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Note

Improved method of preparing L-ascorbic acid 2-sulfate*

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The Note by Mumma *et al.*[1] on the chemical synthesis and characterization of L-ascorbic acid 2-sulfate** (**1**) prompts us to report our findings on the preparation and some properties of the dipotassium (**2**), barium (**3**), and monopyridinium (**4**) salts of **1**. We have found an improved method of preparing **1** whereby the salts of **1** are obtained as sharp-melting, crystalline solids in yields of 40 to 50%, starting from 5,6-*O*-isopropylidene-L-ascorbic acid (**5**). In addition, we have examined the 100-MHz, p.m.r. spectra of L-ascorbic acid, potassium L-ascorbate, dehydro-L-ascorbic acid, and two salts of L-ascorbic acid 2-sulfate. We have also determined the stabilities of **2** and potassium L-ascorbate to oxygen in boiling water.

We are interested in **1** as a potential source of vitamin C in processed foods, because its potassium salt (**2**) is reportedly[2] very stable in acidic and alkaline media, and because L-ascorbate 2-sulfate has been found[3] to be a normal metabolite of L-ascorbate in humans and other animals. The lability of L-ascorbic acid to oxygen, especially in alkaline media, is well known[4–7]. Furthermore, L-ascorbic acid is rapidly decomposed when a food substance (such as wheat flour[8]) contains the enzyme L-ascorbic acid oxidase, which converts L-ascorbic acid into dehydro-L-ascorbic acid, a labile[9] compound whose irreversible decomposition corresponds directly to loss of antiscorbutic activity in the food. Thus, a major proportion of the L-ascorbic acid is lost in cereal products manufactured at elevated temperatures[10]. It is not known whether the 2-sulfuric ester **1** is a substrate for L-ascorbic acid oxidase, but polarographic studies showed[11] that, at pH 4.5, L-ascorbate 2-sulfate is more difficult to oxidize than L-ascorbate.

The reaction sequence that we used for preparing **1** was that used by other investigators[1,12], namely, sulfation of 5,6-*O*-isopropylidene-L-ascorbic acid (**5**) or 5,6-*O*-benzylidene-L-ascorbic acid with pyridine–sulfur trioxide in *N*,*N*-dimethylformamide, followed by hydrolytic removal of the isopropylidene group. However,

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**Until 1972, the product isolated from sulfation of 5,6-acetals of L-ascorbic acid was assigned the 3-sulfate structure. However, X-ray crystallographic determination of structure has shown that the derivative is the 2-sulfuric ester [A. D. BOND, B. W. MCCLELLAND, J. R. EINSTEIN, AND F. J. FINNAMORE, *Arch. Biochem. Biophys.*, 153 (1972) 207.]

we have so improved the procedure that the barium salt (3) of **1** is isolated from the sulfation mixture in ~50% yield as a crystalline, sharp-melting solid, which is readily converted (in 77% yield) into the dipotassium salt by use of a cation-exchange resin. Mumma and co-workers[1] reported a 15% yield of dipotassium L-ascorbate 2-sulfate methanolate, whereas Chu and Slaunwhite[1,2] isolated **2** in 41% yield as an amorphous monohydrate having an unsharp melting-point; both groups of workers used an isolation technique more tedious than that reported here. In our hands, the dipotassium salt (**2**) had m.p. 87–89.5° and contained no solvent of crystallization, as indicated by elemental analysis and by the absence of the methyl signal (τ 6.5) of methanol in the n.m.r. spectrum of the compound. Tolbert *et al.*[2] prepared the barium salt (**3**) by way of sulfation of 5,6-*O*-benzylidene-L-ascorbic acid with a mixture of sulfuric acid and *N,N'*-dicyclohexylcarbodiimide in *N,N*-dimethylformamide[1,3], and purified it by ion-exchange chromatography, but they have not yet published the details of their procedure.

Mumma and co-workers[1] recommended preparation of **2** by a method involving sulfation of **5** with pyridine sulfate–acetic anhydride–pyridine. We have repeated that procedure on four occasions, but obtained only 3% of the desired product (**2**) each time. T.l.c. analysis of the sulfation reaction indicated that **5** was completely used up, but fractional extraction of **2** from the mixture of salts formed when the reaction mixture was made neutral with potassium hydroxide was complicated by the presence of potassium acetate. We found that **2** is almost insoluble (<0.3%) in the solvent (17:3 methanol–water) recommended for extracting **2**, and that increasing the polarity of the extracting solvent only further contaminated the desired product with potassium acetate. We found that **2** could readily be fractionally recrystallized (from potassium acetate) in 17:3 methanol–water.

TABLE I

P M.R. DATA FOR L-ASCORBIC ACID AND SEVERAL OF ITS DERIVATIVES[a]

Derivative of L-*ascorbic acid*	*Chemical shifts* (τ)			*First-order coupling,* $J_{4,5}$ (*Hz*)
	H-4	*H-5*	*H-6, H-6'*	
Free acid	5.03	5.91	6.24	1.9
potassium salt	5.50	5.98	6 26	2.0
2-Sulfate				
dipotassium salt (**2**)	5.45	5.94	6.26	2.0
monopyridinium salt (**4**)	4.95	5.86	6.23	1.9
Dehydro-L-ascorbic acid[b]	5.23	5.41	5.71	0.9

[a]Measured for a 10% solution in deuterium oxide, with values on the τ scale. [b]$J_{6,6'}$ 10.3 Hz; long-range $J_{4,6}$ coupling also observed.

The p.m.r. spectra at 100 MHz of L-ascorbic acid and several of its derivatives in deuterium oxide were examined. The spectrum of dipotassium L-ascorbate 2-sulfate (**2**) showed the H-4 signal as a doublet ($J_{4,5}$ 2.0 Hz) at lowest field (τ 5.45), and the

H-5 signal at τ 5.94 as a multiplet through coupling with H-4 and the protons at C-6. The chemical shifts of H-6 and H-6′ were almost identical (in all cases, $\Delta\nu \leq 0.8$ Hz), so that the anticipated ABX system for H-6,6′ and H-5 displayed only the strong, inner transitions of the AB portion. The n.m.r. data are given in Table I.

It has been reported[2,11] that salts of L-ascorbic acid 2-sulfate are more stable than those of L-ascorbic acid, but little information thereon has been published. We measured the stability of **2** and of potassium L-ascorbate to oxygen in boiling water, and found that each compound is decomposed; the loss of the H-4 signal was readily observed by n.m.r. spectroscopy. The reaction followed zero-order kinetics, as shown in Fig. 1; dipotassium L-ascorbate 2-sulfate (**2**) and potassium L-ascorbate had half-lives of 140 and 7.6 h, respectively. The sulfate is ~18 times as stable under the conditions of this experiment.

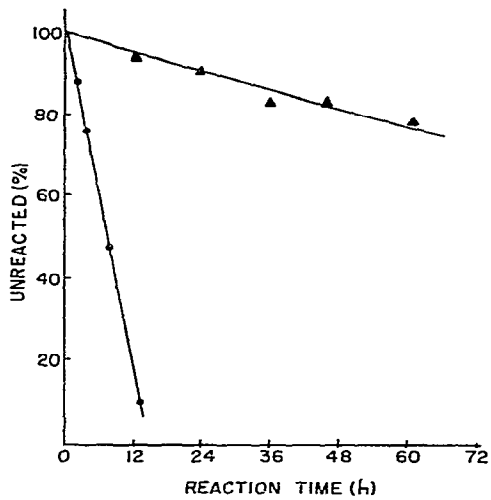

Fig. 1. Decomposition of dipotassium L-ascorbate 2-sulfate (**2**) (▲) and potassium L-ascorbate (●) by oxygen–water at 100°.

EXPERIMENTAL

General. — Melting points were determined with a Thomas–Hoover apparatus. Optical rotations were measured with a Swiss-made, Kern polarimeter, and all evaporations were conducted under diminished pressure below 50°. T.l.c. was performed on plates coated with Silica Gel G (Brinkmann Instruments, Inc., Westbury, New York); developing solvents (v/v) are given in parentheses. The components were located by spraying with 50% aqueous sulfuric acid, followed by charring on a hot plate. Compounds containing enolic hydroxyl groups were located by spraying with 1% ferric chloride in 95% methanol[14]. N.m.r. spectra were recorded for solutions (10% w/v) in deuterium oxide, with a Varian XL-100 or a Varian T-60 spectrometer, at the normal operating temperature of the instrument. Chemical shifts are reported

in τ-values from the reference signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate. A Beckman DB-G spectrophotometer was used for recording u.v. spectra.

Barium L-*ascorbate 2-sulfate dihydrate* (**3**). — To 45 g (208 mmoles) of 5,6-*O*-isopropylidene-L-ascorbic acid[15] (**5**) in *N*,*N*-dimethylformamide (80 ml) at 25° was added dropwise, with stirring, pyridine–sulfur trioxide[15,16] (45 g, 283 mmoles) in *N*,*N*-dimethylformamide (165 ml) during 7 h. The mixture was stirred an additional 3 h; t.l.c. with 17:3 methanol–ethyl acetate then showed that the starting material (R_F 0.7) had reacted completely. The mixture was evaporated to a thick syrup (at 25°), water (150 ml) was added to lower the pH to 0.9, the mixture was kept for 1 h at 25°, and the pH was adjusted to 7.0 by adding a saturated, aqueous solution of barium hydroxide. The solution was concentrated to ~700 ml, and filtered through a bed of charcoal on a filter-aid pad prepared from Fibra-Flo 4-C (Johns–Manville, New York, N.Y.). Methanol (700 ml) was added to the filtrate, the mixture was cooled, and the resulting crystals were collected by filtration, and dried over phosphorus pentaoxide; yield 43.5 g (49%), m.p. 215–225° (dec.). Three recrystallizations from 50% aqueous methanol gave pure **3** as flat prisms, m.p. 235–240° (dec.), $[\alpha]_D^{25}$ +55° (*c* 1.0, water).

Anal. Calc. for $C_6H_6BaO_9S \cdot 2H_2O$: C, 16.86; H, 2.36; S, 7.50. Found: C, 16.50; H, 2.32; S, 7.00.

Dipotassium L-*ascorbate 2-sulfate* (**2**). — Compound **2** was prepared in the usual way from barium L-ascorbate 2-sulfate (**3**) (6.0 g) by using Amberlite IR-120 (K^+) ion-exchange resin. The compound crystallized readily from cold, 50% aqueous methanol in 77% yield (3.6 g), m.p. 87–89.5°, $[\alpha]_D^{25}$ +55° (*c* 1.0, water; u.v. data: λ_{max} 255 nm (ε_{mM} 21.7) at pH 7.2, and 232 nm (ε_{mM} 14.1) at pH 2.1 [lit.[1]: λ_{max} 254 nm (ε_{mM} 29.35) at pH 7, and 233 nm (ε_{mM} 21.9) at pH 2].

Anal. Calc. for $C_6H_6K_2O_9S$: C, 21.68; H, 1.82; S, 9.65. Found: C, 21.05; H, 2.14; S, 9.60.

We also obtained **2** in low yield by using the procedure (Method B) recommended by Mumma and co-workers[1]. In our hands, this procedure gave, on four separate occasions, yields of 3%, or less, of **2**, m.p. 84–88°.

Monopyridinium L-*ascorbate 2-sulfate* (**4**). — A column of Amberlite IR-120 (H^+) ion-exchange resin was converted into the pyridinium form, and an aqueous solution (100 ml) of barium L-ascorbate 2-sulfate dihydrate (5.0 g) was passed through the column. The eluate was concentrated to ~10 ml, absolute ethanol (20 ml) was added, and the solution was kept at −10°. The resulting, crystalline solid was removed by filtration; yield 3.7 g (94%), m.p. 119–120.5°, $[\alpha]_D^{25}$ +40° (*c* 1.0, water). The product appeared to be a monopyridinium salt from evidence of the integrated intensities of the proton signals in its spectrum.

Anal. Calc. for $C_{11}H_{12}NO_9S$: C, 39.52; H, 3.62; S, 9.59. Found: C, 39.69; H, 3.72; S, 9.57.

Stability of dipotassium L-*ascorbate 2-sulfate* (**2**) *and potassium* L-*ascorbate*. — Dipotassium L-ascorbate 2-sulfate (500 mg) was dissolved in 100 ml of preboiled water, and a stream of air (24 ml/min at 1 atmosphere and 25°) was bubbled through the solution while it was heated to reflux. Aliquots (10.0 ml) of the mixture were

withdrawn at intervals, and each was mixed with 5.0 ml of water containing sodium α-toluenesulfonate (10.81 mg) as the reference standard; the resulting solution was evaporated to dryness. The residue was dissolved in deuterium oxide (0.5 ml), and the ratio of the H-4 signal at τ 5.45 to the principal signal (τ 2.37) of the reference standard was used for monitoring the disappearance of **2**. The H-4 region of the spectrum of both compounds (τ 5.38–5.75) showed no signals after complete oxidation of the compounds. The Varian T-60 spectrometer was used to measure stability. The data are given in Table II.

TABLE II

DECOMPOSITION OF DIPOTASSIUM L-ASCORBATE 2-SULFATE (2) AND POTASSIUM L-ASCORBATE BY OXYGEN–WATER AT 100°

Compound	*Time (h)*	*pH*	*% Remaining*
Dipotassium L-ascorbate 2-sulfate	0	7.0	100
	1	6.7	97
	12	5.6	94.5
	24	5.7	91.5
	36	5.4	83.3
	46	5.3	83.3
	61	5.0	78.5
Potassium L-ascorbate	0	7.50	100
	2	6.85	88.2
	4	6.70	76 6
	8	6.30	48.3
	13	6.20	9.5
	25	5.30	0

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REFERENCES

1 R. O. MUMMA, A. J. VERLANGIERI, AND W. W. WEBER, II, *Carbohyd. Res.*, 19 (1971) 127.
2 B. M. TOLBERT, D. J. ISHERWOOD, R. W. ATCHLEY, AND E. M. BAKER, III, *Fed. Proc.*, 30(2) (1971) Abstr. No. 1819.
3 E. M. BAKER, III, D. C. HAMMER, S. C. MARCH, B. M. TOLBERT, AND J. E. CANHAM, *Science*, 173 (1971) 826.
4 M. OLLIVER, in W. H. SEBRELL, JR. AND R. S. HARRIS (Eds.), *The Vitamins*, Vol. I, Academic Press, New York, 1954, pp. 261–268.
5 Ref. 4, p. 245.
6 M. S. CHANEY AND M. L. ROSS, *Nutrition*, Houghton Mifflin Co., New York, 1971, pp. 246–249.
7 R. W. HERBERT, E. L. HIRST, E. G. V. PERCIVAL, R. J. W. REYNOLDS, AND F. SMITH, *J. Chem. Soc.*, (1933) 1270.
8 J. C. BAUERNFIEND AND D. M. PINKERT, *Advan. Food Res.*, 19 (1970) 272–277.

9 F. SMITH, in ref. 4, pp. 202–205.
10 J. C. BAUERNFIEND AND D. M. PINKERT, *Advan. Food. Res.*, 19 (1970) 275.
11 E. A. FORD AND P. M. RUOFF, *Chem. Commun*, 24 (1965) 630.
12 T. M. CHU AND W. R. SLAUNWHITE, JR., *Steroids*, 12 (1968) 309.
13 R. O. MUMMA, C. P. HOIBERG, AND R. SIMPSON, *Carbohyd. Res.*, 14 (1970) 119.
14 C. S. VESTLING AND M. S. REBSTOCK, *J. Biol. Chem.*, 161 (1945) 285; F. ARNDT, L. LOEVRE, AND E. AGEN, *Chem. Ber.*, 85 (1952) 1150.
15 L. L. SALOMON, *Experientia*, 19 (1963) 619.
16 K. B. GUISELEY AND P. M. RUOFF, *J. Org. Chem.*, 26 (1961) 1248.