135. *Tetra-acetyl Glucosone Hydrate. A Novel Route to the Syntheses of Analogues of Ascorbic Acid and a Possible Mechanism for the Transformation of Hexoses into Kojic Acid.*

By M. Stacey and Lilian M. Turton.

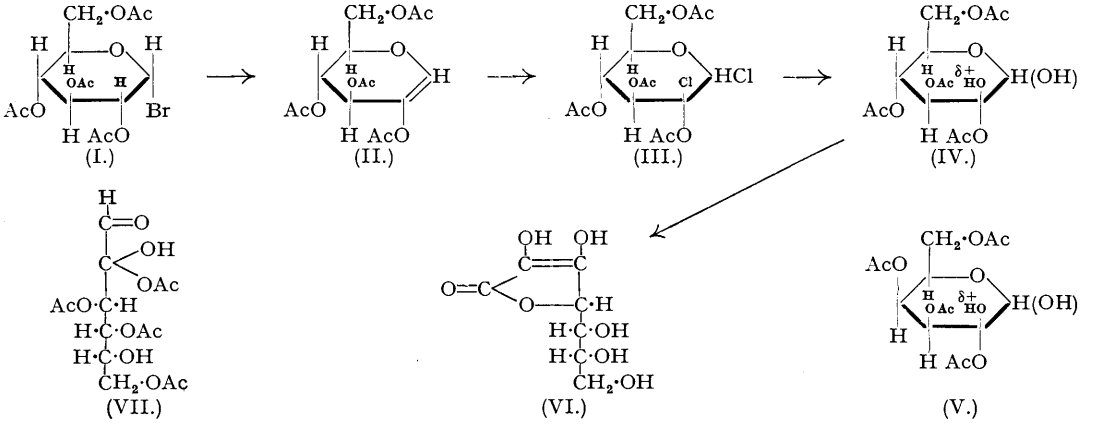
A reinvestigation of the reactions of tetra-acetyl glucosone hydrate has given support to Maurer's formulation of it as 2 : 3 : 4 : 6-tetra-acetyl glucosone hydrate (IV). The compound possesses one incipiently ionic hydrogen atom and forms a *monomethyl* and a dimethyl *ether*. It provides convenient initial material for the syntheses of analogues of ascorbic acid by two routes. Observations have been made on the absorption spectra of kojic acid and related substances and a mechanism for the conversion of (IV) and its hexose analogues into kojic acid is suggested.

Maurer (*Ber.*, 1929, **62**, 332; 1930, **63**, 25; Maurer and Petsch, *Ber.*, 1931, **64**, 2011) has described the synthesis from glucose of a remarkable crystalline glucosone derivative which he formulated as 2 : 3 : 4 : 6-tetra-acetyl glucosone hydrate (IV). The steps in his synthesis were as follows :

Penta-acetyl glucose $\xrightarrow[\text{acetic acid}]{\text{HBr in}}$ acetobromoglucose (I) $\xrightarrow{\text{diethylamine}}$ 2 : 3 : 4 : 6-tetra-acetyl 1 : 2-glucosene (II) $\xrightarrow{Cl_2}$ 1 : 2-dichloro 3 : 4 : 6-triacetyl 2-acetoxy glucal (III) $\xrightarrow{Ag_2CO_3}$ 2 : 3 : 4 : 6-tetra-acetyl glucosone hydrate (IV).

In a similar manner an analogous compound, 2 : 3 : 4 : 6-tetra-acetyl galactosone hydrate (V), was obtained from galactose (Maurer and Müller, *Ber.*, 1930, **63**, 2069). Both of these compounds on being treated with acetic anhydride in pyridine lost two molecules of acetic acid and underwent an intramolecular change with loss of configuration to form diacetyl kojic acid (XX).

Since osones have proved of considerable value as initial material for the syntheses of analogues of ascorbic acid, and because some points in regard to the structure of hexosone hydrates were still obscure, it was deemed of interest to reinvestigate Maurer's compound.



As it was clear that the stages (II) ⟶ (III) ⟶ (IV) entailed an oxidation process, a reagent was sought which would accomplish the conversion of (II) ⟶ (IV) in one step. Perbenzoic acid was found to be eminently suitable since by its action in ethereal solution (II) was converted in *ca.* 30% yield into a crystalline substance of m. p. 151°, $[\alpha]_D$ + 8° in ethanol. Although these properties of (IV) differed from those recorded by Maurer (m. p. 118°, $[\alpha]_D$ + 14° ⟶ + 55°) there would appear to be no doubt that we had obtained (IV) in a highly purified form. Thus it reduced Fehling's solution in the cold, it rapidly decolorised potassium permanganate, and after cautious deacetylation and treatment with phenylhydrazine in the cold it gave glucose phenylosazone. On acetylation at room temperature with acetic anhydride in pyridine it was converted in 80% yield into diacetyl kojic acid (XX) which was identical with diacetyl kojic acid prepared from the kojic acid of *Aspergillus oryzæ*. The synthetic diacetyl kojic acid could be smoothly deacetylated to authentic kojic acid (XV).

Conclusive proof of the structure of (IV) as a derivative of glucosone was furnished by the fact that on treatment in aqueous solution with potassium cyanide and calcium chloride, using the method of Baird *et al.* (*J.*, 1934, 62), it was converted into glucoascorbic acid (VI). The alkaline reagents effected deacetylation, the course of the reaction could be followed iodometrically and spectrophotometrically, and the yield of glucoascorbic acid was reasonably high. It is known that hexosones can be directly oxidised with hypobromous acid to 2-keto hexonic acids (Neuberg and Kitasato, *Biochem. Z.*, 1927, **183**, 485). Since the success of this method depends upon the purity of the hexosones (F. Smith, " Advances in Carbohydrate Chemistry," in the press) it would seem that the crystalline tetra-acetyl hexosone hydrates form eminently suitable material for the syntheses of analogues of ascorbic acid by two routes. In our hands an oxidation of (IV) with bromine

water, followed by heating of the oxidation product with acid, gave a substance in solution which reduced ammoniacal silver nitrate at room temperature and had an absorption band at λ 2450 A., but on the small scale on which the reaction was carried out the *d*-araboascorbic acid which was probably produced was not isolated in crystalline form.

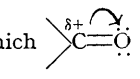
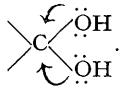
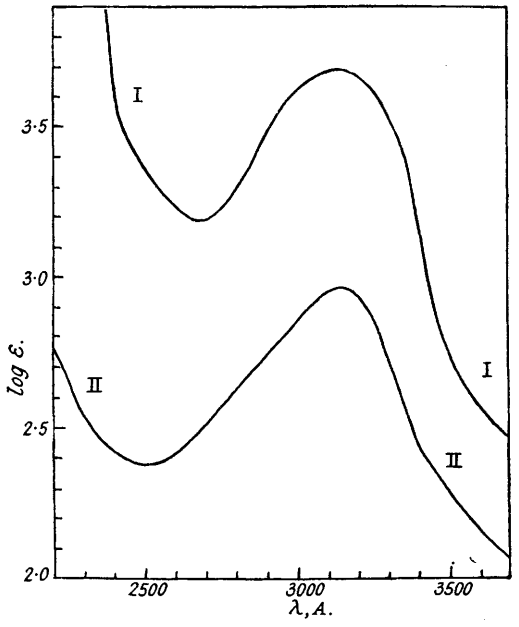
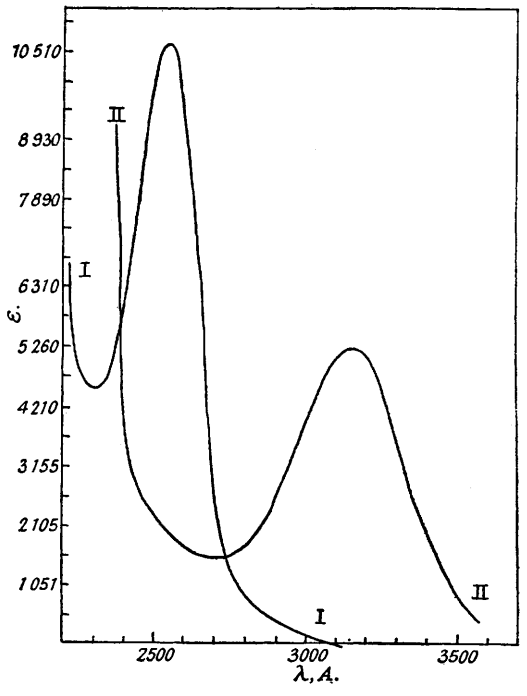
In reactions leading to the synthesis of ascorbic acid analogues the tetra-acetyl glucosone hydrate evidently reacts in the acyclic form (VII), and it is of interest to inquire into the reasons for the stability of such hexosone derivatives and to note the conditions under which  tends to become hydrated to  . The best known example is the conversion of chloral into the relatively stable chloral hydrate. Certain halogenated ketones, in particular trifluoroacetyl derivatives (Swartz, *Bull. Sci. Acad. Roy. Belge*, 1927, **13**, 175; Stacey and Turton, unpublished results), have been observed to behave similarly. It would appear that when an electrophilic group (R) is attached to the carbonyl group the latter tends to be modified so that it no longer is an acceptor of electrons. Such a condition would appear to obtain in tetra-acetyl glucosone

FIG. 1.

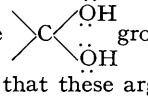


I. *Kojic acid in aqueous neutral or alkaline solution* (λ 3150 A., $\epsilon_{max.}$ ca. 5000; *concn.* 3·7 *mg.*-%).
II. *Solution obtained by dissolving* 2·7 *mg. of* (IV) *in* 0·5 *c.c. of boiling* 0·67N-*sodium hydroxide and diluting to* 90 *mg.*-% *with water* (λ 3150A., $\epsilon_{max.}$ ca. 850).

FIG. 2.



I. *Diacetylkojic acid* (*concn.* 4·3 *mg.*-% *in water;* λ 2550 A., ε ca. 10,000).
II. *Solution I made alkaline* (λ 3150A., ε ca. 5000).

hydrate. When, in such compounds, the electron attracting property of the group (R) can be satisfied by some other means, the  group will be rendered unstable and will tend to revert to the ketonic form. It will be seen below that these arguments are pertinent in considering the formation of the keto group in kojic acid (cf. Isbell, *Ann. Rev. Biochem.*, 1943, **12**, 205; *N.B.S. U.S.A. J. Res.*, 1944, **32**, 45).

There have been but few investigations conducted with the object of interpreting transformations in the carbohydrate group in terms of the electronic theory, though there have been recently some attractive suggestions along these lines in regard to well-known reactions. In particular Isbell (1944, *loc. cit.*) has suggested a mechanism to explain the formation of diacetyl kojic acid from (IV). Our experimental observations agree in some measure with this author's theoretical considerations, but we do not consider that some of his postulations, particularly those envisaging the formation of ortho-acetate structures, are necessary since even simple alkali treatment converts (IV) into (XV) (see Fig. 1, curves I and II).

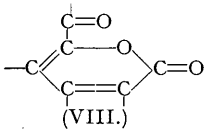
Kojic acid is of some considerable biological interest for it can be produced in high yields by various mould species such as *A. oryzæ* (Challenger, Klein, and Walker, *J.*, 1929, 1498) not only from disaccharides but also from pentoses, tetroses, etc., and even from glycerol as the sole source of carbon. Its formulation as a γ-pyrone (XV) was worked out by Yabuta (*J.*, 1924, **125**, 575) though its derivatives have as yet been but

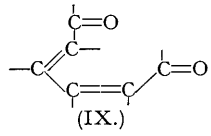
little investigated. It is unlikely that the mechanism of its formation from (IV) will have any bearing on the biological mode of synthesis.

Acetyl determinations on (IV) using the method of adding excess sodium hydroxide followed by back titration (cf. Maurer, *loc. cit.*) indicated the apparent presence of five acetyl residues, whereas acetyl estimations by determining the volatile acetic acid liberated on acid hydrolysis revealed only four. The reason for the discrepancy was clear when it was shown that titration of (IV) with sodium carbonate required one equivalent, thus indicating that one hydrogen atom could be liberated as a proton. Acidification of the solution containing the sodio derivative followed by ether extraction recovered (IV) unchanged. Methylation of (IV) with silver oxide and methyl iodide gave a crystalline *monomethyl ether*, together with a syrupy product which was essentially a dimethyl ether, thus showing that (IV) possessed two free hydroxyl groups. These facts can all be accommodated by the structure (IV) or by the less likely acyclic structure (VII).

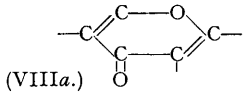
Spectrophotometric measurements have provided strong additional evidence for the readiness with which the glucosone derivative is converted into kojic acid. Fig. 1 (curve II) shows the ultra-violet absorption of a solution obtained by treatment with aqueous alkali of (IV) which itself in neutral solution does not absorb light selectively. For comparison the absorption curve of an authentic specimen of mould kojic acid is given in Fig. 1 (curve I). In Fig. 2 (curve I) is shown the ultra-violet absorption of the product obtained by acetylating (IV) with acetic anhydride in pyridine. That this was a diacetyl kojic acid was confirmed by the fact that, after treatment with alkali, it gave an absorption band (Fig. 2, curve II) identical with that of authentic kojic acid.

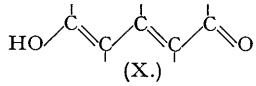
In connection with these absorption curves it is of interest to draw attention to some observations made on certain other ketonic compounds (cf. Haworth, Hirst, and Jones, *J.*, 1938, 710; Haworth, Heslop, Salt, and Smith, *ibid.*, 1944, 217). In particular 6-carbomethoxy-3-methoxy-α-pyrone shows a band at λ 3070 A., $\epsilon_{max.}$ 12,000, and dimethyl 2 : 5-dimethoxymuconate (obtained by alkaline treatment of a methyl tetramethylglucosaccharate, L. F. Wiggins, private communication) has a maximum absorption at λ 3020 A., the intensity varying with the concentration (*e.g.*, *c*, 1·65 mg.-%, ε 25,000). In these cases the absorbing systems must be (VIII) and (IX) respectively.


(VIII.)

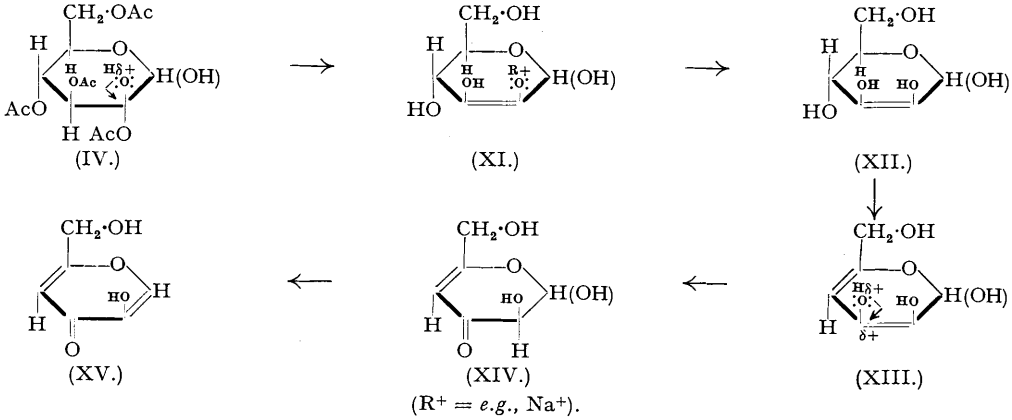

(IX.)

Comparable with the band shift between the neutral and alkaline solutions of diacetyl kojic acid are the values found for the absorption maxima of the mono-enol of 3 : 7-diketocholestene (Barnett, Ryman, and Smith, this vol., p. 526) in ethanolic solution. The monoacetate of this enol displays a band at λ 2840 A., log ε 4·4, in neutral solution and on making the solution alkaline this band moves to λ 3900 A., log ε 4·78. The unsubstituted enol and its ethers show a maximum absorption at λ 3120 A., log ε 4·4, which in the case of the enol itself is displaced in alkali to λ 3900 A. with a slight increase in intensity. The similarity in the position of the band to that of kojic acid is quite striking; the absorbing system in kojic acid is (VIII*a*),


(VIII*a*.)


(X.)

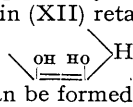
while in the sterol derivative it is presumably (X).

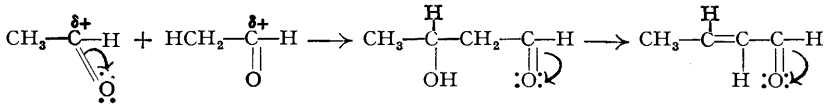

(IV.) (XI.) (XII.) (XIII.) (XIV.) (XV.)

(R^+ = *e.g.*, Na^+).

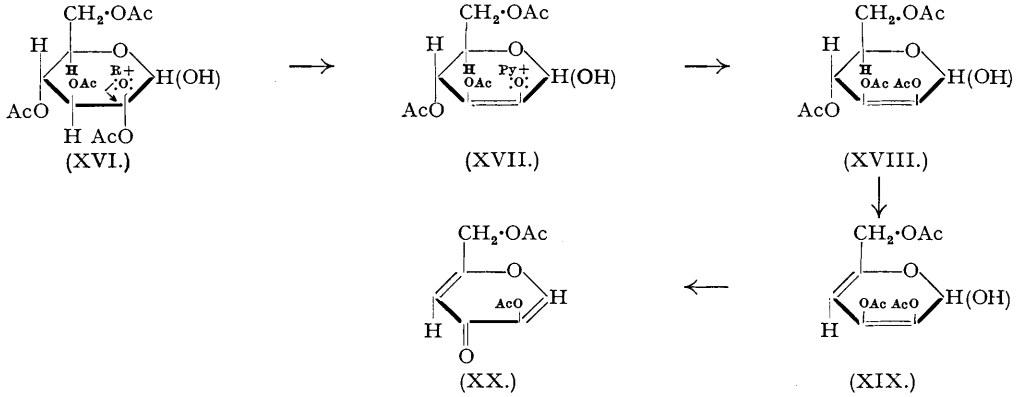
It is considered that treatment of (IV) with aqueous alkali, in addition to effecting deacetylation, will neutralise on C_2 the hydrogen atom which is incipiently ionic (cf. VII). The transformation of (IV) then

probably proceeds in the initial stages essentially as suggested by Isbell (1944, *loc. cit.*) involving a loss of water between C_3 and C_2 with formation of the ene-diol (XI). From this stage we disagree with Isbell's mechanism inasmuch as we consider that a proton acceptor group (*e.g.*, hydroxyl or pyridine) is essential to promote the successive stages such as the conversion (IV) ⟶ (XII). In aqueous solution R^+ in (XI) would readily be replaced by H^+ giving (XII).

Since C_1 in (XII) retains some of the reducing properties of a free aldehyde group (as in all reducing sugars) the system  is in effect analogous to the "ene-one" system in crotonaldehyde. This substance can be formed by an aldol condensation by a mechanism similar to the one leading to (XII) thus:



Crotonaldehyde may undergo further condensation because there is an electron drift towards the CHO group and thus the linkage of the hydrogen atoms to C_4 becomes less firm. In the same way the linkages of both the hydrogen atom and the hydroxyl group on C_4 in (XII) are weakened. Formation of (XIII) by loss of water between C_4 and C_5 can remedy this since there results thereby a conjugated system in which there is a possibility of resonance. As in crotonaldehyde, the aldehydic carbon, C_1, will retain electron acceptor properties and this will cause a tendency in the hydrogen of the C_3 enolic group to ionise with the possible intermediate formation of (XIV). Alternatively the proton may attack directly the aldehydic hydroxyl group on C_1 with the result that kojic acid (XV) is formed. Such a mechanism is independent of the geometry of the hydroxyl groups in respect to the ring and thus, as already shown experimentally in the case of tetra-acetyl galactosone hydrate, the interpretation will hold for any hexosone derivative.



(XVI.) (XVII.) (XVIII.) (XIX.) (XX.)

(Py⁺ = Pyridinium ion)

In the acetylation reactions in pyridine solution the transformation is less simple though a similar interpretation is possible. In pyridine–acetic anhydride solution there can be a donation of electrons by the pyridine to the incipiently ionic proton on C_2 (XVI) followed by loss of acetic acid between C_2 and C_3 giving the acetylated ene-diol (XVII). From this the pyridinium ion will be removed by acetic acid to form (XVIII). A further loss of one molecule of acetic acid by the electronic mechanism suggested above for the transformation in aqueous solution gives (XIX), and finally by loss of a third molecule of acetic acid there results diacetyl kojic acid (XX).

A somewhat similar mechanism has been suggested by Isbell (1943, *loc. cit.*) for the formation by means of acetic anhydride–pyridine treatment of 1 : 2 : 3 : 5-tetra-acetoxybenzene from both of two ketopentaacetates derived from oxidised inositol. Here Isbell again does not specify that a proton acceptor must be used to promote the reaction whereas we regard this as one of the essential factors in such a transformation.

Experimental.

2 : 3 : 4 : 6-*Tetra-acetyl* 1 : 2-*Glucosene*.—Acetobromoglucose (62 g.) was dissolved in benzene according to the method of Maurer and Mahn (*Ber.*, 1927, **60**, 1316). The product was recrystallised by dissolving it in warm alcohol, then adding a few drops of water and agitating vigorously. Yield 22 g., m. p. 61—62°.

Oxidation of Tetra-acetyl 1 : 2-*Glucosene*.—Perbenzoic acid (14 g. in 200 c.c. of ether) was prepared from benzoyl peroxide by the method of Tiffeneau (*Org. Synth.*, **8**, 30). Tetra-acetyl 1 : 2-glucosene (22·5 g.) was added to the ethereal solution which was cooled in ice and shaken until all the solid was dissolved. After 20 hours there separated a crystalline substance which was washed repeatedly on the filter with ether to remove perbenzoic and benzoic acids and then dried. It was recrystallised several times from water and had m. p. 150—151°. From the mother liquor there were separated several further crops of this material giving a total yield of 6·0 g. An unidentified substance having m. p. 93° was separated from the final mother liquor. The main bulk of material had m. p. 151°, $[\alpha]_D^{20°}$ + 8·4° in 20% aqueous alcohol (*c*, 1·3) [Found : C, 46·3; H, 5·5. Calc. for tetra-acetyl glucosone hydrate $C_{14}H_{20}O_{11}$: C, 46·2; H, 5·5%. *O*-Ac (by direct

titration), 59·7; *O*-Ac (by an acid distillation), 52·0. Calc. for 4 *O*-Ac residues : *O*-Ac, 47·3; for 5 *O*-Ac residues, *O*-Ac, 59·1%. Replaceable hydrogen (titration with 0·05N-sodium carbonate), 2·77. Calc. for $C_{14}H_{20}O_{11}$ containing one replaceable hydrogen: 2·74%. On acidification of the titrated solution, followed by extraction with ether, tetra-acetyl glucosone hydrate was recovered unchanged].

Methylation of Tetra-acetyl Glucosone Hydrate.—The hydrate (3·0 g.) was methylated three times with silver oxide and methyl iodide in the usual manner. The product was extracted by chloroform and there was obtained a syrup which partly crystallised. Recrystallised from chloroform–ligroin the crystals (0·2 g.) had m. p. 120°, $[\alpha]_D^{18°}$ + 20° in chloroform (*c*, 1·0) (Found: C, 47·6; H, 5·8; OMe, 8·5. *Monomethyl tetra-acetyl glucosone hydrate*, $C_{15}H_{22}O_{11}$, requires C, 47·6; H, 5·8; OMe, 8·2%). The syrupy residue had OMe, 14·6% (Calc. for dimethyl tetra-acetyl glucosone hydrate, $C_{16}H_{24}O_{11}$: OMe, 15·8%).

Conversion of Tetra-acetyl Glucosone Hydrate into Diacetyl Kojic Acid.—The hydrate (3·0 g.) was shaken with pyridine (20 c.c.) and acetic anhydride (20 c.c.) and then kept at room temperature for 3 days. The solvents were then distilled off under diminished pressure at 35° and the solid residue was recrystallised (1·3 g.) from alcohol–ligroin to constant m. p. 103° alone or in admixture with a specimen prepared by acetylating with the same reagents a specimen of kojic acid obtained from glucose with *A. oryzæ*. On treatment with ammonia both the synthetic and the mould specimen of diacetyl kojic acid were smoothly deacetylated to kojic acid, m. p. 153° alone or in admixture with a specimen from *A. oryzæ*.

Synthesis of Glucoascorbic Acid from Tetra-acetyl Glucosone Hydrate.—Tetra-acetyl glucosone hydrate (4 g.) was dissolved in oxygen-free hot water (250 c.c.) and cautiously cooled (avoiding crystallisation) to 37°. To the solution potassium cyanide (2·5 g.) and calcium chloride (3 g.) were added. Oxygen-free nitrogen was passed through the solution which was kept at room temperature. Titration of small portions from time to time with N/50-iodine showed that the reaction was complete in 1 hour. The calcium was removed quantitatively as oxalate and the solution was acidified with dilute acetic acid and concentrated under diminished pressure in a stream of carbon dioxide to a syrup. This was dissolved in 8% hydrochloric acid (50 c.c.) and the solution digested at 50° for 20 hours; the head of the absorption band at λ 2750 A. was then completely replaced by a band at λ 2450 A. The product in solution was worked up as described previously (Baird, *et al.*, *loc. cit.*) and there resulted glucoascorbic acid monohydrate (1·5 g.), m. p. 138°, $[\alpha]_D^{19°}$ − 22° in methyl alcohol (*c*, 1·1 as monohydrate). After oxidation with iodine it gave a yellow phenylhydrazine derivative, m. p. 222°.

Oxidation of Tetra-acetyl Glucosone Hydrate with Bromine.—The glucosone hydrate (1 g.) was dissolved in boiling water (100 c.c.) and cautiously cooled to 40°, and bromine (0·5 g.) was added. The solution was kept at 40° for 30 hours; the last traces of bromine were then removed in a stream of nitrogen. The solution was concentrated (diminished pressure in carbon dioxide) to 50 c.c. Glacial acetic acid was added and the solution digested at 80° for 24 hours. On working up the product in the usual manner a syrup, $[\alpha]_D^{20°}$ − 20° (*c*, 0·6 in water), was obtained. This had a strong absorption band at λ 2450 A., reduced Fehling's solution readily, reduced ammoniacal silver nitrate in the cold, and after oxidation with iodine gave a yellow phenylhydrazine derivative, m. p. 200°.

Absorption Measurements.—The following data were obtained. (i) Kojic acid in aqueous neutral or alkaline solution : $\epsilon_{max.}$ *ca.* 5000, λ 3150 A. (*c.*, 3·7 mg.-%).

(ii) Tetra-acetyl glucosone hydrate shows no band in neutral solution. On dissolving 2·7 mg. in 0·5 c.c. of 0·67N-sodium hydroxide a deep yellow solution was obtained. This was diluted with water to *c*, 90 mg.-% and showed $\epsilon_{max.}$ *ca.* 850, λ 3150 A. The ε value is calculated assuming *M* to be 364 (*i.e.*, *M* of tetra-acetyl glucosone hydrate).

On keeping the solution (*c*, 90 mg.-%) for 2 hours the ε value had decreased to $\epsilon_{max.}$ *ca.* 700. Thus the absorbing substance was undergoing some decomposition.

6-Carbomethoxy-3-methoxy-α-pyrone (Haworth *et al.*, *loc. cit.*) shows a band at λ 3090 A., $\epsilon_{max.}$ 12,000 (*c*, 2·0 mg.-% in water).

It was difficult to show by ultra-violet absorption methods that pyridine alone would effect the transformation (IV) ⟶ (XV). Nevertheless when an alcohol–pyridine solution of (IV) was compared with alcohol–pyridine alone there was a slight modification in the region of λ 3000—3300 A. of the pyridine band.

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