18. *Ascorbic Acid and Synthetic Analogues.*

By D. K. Baird, W. N. Haworth, R. W. Herbert, E. L. Hirst, F. Smith, and M. Stacey.

By methods similar to those we have already employed in the synthesis of *d*- and *l*-ascorbic acid we have now prepared synthetic specimens of analogues by ascending the series from arabinosone and galactosone. Each of the latter when placed in contact with potassium cyanide and calcium chloride in aqueous solution gives rise to a ψ-product analogous to ψ-ascorbic acid, and conversion into the true ascorbic acid type is effected by digestion with aqueous hydrochloric acid. These two new products may be described as 3-*keto*-l-*glucofuranolactone* and 3-*keto*-d-*galactoheptonofuranolactone* respectively. In view of the expanding nomenclature of substances of this type we propose, however, to describe for the time being the product from arabinosone as *arabo-ascorbic acid* and that from galactosone as *galacto-ascorbic acid*. These terms suggest more readily the relationship to ascorbic acid and also to the osones from which they are synthetically derived. The new products are being submitted to physiological tests.

We have also described improved methods for the preparation of gluco-ascorbic acid (3-keto-*d*-glucoheptonofuranolactone), the synthesis of which was outlined in our earlier paper (J., 1933, 1422). We have, however, to correct the signs given in the earlier paper of the rotation value which, by a typographical error, was expressed as positive instead

of negative. These should read $[\alpha]_{5780}^{18°}$ −14° (in water; *c*, 1), and −22° (in methyl alcohol) respectively.

The same substance has also been obtained by an alternative method. We submitted lactosone to the above reagents and obtained in solution ψ-lacto-ascorbic acid. Under the conditions we employed for the conversion of this substance into the true ascorbic acid type the galactose residue underwent scission and we isolated again the crystalline gluco-ascorbic acid. Similarly we have obtained the corresponding ψ-malto-ascorbic acid.

The properties of these synthetic analogues of ascorbic acid resemble very closely those of ascorbic acid itself. They readily undergo oxidation with aqueous iodine and have the reducing properties and colour reactions associated with the first member of the series. Details are given in the experimental section.

The absorption spectra of these substances have been measured in dilute and in concentrated solution by means of a Hilger Spekker Photometer. In view of the greater accuracy obtainable with this instrument, especially with solutions very liable to oxidation, the absorption spectra of *d*- and *l*-ascorbic acid have been redetermined. The new values of the molecular extinction coefficient are very little different from the approximate values we have already published (J., 1933, 1270). All the substances, excluding the ψ-compounds, show in dilute aqueous solution (2 g. per 100 c.c.) an intense band at λ265 mμ. In more concentrated aqueous solution the band is at λ240—245 mμ and the neutral sodium salts of the acids have a band at λ265 mμ. In all cases log ε is very close to 4·0.

A comparison of the rotation values shown by xylo-, arabo-, gluco-, and galacto-ascorbic acids indicates that in those cases where, as in the Fischer projection formula, the hydroxyl group attached to the fourth carbon atom is on the right, the sodium salt is much more dextrorotatory than the acid and *vice versa*.

The extraordinary rotational behaviour of *l*-arabo-ascorbic acid after oxidation with iodine runs parallel with that of *l*-ascorbic acid (J., 1933, 1270). In *l*-arabo-ascorbic acid the hydroxyl group is on the left and during the formation of the primary oxidation product the rotation moves in the negative direction. Mutarotation then proceeds and after 90 hours a constant small positive rotation is observed. On neutralisation the sodium salt of the oxidation product has a strong positive rotation.

From the properties of the above *l*-arabo-ascorbic acid it emerges that the synthetic product isolated by Maurer and Schiedt (*Ber.*, 1933, **66**, 1054) by interconversion of 2-keto-gluconic acid is actually the *d*-arabo-ascorbic acid. These authors ascribed to it the furan type of structure originally suggested by Micheel and Kraft (*Z. physiol. Chem.*, 1933, **215**, 215; compare Reichstein, Grüssner, and Oppenauer, *Helv. Chim. Acta*, 1933, **16**, 1019) for ascorbic acid, but this should now be brought into line with the formula we have assigned (J., 1933, 1270).

Experimental.

d-*Gluco-ascorbic Acid* (3-*Keto*-d-*glucoheptonofuranolactone*).—The following is an improved method for the preparation of this substance. *d*-Glucosone (1 g.) is allowed to react in oxygen-free aqueous solution with potassium cyanide (0·8 g.) and calcium chloride (1 g.). The reaction is complete in about 10 minutes. The calcium is removed as oxalate, and the solution made lightly acid with acetic acid and concentrated to small volume (diminished pressure; atmosphere of carbon dioxide). If any precipitate forms at this stage, a sample of the solid is titrated against acid iodine. If it shows no activity, it is discarded. If active, it is added to the precipitate obtained on the addition of alcohol to the aqueous portion. The solids, which contain 0·8 g. of active material, are dissolved in 8% ethyl-alcoholic hydrogen chloride (20 c.c.), to which some water (5 c.c.) is added. The mixture is warmed to 50°, filtered to remove inorganic impurities, and then kept at 50° for 24 hours. The reaction is complete when the head of the absorption band, originally at λ275 mμ, has become stationary at λ245 mμ. A slight excess of alcoholic lead acetate is now added and the precipitate (which must contain no active organic material) is separated as quickly as possible on the centrifuge (nitrogen atmosphere). Some water is added to the clear solution and the dissolved lead is removed as sulphide. The filtrate is concentrated at 35° to a thin syrup (diminished pressure; atmosphere of carbon dioxide). By the careful addition of acetone at 0° (about 200 c.c., added at intervals of 45 minutes in

4 portions of 50 c.c. each) inorganic impurities can be precipitated in a crystalline condition. The clear solution is concentrated to a syrup, which is dissolved in acetone containing a little water. On addition of ether the remaining inorganic impurities together with a little syrup of negligible activity are precipitated. The decanted colourless solution on evaporation gives a solid mass of crystals of gluco-ascorbic acid (0·5 g.). The product is usually analytically pure after being washed with dry acetone, but may be recrystallised if necessary from acetone–methyl alcohol–light petroleum. The crystals thus obtained (clusters of rods with pointed ends) consist of the monohydrate of *d*-gluco-ascorbic acid, m. p. 138° after sintering at 128°, $[\alpha]_D^{20°}$ −14° in water (*c*, 1·0 as hydrate); −22° in methyl alcohol (*c*, 1·0 as hydrate). The sodium salt of gluco-ascorbic acid had $[\alpha]_D^{20°}$ −80° in neutral aqueous solution (*c*, 0·75). Hydrated *d*-gluco-ascorbic acid (100 mg.) required for neutralisation 4·45 c.c. *N*/10-sodium hydroxide (calc., 4·47 c.c.) and in acid solution reacted with 8·9 c.c. *N*/10-iodine (calc., 8·9 c.c.) (Found : C, 37·2; H, 5·4. Calc. for $C_7H_{10}O_7,H_2O$: C, 37·5; H, 5·4%).

When the substance is heated below the m. p., the water of crystallisation is lost and the product, m. p. 191° (decomp.), previously described is obtained. *d*-Gluco-ascorbic acid possesses very strong reducing properties and in many ways closely resembles natural ascorbic acid. For example, after oxidation of a sample with acid iodine, neutralisation of the solution, and immediate re-acidification with acetic acid, the product reacted with phenylhydrazine (2 mols.), giving a *compound* which crystallised from alcohol in yellow needles, m. p. 222° (Found : C, 59·4; H, 5·2; N, 14·6. $C_{19}H_{20}O_5N_4$ requires C, 59·4; H, 5·2; N, 14·6%). This substance corresponds to the yellow osazone, m. p. 210°, obtainable under similar conditions from ascorbic acid.

l-*Arabo-ascorbic Acid* (3-*Keto*-l-*gluconofuranolactone*).—(1) *Preparation of* l-*arabinosazone*. To a solution of arabinose (10 g.) in water (200 c.c.) and glacial acetic acid (60 c.c.), phenylhydrazine (18 g.) was added. The solution was heated at 75° until a dark red colour developed. It was then cooled quickly, whereupon the osazone crystallised and the contents of the flask set to a semi-solid mass. After addition of a little water (20 c.c.) the mixture was cooled to −5° and filtered. The product was washed with dilute acetic acid and water until the washings were colourless. Yield, 12 g. Arabinosazone prepared in this way can be dried without decomposition. (2) *Arabinosone*. This can be prepared either by Fischer's method (*Ber.*, 1889, **22**, 87) or by treatment of arabinosazone (brought into solution in water by addition of sufficient alcohol and acetic acid; compare Reichstein, Grüssner, and Oppenauer, *loc. cit.*) with benzaldehyde at 90—95° (vigorous stirring; atmosphere of nitrogen). The precipitated benzaldehydephenylhydrazone was filtered off, and the solution extracted with ether, concentrated to small volume (600 c.c. for each 100 g. of osazone used), and again extracted with ether, the extraction being repeated until the ethereal layer remained colourless. The aqueous portion now contained about 8 g. of arabinosone for each 100 g. of original osazone. (3) *ψ*-l-*Arabo-ascorbic acid*. The above solution of arabinosone (8 g.) was neutralised with ammonia and treated with potassium cyanide (7 g.) and calcium chloride (9·5 g.). Titration of a sample indicated the formation of 7 g. of *ψ*-arabo-ascorbic acid (absorption band at λ275 mμ in acid or neutral solution). The *ψ*-arabo-ascorbic acid was converted into arabo-ascorbic acid (absorption band at λ245 mμ in acid solution) by the method already described for the transformation of *ψ*-ascorbic acid into ascorbic acid. In the present instance the conversion required 48 hours' treatment with 8% aqueous hydrochloric acid [active material (by iodine titration) at this stage, 5·0 g.]. The isolation of the arabo-ascorbic acid was carried out as follows. The hydrochloric acid was removed by addition of lead acetate (not basic) in slight excess. The lead chloride was collected on the centrifuge (nitrogen atmosphere), and the dissolved lead was removed as sulphide. The solution was then concentrated to a thin syrup under diminished pressure (atmosphere of carbon dioxide). Addition of alcohol served to precipitate most of the inorganic impurities. The solution was concentrated to half volume, and more inorganic material precipitated by the addition of a large volume of acetone. The decanted clear solution was concentrated at 30° under diminished pressure, the pale brown syrup (containing 4 g. of active material) dissolved in water (100 c.c., oxygen-free), and the solution extracted with ether. A saturated solution of basic lead acetate (about 5 c.c.) was then added until a faint permanent precipitate (chiefly lead chloride) remained. The filtered solution was treated with an excess of basic lead acetate until only a negligible amount of active material remained in solution (at this stage the solution was slightly alkaline). The curdy white precipitate was separated immediately on the centrifuge (nitrogen atmosphere essential). (If this operation is delayed the product is contaminated by an inactive precipitate which forms more slowly than the highly characteristic curdy precipitate just mentioned.) The lead complex

was suspended in water (oxygen-free), and the lead removed as sulphate. Any excess of sulphuric acid was removed by careful addition of a very slight excess of neutral lead acetate. After filtration the small quantity of lead still in solution was removed as sulphide. The solution was concentrated (atmosphere of carbon dioxide) and the treatment with acetone and ether described above was repeated. Evaporation of the final acetone–ether solution left *l-arabo-ascorbic acid* as a crystalline mass which gave, on recrystallisation from acetone–methyl alcohol–light petroleum, small prisms (2 g.), m. p. 168° (with evolution of gas; decomp. with blackening at 190°), $[\alpha]_D^{20°}$ + 19° in water (*c*, 0·9), + 17° in methyl alcohol (*c*, 0·6). Rotation of sodium salt, $[\alpha]_D^{20°}$ − 94° in neutral aqueous solution (*c*, 0·7). 100 Mg. of *l*-arabo-ascorbic acid required 5·65 c.c. *N*/10-sodium hydroxide for neutralisation (calc., 5·7 c.c.) and reacted with 11·4 c.c. *N*/10-iodine in acid solution (calc., 11·4 c.c.) (Found: C, 40·8; H, 4·7. $C_6H_8O_6$ requires C, 40·9; H, 4·6%).

The primary oxidation product of *l*-arabo-ascorbic acid showed $[\alpha]_D^{20°}$ − 100° (observed in presence of hydriodic acid immediately after oxidation with iodine): −92° (30 mins.); −85° (1 hr.); −78° (2 hrs.); −63° (5 hrs.); −56° (7 hrs.); −32° (18 hrs.); −23° (24 hrs.); −15° (30 hrs.); −1° (45 hrs.); + 4° (55 hrs.); + 12° (90 hrs., constant value). At this stage the solution on neutralisation with sodium hydroxide behaved as a free acid. $[\alpha]_D^{20°}$ of sodium salt + 70° (approx.) in aqueous solution immediately after neutralisation. The behaviour is analogous with that of *l*-ascorbic acid.

d-*Galacto-ascorbic Acid* (3-*Keto*-d-*galacto-heptonofuranolactone*).—*d*-Galactosazone was prepared by the method given under *l*-arabinosazone. From this, galactosone (in aqueous solution) was prepared, both by Fischer's method using hydrochloric acid and by the benzaldehyde method described above, the yield in the latter reaction being superior. The conversion of the *d*-galactosone into d-*galacto-ascorbic acid* was carried out by the method given for the preparation of *l*-arabo-ascorbic acid. Attention should be paid to the following particulars. The ψ-compound, which shows the usual absorption band at λ275 mμ, is converted into galacto-ascorbic acid by heating for 24 hours at 50° in 8% aqueous hydrochloric acid, the course of the reaction being followed spectrophotometrically. At the final stage of the isolation process a clear but viscid syrup is obtained. This crystallises readily on trituration with a small quantity of water, giving the monohydrate of *d*-galacto-ascorbic acid, m. p. 109° (with evolution of gas; blackening at 190°). This is usually pure, but may be recrystallised if necessary from acetone–methyl alcohol–light petroleum. The m. p. remains unaltered (yields: 33 g. of galactosazone gave 4 g. of the ψ-compound, from which 2·5 g. of galacto-ascorbic acid were obtained in solution. The final yield of crystalline material was 1·7 g.). The acid is soluble in water and methyl alcohol, less soluble in acetone and ethyl alcohol, and almost insoluble in ether and light petroleum. In water and methyl alcohol the substance showed no appreciable optical rotatory power. The sodium salt had $[\alpha]_D^{20°}$ − 77° in neutral aqueous solution (*c*, 0·7). 100 Mg. of the monohydrate required 4·45 c.c. *N*/10-sodium hydroxide for neutralisation (calc., 4·47 c.c.) and reacted with 8·8 c.c. *N*/10-iodine in acid solution (calc., 8·9 c.c.) (Found: C, 37·4; H, 5·4. $C_7H_{10}O_7,H_2O$ requires C, 37·5; H, 5·4%).

ψ-*Lacto-ascorbic Acid.*—An aqueous solution of lactosone was prepared from lactosazone by treatment of the latter with benzaldehyde (see above). The reaction between lactosone and potassium cyanide in the presence of calcium chloride took the normal course and from 80 g. of lactose the yield of ψ-lacto-ascorbic acid was 12 g. (estimated by titration with acid iodine and spectrophotometrically from the intensity of the absorption band at λ275 mμ). The calcium was precipitated quantitatively as oxalate, and the solution rendered slightly acid with acetic acid and concentrated. On addition of alcohol a crystalline precipitate was obtained, consisting of ψ-lacto-ascorbic acid, the ammonium and potassium salts, together with other impurities. Considerable purification was effected by fractional precipitation from aqueous alcohol, but owing to the unfavourable solubilities of both the free ψ-lacto-ascorbic acid and the salts it has not been possible up to the present to obtain a product completely free from mineral impurities. ψ-Lacto-ascorbic acid behaves similarly to the ψ-compounds mentioned above. It is very strongly reducing, reacts with two atomic proportions of iodine in acid solution, and has an intense absorption band at λ275 mμ in acid solution. Neutral aqueous solutions show a similar band at the same wave-length. On treatment of the ψ-compound with 8% aqueous hydrochloric acid at 50° hydrolysis of the glucosidic biose link occurred simultaneously with the transformation of the ψ-compound. The crystalline product, which was isolated by the method described under galacto-ascorbic acid, was the monohydrate of *d*-gluco-ascorbic acid, m. p. 138°, alone or when mixed with an authentic sample (100 mg. required 4·47 c.c. *N*/10-iodine in acid solution. Calc., 4·47 c.c.). Yield, 2 g.

from 50 g. of lactose. Owing to the high yields of lactosazone and lactosone obtainable from lactose, this method compares favourably with the direct method already described for the production of *d*-gluco-ascorbic acid.

Similar experiments with maltosone (prepared in aqueous solution by Fischer and Armstrong's method, *Ber.*, 1902, **35**, 3141) gave quantitative yields of ψ-malto-ascorbic acid (absorption band in acid solution at 275 mμ). This substance reacted with acid iodine (2 atomic proportions) in the usual way (yield, 20 g. from 100 g. of maltose). The chemical and physical properties and molecular structure of the ψ-acids are now being investigated in detail.

Absorption Spectra of Ascorbic Acid and of Analogues of Ascorbic Acid.—Extremely dilute solutions (*c*, 0·002) of these substances are unstable (decomposition by oxidation) and the use of the Spekker photometer is particularly advantageous in that a complete record of the absorption spectrum is obtainable within a few seconds after dissolution of the substance. We have re-determined the absorption spectra of ascorbic acid under various conditions with this instrument. The characteristic shape of the absorption band and the value of λ at the head of the band remain as before, but we now find that, for solutions of ascorbic acid containing 2 mg. per 100 c.c. measured in a 1 cm. cell, the value of log I_0/I is 1·0—1·05, equally for alcoholic, aqueous, or acidified aqueous solutions of ascorbic acid. The value of log I_0/I for neutral aqueous solution of the sodium salt of the same concentration is 1·10 (the values previously given for log I_0/I, measured with a sector photometer, ranged from 0·9 to 0·95). The various measurements are summarised in the following table.

Substance.	Solvent.	*c* (mg. per 100 c.c.).	λ at head of band (mμ).	Log I_0/I (*l*, 1 cm.).	Log ε.
l-Ascorbic acid	Water	2·0	265	1·0	3·97
l-Ascorbic acid	Water (containing a little mineral acid)	2·0	245	1·10	3·98
l-Ascorbic acid	Alcohol	2·0	245	1·10	3·98
Sodium salt of *l*-ascorbic acid	Water	2·28	265	1·25	4·04
d-Ascorbic acid	Water (containing a little mineral acid)	2·61	245	1·4	3·98
Sodium salt of *d*-ascorbic acid	Water	2·76	265	1·4	4·0
d-Gluco-ascorbic acid (monohydrate)	Water	2·24	265	0·85	3·93
d-Gluco-ascorbic acid (monohydrate)	Water (containing a little mineral acid)	2·83	245	1·15	3·94
Sodium salt of *d*-gluco-ascorbic acid	Water	2·22	265	0·95	3·99
d-Galacto-ascorbic acid (monohydrate)	Water	2·9	265	1·05	3·90
d-Galacto-ascorbic acid (monohydrate)	Water (containing a little mineral acid)	2·44	240—245	0·85	3·90
Sodium salt of *d*-galacto-ascorbic acid	Water	2·96	265	1·15	3·93
l Arabo-ascorbic acid	Water	2·1	265	1·05	3·94
l-Arabo-ascorbic acid	Water (containing a little mineral acid)	2·1	245	1·1	3·96
Sodium salt of *l*-arabo-ascorbic acid	Water	2·36	265	1·15	3·96

In more concentrated aqueous solutions (1000 mg. per 100 c.c.) *d*-ascorbic acid, *l*-ascorbic acid, *d*-gluco-ascorbic acid, *d*-galacto-ascorbic acid, and *l*-arabo-ascorbic acid show absorption bands of the same shape and approximately the same intensities as those referred to above and in each case the head of the band is at λ240—245 mμ.

Addendum to Synthesis of d- *and* l-*Ascorbic Acid* (see J., 1933, 1421).—During the synthesis of *d*- or *l*-ascorbic acid, after treatment of the ψ-ascorbic acid with 8% hydrochloric acid, the solution, which now contained ascorbic acid, was oxidised with the exact equivalent of iodine. The characteristic yellow phenylhydrazine derivative, m. p. 210° (decomp.), was then prepared by the method previously described (J., 1933, 1283).

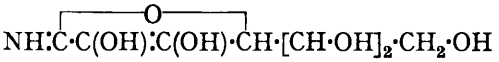
Crystallographic, including *X*-ray, examination by Mr. E. G. Cox proved that the *d*- and the *l*-compound so isolated were identical in structure with the corresponding derivative obtained from natural ascorbic acid.

Our thanks are expressed to Imperial Chemical Industries Limited for a grant.

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, November 29th*, 1933.]

(*Addendum, January 11th*, 1934.) In elucidation of the nature of the above ψ-products we have found that, whilst under our experimental conditions the addition of hydrogen

cyanide to the osone occurs almost instantaneously and is accompanied by evolution of ammonia, yet the hydrolysis to a nitrogen-free compound does not proceed to completion at this stage. For instance, during the synthesis of the gluco-ascorbic acid we have been able to isolate a crystalline intermediate compound, $C_7H_{11}O_6N$, and find that it is this compound which contributes the absorption band at 275 mμ. In view of the chemical properties of this crystalline intermediate product we believe its formula is best expressed by the cyclic structure :



It is transformed into the true gluco-ascorbic acid by hydrolysis even with 20% aqueous acetic acid at 80°.