122. *Gluco-ascorbic Acid.*

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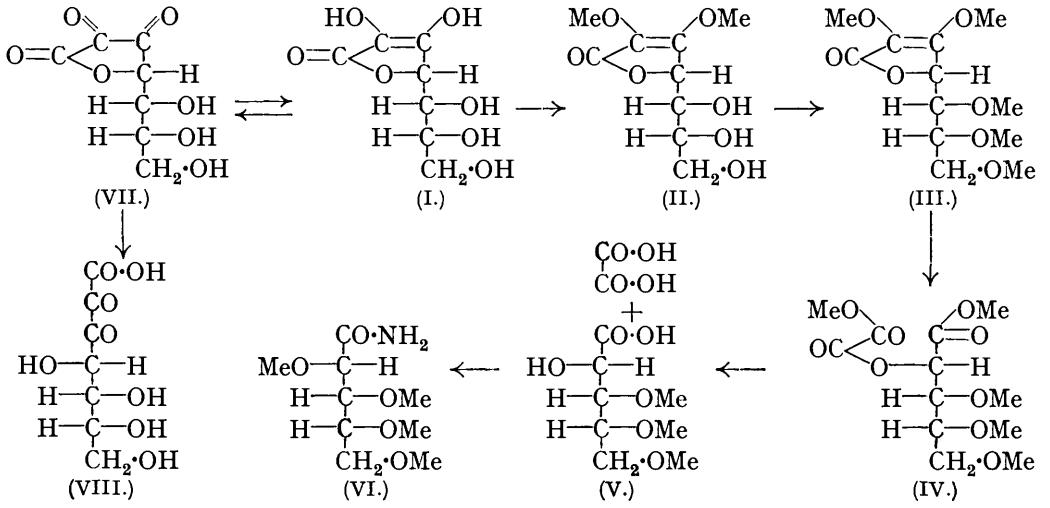
The constitution of *l*-ascorbic acid (vitamin C) as the enolic form of 2-keto-*l*-gulofurano-lactone has been definitely established by ozonisation of its tetramethyl ether and identification of the oxidation product (Herbert, Hirst, Percival, Reynolds, and Smith, J., 1933, 1270). It was shown that a γ-lactone ring is present in *l*-ascorbic acid and by analogy the same ring system has been assigned to the numerous analogues of ascorbic acid which have been obtained synthetically. Although this assumption is in full agreement with the properties of the various analogues, the possibility that ring systems of the pyranose type also may occur in the ascorbic acid series has to be kept in mind and in a subsequent paper it will be shown that such structures do in fact exist in the series. It is necessary, therefore, to establish by definite chemical transformations the nature of the ring system present in any particular analogue and this paper is concerned with the constitution and properties of *d*-gluco-ascorbic acid. Proof is now given of the correctness of the structure (I) provisionally assigned to this substance in earlier communications.

The different stages of the investigation may be summarised as follows. Improved methods are given for the preparation of *d*-gluco-ascorbic acid from *d*-glucosone *via* the imino-glucoascorbic acid obtained by addition of hydrogen cyanide to the osone (Haworth, Hirst, Jones, and Smith, J., 1934, 1192). It is shown that *d*-gluco-ascorbic acid reacts with diazomethane, giving successively the 3-*monomethyl* ether and the 2 : 3-*dimethyl* ether (II), the mode of formation and the properties of both derivatives being such as to make it certain that no structural change occurs during their formation from *d*-gluco-ascorbic acid. The remaining hydroxyl groups can be methylated without opening of the lactone ring and the product is the fully substituted 2 : 3 : 5 : 6 : 7-*pentamethyl* d-*gluco-ascorbic acid* (III). The latter substance reacts with ozone, giving a neutral ester (IV) which on hydrolysis gives oxalic acid and 3 : 4 : 5-*trimethyl arabonic acid* (V). The identity of the last substance was proved in the following way. It was a derivative of *d*-arabonic acid, because methylation of the corresponding ester (*methyl* 3 : 4 : 5-*trimethyl arabonate*) gave *methyl* 2 : 3 : 4 : 5-*tetramethyl* d-*arabonate*, recognised as the crystalline *amide* (VI), which was compared with its enantiomorph (2 : 3 : 4 : 5-*tetramethyl* l-*arabonamide*), prepared specially for this work by methylation of *l*-arabonic acid. Since the *trimethyl* d-*arabonamide* obtained from *d*-gluco-ascorbic acid gave a strong positive Weerman reaction with sodium hypochlorite, it contained a hydroxyl group attached to C_2. It follows that the three methyl groups must of necessity be attached to C_3, C_4, and C_5, and the identity of the amide is thereby established.

The location of the hydroxyl group at C_4 in the trimethyl *d*-arabonamide isolated from methylated gluco-ascorbic acid fixes the point of attachment of the ring in gluco-ascorbic acid and shows that this analogue of ascorbic acid is a γ-lactone (I). The various transformations which furnish the proof of structure are summarised in the scheme on p. 550.

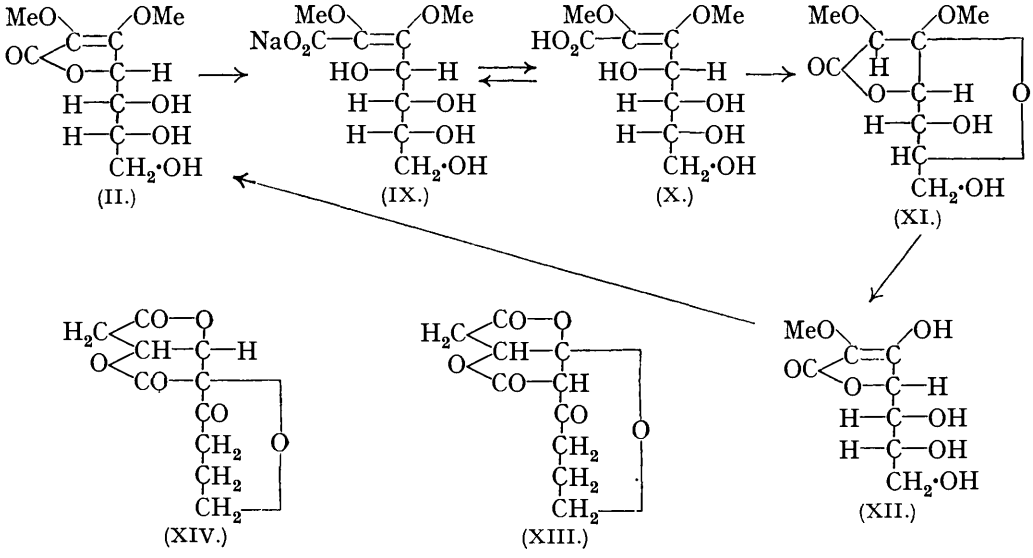
It follows that a γ-lactonic structure must apply also to the enantiomorph of *d*-gluco-ascorbic acid, *l*-gluco-ascorbic acid, the preparation of which from *l*-arabinose is described in the experimental section. The *d*-form has no antiscorbutic activity, but the *l*-form is

slightly active (Reichstein, *Nature*, 1934, **134**, 724; Zilva, *Biochem. J.*, 1935, **29**, 1612). The active isomeride has one feature in common with all the other analogues of ascorbic acid which show antiscorbutic powers, namely, that the lactone ring is on the right when the formula is written in accordance with the Fischer convention.



Further details of the properties of the substances referred to above are recorded in the experimental section. These require no further comment except in the case of 2 : 3-dimethyl gluco-ascorbic acid, the behaviour of which is anomalous in certain respects. The dimethyl derivative does not react with alcoholic ammonia and no derivative can be obtained corresponding to the amide readily formed from dimethyl ascorbic acid. The reaction of 2 : 3-dimethyl gluco-ascorbic acid with alkali proceeds normally with opening of the lactone ring and salt formation (IX), and the open-chain derivative possesses no selective absorption. On acidification, an acid (X) is temporarily produced, but if its isolation is attempted, lactonisation occurs and a substance is obtained with properties similar to those of the *iso*dimethyl ascorbic acid which is formed from dimethyl ascorbic acid under similar experimental conditions (Micheel, *Annalen*, 1935, **519**, 70; **525**, 66). This lactone (iso*dimethyl gluco-ascorbic acid*) is a structural isomeride of dimethyl gluco-ascorbic acid. It shows no selective absorption and differs in rotation and chemical properties from true dimethyl gluco-ascorbic acid. It has been converted into the latter substance in two ways. In the first, the isomeride is kept for some hours in acetone containing sulphuric acid. Rearrangement of the structure is thereby brought about and crystalline 2 : 3-dimethyl gluco-ascorbic acid is regenerated. This process gives no evidence as to the nature of the change involved, but information on this point is provided by the second mode of transformation. When *iso*dimethyl gluco-ascorbic acid is boiled with methyl-alcoholic hydrogen chloride, a strong absorption band at λ 2400 A. gradually makes its appearance and the product on isolation is a monomethyl gluco-ascorbic acid which does not give a blue colour with ferric chloride. It is not 3-methyl gluco-ascorbic acid (the properties of which are fully described below), but the absorption spectrum and the fact that it reacts smoothly with diazomethane, giving 2 : 3-dimethyl gluco-ascorbic acid, indicate that the ring structure of the ordinary form of gluco-ascorbic acid is present. The substance must, therefore, be 2-*methyl gluco-ascorbic acid* (XII). It follows that the methyl group at C_3 is the one involved in the reaction with methyl-alcoholic hydrogen chloride and the series of reactions can best be explained by ascribing the structure (XI) to *iso*dimethyl gluco-ascorbic acid. This differs from (II) in that the hydroxyl group on C_6 has saturated the double bond by the attaching of the hydrogen atom to C_2 and the oxygen to C_3. The methoxyl group at C_3 is then glucosidic in type and its removal during intramolecular rearrangement becomes explicable. This remarkable circumstance leads to the conclusion that the new ring formation is equivalent to the ring closure of a ketose

to give a furanoside. The work of Micheel and our own experiments on dimethyl ascorbic acid show clearly that it is the hydroxyl on C_6 in that substance which is involved in addition at the double bond, and for this reason we have provisionally formulated the second ring in (XI) as five-membered, the ring junctions being at C_3 and C_6. It is possible on stereochemical grounds for a six-membered ring to be formed involving the saturation of the double bond by the primary hydroxyl on C_7, but in view of the close analogy with dimethyl ascorbic acid we are inclined to prefer the structure (XI). An example of this type of isomerisation, which involves simultaneous lactonisation and ring formation by saturation of a double bond, may be held to explain the type of ring closure which occurs when hydrated carlic acid gives rise to carlic acid (Clutterbuck, Raistrick, and Reuter, *Biochem. J.*, 1935, **29**, 871). On this view the formulation of carlic acid would be that shown in (XIV). This structure contains a six-membered ring formed by saturation of the double bond by the hydroxyl group. A possible alternative is given in (XIII), which, however, involves the formation of a seven-membered ring by saturation of the double bond. Clutterbuck, Raistrick, and Reuter (*loc. cit.*) suggested (XIII) as the probable formula for carlic acid, but in view of the work now described it would appear that (XIV) is at least equally probable.



Experimental.

d-*Gluco-ascorbic Acid.*—Imino-*d*-gluco-ascorbic acid was prepared by an improved method from glucosone (compare J., 1934, 1194). Glucosazone (100 g.) was decomposed with concentrated hydrochloric acid (900 c.c.) and filtered from phenylhydrazine hydrochloride. Hydrochloric acid was removed from the filtrate by addition of lead carbonate, and the solution filtered and extracted three times with ether. Potassium cyanide (16 g.) was then added to the clear yellow aqueous solution. The reaction was complete in 1 hour. The solution was centrifuged, exactly neutralised with hydrochloric acid, and concentrated under reduced pressure at 35° to 200 c.c. Crystallisation of the imino-gluco-ascorbic acid was complete in 12 hours; it was filtered off and washed with water, acetone, and ether. Yield, 15—20%. Imino-*d*-gluco-ascorbic acid (6·9 g.), dissolved in *N*-hydrochloric acid (70 c.c.), was kept at room temperature for a week until the absorption band at λ 2750 A. had disappeared and had been replaced by a band at λ 2450 A. Lead acetate solution was added until the reaction to Congo-red was faintly acid. After filtration, dissolved lead was removed as sulphide and the solution was concentrated to 10 c.c. by evaporation at 35° under carbon dioxide (pressure 15 cm.). Some *d*-gluco-ascorbic acid separated (4·8 g.) and the mother-liquors were further concentrated and the residual syrup was dried by distilling through it dry methyl alcohol. The ammonium chloride which separated was filtered off and the solution on evaporation deposited a further

1·4 g. of *d*-gluco-ascorbic acid. This substance after recrystallisation from methyl alcohol, acetone, and light petroleum still contained one molecule of water of crystallisation and had m. p. 140°; $[\alpha]_D^{20°}$ − 22° in water (*c* = 1) (Found: C, 37·2; H, 5·4. Calc. for $C_7H_{10}O_7,H_2O$: C, 37·4; H, 5·4%. 0·052 G. required 5·1 c.c. of *N*/10-iodine when titrated in acid solution). The details of the absorption spectrum and rotatory dispersion have been recorded previously. When the acid was kept in a vacuum desiccator over calcium chloride, the water of crystallisation was slowly given up and the anhydrous substance was obtained.

d-Gluco-ascorbic acid requires one molecular proportion of alkali for neutralisation (100 mg. required 4·45 c.c. of *N*/10-sodium hydroxide. Calc., 4·47 c.c.). The sodium salt had $[\alpha]_D^{20°}$ − 80° in water (*c* = 1·0) (calculated as gluco-ascorbic acid). On acidification, the rotation immediately changes back to − 22°.

d-Gluco-ascorbic acid is oxidised by an aqueous solution of iodine or chlorine (2 atomic proportions: 52 mg. require 5·1 c.c. of *N*/10-iodine), giving a neutral substance (VII) which reacts as a lactone. On titration of the solution containing the oxidation product, two equivalents of alkali are taken up by the hydriodic acid formed during the oxidation, after which the oxidation product takes up one equivalent of alkali slowly and in the manner of a lactone. The primary oxidation product shows no selective absorption, and has $[\alpha]_D$ − 31° (initial value), changing slowly in acid or neutral solution to the equilibrium value $[\alpha]_D$ + 13°. The substance (VII) having $[\alpha]_D$ − 31° can be reduced to *d*-gluco-ascorbic acid by hydrogen sulphide as shown by reappearance of the absorption band and by isolation of the crystalline product, m. p. 138°. After mutarotation has taken place, the acid formed (VIII) is not reducible by hydrogen sulphide to gluco-ascorbic acid. The neutral sodium salt formed from the primary oxidation product has $[\alpha]_D$ + 27° (*c* 1·0, calc. on weight of gluco-ascorbic acid), but on addition of excess of alkali this value rises to + 120° and then rapidly falls (decomposition). The primary oxidation product reduces Fehling's solution readily and is rapidly attacked by permanganate. When oxidised by sodium hypoiodite in alkaline solution, it gives oxalic acid (yield, 80% of the theoretical). The primary oxidation product (both before and after mutarotation has taken place) reacts with phenylhydrazine, giving the yellow substance, m. p. 225° (decomp.) (see J., 1934, 64), which by analogy with the corresponding derivative of *l*-ascorbic acid probably has the structure of a pyrazolone (Ohle, *Ber.*, 1934, **67**, 1750; see *Ann. Reports*, 1934, 182). A red *substance*, m. p. 215°, probably structurally a true osazone, is obtainable from *d*-gluco-ascorbic acid by the action of phenylhydrazine in dilute acetic acid (Found: N, 14·6. $C_{19}H_{20}O_5N_4$ requires N, 14·6%).

l-*Gluco-ascorbic Acid.*—*l*-Gluconolactone was prepared from *l*-arabinose (compare Hudson, Hartley, and Purves, *J. Amer. Chem. Soc.*, 1934, **56**, 1248). The lactone was reduced by sodium amalgam by the standard procedure and the aqueous solution of *l*-glucose so obtained was treated with phenylhydrazine and dilute acetic acid at 70° for 4 hours. *l*-Glucosazone separated and was washed successively with 2% acetic acid, water, benzene, and methylated spirit. The bright yellow crystals obtained had m. p. 206° and were of the high standard of purity required in subsequent operations. *l*-Glucosazone was then converted into *imino*-l-*gluco-ascorbic acid* by the method given above for *d*-glucosazone. Imino-*l*-gluco-ascorbic acid had $[\alpha]_{5780}^{14°}$ + 148° in water (*c*, 1·0); it began to darken at about 215°, and in aqueous solution it showed an intense absorption band at λ 2750 A. (ε, 17,000) (Found: C, 41·0; H, 5·5; N, 6·7. $C_7H_{11}O_6N$ requires C, 40·9; H, 5·4; N, 6·8%). Its chemical properties were identical with those observed for the *d*-enantiomorph and on digestion with *N*-hydrochloric acid at room temperature for 7 days it was quantitatively transformed into *l*-gluco-ascorbic acid (for details of purification, see above), m. p. (hydrate) 140°, $[\alpha]_D^{22°}$ + 24° in water (*c*, 0·7) as hydrate (Found for the anhydrous substance: C, 40·5; H, 4·7. Calc. for $C_7H_{10}O_7$: C, 40·8; H, 4·9%).

3-*Methyl* d-*Gluco-ascorbic Acid.*—*d*-Gluco-ascorbic acid monohydrate (1 g.) was dissolved in dry methyl alcohol (20 c.c.) and titrated with an ethereal solution of diazomethane at − 10°. At first the yellow colour of the diazomethane was discharged immediately, and the reaction was stopped when, after addition of a drop of reagent, the colour persisted for several minutes. Evaporation of the solvent left a colourless syrup, which was dissolved in acetone (10 c.c.) and precipitated by addition of light petroleum. The syrup so obtained slowly crystallised when kept over calcium chloride in a vacuum desiccator. The crystals were triturated with and recrystallised from acetone–light petroleum, giving 3-*methyl* d-*gluco-ascorbic acid*, m. p. 142°, $[\alpha]_D^{20°}$ − 25° in water (*c*, 0·8) (Found: C, 43·2; H, 5·2; OMe, 14·1. $C_8H_{12}O_7$ requires C, 43·4; H, 5·4; OMe, 14·1%). This substance is acid to litmus in aqueous solution (9·0 mg. required 4·0 c.c. of *N*/100-sodium hydroxide for neutralisation. Calc., 4·1 c.c.). It reduces hot Fehling's solution and gives a permanent deep blue-violet colour with aqueous ferric chloride. It reacts

slowly with iodine in acid solution. It shows a strong absorption band at λ 2450 A. (ε, 10,000) in acidified aqueous solution and at λ 2750 A. in dilute alkaline solution. On treatment with diazomethane in methyl-alcoholic solution, it gives quantitatively 2 : 3-dimethyl gluco-ascorbic acid (see below). A small quantity of the crude syrupy monomethyl gluco-ascorbic acid failed to dissolve in acetone. This crystallised slowly and was washed with acetone and dried in a vacuum desiccator. It was acidic, gave no colour with ferric chloride, reduced hot Fehling's solution, did not react with iodine in acidic solution, and had a strong absorption band in acidified aqueous solution at λ 2800 A. and in dilute alkaline solution at λ 3200 A. The yield was insufficient to permit purification, but the substance appeared to have the composition of a monomethyl gluco-ascorbic acid and corresponded in properties to a similar substance obtained by partial methylation of *l*-ascorbic acid (Haworth, Hirst, and Smith, *loc. cit.*).

2 : 3-*Dimethyl Gluco-ascorbic Acid.*—Anhydrous *d*-gluco-ascorbic acid (5 g.) was dissolved in dry methyl alcohol, and dry ether added until a precipitate just began to form. The calculated quantity of diazomethane was then passed into the cooled solution (ice–salt mixture), which was kept overnight in the refrigerator. Next morning the clear yellow solution was evaporated at 35° under diminished pressure to a syrup, which crystallised when rubbed with a little water. The *monohydrate* of 2 : 3-*dimethyl* d-*gluco-ascorbic acid* after recrystallisation from acetone–light petroleum had m. p. 94°, $[\alpha]_D^{20°}$ — 22° in water (*c*, 4·0), — 7° in methyl alcohol (*c*, 0·8). In aqueous solution it showed an intense absorption band with head at λ 2300 A. (ε = 11,000), measurement being made with a solution containing 4 mg. per 100 c.c. (Found : C, 43·0; H, 6·5; OMe, 24·2. $C_9H_{14}O_7,H_2O$ requires C, 42·8; H, 6·4; OMe, 24·6%). Dimethyl gluco-ascorbic acid does not react with acid iodine solution and gives no colour with ferric chloride. It reacts smoothly with sodium hydroxide in aqueous solution, giving a salt which does not display selective absorption. It does not react with methyl-alcoholic ammonia or with liquid ammonia (contrast 2 : 3-dimethyl ascorbic acid).

2 : 3 : 5 : 6 : 7-*Pentamethyl* d-*Gluco-ascorbic Acid.*—Anhydrous dimethyl gluco-ascorbic acid (2 g.) was dissolved in methyl iodide and methyl alcohol and the solution was boiled with silver oxide in the usual way. The syrupy product crystallised, giving 1·5 g. of the fully methylated product. The reaction is, however, sensitive to traces of impurity and frequently little or no methylation can be effected. We have had similar experiences in the methylation of 1 : 3 : 4 : 5-tetra-acetyl fructose, which sometimes is completed in one operation and on other occasions does not take place at all. The factor responsible for stopping the action of the Purdie reagents is at present unrecognised. In the present instance, we found it more satisfactory to proceed as follows. The monohydrate of dimethyl gluco-ascorbic acid (8 g.) was dissolved in methyl alcohol, and the solution evaporated to dryness under reduced pressure. The anhydrous dimethyl gluco-ascorbic acid so obtained was dissolved in dry acetone and treated with methyl iodide and silver oxide in the usual way. The methylation in acetone solution was repeated until the product was completely soluble in methyl iodide (eight treatments). Methylation operations were stopped when the refractive index of the product had become constant at $n_D^{16°}$ 1·4795; on distillation at 150° (bath temp.)/0·04 mm., a colourless syrup was obtained which consisted mainly of *trimethyl gluco-ascorbic acid monoacetone*. This was a colourless syrup soluble in organic solvents but almost insoluble in water, $n_D^{20°}$ 1·4745, $[\alpha]_D^{21°}$ — 1·6° in methyl alcohol (*c*, 4·2). The absorption band in methyl alcohol is at λ 2350 A. (ε, 12,000) (Found : C, 54·3; H, 7·4; OMe, 36·3. $C_{13}H_{20}O_7$ requires C, 54·8; H, 7·4; OMe, 36·3%). The analytical figures indicate slight contamination with pentamethyl gluco-ascorbic acid.

The acetone derivative was hydrolysed by heating with water for 12 hours at 95°. The solution was concentrated under diminished pressure and the syrupy product ($n_D^{22°}$ 1·4925) was methylated six times by silver oxide and methyl iodide. The product ($n_D^{20°}$ 1·4720) crystallised partly * on nucleation with authentic pentamethyl *d*-gluco-ascorbic acid. The solid material was triturated with ether and then recrystallised from ether, giving 2 : 3 : 5 : 6 : 7-*pentamethyl gluco-ascorbic acid*. Yield, 2·5 g. (Found : C, 52·4; H, 7·5; OMe, 56·4. $C_{12}H_{22}O_7$ requires C, 52·2; H, 7·4; OMe 56·2%). M. p. 80°. $[\alpha]_D^{21°}$ — 5° in methyl alcohol (*c*, 1·4); + 21° in carbon tetrachloride (*c*, 3·65). The substance was soluble in the usual organic solvents with the exception of light petroleum, in which it was slightly soluble, and almost insoluble in water. It displayed in methyl-alcoholic solution a strong absorption band with head at λ 2350 A. (ε = 10,000). Pentamethyl gluco-ascorbic acid does not react with *N*/10-sodium hydroxide

* The non-crystalline portion had the composition of a tetramethyl gluco-ascorbic acid, showed zero rotation, did not react with dilute alkali, did not show selective absorption, and could not be further methylated. It did not possess the typical ascorbic acid structure and was not further examined.

in the cold (contrast dimethyl gluco-ascorbic acid) and slowly undergoes profound decomposition when heated with alkali.

Ozonisation of Pentamethyl Gluco-ascorbic Acid.—The acid (1·19 g.) was dissolved in dry freshly distilled carbon tetrachloride (60 c.c.) and a rapid stream of ozonised oxygen was passed through the cooled solution (— 5°) for 4 hours. The rotation changed from $[\alpha]_D^{20°}$ + 21° to — 2·5° (constant value). Water (20 c.c.) was added and the mixture was heated for 30 minutes on the boiling water-bath. The solution was then concentrated under diminished pressure at 30°. The resulting syrup (1·15 g., $n_D^{19°}$ 1·4492) was dissolved in *N*/3-barium hydroxide (50 c.c.) and heated at 60° for 30 minutes, barium oxalate being precipitated. The excess of barium hydroxide was precipitated as the carbonate, and the mixture of oxalate and carbonate was removed by filtration, acidified with sulphuric acid, and titrated with *N*/10-potassium permanganate, 120·1 c.c. being used. This corresponds to a yield of barium oxalate equivalent to 70% of the theoretical value.*

The barium in the clear filtrate was removed by addition of the exact quantity of sulphuric acid. The filtered solution was then concentrated to a syrup, which was boiled with a mixture of ether and chloroform in equal volumes. Evaporation of the solution gave crystalline 3 : 4 : 5-*trimethyl* d-*arabonic acid* in the form of its *monohydrate*, which after recrystallisation from ether had m. p. 67°, $[\alpha]_D^{19°}$ + 5° in methyl alcohol (*c*, 1·0) (Found: OMe, 42·0. $C_8H_{16}O_6,H_2O$ requires OMe, 41·1%). The water of crystallisation was lost slowly when the substance was kept over calcium chloride in a desiccator (Found for the anhydrous substance: C, 46·1; H, 7·8; OMe, 44·3. $C_8H_{16}O_6$ requires C, 46·1; H, 7·8; OMe, 44·7%. 10·2 Mg. required 4·75 c.c. of *N*/100-sodium hydroxide for neutralisation; hence *M*, 211. Calc. for the anhydrous substance: *M*, 208).

Methyl 3 : 4 : 5-*trimethyl* d-*arabonate* was formed by boiling the acid (0·54 g.) with methyl-alcoholic hydrogen chloride, isolated in the usual way, and purified by distillation, being obtained as a colourless syrup (0·52 g.), b. p. (bath temp.) 110°/0·03 mm., $n_D^{15°}$ 1·4425, $[\alpha]_{5461}^{18°}$ — 17·5° in methyl alcohol (*c*, 3·1) (Found: C, 48·3; H, 8·1; OMe, 54·2. $C_9H_{18}O_6$ requires C, 48·5; H, 8·2; OMe, 55·8%). Treatment of this ester with methyl-alcoholic ammonia gave the corresponding amide, which crystallised as the *monohydrate*; the latter on recrystallisation from acetone–ether gave long needles, m. p. 51°, $[\alpha]_{5461}^{17°}$ — 30° in water (*c*, 1·0) (Found: C, 42·7; H, 8·9; N, 6·2; OMe, 41·2. $C_8H_{17}O_5N$ requires C, 42·6; H, 8·5; N, 6·2; OMe, 41·3%). This amide gave a strong positive Weerman reaction, the sodium cyanate liberated being recognised after conversion into hydrazodicarbonamide, m. p. 256° alone or on admixture with an authentic specimen (yield, 26 mg. from 25 mg. of the amide). Control experiments with 2 : 3 : 4-trimethyl *l*-arabonamide and 2 : 3 : 5-trimethyl *l*-arabonamide gave no hydrazodicarbonamide.

Anhydrous 3 : 4 : 5-trimethyl arabonic acid was methylated three times by Purdie's reagents. The product was purified by distillation, giving *methyl* 2 : 3 : 4 : 5-*tetramethyl* d-*arabonate*, b. p. 100°/0·1 mm. (bath temp.), $n_D^{16·5°}$ 1·4355 (yield, almost quantitative) (Found: OMe, 62·0. $C_{10}H_{20}O_6$ requires OMe, 65·6%). Treatment of this ester with methyl-alcoholic ammonia gave the corresponding *amide*, which after recrystallisation from ether–light petroleum had m. p. 101°, $[\alpha]_D^{20°}$ + 33° in methyl alcohol (*c* = 1·5; observations taken in a micro-polarimeter tube) (Found: OMe, 55·1. $C_9H_{19}O_5N$ requires OMe, 56·1%). The identity of this substance was confirmed by crystallographic (including *X*-ray) examination and comparison with the corresponding derivative of *l*-arabonic acid. The latter was prepared from calcium *l*-arabonate, which was methylated by methyl sulphate and sodium hydroxide. The product was isolated in the usual way, and methylation completed by use of Purdie's reagents. The fully methylated ester was treated with methyl-alcoholic ammonia, giving 2 : 3 : 4 : 5-*tetramethyl* l-*arabonamide*, m. p. (after recrystallisation from acetone–light petroleum) 101°, $[\alpha]_D^{17°}$ + 34·0° in methyl alcohol (*c*, 1·0) (Found: C, 48·6; H, 9·0; OMe, 56·2. $C_9H_{19}O_5N$ requires C, 48·8; H, 8·9; OMe, 56·1%).

Isomerisation of 2 : 3-*Dimethyl* d-*Gluco-ascorbic Acid.*—This substance reacts in the cold with *N*/10-sodium hydroxide, giving a sodium salt having $[\alpha]_D^{20°}$ + 40° (calc. on concentration of dimethyl gluco-ascorbic acid hydrate), and with *N*/10-barium hydroxide, giving the corresponding barium salt. The sodium salt does not display selective absorption. On addition of excess of mineral acid to the aqueous solution the rotation drops immediately to + 31° and the product does not display selective absorption. The rotation gradually falls to zero (constant

* In other experiments the ozonisation was carried out in glacial acetic acid solution. The product was a neutral ester which on treatment with methyl-alcoholic ammonia gave oxamide (yield, 85% of the theoretical).

value) during 24 hours, and at this stage also no selective absorption is shown. If the solution containing mineral acid is heated at 60°, a weak band develops at λ 2300 A., together with a much stronger band at λ 2800 A. Colorimetric tests then indicate the presence of hydroxymethylfurfuraldehyde and the characteristics of the band at λ 2800 A. are in agreement with the view that it is due to hydroxymethylfurfuraldehyde produced by decomposition. The intensity of the band at λ 2300 A. reaches a maximum corresponding to the presence of a mono- or di-methyl gluco-ascorbic acid equal in amount to some 10% of the weight of dimethyl derivative originally used. On further heating, the band at λ 2300 A. disappears and that at λ 2800 A. reaches an intensity corresponding to nearly complete conversion of dimethyl gluco-ascorbic acid into hydroxymethylfurfuraldehyde.

The substance having zero rotation was isolated as follows: dimethyl gluco-ascorbic acid hydrate (1·4 g.) was dissolved in air-free distilled water (50 c.c.), and a slight excess of barium hydroxide added. After 30 minutes, the excess of barium hydroxide was removed by passing carbon dioxide at 60°. The calculated quantity of sulphuric acid was added to the filtered solution and after separation of barium sulphate in the centrifuge the clear solution was evaporated to dryness under diminished pressure at 40°, leaving a syrup, which was purified by solution in methyl alcohol and removal of the solvent. iso*Dimethyl gluco-ascorbic acid* was then obtained as a colourless glass (1·32 g.), $[\alpha]_D^{20°} \pm 0°$ in water. Its solutions were remarkably transparent to ultra-violet light and did not display selective absorption (tested in concentrated solution) (Found: OMe, 26·3. $C_9H_{14}O_7$ requires OMe, 26·5%). *iso*Dimethyl gluco-ascorbic acid titrates as a lactone and required 1 equiv. of alkali for neutralisation. With methyl-alcoholic ammonia it gave a syrup, which contained combined nitrogen and behaved as an amide, liberating ammonia when heated with dilute alkali (contrast dimethyl gluco-ascorbic acid).

Regeneration of 2 : 3-*Dimethyl Gluco-ascorbic Acid from* iso*Dimethyl Gluco-ascorbic Acid.*—(a) *Treatment with acetone and sulphuric acid.* To the *iso*-derivative (0·1 g.), dissolved in acetone (100 c.c.), concentrated sulphuric acid (10 c.c.) was carefully added and the solution was left at room temperature for 24 hours. The mineral acid was then neutralised by sodium carbonate and the filtered solution was concentrated to a syrup, which was heated with a little water for 30 minutes. The solution was extracted with ether and then concentrated to a syrup, which was distilled under diminished pressure, b. p. 230°/0·04 mm. (bath temp.). The distillate crystallised on trituration with water, giving the monohydrate of 2 : 3-dimethyl gluco-ascorbic acid, m. p. and mixed m. p. with an authentic specimen, 94°, absorption band in aqueous solution at λ 2300—2350 A. (ε, 11,400). *iso*Dimethyl gluco-ascorbic acid distils unchanged at 230°/0·01 mm. and does not regenerate 2 : 3-dimethyl gluco-ascorbic acid.

(b) *Treatment with methyl-alcoholic hydrogen chloride.* *iso*Dimethyl gluco-ascorbic acid (0·5 g.) was boiled with 3% methyl-alcoholic hydrogen chloride (50 c.c.) for 24 hours. The rotation changed from $[\alpha]_D^{20°} \pm 0°$ to − 13° (constant value). The solution darkened in colour; it showed no selective absorption at the beginning of the experiment, but, as the rotation changed, a band developed at λ 2400 A. (in acidified methyl-alcoholic solution) and when the rotation had become constant, its intensity (calc. on the weight of original material used) was $\varepsilon = 11{,}000$. 43 C.c. of this solution were diluted to 100 c.c. with methyl alcohol and 20 c.c. of this dilute solution (A) were used for titration with silver nitrate (weight of silver chloride obtained was equivalent to 6·6 c.c. of *N*/10-hydrochloric acid); 50 c.c. of solution were titrated with an ethereal solution of diazomethane (equivalent to 26·2 c.c. of *N*/10-diazomethane, the ethereal solution of the latter reagent having been standardised against iodine immediately before the experiment). From these figures, it appears that more diazomethane had been used up than would be accounted for by the hydrochloric acid. *iso*Dimethyl gluco-ascorbic acid does not react with diazomethane and it follows that the reaction with methyl-alcoholic hydrogen chloride had resulted in the formation of a substance containing a hydroxyl group capable of reacting with diazomethane (see below). The solution after titration with diazomethane was concentrated under reduced pressure, giving a syrup which slowly crystallised when left in a moist atmosphere. The crystals, after being washed with acetone, had m. p. 94° alone or on admixture with authentic 2 : 3-dimethyl gluco-ascorbic acid monohydrate and in aqueous solution they had an absorption band at λ 2300—2350 A. ($\varepsilon = 11{,}000$), which disappeared on the addition of alkali.

20 C.c. of the solution (A) (see above) were neutralised with barium carbonate, filtered, and concentrated to a mixture of syrup and solid. The organic material was extracted with acetone, and the acetone solution concentrated to a syrup, which consisted mainly of 2-*monomethyl gluco-ascorbic acid* (Found: OMe, 14·2. $C_8H_{12}O_7$ requires OMe, 14·1%). This gave no colour with ferric chloride (distinction from 3-monomethyl gluco-ascorbic acid), was acidic, and had an

absorption band at λ 2400 A. (ε, 11,000) both in acidified aqueous and in methyl-alcoholic solution, and at about λ 2650 A. (ε, 5000) in dilute alkaline solution. This substance was treated with diazomethane in ethereal solution and after 12 hours the solvent was removed by evaporation, leaving a syrup which slowly crystallised. The solid, after being washed with acetone, had m. p. 94° alone or on admixture with 2 : 3-dimethyl gluco-ascorbic acid hydrate, $[\alpha]_D$ — 7° in methyl alcohol (*c*, 1·0). The absorption spectrum was identical with that of 2 : 3-dimethyl gluco-ascorbic acid. The yield of crystalline material (60% of the theoretical) was confirmed in later experiments carried out on a larger scale.

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