## 522. iso*Propylidene Derivatives of Glucosone.*

By S. Bayne, G. A. Collie, and J. A. Fewster.

When glucosone condenses with acetone in the presence of sulphuric acid crystalline 1 : 2-2 : 3-5 : 6-tri*iso*propylidene glucosone hydrate can be isolated. On partial hydrolysis 1 : 2-2 : 3-di*iso*propylidene glucosone hydrate is obtained; this is characterised as its crystalline diacetate, and its constitution proved by (*a*) periodate oxidation and (*b*) methylation and identification of 5 : 6-dimethyl glucosone by formation of the *p*-bromophenylosazone of 5 : 6-dimethyl glucose and by oxidation to αβ-dimethoxypropaldehyde.

Glucosone was prepared by Fischer (*Ber.*, 1889, **22**, 87) by the action of concentrated hydrochloric acid on glucose phenylosazone. Hynd (*Proc. Roy. Soc.*, 1927, *B*, **101**, 244) suggested that the benzaldehyde method, applied by Fischer and Armstrong (*Ber.*, 1902, **35**, 3141) to lactosazone and maltosazone, might have certain advantages in the preparation of glucosone and both methods were widely employed in the synthesis of ascorbic acid and its analogues. Although the benzaldehyde method was largely used in the preparation of the pentosones it was considered (Haworth, Hirst, Jones, and Smith, *J.*, 1934, 1192) that the hydrochloric acid method was to be preferred for the less soluble hexosazones. Smith (*Adv. Carbohydrate Chem.*, 1946, **2**, 82) has remarked that the purity of the osone is largely dependent on the initial isolation of a pure osazone. The quoted m. p.s of glucosazone range from 205° to 212°, and the m. p. affords little indication of purity, which is best determined by the observation of a constant final rotation on repeated recrystallisation. This criterion is not applicable to those osazones which readily undergo anhydride formation, *e.g.*, lactosazone (Montgomery and Hudson, *J. Amer. Chem. Soc.*, 1930, **52**, 2105) and sedoheptulosazone. For the present work glucosone has been prepared in good yield by an adaptation of the benzaldehyde method; exhaustive attempts to purify the syrup do not alter its chemical properties or cause variation in its toxicity to animals (Bayne, *Biochem. J.*, 1952, **50**, xxvii) or in its inhibition of yeast fermentation (Mitchell and Bayne, *ibid.*, 1952, **50**, xxvii).

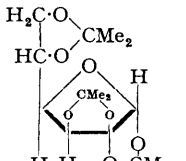
Preparation by direct oxidation of glucose or preferably fructose by using hydrogen peroxide in the presence of a ferrous salt (Morrell and Crofts, *J.*, 1899, 786), selenium dioxide (Dixon and Harrison, *Biochem. J.*, 1932, **26**, 1954), or copper acetate (Evans,

Nicoll, Strause, and Waring, *J. Amer. Chem. Soc.*, 1928, **50**, 2267) has the disadvantage that the oxidation process is not specific and the varied by-products are not easily separated from the osone.

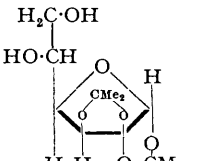
None of the osones has been crystallised, and there is no simple method of assessing their purity. Glucosone reduces the copper reagents, including Fehling's solution, in the cold and has been shown (Hynd, personal communication) to react with alkali cyanide, forming a product which gives an intense blue colour with Benedict's arsenophosphotungstic acid reagent for uric acid (Ariyama, *J. Biol. Chem.*, 1927, **74**, xiv). The rapid formation of a quinoxaline with *o*-phenylenediamine (Fischer, *loc. cit.*; Ohle, *Ber.*, 1934, **67**, 155), and the reaction at ordinary temperature with phenylhydrazine or substituted phenylhydrazines (Morrell and Crofts, *loc. cit.*) have been used in the detection of glucosone. Attempts to use these reactions quantitatively (Dixon and Harrison, *loc. cit.*; Berkeley, *Biochem. J.*, 1933, **27**, 1357; Bond, Knight, and Walker, *ibid.*, 1937, **31**, 1033) have been hampered by the lack of a glucosone standard or a crystalline derivative from which glucosone might be readily prepared in pure form.

By treatment of a purified glucosone syrup with acetone there was obtained in fair yield a crystalline derivative which was fully substituted and insoluble in water. The compound was identified analytically as a tri*iso*propylidene glucosone hydrate, at least one of the *iso*propylidene groups of which was labile to dilute acid, the crystalline compound not being recoverable after brief treatment with 0·1N-sulphuric acid. It has been pointed out by Bell (*J.*, 1947, 1461) that this behaviour is characteristic of those *iso*propylidene sugars which possess a furanose ring, a possible exception being 1 : 2-4 : 5-di*iso*propylidene fructose which, although partially hydrolysed by cold dilute acid (Irvine and Garrett, *J.*, 1910, 1283), is not generally considered to have a furanose structure. The labile *iso*propylidene group is generally attached to the primary alcoholic group of the sugar (Bell, *loc. cit.*).

From the graded hydrolysis of the tri*iso*propylidene glucosone hydrate a di*iso*propylidene derivative was separated and characterised as its crystalline acetate. Two acetyl groups were shown to be present by direct titration, this result demonstrating that the *iso*propylidene group removed was not attached to the hydrated keto-group of the sugar. Stacey and Turton (*J.*, 1946, 661) have shown that the free hydroxyl group on the second carbon atom of tetra-acetyl glucosone hydrate (III) has an incipiently ionic hydrogen atom; thus, if the labile *iso*propylidene group in tri*iso*propylidene glucosone hydrate were attached in such a position the direct titration of di*iso*propylidene glucosone hydrate diacetate should have required 3 equivalents of acid. The assignment of the structure 1 : 2-2 : 3-di*iso*propylidene D-glucosone hydrate (II) to the di*iso*propylidene compound and 1 : 2-2 : 3-5 : 6-tri*iso*propylidene D-glucosone hydrate (I) to the parent compound depends on the demonstration that the former, prepared by deacetylation of its diacetate, may be oxidised by one mol. of periodate with the production of one mol. of formaldehyde. Confirmation has been obtained by removal of the *iso*propylidene groups from dimethyldi*iso*propylidene D-glucosone hydrate (IV), prepared by methylation of di*iso*propylidene glucosone hydrate; the resulting partially methylated osone forms 5 : 6-dimethyl glucose *p*-bromophenylosazone, and may be oxidised by periodate to αβ-dimethoxypropaldehyde (dimethyl glyceraldehyde), characterised as the *p*-bromophenacyl derivative of αβ-dimethoxypropionic acid (dimethyl glyceric acid).



(I)



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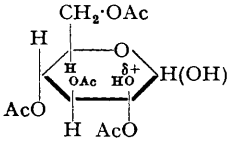

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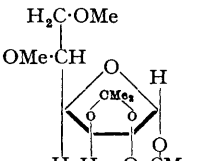


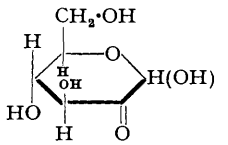
(IV)

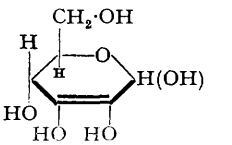
Tetra-acetyl glucosone hydrate was first prepared from acetobromoglucose and defined structurally as (III) by Maurer (*Ber.*, 1929, **62**, 332; *ibid.*, 1930, **63**, 25), although in a later

publication Maurer and Bohrne (*Ber.*, 1936, **69**, 1399) suggested that the corresponding benzoyl derivative was 2 : 3 : 4 : 6-tetrabenzoyl glucosone, having a 1 : 2-ethylene oxide ring. The possible reasons for the hydration of the keto-group in tetra-acetyl glucosone hydrate have been specified by Stacey and Turton (*loc. cit.*). The ring structure in 2 : 3 : 4 : 6-tetra-acetyl D-glucosone hydrate (III) is established as pyranose as no ring displacement is likely in the transformation of acetobromoglucose to the glucosone derivative. In 1 : 2-2 : 3-5 : 6-tri*iso*propylidene D-glucosone hydrate (I), on the other hand, a furanose ring is considered to be present, and while this in no way implies a similar structure for free glucosone it is apparent that, in acid conditions, conversion into the 1 : 4-furanose modification with a free or hydrated keto-group is facilitated.

Fleury and Fievet-Guinard (*Ann. Pharm. franc.*, 1947, **5**, 404) showed that glucosone was oxidised regularly by periodic acid to formaldehyde, formic acid, and glyoxylic acid which was further degraded to formic acid and carbon dioxide. It had been noted (Hynd, *loc. cit.*) that glucosone might contain one or possibly two oxidic rings, the first evidence in this direction being supplied by Becker and May (*J. Amer. Chem. Soc.*, 1949, **71**, 1941) from the selective oxidative action of lead tetra-acetate on glucosone. These workers observed a fairly rapid initial utilisation of two moles of oxidant per mole of glucosone, followed by a slow progressive oxidation over several days, no significant amount of formaldehyde being formed. They suggest various possible ring structures for glucosone but are unable to differentiate between them on the available evidence. The absence of formaldehyde as an oxidation product would seem to exclude the possibility that the 1 : 4-furanose modification present in the *iso*propylidene derivative is a quantitatively important component of the free sugar. The same authors have also noted that rotational changes take place in an aqueous solution of glucosone. It is surprising that there is no previous record of this observation, which we have now confirmed.

Various other structural possibilities for glucosone have been advanced but it appears that definitive proof is lacking in all cases. From the reaction with Schiff's reagent and sodium sulphite Dixon and Harrison (*loc. cit.*) postulated a free aldehyde group. Their method of preparation, involving oxidation of fructose by selenium dioxide, is not sufficiently specific to exclude the possibility that impurities may account for these results; glucosone prepared by other methods does not show the same properties. Furthermore in the present work it has not been possible to prepare a semicarbazone or oxime from glucosone, and the sugar does not react with dimedone in aqueous solution. Bednarczyk and Marchlewski (*Bull. intern. Acad. polon. Sci.*, 1938, *A*, 524) from a study of the ultra-violet absorption considered that glucosone in aqueous solution might contain a free carbonyl group, although they admit the possible impurity of their material which was prepared by Fischer's (*loc. cit.*) original method. The same authors record similar observations for fructose (*Biochem. Z.*, 1938, **300**, 42) and sorbose. From other evidence it is known that the free keto-form of these sugars is in fact present in aqueous solution to a limited extent. Evans, Nicoll, Strause, and Waring (*loc. cit.*) proposed a $\Delta^2$-diol structure for glucosone, regarding

(V) 

(VI)

Fischer's lead–glucosone complex as the salt of this structure. Brüll (*Ricerca scient.*, 1937, **8**, I, 527) drew an analogy between the reductones and the osones and assigned to glucosone the hemiacetal structure (V) in equilibrium with the tautomeric (VI).

## Experimental

M.p.s are uncorrected. Microanalyses for carbon and hydrogen are by Drs. Weiler and Strauss, Oxford.

D-*Glucose Phenylosazone*.—(*a*) The preparation was based on Weygand's (*Ber.*, 1940, **73**, 1259) utilisation of *p*-toluidine as a catalyst in osazone formation. D-Glucose hydrate (100 g.)

gave D-glucose phenylosazone (130 g., 72·5%) as pale yellow crystals, m. p. 208° (decomp.), $[\alpha]_D^{18}$ −58·5° ⟶ −36° (24 hours) [*c*, 1·00 in pyridine–ethanol (2 : 3)].

(*b*) *N*-*p*-Tolyl-D-*iso*glucosamine was prepared according to Weygand's method (*loc. cit.*). Reaction with phenylhydrazine gave glucose phenylosazone, m. p. 208°, in almost quantitative yield.

D-*Glucosone.*—The method was essentially that of Reichstein, Grüssner, and Oppenauer (*Helv. Chim. Acta*, 1933, **16**, 561, 1069). D-Glucose phenylosazone (10 g.) gave a pale yellow syrup (2·8 g.) which was further purified by extraction with hot 96% ethanol. D-Glucosone (2·2 g., 44·5%) was obtained as a very pale yellow, thick syrup, $[\alpha]_D^{15}$ −10·6° ⟶ +7·9° (275 hours) (*c*, 8·52 in water). It did not restore the colour to Schiff's reagent, it reduced Fehling's solution in the cold, and it gave a blue colour with Benedict's arsenophosphotungstic acid reagent for uric acid (Hynd, personal communication) and with Adler's benzidine reagent.

*Treatment of* D-*Glucosone with Acetone.*—Glucosone (4·5 g.) was shaken for 8 hours with acetone (150 c.c.) containing concentrated sulphuric acid (5 c.c.) and the mixture cooled and neutralised with anhydrous sodium carbonate. The filtrate was evaporated to dryness and the residual semi-crystalline syrup was crystallised from methanol; further crops were obtained by concentration of the mother liquor and chromatographic analysis of the concentrate on an alumina column; the total yield was 1·07 g. (13·5%). Recrystallisation from methanol gave *tri*iso*propylidene* D-*glucosone hydrate*, m. p. 125°, $[\alpha]_D^{15}$ −6·6° (*c*, 2·12 in methanol) (Found: C, 57·0; H, 7·5; $CMe_2$, 39·5. $C_{15}H_{24}O_7$ requires C, 56·9; H, 7·6%; $3CMe_2$, 39·9%).

L-*Glucosone.*—The mixture of L-glucose and L-mannose obtained by acid decomposition of the product of the condensation of L-arabinose with nitromethane (Sowden and Fisher, *J. Amer. Chem. Soc.*, 1947, **69**, 1963) was treated with phenylhydrazine acetate, giving L-glucosazone, m. p. 205—206°, $[\alpha]_D^{16}$ +57° ⟶ +36° (24 hours) [*c*, 1·00 in pyridine–ethanol (2 : 3)]. L-Glucosazone (5 g.) was refluxed with benzaldehyde in aqueous ethanol and the resulting solution of osone purified as described for D-glucosone. L-Glucosone, a colourless syrup (1·0 g., 40·0%), showed the same chemical properties as D-glucosone.

*Tri*iso*propylidene* L-*Glucosone Hydrate.*—When L-glucosone (1·0 g.) was treated with acetone as described above, crystalline *tri*iso*propylidene* L-*glucosone hydrate* (0·2 g., 11·5%) was obtained, m. p. 125°, $[\alpha]_D^{16}$ +6·8° (*c*, 2·00 in methanol) (Found: C, 57·1; H, 7·6%).

*Attempted Acetylation of Tri*iso*propylidene* D-*Glucosone Hydrate.*—(*a*) The compound was recovered unchanged when treated with pyridine (3 c.c.) and acetic anhydride (3 c.c.).

(*b*) The derivative (0·5 g.) was heated with acetic anhydride (5 c.c.) and anhydrous sodium acetate (0·1 g.) for 5 hours at 75°, but was recovered unchanged.

*Attempted Methylation of Tri*iso*propylidene* D-*Glucosone Hydrate.*—(*a*) The compound (0·75 g.) was subjected to four methylations with silver oxide (1·0 g.) and methyl iodide (3 c.c.) (Purdie and Irvine, *J.*, 1903, 1021). The residual semi-crystalline syrup was unchanged starting material.

(*b*) The derivative (0·5 g.) dissolved in dry ether (10 c.c.) was refluxed with sodium wire (0·5 g.) for 6 hours; no reaction was apparent. The solution was decanted from the sodium and treated with methyl sulphate (0·3 c.c.) (Vischer and Reichstein, *Helv. Chim. Acta*, 1944, **27**, 1332), but only unchanged material (0·4 g.) was recovered.

*Hydrolysis of Tri*iso*propylidene* D-*Glucosone Hydrate.*—(*a*) The compound (0·2 g.) was refluxed with acetic acid (5 c.c.), water (5 c.c.), and 0·1 N-hydrochloric acid (0·1 c.c.) for 2 hours. D-Glucosone was identified by the rapid formation in the cold of D-glucose phenylosazone (0·1 g.), m. p. 205° after recrystallisation from ethanol and unchanged by admixture with authentic D-glucose phenylosazone. The identity of the osazone was confirmed by its conversion into 2-phenyl-4-D-*arabo*tetrahydroxybutyl-2 : 1 : 3-triazole, m. p. 194° (cf. " D-glucose phenylosotriazole "; Hahn and Hudson, *J. Amer. Chem. Soc.*, 1944, **66**, 735).

(*b*) The derivative (0·48 g.) was dissolved in 85% acetic acid (10 c.c.), the whole kept at 50°, and the hydrolysis followed polarimetrically (Fig., p. 2770). After 3 hours a slight coloration with the arsenophosphotungstic acid reagent and a slight reduction of Fehling's solution were observed. After 20 hours a strong coloration was obtained with the arsenophosphotungstic acid reagent, and the solution strongly reduced Fehling's solution.

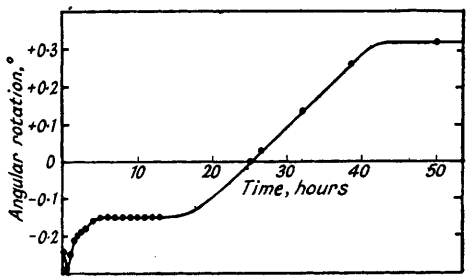
*Partial Hydrolysis of Tri*iso*propylidene* D-*Glucosone Hydrate.*—The compound (1·32 g.) was dissolved in 85% acetic acid (26 c.c.), and the solution kept at 50° for 10 hours. The solution was evaporated at 40° to a pale yellow syrup (1·1 g.), from which a small crystalline fraction (0·1 g.), identified as unchanged compound, separated. The syrup, which gave only a very faint colour with the arsenophosphotungstic acid reagent and did not reduce Benedict's solution, was purified by extraction with acetone.

*Diacetyl Di*iso*propylidene* D-*Glucosone Hydrate.*—The syrup (0·39 g.) obtained by partial hydrolysis of the tri*iso*propylidene derivative was dissolved in acetic anhydride (4·0 c.c.) and warmed at 75° for 5 hours with anhydrous sodium acetate (0·1 g.). The syrup (0·47 g.), from a chloroform extract of the product, crystallised from ether–light petroleum as colourless aggregated prisms (0·38 g., 75·0%), m. p. 69°. Recrystallisation from aqueous methanol gave *diacetyl di*iso*propylidene* D-*glucosone hydrate*, m. p. 70°, $[\alpha]_D^{18}$ +15·9° (*c*, 1·44 in methanol) [Found: C, 53·2; H, 6·65; $CMe_2$, 23·4; OAc, 24·8 (by direct titration). $C_{16}H_{24}O_9$ requires C, 53·3; H, 6·7; $2CMe_2$, 23·2; 2OAc, 23·9%].

*Oxidation of Di*iso*propylidene* D-*Glucosone Hydrate by Periodate at Room Temperature.*—(a) *Reduction of periodate.* Diacetyl di*iso*propylidene D-glucosone hydrate (0·134 g.) was dissolved in 0·1N-sodium hydroxide (12 c.c.) at 100°. The solution was cooled and neutralised (phenolphthalein) by addition of 0·1N-hydrochloric acid. 0·265M-Sodium periodate (2 c.c.) was added and the volume adjusted to 20 c.c. The periodate was determined on samples by the usual iodine–arsenite method; 0·92 mole of periodate was reduced per mole of the sugar in 2 hours, and 0·98 mole in 6 hours.

(b) *Formic acid production.* Titration of a 6-hour sample (10 c.c.) showed that no acid had been formed.

(c) *Formaldehyde production.* The technique employed was that described by Bell (*J.*, 1948, 992) and Bell and Greville (*J.*, 1950, 1902). When the solution of di*iso*propylidene D-glucosone hydrate obtained as in (*a*) was oxidised under these conditions the product formed (32·2 mg. in 2 hours, 34·8 mg. in 24 hours; from 23·2 mg. of the diacetyl compound) on addition




1 : 2-2 : 3-5 : 6-*Tri*iso*propylidene* D-*glucosone hydrate* (c, 4·80 *in* 85% *acetic acid*).

of dimedone had m. p. 140—150°. After a single recrystallisation from ethanol (1 c.c.) the formaldehyde derivative, m. p. 184—185° alone and mixed with an authentic sample, was obtained. 0·79 Mole of formaldehyde was formed per mole of the sugar in 2 hours, and 0·80 mole in 24 hours.

By addition of water to the ethanolic mother liquor a crystalline product, m. p. 158—159° after two recrystallisations from 50% ethanol, was isolated. The expected carbohydrate product is 1 : 2-2 : 3-di*iso*propylidene 5-*aldo*-D-xylosone hydrate; it would appear that its dimedone derivative is insoluble in water. Bell (*loc. cit.*) did not observe the precipitation of carbohydrate-dimedone derivatives following periodate oxidation of partially methylated sugars, and oxidation of 1 : 2-*iso*propylidene D-glucose, under the same conditions, yielded only the dimedone derivative of formaldehyde, the oxidation product, 1 : 2-*iso*propylidene 5-*aldo*-D-xylose (Iwadare, *Bull. Chem. Soc. Japan*, 1941, **16**, 40), remaining in solution, presumably as its dimedone derivative.

*Methylation of Di*iso*propylidene* D-*Glucosone Hydrate.*—(a) *Deacetylation.* The diacetate (1·0 g.) was subjected to catalytic deacetylation by sodium in methanol.

(b) *Methylation.* The usual procedure with methyl iodide and silver oxide was employed in four methylations. The product was distilled in a vacuum (90—110°/0·05 mm.) to yield syrupy *dimethyl di*iso*propylidene* D-*glucosone hydrate* (0·37 g., 43%), $[\alpha]_D^{20}$ +1·2° (*c*, 3·3 in methanol) (Found: OMe, 19·5; $C_{14}H_{24}O_7$ requires 2OMe, 20·4%).

*Hydrolysis of Dimethyl Di*iso*propylidene* D-*Glucosone Hydrate.* A solution of the hydrate (50 mg.) in methanol was evaporated to a small volume (0·25 c.c.), water (1 c.c.) and concentrated hydrochloric acid (2 drops) were added, and the mixture was heated at 100° for 10 minutes. The hydrolysate was neutralised by addition of solid sodium acetate. With *p*-bromophenylhydrazine hydrochloride (0·2 g.) and sodium acetate (0·2 g.) a crystalline derivative, m. p. 154°, was obtained. Salmon and Powell (*J. Amer. Chem. Soc.*, 1939, **61**, 3507) give m. p. 156° for 5 : 6-dimethyl glucose *p*-bromophenylosazone.

*Oxidation of* 5 : 6-*Dimethyl* D-*Glucosone.*—To N-sulphuric acid (2 c.c.) in an open dish heated

on the boiling-water bath was added dropwise a solution of the di*iso*propylidene derivative (150 mg.) in methanol (4·5 c.c.). After 15 minutes' heating to remove methanol the yellow solution was transferred to a conical flask and cooled. 10% Sodium metaperiodate (6 c.c.) was added, and the mixture set aside at room temperature for 72 hours. From the reaction product, by Salmon and Powell's method (*loc. cit.*), there was obtained a solution of αβ-dimethoxypropionic acid, identified as its *p*-bromophenacyl derivative (150 mg., 64·5%).

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Department of Biochemistry,
United College, St. Andrews, Fife. [*Received, February 13th,* 1952.]