260. *Synthesis of Ascorbic Acid and its Analogues: The Addition of Hydrogen Cyanide to Osones.**

By W. N. Haworth, E. L. Hirst, J. K. N. Jones, and F. Smith.

The experimental methods originally employed in the synthesis of natural ascorbic acid from *l*-xylosone differed principally in the choice of reagents to effect the addition of hydrogen cyanide. Reichstein and his co-workers (*Helv. Chim. Acta*, 1933, **16**, 1019) employed liquid hydrogen cyanide and an elevated temperature in the presence of some potassium cyanide, whilst in this laboratory we made use of dilute aqueous potassium cyanide containing some calcium chloride. The reaction, which was carried out in the cold, was complete in a few minutes and gave in almost quantitative yield the cyanohydrin of xylosone. When potassium cyanide was used alone, the reaction proceeded similarly but less rapidly, and unless the solution of xylosone was rendered exactly neutral before addition of the cyanide, the yield in routine experiments was slightly inferior. We noticed

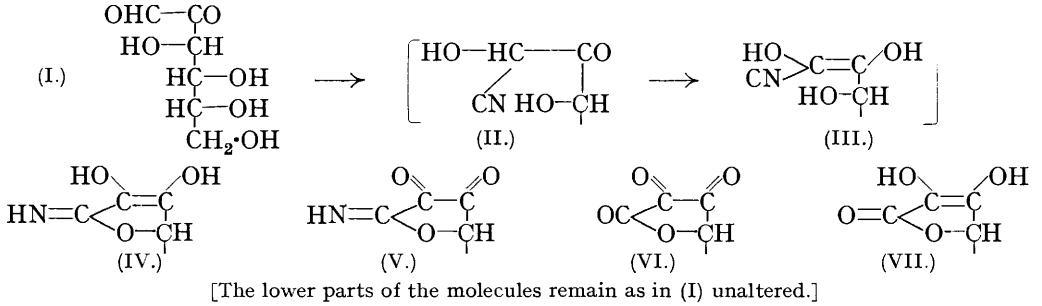
* A preliminary account of part of this work was given in *Helv. Chim. Acta*, 1934, **17**, 520.

that in the presence of calcium chloride some ammonia was liberated, and the reaction appeared to fall into line with our previous experience with this mixture of reagents in the ascent of the series from glucose to glucoheptose. In the latter instance, hydrolysis of the intermediate compound takes place with liberation of ammonia and formation of the monobasic acid, which is then isolated as the calcium salt.

We interpreted in the same way the reaction between xylosone and potassium cyanide in the presence of calcium chloride. The primary product was not ascorbic acid but was convertible into it by simple treatment with dilute hydrochloric acid. We provisionally designated the intermediate phase ψ-ascorbic acid, and since this ψ-compound, which had not then been isolated, possessed many interesting properties, we decided to investigate it more fully. Owing to unfavourable solubilities, we have not yet succeeded in isolating a crystalline primary product in the case of the synthesis from xylosone, but have done so readily in the analogous cases of *d*-glucosone (I) and *d*-galactosone, which give rise to *d*-gluco- (VII) and *d*-galacto-ascorbic acids, respectively, when submitted to the reactions outlined above. As shown previously, these substances are exact analogues of the natural ascorbic acid, and as the result of our new observations we are able to give a precise interpretation of the mechanism of the reaction which leads to the synthesis of ascorbic acid.

In our earlier work we had noticed that the primary product, which was characterised by an intense absorption band at λ 275 mμ, changed gradually on digestion with mineral acid into ascorbic acid, which has a band at λ 245 mμ in acid solution. This behaviour is characteristic, and may be utilised for all analogues of ascorbic acid in following the transformation from the primary synthetic product into the final substance. The operations leading to the synthesis of gluco-ascorbic acid may be cited as typical. The primary product when isolated was a crystalline substance of formula $C_7H_{11}O_6N$, corresponding to the nitrile (II or III) which would be expected to result from the addition of hydrogen cyanide to glucosone. The substance is, however, neutral, and a detailed study of its properties leads us to the view that it is best formulated as a cyclic compound (IV) and for this reason we designate it *imino-gluco-ascorbic acid*. It is an internal salt in which the basic imino-group neutralises the strongly acidic enolic hydroxyl group.

The primary product is converted on digestion with warm (or cold) dilute mineral acid into *d*-gluco-ascorbic acid, with liberation of the ammonium salt of the mineral acid. A similar conversion can be effected by hot aqueous acetic acid. This ease of hydrolysis in aqueous solution to a compound possessing a closed-ring (lactone) system is a strong argument in favour of the presence of a cyclic structure in the primary product itself. This view receives strong support from a consideration of the other properties of this acid. For instance, its absorption spectrum resembles that of ascorbic acid, whereas the open-chain derivatives so far investigated (*e.g.*, the sodium salt derived from dimethyl ascorbic acid) do not display selective absorption. Again, it is oxidised instantaneously by 2 atoms of iodine in dilute acidified aqueous solutions, with liberation of 2 mols. of hydriodic acid. The product is a base (V) which combines with 1 mol. of mineral acid, giving a neutral product. The imino-group in this is particularly labile, and is removed by hydrolysis in



[The lower parts of the molecules remain as in (I) unaltered.]

the cold in very dilute acid solutions. As demonstrated on p. 1195 the initial product in the latter hydrolysis is the lactone form of the diketo-acid (VI), produced by the action of

iodine on gluco-ascorbic acid. The lactone ring gradually opens in aqueous solution, and at the final stage we had present the first oxidation product of gluco-ascorbic acid in its equilibrium condition. At present we are unable to say whether (V) is capable of opening up its lactone ring without preliminary hydrolysis of the imino-group, but the change occurs, if at all, only to a very small extent. These observations find a reasonable interpretation only on the hypothesis that imino-gluco-ascorbic acid has a cyclic structure.

Other cogent arguments may be advanced on the ground of the physical properties of the substance. The high m.p. (*ca.* 236°) is much more appropriate for an internal salt than for an open-chain nitrile. Furthermore, the specific rotation of the substance in aqueous solution is quite unusually large ($[\alpha]_D^{20°}$ — 145°) and is very similar to that of the ionised salts of gluco-ascorbic acid. On the other hand, when the ionisation of the organic acid is suppressed in acidified solutions, the rotation (— 17°) becomes similar to that of free gluco-ascorbic acid. Exactly similar behaviour is, of course, observed with ascorbic acid and all its analogues, the rotation changes being so great that the exact analogy with the present instance becomes highly significant. The cyclic form receives additional support from the observation that open-chain forms of *d*-gluco-ascorbic acid have positive rotations.

We have obtained yet further evidence in favour of the cyclic structure from a study of the optical rotatory dispersion of imino-gluco-ascorbic acid in neutral and in acid solutions. We had shown previously (Herbert, Hirst, and Wood, J., 1933, 1564; cf. Lowry and Pearman, *ibid.*, p. 1444) that a remarkable difference in the character of the rotatory dispersion is encountered when the dispersion of *l*-ascorbic acid is compared with that of its salts. In this instance highly anomalous rotatory dispersion with a negative low-frequency term gives place, on ionisation of the ascorbic acid, to simple dispersion with a positive low-frequency term. Exactly similar phenomena are found when aqueous solutions (ionised form) are compared with strongly acid solutions (un-ionised form) of imino-gluco-ascorbic acid. The rotations of the un-ionised forms are expressible by two-term Drude equations of the type $\alpha_\lambda = k_1/(\lambda^2 - \lambda_1)^2 + k_2/(\lambda^2 - \lambda_2^2)$, and those of the ionised substances by single-term equations of the type $\alpha_\lambda = k_2/(\lambda^2 - \lambda_2^2)$. The values of the various constants are collected in the accompanying table, reference being made in each case, for ease of comparison, to the *d*-isomeride. The concentration of the solution in the case of the $[\alpha]_D^{20°}$ values is *ca.* 1.

Substance.	λ_1.	λ_2.	k_1/k_2.	$[\alpha]_D^{20°}$.
Imino-gluco-ascorbic acid (in acid solution)	0·024	0·065	—1·66	— 17° *
Ascorbic acid (in acid solution)	0·0195	0·063	—1·81	— 22 *
Sodium ascorbate (in water)	—	0·065	—	—102
Imino-gluco-ascorbic acid (in water)	—	0·064	—	—145

* In 8% hydrochloric acid.

The resemblances and differences are remarkably characteristic, and provide strong evidence in support of the idea that imino-gluco-ascorbic acid is to be formulated as the ionised internal salt (IV) of a cyclic acid containing the special structure found in ascorbic acid.

Similar arguments apply to the formulation of *imino-galacto-ascorbic acid* as a cyclic structure. The properties of this substance are described on p. 1197.

Experimental.

Reaction between Glucosone and Potassium Cyanide : Isolation of Primary Product (Imino gluco-ascorbic Acid).—To a neutral aqueous solution of glucosone (3 g., in water, 100 c.c.) potassium cyanide (2·5 g.) and calcium chloride (3 g.) were added. A stream of oxygen-free nitrogen was passed through the solution, which was kept at room temperature. Slight alkalinity developed, and some ammonia was liberated (no ammonia was evolved in the absence of glucosone). The formation of the addition product was complete in a few minutes. When the yield of the primary product had reached the maximum (estimated by titration of a portion of the solution with iodine), the reaction was arrested by addition of sufficient oxalic acid to render the solution neutral to litmus. Continuation of the treatment under slightly alkaline conditions causes progressive loss of product. The reaction proceeds in a similar manner in the

absence of calcium chloride, but somewhat less rapidly, and the yield is slightly inferior. No ammonia is liberated in the absence of calcium chloride.

After filtration, the solution was diluted with an equal volume of alcohol and left for 30 hours at −5°. The solid which separated was recrystallised from aqueous alcohol, giving the primary *product* (IV) as a mass of minute colourless crystals, sparingly soluble in cold water, moderately soluble in hot water, readily soluble in dilute aqueous alkali and in dilute mineral acids, and almost insoluble in organic solvents. The aqueous solution was neutral to litmus (yield, almost theoretical) (Found: C, 40·9; H, 5·7; N, 6·6. $C_7H_{11}O_6N$ requires C, 40·9; H, 5·4; N, 6·8%).

Properties of imino-gluco-ascorbic acid. When heated, the substance darkened at 220° and slowly decomposed at about 236°; $[\alpha]_D^{20°}$ − 145° in water (*c*, 0·8); − 181° in 0·03*N*-sodium hydroxide (*c*, 0·2); − 60° in 0·1*N*-sulphuric acid (*c*, 1·0); − 40° in 0·35*N*-sulphuric acid; − 17° in 8% aqueous hydrochloric acid. The rotation value depends markedly on the p_H of the solution.

Aqueous-alcoholic solutions of the primary product containing 1 mol. of hydrochloric acid deposit, although in poor yield, the unchanged material (not the hydrochloride). The substance displays in aqueous solution an intense absorption band with head at λ 275 mμ. The molecular extinction coefficient for a solution containing 20 mg. per litre is 17,000. Neither the intensity nor the position of the head of the band is appreciably changed in dilute acidic solutions.

0·1 G. in acid solution was oxidised by 9·7 c.c. of *N*/10-iodine (see p. 1193) under the conditions used for ascorbic acid ($C_7H_{11}O_6N$ requires 9·75 c.c.). The oxidation product so obtained has $[\alpha]_D^{20°}$ − 35° in dilute hydriodic acid. It cannot be reduced to the original substance by hydrogen sulphide under the conditions appropriate for the reduction of oxidised ascorbic acid. Of the 2 mols. of hydriodic acid formed during the oxidation, one can be titrated directly with sodium hydroxide, but the other is neutralised by the basic portion of the molecule (the solution obtained by oxidising 100 mg. of the primary product required 5·0 c.c. *N*/10-sodium hydroxide for neutralisation). On addition of more alkali a second molecular proportion of sodium hydroxide is taken up slowly with elimination of ammonia, and the product, which has $[\alpha]_D^{20°}$ + 30°, is the same as that obtained by addition of alkali to gluco-ascorbic acid which has been reversibly oxidised by iodine (or chlorine).

When imino-gluco-ascorbic acid is oxidised with iodine (or chlorine) the acid solution of the product so obtained is unstable at room temperature. This oxidation product then slowly undergoes hydrolysis with elimination of ammonia as ammonium iodide (or chloride) and formation of the primary oxidation product of gluco-ascorbic acid. This transformation can be followed polarimetrically ($[\alpha]_D^{20°}$ − 35° ⟶ +13°), the end value reached after several days being that of reversibly oxidised gluco-ascorbic acid which has reached equilibrium (cf. Herbert, Hirst, Percival, Reynolds, and Smith, J., 1933, 1270). If the solution undergoing transformation is examined before the equilibrium stage has been reached, it contains the oxidised gluco-ascorbic acid in its true lactone form, recognisable by its reduction to gluco-ascorbic acid. The absorption band at λ 245 mμ (in acid solution) can then be observed, and the presence of gluco-ascorbic acid can be recognised also by the reaction with iodine. The oxidised form of the imino-compound does not display selective absorption in the ultra-violet region.

Imino-gluco-ascorbic acid liberates ammonia in the cold in presence of dilute aqueous alkali. During this reaction the characteristic absorption band at λ 275 mμ disappears, together with the power of reducing iodine in acid solution. No absorption band characteristic of gluco-ascorbic acid or of its salts appears during the alkaline hydrolysis.

After oxidation with iodine, the imino-gluco-ascorbic acid gave the same osazone (yellow), m. p. 225°, as that obtained from oxidised gluco-ascorbic acid (Found: N, 14·6. Calc. for $C_{19}H_{20}O_5N_4$: N, 14·6%). The identity of the two substances was confirmed by *X*-ray crystallographic examination by Mr. E. G. Cox. Under the above treatment, hydrolysis of the imino-group evidently occurs during the reaction with phenylhydrazine in acetic acid solution. The imino-group of the compound in its oxidised condition is very labile.

Oxidation of imino-gluco-ascorbic acid with iodine in acid solution, followed by treatment with sodium hypoiodite, gives rise to some 70% of the theoretical amount of oxalic acid, corresponding to loss of the first two carbon atoms of the chain. This reaction is complicated by interaction between the liberated ammonia and sodium hypoiodite and is therefore not suitable for quantitative estimations.

Transformation of Imino-gluco-ascorbic Acid into Gluco-ascorbic Acid.—Digestion of a solution of the imino-substance (1 g.) in 8% aqueous hydrochloric acid (25 c.c.) at 50° for 16 hours resulted in the quantitative formation of gluco-ascorbic acid, which was isolated (yield

quantitative) and determined iodimetrically and by spectrophotometric methods. The characteristic absorption band at λ 245 mμ appears during the digestion, and gradually replaces the band at λ 275 mμ, no trace of the latter being observed at the conclusion of the reaction. A similar transformation can be effected by heating the imino-gluco-ascorbic acid in 20% acetic acid at 80° for 12 hours. The product, which was isolated by the procedure described previously (J., *loc. cit.*), was the monohydrate of gluco-ascorbic acid, m. p. 138°, $[\alpha]_D^{20°}$ − 22° in water (*c*, 1·0 as monohydrate); m. p. of the anhydrous form 192°. Digestion of the imino-product with water at 100° did not bring about the transformation into gluco-ascorbic acid.

Rotatory Dispersion of Imino-gluco-ascorbic Acid (with R. W. Herbert and C. E. Wood).—The measurements were made by the methods previously described (Harris, Hirst, and Wood, J., 1932, 2112). An aqueous solution of imino-gluco-ascorbic acid containing 8·386 g. per litre was prepared, and its rotation (l = 2 dm.) determined at 25°. Observations were obtained over the range of wave-lengths from λ 6708 to λ 3510. Throughout this region, the rotatory dispersion was approximately simple (λ_0^2 = 0·064) and corresponded exactly to that of sodium *l*-ascorbate (λ_0^2 = 0·060) in aqueous solution. The results are summarised in Table I. A solution of the imino-substance was then acidified, the composition of the solution examined being 20·656 g. of imino-gluco-ascorbic acid and 30·4 g. (3 mols.) of sulphuric acid in 1 litre of solution. The added mineral acid depressed the ionisation of the acidic enol group of the imino-compound, and the rotatory dispersion was now exactly analogous with that of ascorbic acid in aqueous or acid solution (Herbert, Hirst, and Wood, J., 1933, 1564). The results, which are summarised in Table II, now show that the rotatory dispersion is anomalous (λ_1^2 = 0·024; λ_2^2 = 0·065; k_1/k_2 = − 1·66), and is very closely similar to that exhibited by *l*-ascorbic acid (λ_1^2 = 0·0195; λ_2^2 = 0·063; k_1/k_2 = − 1·811). When the observations are expressed by the two-term Drude equation and this is compared with the single-term equation applicable to the rotatory dispersion of the imino-compound in water, it is evident that in the case of imino-gluco-ascorbic acid the sign of the low-frequency term is reversed (without appreciable change in the nature or intensity of the observed absorption band) on passage of the substance from the ionised to the un-ionised condition. Exactly similar behaviour is exhibited by *l*-ascorbic acid, and further comments on this phenomenon will be made in subsequent communications.

Table I.

Imino-gluco-ascorbic Acid in Water.

c, 0·8386 g. in 100 c.c.; l = 2 dm.; *t*, 25°; $[\alpha]_\lambda = 59{\cdot}6a_\lambda$; $a_\lambda = -0{\cdot}67005/(\lambda^2 - 0{\cdot}064)$.

	a.				*a*.				*a*.		
λ.	Obs.	Calc.	Diff.	λ.	Obs.	Calc.	Diff.	λ.	Obs.	Calc.	Diff.
6708	−1·76°	−1·78°	+0·02°	5515	−2·77°	−2·79°	+0·02°	4294	−5·56°	−5·57°	+0·01°
6292	2·06	2·02	−0·04	5225	3·16	3·21	−0·05	3910	7·56	7·54	−0·02
6104	2·16	2·17	+0·01	4891	3·79	3·82	+0·03	3669	9·56	9·49	−0·07
5893	2·35	2·37	+0·02	4887	3·80	3·83	+0·03	3510	11·56	11·32	−0·24
5805	2·46	2·45	−0·01								

Table II.

Imino-gluco-ascorbic Acid in Dilute Sulphuric Acid.

c, 2·0656 g. in 100 c.c. aqueous H_2SO_4 (3·04 g. H_2SO_4 per 100 c.c.); l = 2 dm.; *t* = 25°; $[\alpha]_\lambda = 24{\cdot}2a_\lambda$; $a_\lambda = -1{\cdot}31863/(\lambda^2 - 0{\cdot}024) + 0{\cdot}79251/(\lambda^2 - 0{\cdot}065)$.

6292	−1·16	−1·15	−0·01	4887	−1·58	−1·58	+0·00	3674	−0·56	−0·55	−0·01
5893	1·24	1·27	+0·03	4272	1·56	1·58	+0·02	3604	−0·26	−0·24	−0·02
5805	1·30	1·30	±0·00	4119	1·46	1·48	+0·02	3565	zero	−0·03	+0·03
5515	1·36	1·40	+0·04	3957	1·31	1·30	−0·01	3494	+0·44	+0·44	±0·00
5225	1·46	1·49	+0·03	3886	1·16	1·17	+0·01	3441	+0·94	+0·87	+0·07
4950	1·56	1·57	+0·01	3749	0·86	0·82	−0·04	3342	+1·94	+1·93	+0·01

Reaction between Galactosone and Sodium Cyanide: Isolation of Primary Product (Imino-galacto-ascorbic Acid).—Galactose (20 g.) was heated with phenylhydrazine (60 c.c.) and glacial acetic acid (50 c.c.) dissolved in water (400 c.c.) for 3·5 hours at 85° (yield of galactosazone, 23 g.). Galactosazone (20 g.) was treated with fuming hydrochloric acid (190 c.c., saturated at 0°) for 30 mins., during which the temperature gradually rose to 15° (cf. Fischer, *Ber.*, 1889, **22**, 87). The phenylhydrazine hydrochloride was filtered off, and the filtrate diluted to 2 litres. The solution, cooled to 0°, was neutralised with lead carbonate, decolorised (charcoal), and rendered slightly alkaline with basic lead acetate. On addition of barium hydroxide, the

osone–lead hydroxide complex was precipitated, and from this the osone was regenerated by the usual procedure (yield, 1·2 g.).

To a solution of galactosone (7 g.) in water (300 c.c.), sodium cyanide (2·8 g.) was added (nitrogen atmosphere). After 20 minutes, the condensation was complete, and titration with iodine in acid solution showed that 7·2 g. of " active " primary product were present. The solution was neutralised by dilute hydrochloric acid and cooled at — 5°. After 14 days, crystals of imino-galacto-ascorbic acid began to separate. In this way a nucleus was provided.

In subsequent preparations the solution immediately after neutralisation was concentrated to 30 c.c., inoculated if necessary, and set aside to crystallise (yield almost quantitative). Recrystallisation from water gave pure *imino-galacto-ascorbic acid,* m. p. 190° (decomp.), $[\alpha]_D^{22°}$ — 95° in water (*c*, 0·8); + 25° in *N*-hydrochloric acid (*c*, 1·0) (Found : C, 41·0; H, 5·4; N, 6·5. $C_7H_{11}O_6N$ requires C, 40·96; H, 5·4; N, 6·8%).

Imino-galacto-ascorbic acid closely resembled the corresponding gluco-derivative in its properties, but was rather more soluble in water. In aqueous solution it exhibits an intense single absorption band at λ 275 mμ (ε approx. 18,000 for a solution containing 20 mg. per litre). In *N*-hydrochloric acid solution at 15° it was hydrolysed in 24 hours to galacto-ascorbic acid. which could be isolated as the monohydrate, m. p. 109°, the properties of which have been described previously (*loc. cit.*). Imino-galacto-ascorbic acid was neutral to litmus, possessed intense reducing power, and underwent oxidation with iodine (2 atoms) in acid solution with formation of a basic product ($[\alpha]_D$ — 125° in aqueous solution containing 1 mol. proportion of hydriodic acid), which combined with one of the two mol. proportions of hydriodic acid liberated during the oxidation. The rotation of the oxidation product ($[\alpha]_D$ — 125°) decreased slowly when the solution was kept at room temperature, the constant value — 69° being reached in 24 days. As in the case of the corresponding product from imino-gluco-ascorbic acid, the rotation became constant (after several weeks) when the solution contained the primary oxidation product of galacto-ascorbic acid in the " equilibrium " condition. Hydrolysis of the imino-group and opening of the lactone ring took place simultaneously.

The authors are grateful for financial assistance from the Government Grant Committee of the Royal Society, and from the Department of Scientific and Industrial Research.

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, July 11th,* 1934.]