Synthesis and Stability of L-Ascorbate 2-Sulphate

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L-Ascorbate 2-sulphate (I) has been isolated in 75—80% yield by two methods. In a modified conventional procedure, tertiary amine salts of 5,6-acetal derivatives of L-ascorbic acid were sulphated with an amine–sulphur trioxide complex in a dipolar aprotic solvent. In an alternative procedure, L-ascorbate **(II)** was sulphated rapidly and almost quantitatively by treatment with trimethylamine–sulphur trioxide in alkali (pH 9·5—10·5) at 70°. L-Ascorbate 2-sulphate (I) was 20 times more stable than L-ascorbate to oxygen (air) in boiling water. In boiling alkali (pH 13·0) it had a half-life of 21 h, whereas in boiling acid (pH 1·0) its half-life was 4·7 min. Loss of sulphate from L-ascorbic acid 2-(hydrogen sulphate) (III) was 1500 times faster in 99% methanol than in water at 50°.

L-ASCORBATE 2-SULPHATE † (I) occurs naturally [2] in urine from man, guinea pig, rat, and fish. It also has been found [3,4] in the dormant embryo of brine shrimp, which contains little,[4] if any, L-ascorbate (II).

The vitamin C potency of (I) appears to be species-dependent. Halver *et al.*[5] reported that it prevents scurvy in fish; Baker *et al.*[6] found it to be the primary urinary and tissue metabolite of L-ascorbate in rainbow trout and rats. On the other hand, several investigators [7-9] recently have found that it has no antiscorbutic activity in the guinea pig, contrary to the report by Mumma and his co-workers.[10] No data concerning the antiscorbutic activity of L-ascorbate 2-sulphate (I) in man

† In most of the literature, L-ascorbate 2-sulphate (I) is incorrectly identified as the 3-sulphate.[1]

[1] A. D. Bond, B. W. McClelland, J. R. Einstein, and F. J. Finamore, *Arch. Biochem. Biophys.*, 1972, **153**, 207.

[2] E. M. Baker, D. C. Hammer, S. C. March, B. M. Tolbert, and J. E. Canham, *Science*, 1971, **173**, 826.

[3] C. G. Mead and F. J. Finamore, *Biochemistry*, 1969, **8**, 2652.

[4] A. L. Golub and F. J. Finamore, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 1972, **31** (2), Abs. No. 2765.

[5] J. E. Halver, C. L. Johnson, R. R. Smith, B. M. Tolbert, and E. M. Baker, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 1972, **31** (2), Abs. No. 2764; J. E. Halver, L. M. Ashley, R. R. Smith, B. M. Tolbert, and E. M. Baker, *ibid.*, 1973, **32** (3), Abs. No. 4010.

[6] E. M. Baker, K. A. Tillotson, J. E. Kennedy, and J. E. Halver, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 1973, **32** (3), Abs. No. 4009.

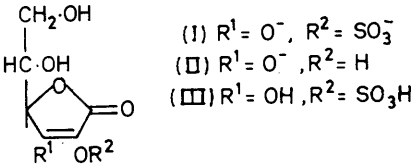
[7] J. D. Campeau, S. C. March, and B. M. Tolbert, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 1973, **32** (3), Abs. No. 4008.

[8] A. D. Bond, personal communication, 1972.

[9] F. S. Julius, C. W. Deyoe, R. C. Hoseney, and P. A. Seib, unpublished results.

[10] R. O. Mumma, E. E. McKee, A. J. Verlangieri, and G. P. Barron, *Nutrition Reports International*, 1972, **6** (3), 133.

and other primates have been published, though there have been some metabolic experiments.[11,12]



We became interested in the sulphate (I) as a possible source of vitamin C to enrich foods, in that it is reported to be more stable [13-16] than L-ascorbate (II), and because it occurs naturally in animals.

The first chemical synthesis of compound (I) involved treatment [13] of a 5,6-acetal of L-ascorbic acid with an amine–sulphur trioxide complex in a dipolar aprotic solvent.* Most investigators since have used that conventional approach [1,13-16,18-20] † though a few have used other sulphating agents.[15,16,19] We found the published procedures to be inadequate for preparing large quantities of pure salts of (I); yields generally were † low (<25%), and the product frequently was contaminated with inorganic sulphates.

We report here two better methods for synthesizing salts of L-ascorbate 2-sulphate (I); one involves selective and almost stoicheiometric sulphation of L-ascorbate in aqueous medium. The other is a modification of the previous approach. We have also developed an analytical procedure to determine L-ascorbate 2-sulphate (I), and have measured its stability under various conditions.

Our first approach [20] involved the conventional sulphation on 5,6-*O*-isopropylidene-L-ascorbic acid with pyridine-sulphur trioxide complex in *NN*-dimethylformamide (DMF). The method of isolation was improved [20] so that barium L-ascorbate 2-sulphate dihydrate was obtained in 48% yield as a sharp-melting, analytically pure solid. We now report that adding one or more equivalents (based on L-ascorbic acid) of a tertiary amine prior to sulphating 5,6-*O*-isopropylidene-L-ascorbic acid with pyridine–sulphur trioxide in DMF further improved the yield to 75%, as a result of avoiding hydrolysis of the 5,6-blocking group during sulphation, which in turn prevents sulphation at the 6-hydroxy-group.[19] Water could be present during sulphation either from degradation of L-ascorbic acid, or as an impurity in the amine–sulphur trioxide reagent. The 3-OH of L-ascorbic acid would serve as hydrolysis catalyst (pK_a 4·3).[21]

The sulphate (I) prepared in our laboratory was identical with that isolated from brine shrimp, which previously had been identified unequivocally [1] by *X*-ray crystallographic analysis of the barium salt. Potassium, zinc (dihydrate), and cobalt salts of (I) also crystallize easily.

A much better method for preparing the sulphate (I) is to treat L-ascorbate (II) with 1·5 equiv. of trimethylamine–sulphur trioxide in alkali (pH 9·5—10·5) at 70° for 0·5 h. The yield is 97% yield and analytically pure salts are easily isolated in 80% yield.

Sulphation of the 2-hydroxy-group of L-ascorbate with trimethylamine–sulphur trioxide in alkali is pH-dependent. U.v. analysis [1] of the products showed that stoicheiometric sulphation (99·2%) at position 2 was obtained only in the optimal pH range 9·5—10·5 (Table). At optimal pH, preparative paper chromatography–u.v. analysis showed that the reaction mixture contained only L-ascorbate 2-sulphate and L-ascorbate 2,6-disulphate in the ratio 40 : 1, respectively. The conversion of (II) into (I) was therefore 96·7%.

The time for disappearance of starting material (II) also showed the same pH dependence as did the degree of 2-*O*-sulphation (Table). The data indicate that the

Sulphation [a] of L-ascorbate (II) in alkali at 70°

pH	Reaction period (min)	Sulphation of 2-OH (%)
8·5	630 [b]	77 [c]
9·0	360	88
9·5	45	99
10·0	30	99 [d]
10·5	35	99
11·0	60	74
11·5	180	35

[a] Ascorbic acid (2·0 g) at 70° in alkali (25·0 ml) with trimethylamine–sulphur trioxide (2·4 g). [b] Time for disappearance of L-ascorbate (II). [c] Determined from absorbance at 255 nm. [d] Reaction mixture contains (I) (96·7%) and L-ascorbate 2,6-disulphate (2·5%) (see Experimental section).

formation of (I) accelerates as the pH of medium increases above 7·0, because ionization of the 2-hydroxy-group (pK_a 11·79) is increased. Conditions that accelerate sulphation of (II) also result in a higher conversion into L-ascorbate 2-sulphate since the latter is more stable to alkali and oxygen than is L-ascorbate (II). However, when the pH of the sulphation medium exceeds 10·5, the higher hydroxide ion concentration destroys [22] the amine–sulphur trioxide, and L-ascorbate is consumed by oxidative alkaline degradation.

* Dimethyl sulphoxide cannot be used as solvent for this reaction because alcohols are oxidized by a mixture of dimethyl sulphoxide and sulphur trioxide.[17]

† Bond *et al.*[1] reported a 50% yield of (I) from sulphation of 5,6-*O*-cyclohexylidene-L-ascorbic acid followed by purification on an ion-exchange column.

[11] E. M. Baker, J. E. Kennedy, B. M. Tolbert, and J. E. Canham, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 1972, **31** (2), Abs. No. 2760.

[12] A. D. Bond, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 1973, **32** (3), Abs. No. 4011.

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[15] B. M. Tolbert, D. J. Isherwood, R. W. Atchley, and E. M. Baker, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 1971, **30** (2), Abs. No. 1819.

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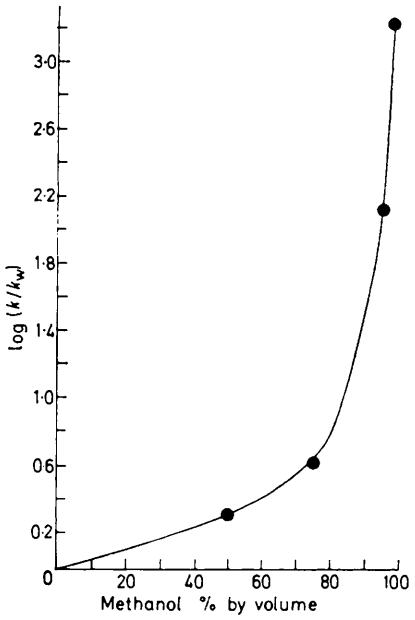
[21] R. P. Bell and R. R. Robinson, *Trans. Faraday Soc.*, 1961, **57**, 965.

[22] E. E. Gilbert, *Chem. Rev.*, 1961, **61**, 550.

L-Ascorbate 2-sulphate (I) is much more stable towards oxygen than is L-ascorbate. In boiling water with a stream of air passing through, potassium L-ascorbate was degraded 20 times faster than potassium L-ascorbate 2-sulphate ($t_{\frac{1}{2}}$ 6·8 and 136 h, respectively); disappearance of both compounds showed zero-order kinetics. When a stream of nitrogen was passed through the solutions in boiling water, potassium L-ascorbate decomposed only three times faster than the 2-sulphate ($t_{\frac{1}{2}}$ 73 and 212 h, respectively). Ford and Ruoff [13] previously reported that (I) is only slightly more difficult to oxidize than (II) at pH 4·5.

In boiling deaerated alkali (pH 13·0), L-ascorbate 2-sulphate (I) and L-ascorbate (II) had half-lives of 21 and



Loss of sulphate from L-ascorbic acid 2-(hydrogen sulphate) (III) in various mixtures of methanol and water; rates are relative to the rate constant (k_w) of 0·0136 h^{-1} in water

15 h, respectively. In boiling M-acid, the sulphate ester group was hydrolysed rapidly ($t_{\frac{1}{2}}$ 4·7 min). Under conditions (pH 1·5 and 37° in a nitrogen atmosphere) which simulate the physiological conditions of the stomach of monogastric animals, compound (I) had a half-life of 150 h. Mumma and his co-workers previously reported [16] a 20% loss of sulphate from (I) after 6·5 h at room temperature and pH 1.

Sulphate can be removed from (I) to give (II) much faster in acidic methanolic than in acidic aqueous solution. The desulphation followed pseudo-first-order kinetics at all solvent ratios; a plot of rate constants obtained at 50° against methanol concentration is shown in the Figure. The rate of disappearance of L-ascorbic acid 2-(hydrogen sulphate) (III) was 1,500 times greater ($t_{\frac{1}{2}}$ 2·0 min) in 99% methanol than in aqueous medium (3000 min), and the reaction accelerated sharply as the solvent medium approached pure methanol.

The ready conversion of the 2-(hydrogen sulphate) (III) into L-ascorbic acid (II) may have important biological significance. It is apparent that compound (I) becomes a potent sulphating agent for alcohols when it is converted into its free acid form in a non-ionic medium. Investigators previously have proposed that (I) may transfer sulphate to biological compounds under *oxidative* conditions.[1,13,14,18]

Information concerning the methanolysis of sulphate esters of carbohydrates is meagre.[23] Several polysaccharide sulphates have been desulphated at room temperature with methanolic hydrogen chloride (<0·1M), but the reactions were allowed to proceed for several days because they were heterogeneous.[23] Therefore, we do not know whether methanolysis of (I) is more rapid than that of other carbohydrate sulphates.

EXPERIMENTAL

M.p.s were determined with a Thomas–Hoover Unimelt apparatus. Elemental analyses were performed by Huffman Lab. Inc. (Wheatridge, Colo.). Solutions were evaporated under reduced pressure below 50°. Paper chromatography was performed on Whatman no. 1 paper at 25° with propan-1-ol–water–trichloroacetic acid (15 : 4 : 1, v/v/w). Components were located by dipping the paper in the following reagents: (A) silver nitrate in aqueous acetone, followed by methanolic sodium hydroxide; [24] (B) 1% ferric chloride in 95% ethanol.[25] T.l.c. was performed on plates coated with silica gel G (Brinkman Instruments, Inc., Westbury, New York). Components were located by spray (B), or by spraying with 50% aqueous sulphuric acid and charring on a hot plate. A Beckman DB-G spectrophotometer was used to record u.v. spectra.

Sulphation of 5,6-O-*Isopropylidene*-L-*ascorbic Acid in DMF.*—To anhydrous *NN*-dimethylformamide * (20 ml) containing 5,6-*O*-isopropylidene-L-ascorbic acid [26] (10 g) and anhydrous pyridine (4 ml) at 25° was added, dropwise with stirring, pyridine–sulphur trioxide [27] (14 g, 1·9 equiv.) in *NN*-dimethylformamide (40 ml). The mixture was stirred for 12 h; t.l.c. [methanol–ethyl acetate (85 : 15)] then showed the absence of starting material (R_f 0·7). Paper chromatography [spray (A)] showed that the mixture contained two components whose mobility differed from that of the starting material with $R_{\text{ascorbic acid}}$ (R_a) = 1·6. The major product (R_a 0·7) was 5,6-*O*-isopropylidene-L-ascorbate 2-sulphate; upon mild acidic hydrolysis it gave material (R_a 0·4) with spectral characteristics identical with those of analytically pure [1,20] salts of L-ascorbate 2-sulphate; $\lambda_{max.}$ (pH 7·0) 255 and (pH 2·0) 232 nm; $\nu_{max.}$ 1265s, 1230s, 1050s, 830m, and 775m cm^{-1}. The other component (R_a 0·1) was not investigated further. Preparative paper chroma-

* Other dipolar aprotic solvents that have been used successfully in this reaction include nitromethane, acetonitrile, acetone, and *NN*-dimethylacetamide. Dimethyl sulphoxide cannot [17] be used.

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tography of a sample (50 μl) of the reaction mixture, followed by u.v. analysis at 255 nm (ε 16,300 at pH 7·0),[20] showed that 92% of the starting material had been converted into L-ascorbate 2-sulphate.

The sulphation reaction was rerun without the added pyridine. A sample (50 μl) was again subjected to preparative paper chromatography followed by u.v. analysis. The results showed that the reaction mixture contained 5,6-*O*-isopropylidene-L-ascorbate 2-sulphate (35%), the unidentified component (R_a 0·1), and L-ascorbate 2,6-disulphate (57%). The mobility of the disulphate (R_a 0·2) did not change upon treatment with aqueous M-hydrochloric acid at 25° for 1 h. The compound gave a brick-red colour reaction (enolic 3-OH) with ferric chloride and its u.v. absorption [$\lambda_{max.}$ (pH 7·0) 255 and (pH 2·0) 232 nm] was consistent [1] only with the 3-OH being the enolic hydroxy-group. The 57% conversion of starting material into the disulphate was calculated from the absorbance at 255 nm (pH 7·0), on the assumption that the 2,6-disulphate has the same extinction coefficient as L-ascorbate 2-sulphate.

The mixture obtained from sulphating 5,6-*O*-isopropylidene-L-ascorbate with added pyridine was warmed (<500°) under reduced pressure to remove DMF. Addition of water (30 ml) to the thick red syrup lowered the pH of the aqueous solution to 1·0, and the mixture was kept for 30 min at 25°. The pH was adjusted to 7·0 by adding saturated aqueous barium hydroxide, and barium sulphate (8·38 g) was filtered off through a glass-fibre filter pad (grade 984H, Arthur H. Thomas Co., Philadelphia). The filtrate was decolourized with charcoal, and concentrated to 50 ml. Methanol (50 ml) was added, the mixture was cooled, and the crystalline solid was filtered off and dried over phosphorus pentaoxide to give barium L-ascorbate 2-sulphate (14·8 g, 75·0%), m.p. 220—225° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water), identical with samples previously synthesized [20] or isolated from extracts of brine shrimp. A sample of the diammonium salt of the naturally occurring L-ascorbic acid sulphate (obtained from A. D. Bond [1]) was converted into the barium salt by Amberlite IR-120 (Ba^{2+}) ion-exchange chromatography.

Sulphation of L-Ascorbate in Alkali.—To a solution of L-ascorbic acid (2 g, 11·5 mmol) in water (25·0 ml) we added aqueous 10M-sodium hydroxide until the pH of the medium reached 10·0. The mixture was heated to 70°, then trimethylamine–sulphur trioxide (2·4 g, 16·2 mmol; Aldrich) was added. The pH of the medium was maintained at 9·5—10·5 by periodically adding aqueous 10M-sodium hydroxide. After the mixture had been stirred for 30 min, t.l.c. (methanol–water, 6 : 4) showed that no starting material remained and u.v. absorbance at 255 nm (pH 7·0) indicated 99·2% 2-*O*-sulphation. The products from a small sample of the mixture were separated by preparative paper chromatography, and the ratio of L-ascorbate 2-sulphate (R_a 0·4) to the 2,6-disulphate (R_a 0·2) was determined as 40 : 1 by u.v. spectroscopy (ratio of absorbance readings at 255 nm; pH 7·0).

The reaction mixture was cooled and passed through Amberlite IR-120 (H^+) resin. The effluent and washings from the column were combined and neutralized with saturated aqueous barium hydroxide. The mixture was filtered to remove barium sulphate and concentrated to 15 ml. An equal volume of methanol was added, the mixture was cooled, and the solid was filtered off and dried under vacuum (P_2O_5); yield 3·9 g (80%), m.p. 212—219°. Recrystallization gave barium L-ascorbate 2-sulphate dihydrate, m.p. 230—235° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water) {lit.,[20] m.p. 235—240° (decomp.), $[\alpha]_D^{25}$ +55° (*c* 1·0 in water)}.

Sulphation of L-Ascorbate (II) *in Aqueous Media at Various Alkalinities; Estimation of L-Ascorbate 2-Sulphate.*—A method for estimating (I) in the presence of L-ascorbate or other weak acids was devised. Samples of standard solutions containing L-ascorbic acid (5·0 mg) and barium L-ascorbate 2-sulphate dihydrate (3·2 mg) were mixed and the solution was adjusted to pH 7·0. It was then added to the top of a pipette (6 × 100 mm) containing a weakly basic, anion-exchange resin (200 mg; type AG3–X4A, Cl^-, 200—400 mesh, BioRad Laboratories, Richmond, California). The column was washed well with water (100 ml) to elute weak acids, and the sulphated compound (I) was then eluted with aqueous M-ammonium sulphate (100·0 ml). The effluent was adjusted to pH 7·0 and the absorbance read at 255 nm. Recovery of (I) was quantitative. By this procedure, accurate determinations can be made on effluents from the resin column containing as little as 2 μg ml^{-1} of L-ascorbate 2-sulphate.

Conversions of (II) into (I) with trimethylamine–sulphur trioxide at various concentrations of alkali were determined as follows. To seven aqueous solutions (20 ml) of L-ascorbic acid (2·0 g) was added aqueous sodium hydroxide such that solutions were obtained with a final volume of 25 ml and pH values of 8·5 to 11·5 (interval 0·5). Each solution was placed in a beaker fitted with pH electrodes, and the solution was warmed with stirring to 70° in a water-bath. Trimethylamine–sulphur trioxide (2·4 g) was added, and the pH of the mixture was adjusted periodically by adding small amounts (syringe) of aqueous 10M-sodium hydroxide. The progress of the reaction was monitored by t.l.c. (methanol–water, 6 : 4), and when starting material had disappeared the mixture was cooled and diluted to 100 ml with water. A sample (500 μl) was analysed for (I) as already described above. A control run on the sulphation reaction in which L-ascorbic acid (2 g) was completely degraded at pH 11·5 and the reaction mixture was subsequently analysed, showed that the ammonium sulphate effluent from the BioRad AG-3(Cl^-) column exhibited negligible absorbance at 255 nm. The results are given in the Table.

Methanolysis of L-Ascorbic Acid 2-(Hydrogen Sulphate) (III).—A sample (200 μl) of an aqueous solution (2·0 ml) of L-ascorbic acid 2-(hydrogen sulphate) (600 mg), which previously had been generated from the barium salt of (I) by passage through Amberlite IR-120 (H^+) followed by freeze-drying, was diluted with various amounts of water and methanol at 50° to give a final volume of 20·0 ml. To follow disappearance of L-ascorbic acid 2-(hydrogen sulphate) at a given methanol concentration, samples (1·0 ml) were removed at various times and analysed as previously described. First-order plots of the data were constructed, and the rate constants were derived from the slopes of the lines. The results are shown in the Figure.

In a separate, large-scale reaction, the barium salt of (I) (2·0 g, 4·7 mmol) was converted into the free acid form and the solution was concentrated below 25° to a thick syrup. The syrup was dissolved in methanol (10 ml) and diethyl ether was added. After cooling, crystalline L-ascorbic acid (0·62 g, 76%) was isolated; m.p. and mixed m.p. 188—190°.

Stability of L-Ascorbate 2-Sulphate (I) *and L-Ascorbate* (II) *in Boiling Water with and without Air.*—Potassium L-ascorbate 2-sulphate (500 mg, 1·5 mmol) (see later) or potassium L-ascorbate (322 mg, 1·5 mmol) was dissolved in water (100 ml) that had been previously heated to the boil. A

stream of nitrogen or air (24 ml min^{-1} at 1 atm and 25°) was bubbled through the solutions while they were heated to reflux. Thus, four reactions were run. Samples (1·0 ml) were withdrawn at various times and analysed for L-ascorbate 2-sulphate as described previously. Analysis for L-ascorbate was done by fluorometry.[28] In all reactions disappearance of the starting compounds followed zero-order kinetics. Rate constants were: L-ascorbate 2-sulphate with air, 0·893 μmol l^{-1} min^{-1}; L-ascorbate 2-sulphate under nitrogen, 0·596 μmol l^{-1} min^{-1}; L-ascorbate with air, 16·4 μmol l^{-1} min^{-1}; L-ascorbate under nitrogen, 1·79 μmol l^{-1} min^{-1}.

Destruction of L-*Ascorbate 2-Sulphate and* L-*Ascorbate with Strong Acid and Strong Base at* 100°.—Potassium L-ascorbate 2-sulphate (200 mg, 0·60 mmol) was dissolved in 100 ml of either aqueous hydrochloric acid at pH 1·0 (or pH 1·5) or aqueous potassium hydroxide (pH 13·0); all alkaline or acidic media had been previously boiled before the organic salt was added. The solutions of pH 1·0 and 13·0 were heated to the boil under nitrogen; the solution at pH 1·5 was held at 37°. Samples (1·0 ml) were withdrawn at various times and analysed for L-ascorbate 2-sulphate as previously described.

Potassium L-ascorbate (130 mg) was treated similarly in hot alkali, and residual (II) was determined by u.v. spectrophotometry as follows. A sample (1·0 ml) was diluted quickly to 100·0 ml with 5% metaphosphoric acid to arrest destruction [29] of (II), and the absorbance was measured at 245 nm and pH 2·0.

Plots of the data followed either zero-order or first-order kinetics up to 70% loss of (I) or (II). The reactions and their half-lives were: L-ascorbate 2-sulphate (I) at 100° and pH 13, $t_{\frac{1}{2}}$ 21 h; compound (I) at 37° and pH 1·5, $t_{\frac{1}{2}}$ 150 h; L-ascorbate at 100° and pH 13·0, $t_{\frac{1}{2}}$ 15 h.

Salts of L-*Ascorbate 2-Sulphate* (I).—The sodium, potassium, ammonium, pyridinium, calcium, magnesium, zinc, cobalt, aluminium, and alanine salts of L-ascorbate 2-sulphate were prepared [16] by one of two procedures [(*A*) or (*B*)], illustrated by the preparation of the magnesium salt.

(*A*) Barium L-ascorbate 2-sulphate dihydrate (2·000 g) was dissolved in water (100 ml), and magnesium sulphate heptahydrate (1·153 g, 1 equiv.) was added. The mixture was stirred for 1 h and barium sulphate (1·270 g) was filtered off. Evaporation gave of a crystalline *solid* (1·269 g, 97·5%), m.p. 205—210° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water) (Found: C, 25·6; H, 2·25; S, 10·85. $C_6H_6MgO_9S$ requires C, 25·9; H, 2·15; S, 11·5%).

(*B*) Barium L-ascorbate 2-sulphate dihydrate (3·0 g) was dissolved in water (100 ml) and passed through Amberlite IR-120 (Mg^{2+}) resin (12 ml). The column was washed with two volumes of water and the effluents were combined and concentrated to dryness. The product (1·48 g, 76% yield) was identical with that isolated by method (*A*).

Potassium L-ascorbate 2-sulphate crystallized* from 50% aqueous methanol with m.p. 96—97°, $[\alpha]_D^{25}$ +50° (*c* 1·0 in water) (Found: C, 21·7; H, 1·8; S, 9·65. Calc. for $C_6H_6K_2O_9S$: C, 21·05; H, 2·15; S, 9·6%), solubility in water 0·795 g ml^{-1} at 25°. The potassium salt also crystallizes [30] as a dihydrate with m.p. 91—92°. *Sodium L-ascorbate 2-sulphate* solidified when all water was removed. The strongly birefringent solid had m.p. 190—210° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water) (Found: C, 23·35; H, 2·1. $C_6H_6Na_2O_9S$ requires C, 24·0; H, 2·0%). *Calcium L-ascorbate 2-sulphate* solidified when all water was removed; m.p. 220—240° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water) (Found: C, 24·25; H, 2·0; S, 10·85. $C_6H_6CaO_9S$ requires C, 24·5; H, 2·05; S, 10·9%). *Zinc L-ascorbate 2-sulphate* was a crystalline solid, m.p. 185—190° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water) (Found: C, 20·25; H, 3·15. $C_6H_6O_9SZn,2H_2O$ requires C, 20·25; H, 2·85%). *Magnesium L-ascorbate 2-sulphate* was a solid, m.p. 205—210° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water) (Found: C, 25·6; H, 2·25; S, 10·85. $C_6H_6MgO_9S$ requires C, 25·9; H, 2·15; S, 11·5%). *Cobalt*(II) *L-ascorbate 2-sulphate* was a crystalline solid, m.p. 196—198° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water) (Found: C, 20·3; H, 3·2. $C_6H_6CoO_9S$ requires C, 20·6; H, 2·9%). The L-alanine salt crystallized from cold aqueous acetone with m.p. 155—160°, $[\alpha]_D^{25}$ +40° (*c* 1·0 in water). N.m.r. analysis confirmed that the salt contained one molecule of L-alanine to one molecule of L-ascorbate 2-sulphate. The ammonium salt formed a crystalline solid when a solution in water was evaporated; m.p. 90—95° (decomp.), $[\alpha]_D^{25}$ +50° (*c* 1·0 in water).

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* This salt has also been isolated with m.p. 162—165°.

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