332. *Synthesis of* d- *and of* l-*Ascorbic Acid and of Analogous Substances.*

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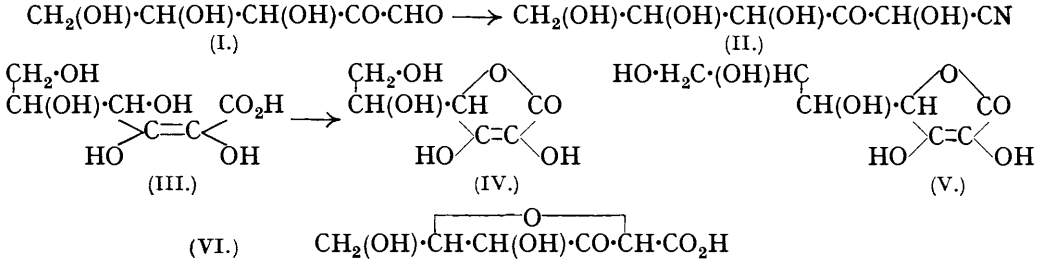
The co-operation of the Birmingham Chemical Laboratories with Professor Szent Györgyi of the University of Szeged, Hungary, has resulted in the publication of the detailed proof of the constitution of ascorbic acid by Herbert, Hirst, Percival, Reynolds, and Smith (this

vol., p. 1270) and we are greatly indebted to Professor Szent Györgyi and his collaborators for the facilities provided in the way of materials. The constitutional formula which is now generally accepted was first proposed by E. L. Hirst (*J. Soc. Chem. Ind.*, 1933, **52**, 221; March 10th). The analytical work of Hirst and his co-workers had revealed the groups associated with each carbon atom, and in the latter communication the new lactone ring structure was advanced for the first time. We then embarked upon a series of experiments directed to the synthetic preparation of both *d*- and *l*-ascorbic acid from *d*- and *l*-xylosone. A preliminary account of these syntheses has already been communicated (Haworth and Hirst, *J. Soc. Chem. Ind.*, 1933, **52**, 645; Aug. 4th): the complete account, including also the synthesis of an analogue of ascorbic acid from glucosone, is now submitted (compare Reichstein, Grüssner, and Oppenauer, *Nature*, 1933, Aug. 19th, p. 280).

The provision of the initial materials, namely, the *d*- and *l*-forms of xylosone, in sufficient quantity for the projected syntheses has involved the labour of a team of workers over many months. The chief losses in the yield of the final product occurred during these stages leading to the preparation of the xylosone. Thereafter, by the methods herein described, it has been found possible to isolate the *d*- and *l*-ascorbic acid in a yield of upwards of 70% of the theoretical. We have found that the best conditions to attain this end are to ascend the series from xylosone (I) to the β-keto-nitrile (II) by placing xylosone in contact with potassium cyanide and calcium chloride. The reaction involving the addition of hydrogen cyanide proceeds to completion in the course of a few minutes with the almost simultaneous hydrolysis to the β-keto-acid (III) and the instantaneous elimination of ammonia. The product (III) can exist in many forms, both enolic and keto, and is also capable of lactonisation. This intermediate product (III), although capable of reducing acid iodine solution, does not possess the properties of ascorbic acid and we have for reasons indicated below designated it *ψ*-ascorbic acid. It exhibits a different absorption spectrum from that of ascorbic acid, and gives rise to a different osazone. Its conversion into ascorbic acid proceeds quantitatively in the presence of 8% aqueous hydrochloric acid at 40—50°. The isolation of the *d*- and *l*-forms of ascorbic acid from the preparation in which either enantiomorph of xylosone has been employed is effected by the removal of such mineral salts as are formed in the process and the product in either case is isolated in good yield and with considerable ease as a highly pure crystalline specimen.



The formulation of natural ascorbic acid (which is the *l*-variety) by the constitutional formula (IV) (Hirst and co-workers, *loc. cit.*) receives strong experimental support from the synthesis which is now communicated.

By an analogous procedure glucosone has given rise to a crystalline product having properties which are almost identical with those of ascorbic acid. Its constitution is represented by (V) on the analogy of the structure which has been determined for ascorbic acid. It may provisionally be claimed to be 3-*keto-glucoheptonofuranolactone*. This structure is dependent upon the similarity in properties to ascorbic acid and the similar procedure involved in its synthesis.

All these synthetic products are being submitted to physiological tests.

A constitutional formula (VI) which we had earlier considered and rejected was at one time advanced by Micheel (*Nature*, 1933, **131**, 274). He has since abandoned this formula in favour of that proposed by Hirst and his co-workers (*loc. cit.*). But in the interim a communication by Reichstein, Grüssner, and Oppenauer (*Helv. Chim. Acta*, 1933, **16**,

561) appeared to give support to this formulation. As was pointed out by one of us (Haworth, *J. Soc. Chem. Ind.*, 1933, **52**, 482), an erroneous interpretation was given to the mechanism of the reaction involved in the synthesis of the *d*-enantiomorph of acetone ascorbic acid, and the Swiss authors have now accepted the interpretation which supports the constitution (IV) for ascorbic acid (private communication from Dr. Reichstein).

Experimental.

Preparation of d- *and* l-*Xylosone.*—As these preparations have not been described in the literature, the following directions are given (compare Fischer, *Ber.*, 1889, **22**, 87).

Finely-powdered dry *d*- or *l*-xylosazone (10 g.) was mixed with 90 c.c. of concentrated hydrochloric acid at 15°, and warmed quickly to 40° (vigorous shaking) until a clear dark red solution was obtained. This was immediately cooled to 25°, kept at this temperature for 10 minutes, and then cooled in a freezing mixture (ice and salt) for 15 minutes. The crystalline phenylhydrazine hydrochloride which separated was removed by filtration through glass wool and washed with a little concentrated hydrochloric acid. The filtrate was diluted with water (1 l.), neutralised with lead carbonate, and filtered. The pale yellow filtrate was cooled to 0° and made just alkaline with barium hydroxide. The lead hydroxide complex of xylosone which was precipitated as a pale yellow compound was washed twice by decantation and collected on a centrifuge. The lead compound was stirred into 60 c.c. of water containing 2·5 g. of sulphuric acid. The excess of acid was neutralised with barium carbonate, charcoal added, and the liquid filtered. The amount of xylosone in solution (estimated as xylosazone) was 0·35 g.

Synthesis of d- *and* l-*ψ-Ascorbic Acid.*—Xylosone (2 g.) in water (350 c.c.) was mixed with an aqueous solution containing calcium chloride (1·6 g.) and potassium cyanide (1·2 g.). A gentle stream of oxygen-free nitrogen was passed continuously through the liquid. There was an immediate evolution of ammonia and the course of the reaction was followed by titration of test samples with *N*/50-iodine in acid solution. The reaction was complete in 20 minutes. The calcium was precipitated with the equivalent of oxalic acid. A few drops of acetic acid were added to ensure that the filtrate was acid. The solution was concentrated at 35° under diminished pressure in an atmosphere of carbon dioxide to a thick syrup containing, as estimated by iodine titration, 1·8 g. of ψ-ascorbic acid. The syrup was extracted with ethyl alcohol, and inorganic material removed by filtration.

The absorption spectrum of the neutral syrup in aqueous solution showed a band at 275 mμ which did not change on acidification (contrast with ascorbic acid). ψ-Ascorbic acid was considerably less stable than ascorbic acid, but was convertible into the latter as indicated below.

A portion of the syrup (0·1 g.) was oxidised with iodine in acid solution, neutralised, then made acid again with acetic acid, and treated at 100° with phenylhydrazine. A yellow crystalline osazone (0·1 g.) was formed, m.p. 210°. *X*-Ray crystallographic examination by Mr. E. G. Cox revealed that it was not identical with the corresponding osazone from ascorbic acid.

Synthesis of d- *and* l-*Ascorbic Acid.*—The syrupy ψ-ascorbic acid (containing 1·8 g. of active material as estimated by the iodine titration) was dissolved in 20 c.c. of 8% hydrochloric acid. This solution was digested for 26 hours at 45—50° in an atmosphere of carbon dioxide. Its absorption spectrum then showed a band at 245 mμ in aqueous acid solution, moving to 265 mμ on formation of the sodium salt. The intensity of the band indicated a concentration of ascorbic acid identical with that estimated by a titration with acid iodine (compare Herbert, Hirst, Percival, Reynolds, and Smith, *loc. cit.*).

The solution was diluted with oxygen-free water, and most, but not quite all, of the hydrochloric acid removed by the addition of lead acetate (not basic). It is essential that the solution should remain acid at this stage. The lead was collected as sulphide, and the pale yellow solution concentrated to a thick syrup under diminished pressure at 35°. The syrup was extracted with dry ethyl alcohol and on the careful addition of dry ether to the alcoholic solution a considerable amount of inorganic material was precipitated. This operation was twice repeated. Evaporation of the solvents yielded a syrup which still contained inorganic impurity, although the whole was completely soluble in a little acetone. Addition of a large excess of dry acetone precipitated more of the mineral impurity. Ether was now added to the acetone solution until a permanent turbidity was observed and the solution was kept at 0° for 3 hours; a little syrup then separated. The decanted solution was concentrated at 30° in an atmosphere of carbon dioxide and gave a colourless crystalline mass of ascorbic acid (1·2 g.), which was pure after being washed with acetone. M.p. 190°. Its absorption spectra in aqueous acid and in neutral solution (*c*, 0·002)

were identical with those of natural ascorbic acid (bands at 245 and at 265 mμ respectively; ε, 7500).

The above process represents the experiments carried out for the synthesis of both the *d*- and the *l*-form of ascorbic acid. The physical properties and analytical data shown by each enantiomorph are as follows :

d-*Ascorbic acid* (synthetic) (Found : C, 40·8; H, 4·9. Calc. for $C_6H_8O_6$: C, 40·9; H, 4·6%). M.p. 190°. $[\alpha]_D^{18°}$ −48°. $[\alpha]_{5780}^{18°}$ −50° (in methyl alcohol; *c*, 0·75), $[\alpha]_{5780}^{18°}$ −24° (in water; *c*, 1).

l-*Ascorbic acid* (natural) (Found : C, 41·0; H, 4·7%). M. p. 190°. $[\alpha]_D^{18°}$ +49°. $[\alpha]_{5780}$ +50° (in methyl alcohol; *c*, 1·0), $[\alpha]_{5780}$ +24° (in water; *c*, 1).

l-*Ascorbic acid* (synthetic), m. p. 190° alone or when mixed with natural *l*-ascorbic acid, was in respect of all its properties indistinguishable from the natural product; its *X*-ray diagram was identical with that of the natural substance, and it had the same crystalline habit and highly characteristic refractive indices (E. G. Cox).

Synthesis of an Analogue of Ascorbic (*from Acid Glucosone*). 3-*Keto*-d-*glucoheptonofurano-lactone*.—A solution of glucosone (3·5 g.) in 300 c.c. of water was mixed with a solution of calcium chloride (3·2 g.) and potassium cyanide (2·4 g.) in 50 c.c. of boiled-out water. A stream of nitrogen (oxygen-free) was passed through the liquid with the immediate evolution of ammonia. Test samples of the product were titrated with *N*/50-acid iodine, and the reaction was complete in about 10 minutes. By iodine titration the amount of ψ-3-keto-*d*-glucoheptonofuranolactone was estimated to be 3·0 g. An equivalent amount of oxalic acid was now added to remove the calcium, a few drops of acetic acid being added to the filtrate to maintain acidity. This was then concentrated to 20 c.c. in a current of carbon dioxide. A considerable volume of alcohol was now added and after keeping over-night a cream-coloured crystalline powder separated. This consisted chiefly of the ammonium and potassium salts of the ψ-acid, showing an absorption band at 275 mμ which did not change in acid solution. After acidification of this salt and oxidation with iodine it gave rise to a yellow osazone, m. p. 215°, which will be described later. The isolation of 3-*keto*-d-*glucoheptonofuranolactone* from the salt of the ψ-compound was carried out as follows : The crystalline salt was dissolved in 40 c.c. of 8% hydrochloric acid and digested at 50° for 24 hours. The solution now showed absorption bands at 245 mμ in acid and 265 mμ in neutral solution. The hydrochloric acid was removed by the addition of lead acetate (not basic) and the lead in solution was precipitated as the sulphide, the pale yellow filtrate being concentrated under diminished pressure at 30°. At this stage also ammonium chloride was collected. Much of the inorganic matter was eliminated by alcohol–ether precipitation and the product was finally purified by trituration of the syrup with a large volume of dry acetone. Removal of the acetone yielded a crystalline mass, which was recrystallised by solution in acetone containing a few drops of ethyl alcohol and light petroleum. The crystals were larger than those of ascorbic acid and consisted of clusters of rods with pointed ends, m. p. 191° (Found : C, 41·0; H, 4·7. $C_7H_{10}O_7$ requires C, 40·8; H, 4·85%). The yield was 2·5 g. $[\alpha]_{5780}^{18°}$ + 14° (in water; *c*, 1), + 22° (in methyl alcohol; *c*, 1). Iodine titration : 0·052 g. required 5·2 c.c. *N*/10-iodine.

Preparation of l-*Xylosephenylosazone*.—The initial material for this preparation was *d*-galacturonic acid. This is obtainable either by the method already described in the literature from citrus pectic acid (Link and Nedden, *J. Biol. Chem.*, 1931, **94**, 307) or from the diacetone galacturonic acid prepared by Ohle and Berend (*Ber.*, 1925, **58**, 2585). The following series of transformations is then required for the conversion of *d*-galacturonic acid into *l*-galactonic acid, lactone, *l*-galactonamide, *l*-lyxose, *l*-xylosephenylosazone.

Reduction of potassium galacturonate to l-*galactonic acid*. The potassium salt (11 g.) of *d*-galacturonic acid in 250 c.c. of water was vigorously stirred at 15° with 480 g. of freshly prepared sodium amalgam (2·5%), the latter being added in 50 g. lots over a period of 5—6 hours. The alkaline solution was carefully neutralised with 50% sulphuric acid before each fresh addition of sodium amalgam. After keeping over-night, the solution showed no reducing action towards Fehling's solution. The mercury was separated, and the solution neutralised with 50% sulphuric acid and then filtered (charcoal). It was concentrated under diminished pressure to a thick syrup, which was poured into 1500 c.c. of 95% alcohol maintained at 65°. The precipitated sodium sulphate was extracted with hot alcohol and the filtrate and extracts were concentrated to 50 c.c., and any slight excess of sulphuric acid neutralised with barium carbonate. After filtration and complete evaporation the syrup was heated for 2 hours at 70° to complete the lactonisation. A portion of the lactone was crystallised for the purpose of identification, but the remainder was utilised as syrup, dissolved in dry methyl alcohol, and converted into *l*-galactonamide as follows :

Conversion of galactonolactone into l-*galactonamide.* When the methyl-alcoholic solution of the lactone was saturated at 0° with dry ammonia, there was an immediate precipitation of the amide. This was kept for several hours, collected, and recrystallised from ethyl alcohol–water, forming fine rods with pointed ends, m. p. 170°, $[\alpha]^{18°}_{5780}$ — 28° (in water; *c*, 1·0).

Conversion of l-*galactonamide into* l-*lyxose.* The method was that already described for the *d*-series by Weerman (*Rec. trav. chim.*, 1917, **36**, 16; compare Haworth and Hirst, J., 1928, 1221). The lyxose was not isolated but was converted immediately by contact with phenylhydrazine into *l*-xylosephenylosazone, m.p. 158—160°. Yield from 10 g. of *l*-galactonamide, 4 g. This was purified by repeated crystallisation, the losses being about 50%.

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