-phenyl-2 : 1 : 3-triazole which are obtained from the action of copper sulphate on sugar phenylosazones, $C_{(1)}$ and $C_{(2)}$ of the parent sugar forming part of the triazole ring, *e.g.*, glucose phenylosazone ⟶ glucose phenylosotriazole (2-phenyl-4-D-*arabo*tetrahydroxybutyl-2 : 1 : 3-triazole).

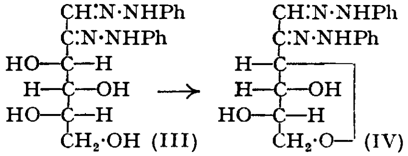
*Physical constants of lactose phenylosazone.*

| Reference | M. p. | $[\alpha]_D$ in methanol | $[\alpha]_D$ in pyridine–ethanol (2 : 3) |
|---|---|---|---|
| Fischer (*Ber.*, 1908, **41**, 76) | 203—215° | — | — |
| Neuberg (*loc. cit.*) | — | — | 0° (initial ?) |
| Votocek and Valentin (*loc. cit.*) | — | $-25{\cdot}4° \longrightarrow -7{\cdot}9°$ | — |
| Klages and Maurenbrecher (*loc. cit.*) | — | $-31°$ (final) | $-17° \longrightarrow -9° \longrightarrow -19°$ |
| Present author | 206—207° | $-13{\cdot}5° \longrightarrow -10°$ | $+10° \longrightarrow -21°$ |

resistant to hot dilute acid; lactosone is hydrolysed to glucosone and galactose under similar conditions (Fischer and Armstrong, *Ber.*, 1902, **35**, 3141; Hynd, *Proc. Roy. Soc.*, 1927, *B*, **101**, 244). The "anhydro"-compound was also investigated by Diels and Meyer (*Annalen*, 1935, **519**, 157). Percival and Percival (*J.*, 1937, 1320), from acetylation studies, favoured structure (I) for Fischer's "anhydro-lactosazone." As this was not in accord with osone formation an aqueous suspension of the anhydro-osazone was heated under reflux with copper sulphate (Hann and Hudson, *J. Amer. Chem. Soc.*, 1944, **66**, 735); the phenylosotriazole of an anhydro-disaccharide was isolated. This compound was readily hydrolysed to galactose and the phenylosotriazole of an anhydrohexose, identical with the phenylosotriazole obtained from Diels and Meyer's "anhydroglucosazone." The latter osazone and its osotriazole have been recently identified by Hardegger and Schrier (*Helv. Chim. Acta*, 1952, **35**, 232) as derivatives of 3 : 6-anhydro-D-allose; thus Fischer's "anhydro-lactosazone" is 3 : 6-anhydro-4-β-D-galactosido-D-allose phenylosazone (II). The "anhydro-cellobiosazone" of Diels, Meyer, and Onnen (*Annalen*, 1936, **525**, 106) (cf. Muir and Percival, *J.*, 1940, 1479) is similarly 3 : 6-anhydro-4-β-D-glucosido-D-allose phenylosazone.


(I)


(II)


(III) (IV)

The statement by Hardegger and Schrier (*loc. cit.*) that $C_{(4)}$ is not involved in the Walden inversion occurring on $C_{(3)}$ when glucose phenylosazone is converted into 3 : 6-anhydroallose phenylosazone is confirmed by this occurrence of a similar inversion with lactose and cellobiose phenylosazones under the same conditions (cf. also Levene, Raymond, and Walti, *J. Biol. Chem.*, 1929, **82**, 191). The prediction by El Khadem, Schrier, Stohr, and Hardegger (*Helv. Chim. Acta*, 1952, **35**, 993) that 3 : 6-anhydrogalactose phenylosazone should be formed when either galactose phenylosazone or gulose phenylosazone is heated in acid alcohol has now been confirmed. The anhydro-osazone from D-galactose phenylosazone forms a phenylosotriazole which differs from those from 3 : 6-anhydro-D-allose and 3 : 6-anhydro-D-glucose; the L-isomer of the same phenylosotriazole is obtained from the anhydro-osazone (IV) prepared from L-gulose phenylosazone (III). The assignment of 3 : 6-anhydro-D- and -L-galactose structures to these derivatives depends on the demonstration by Percival (*J.*, 1945, 783) that the phenylosazone from 3 : 6-anhydrogalactose is identical with Diels and Meyer's (*loc. cit.*) anhydrogalactosazone, now reinforced by the preparation of 3 : 6-anhydro-D-galactose phenylosotriazole from both osazones.

## EXPERIMENTAL

M. p.s are uncorrected. Analyses by Drs. Weiler and Strauss, Oxford.

*Lactose Phenylosazone and* 3 : 6-*Anhydro*-4-β-D-*galactosido*-D-*allose Phenylosazone*.—A freshly prepared sample of lactosazone (10 g.) was recrystallised four times from hot 95% ethanol with addition of decolorising charcoal. The product (7 g.), m. p. 211—213°, $[\alpha]_D^{16}$ $-93° \longrightarrow -80°$ (after 24 hr.) [*c*, 2·06 in pyridine–ethanol (2 : 3)], was fractionated by rapid extraction with hot water into lactose phenylosazone (1·4 g.), m. p. 206—207°, $[\alpha]_D^{15}$ $+10° \longrightarrow -21°$ (after 168 hr.) [*c*, 0·5 in pyridine–ethanol (2 : 3)], $[\alpha]_D^{16}$ −13·5° $\longrightarrow$ −10° (after 72 hr.) (*c*, 0·42 in MeOH), and 3 : 6-anhydro-4-β-D-galactosido-D-allose phenylosazone (4·3 g.) (Fischer's “ anhydro-lactosazone ”), m. p. 226—227°, $[\alpha]_D^{16}$ −172·0° [*c*, 0·69 in pyridine–ethanol (2 : 3)].

3 : 6-Anhydro-4-β-D-galactosido-D-allose phenylosazone (4·0 g.) with benzaldehyde under reflux (Fischer and Armstrong, *loc. cit.*) gave an *osone* (0·5 g.), $[\alpha]_D^{15}$ −3·25° (initial) (*c*, 4·0 in $H_2O$), identified by the characteristic reducing action (cf. Bayne, Collie, and Fewster, *J.*, 1952, 2766) and by reconversion into the original anhydro-osazone on treatment with phenylhydrazine in the cold. The osone was heated at 100° for 2 hours with 0·2N-sulphuric acid; neutralisation with sodium acetate and treatment with phenylhydrazine gave only 3 : 6-anhydro-4-β-D-galactosido-D-allose phenylosazone in the cold and no further osazone was formed on heating.

3 : 6-*Anhydro*-4-β-D-*galactosido*-D-*allose Phenylosotriazole*.—3 : 6-Anhydro-4-β-D-galactosido-D-allose phenylosazone (10·0 g.) was refluxed for 2 hours with copper sulphate (5·5 g.) in water (1 l.). Further procedure was essentially that employed by Haskins, Hann, and Hudson (*J. Amer. Chem. Soc.*, 1945, **67**, 941) in the preparation of lactose phenylosotriazole. Recrystallised from ethanol in which it is less soluble than the lactose derivative, 3 : 6-*anhydro*-4-β-D-*galactosido*-D-*allose phenylosotriazole* (5·3 g.) had m. p. 160°, $[\alpha]_D^{20}$ −67·8° (*c*, 0·80 in $H_2O$) (Found : C, 51·5; N, 5·9; N, 10·4. $C_{18}H_{23}O_8N_3$ requires C, 51·6; H, 5·7; N, 10·3%).

*Hydrolysis of* 3 : 6-*Anhydro*-4-β-D-*galactosido*-D-*allose Phenylosotriazole*.—The phenylosotriazole (2·0 g.) was heated under reflux for 3 hours with 0·5N-hydrochloric acid (100 ml.), cooled, neutralised, and evaporated under reduced pressure to 20 ml. On cooling overnight there separated 3 : 6-anhydro-D-allose phenylosotriazole (0·8 g.), m. p. 91—92°, $[\alpha]_D^{18}$ −72·3° (*c*, 0·45 in $H_2O$), −54° (*c*, 0·336 in pyridine) (Found : C, 58·6; H, 5·3; N, 17·2. Calc. for $C_{12}H_{13}O_3N_3$ : C, 58·3; H, 5·3; N, 17·0%). Mixed m. p. with 3 : 6-anhydro-D-glucose phenylosotriazole and 3 : 6-anhydro-D-allose phenylosotriazole prepared from 3 : 6-anhydro-D-allose phenylosazone (Diels and Meyer's “ anhydro-glucosazone ”) were 78—81° and 90—92° respectively. Galactose was identified by evaporation of the mother-liquor to dryness and crystallisation from acetic acid.

3 : 6-*Anhydro*-4-β-D-*glucosido*-D-*allose Phenylosotriazole*.—Cellobiose phenylosazone (5·0 g.), m. p. 195—197°, by Diels and Meyer's method (*loc. cit.*) gave 3 : 6-anhydro-4-β-D-glucosido-D-allose phenylosazone (“ anhydrocellobiosazone ”) (2·25 g.), m. p. 221—222° (from ethanol), $[\alpha]_D^{20}$ −145° (*c*, 0·25 in MeOH) (cf. Muir and Percival, *loc. cit.*). 3 : 6-Anhydro-4-β-D-glucosido-D-allose phenylosazone (1·5 g.) was heated for 2 hours with copper sulphate (0·85 g.) in boiling water (150 ml.). 3 : 6-*Anhydro*-4-β-D-*glucosido*-D-*allose phenylosotriazole* (0·5 g.), isolated as was the derivative from lactose and crystallised from ethanol, had m. p. 174—176°, $[\alpha]_D^{20}$ −77·7° (*c*, 0·90 in $H_2O$) (Found C, 52·0; H, 5·8; N, 10·5%).

*Hydrolysis of* 3 : 6-*Anhydro*-4-β-D-*glucosido*-D-*allose Phenylosotriazole*.—This phenylosotriazole (0·25 g.), hydrolysed as above, gave 3 : 6-anhydro-D-allose phenylosotriazole (0·12 g.), m. p. and mixed m. p. 89—91°, and glucose was recovered from the mother-liquor.

3 : 6-*Anhydro*-D-*glucose Phenylosotriazole*.—3 : 6-Anhydro-D-glucose phenylosazone (0·75 g.), m. p. 184—186° (recrystallised twice from acetonitrile), was treated for 1½ hours with copper sulphate (0·6 g.) in boiling water (50 ml.), filtered, and cooled. The precipitate, recrystallised from water, gave 3 : 6-anhydro-D-glucose phenylosotriazole (0·28 g.), m. p. 95—97°, $[\alpha]_D^{16}$ −105·9° (*c*, 0·831 in pyridine) (Found : C, 54·0; H, 5·7; N, 15·8. Calc. for $C_{12}H_{13}O_3N_3,H_2O$ : C, 54·3; H, 5·7; N, 15·8%). Hardegger and Schrier describe an anhydrous form (*loc. cit.*) and a monohydrate (*Helv. Chim. Acta*, 1952, **35**, 623), and El Khadem *et al.* (*loc. cit.*) describe a hemihydrate.

3 : 6-*Anhydro*-D-*galactose Phenylosazone*.—(*a*) D-Galactose phenylosazone (10 g.) by Diels and Meyer's method (*loc. cit.*) gave 3 : 6-anhydro-D-galactose phenylosazone (3·3 g.), m. p. 214—216°, $[\alpha]_D^{22}$ +56° (*c*, 0·50 in MeOH). (*b*) 3 : 6-Anhydro-D-galactose (4·6 g.) (Haworth, Jackson, and Smith, *J.*, 1940, 620) gave the same osazone (2 g.), m. p. and mixed m. p. 214—216°.

3 : 6-*Anhydro*-D-*galactose Phenylosotriazole*.—3 : 6-Anhydro-D-galactose phenylosazone (4·0 g.) was heated for 2 hours with copper sulphate (1·54 g.) in water (200 ml.). The solution was evaporated to 50 ml., filtered, and extracted with ether. The extract was washed with a little 20% sulphuric acid, then saturated sodium carbonate solution, and water, dried ($Na_2SO_4$), and evaporated. The residue, recrystallised twice from water, gave 3 : 6-*anhydro*-D-*galactose phenylosotriazole* (1·8 g.), m. p. 101—102°, $[\alpha]_D^{17}$ −6·0° (*c*, 0·806 in pyridine) (Found : C, 58·5; H, 5·2; N, 17·2%). 1 Mole reacted with 1·075 moles of sodium metaperiodate at room temperature in 24 hours, no formaldehyde being formed.

3 : 6-*Anhydro*-L-*galactose Phenylosazone*.—L-Gulose phenylosazone (10 g.) with sulphuric acid (1·5 ml.) in boiling methanol (500 ml.) (5 hours) gave 3 : 6-*anhydro*-L-*galactose phenylosazone* (5·5 g.), m. p. 215—217° (from methanol and then acetonitrile), $[\alpha]_D^{22}$ −54° (*c*, 0·520 in MeOH)

(Found: C, 63·2; H, 5·75; N, 17·5. $C_{18}H_{20}O_3N_4$ requires C, 63·5; H, 5·9; N, 16·5%). Mixed with 3 : 6-anhydro-D-galactose phenylosazone this melted at 200—202°.

3 : 6-*Anhydro*-L-*galactose Phenylosotriazole.*—3 : 6-Anhydro-L-galactose phenylosazone (5·0 g.) gave 3 : 6-*anhydro*-L-*galactose phenylosotriazole* (2·0 g.), m. p. 101—102°, $[\alpha]_D^{20}$ +5·0° (*c*, 2·00 in pyridine) (Found: C, 58·4; H, 5·3; N, 17·1%).

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