338. *Methyl Ethers of Ascorbic Acid.*

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Important evidence concerning the structure of ascorbic acid was obtained from a study of the dimethyl and tetramethyl derivatives (J., 1933, 1270) and during the course of the work it became evident that these had respectively the structures (III) and (IV). There are, however, various questions connected with the formation and properties of these derivatives which are not completely answerable by the evidence already published. They include, for example, the relative rates of methylation by diazomethane of the hydroxyl groups at positions 2 and 3, the behaviour of the dimethyl and tetramethyl derivatives towards alkali, and the observation that the specimen of tetramethyl ascorbic acid employed in our earlier work gave after ozonisation, in addition to the main product (3 : 4-dimethyl *l*-threonic acid), a small amount of 3 : 4-dimethyl *l*-erythronic acid. In the present paper we describe further experiments which elucidate these various points and confirm in all particulars the structural formula previously advanced.

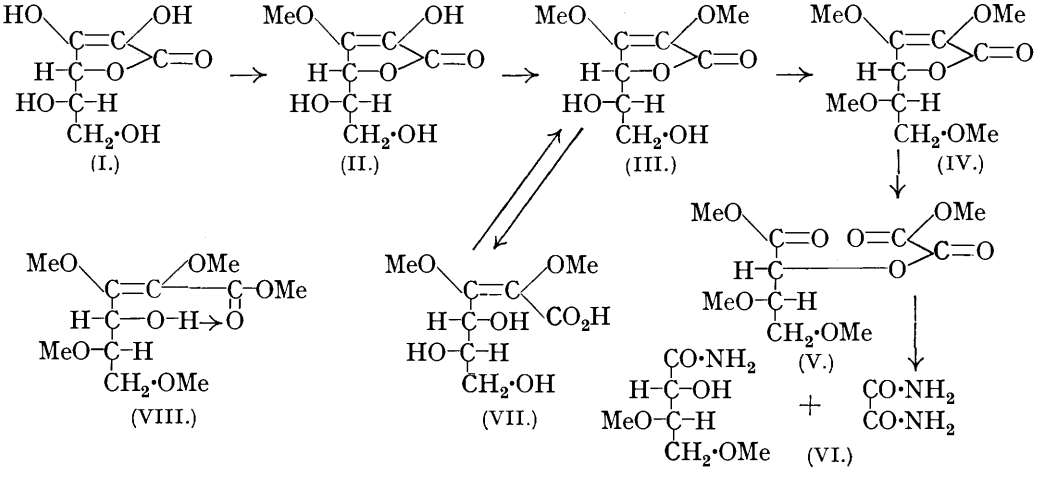
The results may be summarised as follows, detailed evidence being given in the experimental section.

(1) Ascorbic acid gives with one molecular proportion of diazomethane mainly 3-*methyl ascorbic acid*, the position of the methyl group being indicated by the observation that the non-reducing methyl derivative gives an intense blue colour with aqueous ferric chloride (compare Reichstein, Grüssner, and Oppenauer, *Helv. Chim. Acta*, 1934, **17**, 510; Haworth and Hirst, *ibid.*, p. 520).

(2) 3-Methyl ascorbic acid yields quantitatively crystalline 2 : 3-dimethyl ascorbic acid on methylation with diazomethane. This proves the structure of the monomethyl derivative (II), and yet further confirmation is provided by the observation that 3-methyl ascorbic acid gives oxalic acid and *l*-threonic acid on ozonisation, followed by hydrolysis (for course of reaction compare formulæ IV—VI).

(3) Crystalline 2 : 3-dimethyl ascorbic acid gives 2 : 3 : 5 : 6-tetramethyl ascorbic acid on methylation by silver oxide and methyl iodide. This tetramethyl derivative when submitted to ozonisation under the conditions previously described is transformed into the neutral ester (V), which on treatment with ammonia yields oxamide and 3 : 4-dimethyl *l*-threonamide. No 3 : 4-dimethyl *l*-erythronamide is formed and confirmation is thus provided for the suggestion previously advanced, namely, that the dimethyl erythronic acid formerly observed was produced by isomerisation at some stage of the methylation.

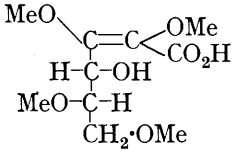
(4) When 2 : 3-dimethyl ascorbic acid is allowed to react with barium hydroxide, the salt of the open-chain acid (VII) is formed. Removal of the barium gives the free acid, which loses water when heated in a vacuum and regenerates the original 2 : 3-dimethyl



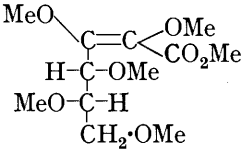
ascorbic acid. Treatment of (VII) with diazomethane in methyl-alcoholic ether, followed by evaporation of the solution at room temperature, also serves for the regeneration of (III). Similar results were obtained with 2 : 3 : 5 : 6-tetramethyl ascorbic acid, lactonisation of the corresponding open-chain acid being in this case more readily effected. The open-chain derivatives do not display selective absorption.

(5) Methylation of 2 : 3-dimethyl ascorbic acid with methyl sulphate and alkali readily yields a tetramethyl derivative of the open-chain acid. This substance on esterification gives the corresponding ester, which resists further methylation. The structure of the latter substance is not quite clear, but in view of our earlier experiences with resistant γ-hydroxy-groups it may be suggested that the structure is (VIII) in which the hydroxyl group on the fourth carbon atom is rendered non-reactive by co-ordination.

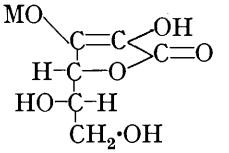
(6) The reaction between 2 : 3 : 5 : 6-tetramethyl ascorbic acid and methyl sulphate in the presence of alkali proceeds somewhat differently. The reaction is sluggish and usually gives a mixture of the corresponding open-chain acid (IX) together with some fully methylated derivative. After treatment with silver oxide and methyl iodide the latter appears to give the corresponding ester (X) whilst the former is reconverted into 2 : 3 : 5 : 6-tetramethyl ascorbic acid. Here again grave difficulty is encountered in attempts to methylate the hydroxyl group at position 4.



(IX.)



(X.)



(XI.)

(7) The preferential formation of 3-methyl ascorbic acid indicates the superior acidity of the 3-hydroxyl group as compared with that at position 2. There is, however, an enormous difference in rotation between 3-methyl ascorbic acid and the salts of ascorbic acid. Whilst, therefore, there is evidence in favour of structure (XI) for the salts, the possibility still remains open that some other structure may be present in ionised ascorbic acid. The position is still further complicated by the fact that accompanying 3-methyl ascorbic acid there is always a small proportion of an isomeric monomethyl derivative (*? 2-methyl ascorbic acid*) which gives only a faint transient colour with ferric chloride and is non-reducing. It is slightly more labile than the 3-methyl derivative. Besides having in all probability the methyl group in another position, this monomethyl derivative differs structurally from 3-methyl ascorbic acid. It possesses a very high rotation and an absorption spectrum different from that of (II) and does not give oxalic acid on ozonisation. The double bond, therefore, may be situated between C_1 and C_2. On treatment of the second monomethyl derivative with diazomethane the corresponding *dimethyl* derivative is formed without structural change. This dimethyl derivative is remarkable in that when heated it undergoes transformation into ordinary 2 : 3-dimethyl ascorbic acid. A similar change occurs when attempts are made to methylate the labile dimethyl derivative with silver oxide and methyl iodide. The product of this reaction seems to consist largely of 2 : 3 : 5 : 6-tetramethyl ascorbic acid (IV). Whilst the structure of these labile derivatives is not yet definitely established, it is now abundantly clear that ascorbic acid may undergo isomeric change and react in more than one structural modification, and full justification is provided for the caution exercised when the constitution of ascorbic acid was first announced (*J. Soc. Chem. Ind.*, 1933, **52**, 221) and alternative structural forms were indicated.

Experimental.

3-Methyl l-Ascorbic Acid.—*l*-Ascorbic acid, dissolved in dry methyl alcohol, was titrated with an ethereal solution of diazomethane at — 10°. Removal of the solvent in a vacuum at 15° left a syrup which slowly crystallised. The solid was triturated with ether containing a little acetone to remove adhering syrup. The crystalline mass was extracted with hot acetone,

leaving a residue (A) (see below). On gradual addition of light petroleum to the acetone solution crystalline 3-*methyl* l-*ascorbic acid* was obtained as fine needles (yield, 90%), m. p. 121°, $[\alpha]_D^{20°}$ + 29° in water (*c*, 0·8) (Found : C, 43·5; H, 5·2; OMe, 17·0. $C_7H_{10}O_6$ requires C, 44·2; H, 5·3; OMe, 16·3%). The substance was acid to litmus and gave an intense permanent blue-violet colour with aqueous ferric chloride. It required one equivalent of sodium hydroxide for neutralisation (indicator : phenolphthalein) ($[\alpha]_D$ + 49·3°, after reaction with one equivalent of sodium hydroxide). 3-Methyl ascorbic acid does not react with acidified aqueous iodine except slowly on long standing (contrast *l*-ascorbic acid). It reduces boiling Fehling's solution. After treatment of the substance with hot alkali, followed by acidification, iodine is reduced. 3-Methyl *l*-ascorbic acid has an intense absorption band in aqueous solution at 245 mμ (ε, 10,000 approx. for *c*, 40 mg. per litre). In dilute alkaline solution the head of the band is at 275 mμ.

3-Methyl ascorbic acid (0·2 g.), dissolved in aqueous acetic acid (10 c.c., containing 1 c.c. of water and 9 c.c. of glacial acetic acid), was allowed to react with ozone until the rotation became constant. The solution was diluted with water and evaporated to dryness under diminished pressure. The product was heated with methyl alcohol, and the solvent removed. The syrupy product was next dissolved in dry methyl alcohol, and dry ammonia passed into the solution. Oxamide separated immediately. After 12 hours the ammonia and solvent were removed by evaporation under diminished pressure at 15°. The mixture of oxamide and *l*-threonamide was separated by extraction with methyl alcohol. Removal of the solvent left l-*threonamide*, which crystallised when kept and was purified by trituration with a mixture of methyl and ethyl alcohol; m. p. 88—90°, $[\alpha]_D^{20°}$ + 58° in water (*c*, 1·0) (Found : C, 35·2; H, 6·8; N, 10·7. $C_4H_9O_4N$ requires C, 35·5; H, 6·7; N, 10·4%).

2 : 3-*Dimethyl* l-*Ascorbic Acid*.—3-Methyl ascorbic acid, dissolved in a small quantity of methyl alcohol, was treated at − 5° for 12 hours with an excess of diazomethane in ethereal solution. On removal of the solvent a pale yellow syrup remained. This readily crystallised on nucleation with a fragment of 2 : 3-dimethyl *l*-ascorbic acid. Recrystallisation from dry ether gave the pure substance, m. p. 63° alone or when mixed with a specimen of the dimethyl derivative prepared directly from ascorbic acid. $[\alpha]_D$ + 30° in water (*c*, 1·3). This crystalline dimethyl derivative on treatment with methyl-alcoholic ammonia readily yielded the amide, $C_8H_{15}O_6N,CH_3{\cdot}OH$, m. p. 124°. 2 : 3-Dimethyl *l*-ascorbic acid was non-reducing towards Fehling's solution and towards iodine in acid solution. As previously reported, it exhibits an absorption band at 230—235 mμ. For purified recrystallised material in water the values are λ235 mμ, ε, 10,300 for *c*, 28 mg. per litre. In alcohol, λ230 mμ, ε, 10,700 (*c*, 30 mg. per litre). On treatment with alkali in the cold the crystalline dimethyl derivative in aqueous solution takes up one equivalent of sodium hydroxide (rotation after completion of reaction, $[\alpha]_D$ − 20°, calc. on conc. of dimethyl ascorbic acid). On acidification the rotation changes to $[\alpha]_D$ − 14° (constant value). Similar experiments were carried out with aqueous barium hydroxide, giving the corresponding barium salt. After removal of the barium as sulphate the aqueous solution containing the free organic acid was evaporated to dryness under diminished pressure, leaving a stiff colourless syrup which was acid to litmus. This was heated at 120°/0·1 mm. for 15 minutes. Lactonisation then occurred and the neutral product consisted almost entirely of 2 : 3-dimethyl ascorbic acid ($[\alpha]_D$ + 27° in water, *c*, 1·0; absorption band at 235 mμ) (Found : OMe, 31. Calc., 30·4%). That no free carboxyl group was present was confirmed by the observation that the substance remained unchanged on treatment with diazomethane.

2 : 3 : 5 : 6-*Tetramethyl* l-*Ascorbic Acid*.—Crystalline dimethyl ascorbic acid was methylated four times in succession with methyl iodide and silver oxide. The product after distillation was a colourless liquid (yield almost quantitative), b. p. 125°/0·1 mm., $n_D^{20°}$ 1·4704, $[\alpha]_D^{20°}$ + 11° in methyl alcohol (*c*, 1·0) (compare Herbert, Hirst, Percival, Reynolds, and Smith, *loc. cit.*) (Found : OMe, 53·0. Calc. for $C_{10}H_{16}O_6$, 53·4%). This sample of tetramethyl *l*-ascorbic acid was submitted to ozonisation by the method previously described (J., 1933, 12). The product when treated with ammonia in methyl-alcoholic solution gave oxamide (yield, 90% of the theoretical) and 3 : 4-dimethyl *l*-threonamide (yield, 85%). The latter substance was recognised by the method given in our earlier paper. A special search was made for the crystalline amide (3 : 4-dimethyl erythronamide) which was encountered in small amount during ozonisation experiments on specimens of tetramethyl ascorbic acid prepared from partly crystalline dimethyl ascorbic acid. In the present experiments this crystalline amide was not found and its occurrence must depend upon the presence of isomeric forms in the partly crystalline dimethyl derivative.

2 : 3 : 5 : 6-Tetramethyl *l*-ascorbic acid shows in aqueous solution an intense absorption

band situated at λ235—240 mμ, the value of ε being approximately 10,000 for a solution containing 4·3 mg. per 100 c.c.

In the cold, tetramethyl *l*-ascorbic acid reacted with 1 mol. proportion of sodium hydroxide (66·1 mg. required 2·84 c.c. of *N*/10-sodium hydroxide. Calc., 2·85 c.c.). No decomposition occurred and the sodium salt thus formed had a negligible specific rotation, and displayed no selective absorption (tested for concentrations up to 60 mg. per 100 c.c.). The barium salt (properties similar to those of the sodium salt) was formed by warming tetramethyl *l*-ascorbic acid with barium hydroxide in a nitrogen atmosphere. The excess of barium was removed as carbonate and the free organic acid was liberated by addition of the calculated quantity of sulphuric acid. On evaporating the solution to dryness, (IX) was momentarily obtained, but lactonisation rapidly ensued with regeneration of 2 : 3 : 5 : 6-tetramethyl *l*-ascorbic acid, $[\alpha]_D^{20°}$ + 11° in methyl alcohol (*c*, 1·0); absorption band at λ235 mμ, ε approx. 9000 (*c*, 2·5 mg. per 100 c.c.).

Methylation with Methyl Sulphate.—(a) 2 : 3-*Dimethyl ascorbic acid and methyl sulphate.* Crystalline 2 : 3-dimethyl *l*-ascorbic acid (3 g.) in water (5 c.c.) and acetone (20 c.c.) was treated for 48 hours with methyl sulphate (5 c.c.) and 40% aqueous potassium hydroxide (15 c.c.) at room temperature in an atmosphere of nitrogen. After acidification the product was extracted with chloroform and remethylated by the above procedure. After the second treatment the product was an acid which had no action on boiling Fehling's solution. After esterification by treatment with silver oxide and methyl iodide the methyl ester was obtained as a colourless oil (yield, almost quantitative), b. p. 105°/0·03 mm., $n_D^{18°}$ 1·4555, $[\alpha]_D^{20°}$ — 51° in methyl alcohol (*c*, 0·8) (Found : C, 49·9; H, 7·7; OMe, 57·0. Calc. for $C_{11}H_{20}O_7$: C, 50·0; H, 7·6; OMe, 58·7%).

This product, which had the elementary composition of methyl tetramethyl ketogulonate, was an ester, since on treatment with warm aqueous sodium hydroxide it lost one mol. proportion of methyl alcohol. The methyl alcohol was removed by distillation in a slow stream of carbon dioxide, trapped in hydriodic acid, and estimated in the usual way as silver iodide (Found : OMe, 10·6. Calc., 11·7%). In another experiment the hydrolysis was performed with barium hydroxide, the methyl alcohol was removed by distillation in a stream of carbon dioxide, and the solution evaporated to dryness under diminished pressure. All these operations were conducted in a Zeisel apparatus. Hydriodic acid was then added to the solid residue (mixture of barium carbonate and the barium salt of the organic acid) and a Zeisel estimation was carried out in the usual way [Found : OMe, 45·6 (calc. on weight of original ester). Calc., OMe, 47·0%].

The ester was soluble in water and in organic solvents. It was a neutral substance which did not reduce Fehling's solution (even on prolonged boiling) or acid iodine. The absorption spectrum showed a weak band at about λ240—245 mμ (ε approx. 500). (It is possible that the selective absorption was due to the presence of some 5% of tetramethyl ascorbic acid as impurity.) The Zerewitinov reaction for active hydrogen was completely negative. The reaction with phenylhydrazine was inconclusive : a quantitative experiment indicated that some phenylhydrazine was taken up, but no crystalline derivative was obtained. After prolonged digestion with methyl-alcoholic ammonia the ester was recovered quantitatively and unchanged ($n_D^{17°}$ 1·4555. Found : N, nil; OMe, 58%).

No decomposition occurred during treatment of the ester with barium hydroxide at 50°. From the barium salt so obtained, the acid was liberated in the usual way. $[\alpha]_D^{20°}$ — 42° in methyl alcohol (*c*, 1·0). It reacted with one equivalent of alkali. On methylation it gave the above methyl ester, $n_D^{18°}$ 1·455, $[\alpha]_D^{20°}$ — 45° in methyl alcohol (*c*, 0·8) (Found : OMe, 57%).

The methyl ester readily gave furfural when heated with 12% hydrochloric acid (yield, 67% of the theoretical, the furfural evolved being weighed as the phloroglucide). It displayed great resistance to attack by ozone, no change being detectable after treatment of the substance for 1 hour in a rapid stream of ozonised oxygen.

The methyl ester (2·0 g.) was oxidised by nitric acid (*d* 1·42) for 30 minutes at 65° and then for 4 hours at 90°. The nitric acid was removed by distillation under diminished pressure and the product was methylated and esterified by treatment with silver oxide and methyl iodide. Fractional distillation of the mixed esters gave some methyl oxalate, followed by a mobile syrup (1·0 g.), b. p. 95°/0·1 mm., $n_D^{18°}$ 1·438, $[\alpha]_D^{18°}$ + 40° in methyl alcohol (*c*, 1·0) (Found : OMe, 59%). That this contained a considerable proportion of methyl *d*-dimethoxysuccinate was shown by the formation of *d*-dimethoxysuccinamide, m. p. about 280° (decomp.), $[\alpha]_D^{17°}$ + 90° in water (*c*, 1·0) (yield, 60%) (Found : OMe, 34·8. Calc. for $C_6H_{12}O_4N_2$: OMe, 35·2%).

(b) *Tetramethyl* l-*ascorbic acid and methyl sulphate.* Tetramethyl ascorbic acid (1 g.) was

treated with methyl sulphate and potassium hydroxide under similar conditions to those given above for dimethyl ascorbic acid. The methylated acid thereby obtained was non-reducing. On esterification with silver oxide and methyl iodide it gave the methyl ester (0·8 g.), b. p. 93°/0·02 mm., $n_D^{17°}$ 1·4608. The specific rotation in methyl alcohol was almost zero (Found : C, 51·0; H, 7·8; OMe, 61·0%). These figures indicate a mixture of tetramethyl ascorbic acid and (X). The ester required for hydrolysis one equivalent of sodium hydroxide. It resembled the methylated product obtained from dimethyl ascorbic acid in being non-reducing and in giving furfural when boiled with 12% hydrochloric acid. It differed from this substance in being insoluble in water, in rotation, and in absorption of ultra-violet light. The substance obtained from tetramethyl ascorbic acid showed in aqueous solution an intense absorption band at λ240—245 mμ, ε approx. 6000 (*c*, 4·5 mg. per 100 c.c.).

2(?)-*Methyl* l-*Ascorbic Acid.*—The residue (A) obtained in the preparation of 3-methyl *l*-ascorbic acid (see p. 1558) was recrystallised from methyl alcohol, giving 2(?)-*methyl* l-*ascorbic acid*, m. p. 162°, $[\alpha]_D^{22°}$ + 200° in water (*c*, 0·6) (Found : C, 44·0; H, 5·5; OMe, 15·7. $C_7H_{10}O_6$ requires C, 44·2; H, 5·3; OMe, 16·3%).

This substance is acidic, requiring an equivalent of sodium hydroxide for neutralisation ($[\alpha]_D^{22°}$ of sodium salt + 223° in water, *c*, 0·5). It reacts slowly with iodine in acid solution. In alkaline solution it readily reduces hot Fehling's solution. It gives a faint transient colour with aqueous ferric chloride. Aqueous solutions of this monomethyl ether display an intense absorption band at λ280 mμ (11,000; *c*, 2·5 mg. per 100 c.c.). In alkaline solutions the head of the band lies further towards the visible (λ320 mμ).

The monomethyl ether was dissolved in methyl alcohol and allowed to react with excess of diazomethane in ethereal solution. The product was a viscid syrup which showed $[\alpha]_D^{20°}$ + 107° in methyl alcohol (*c*, 10) (Found : OMe, 31. $C_8H_{12}O_6$ requires OMe, 30·4%). This *dimethyl* ether readily occludes methyl alcohol, which can be removed only by heating for some hours in a vacuum at 60°. The heating affects the dimethyl ether in that the specific rotation and the intensity of the absorption steadily diminish as heating is prolonged. The head of the absorption band in aqueous solution is at λ270 mμ and for the freshly prepared unheated substance the value of ε is about 10,000; *c*, 4 mg. per 100 c.c. When the substance is heated, the band at λ270 mμ diminishes in intensity and a strong band at λ235—240 mμ makes its appearance and the rotation diminishes to that of 2 : 3-dimethyl ascorbic acid ($[\alpha]_D$ + 27° in water). It is evident, therefore, that isomerisation to 2 : 3-dimethyl ascorbic acid has taken place.

Methylation of the second dimethyl ether with silver oxide and methyl iodide gave a syrup which distilled at 105°/0·02 mm., giving a colourless oil (0·5 g.), $n_D^{17°}$ 1·462, $[\alpha]_D^{22°}$ − 10° in methyl alcohol (*c*, 0·9) (Found : C, 51·2; H, 7·6; OMe, 55%). This product had an intense absorption band at λ240 mμ (ε, approx. 5000; *c*, 20 mg. per 100 c.c.). The analytical data indicated a mixture of substances and from the nature of the absorption and the general properties of the material it seems likely that it consisted largely (*ca.* 50%) of 2 : 3 : 5 : 6-tetramethyl ascorbic acid. On ozonisation it gave oxalic acid (150 mg. gave 20 mg. of oxalic acid, weighed as oxamide).

The authors are grateful for a grant to one of them from the Department of Scientific and Industrial Research.

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, August 17th*, 1934.]