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Note

The preparation of L-ascorbic acid 2-sulfate-^{35}S having a high specific activity

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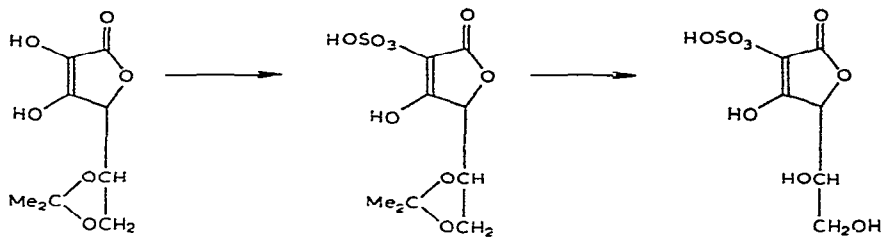
The use of L-ascorbic acid 2-sulfate (**3**) as a potential derivative of, and possible source of vitamin C has caused considerable interest. In addition to reports[1,2] that **3** is more stable than its parent, L-ascorbic acid, there have been claims[2–6] that it may act as a biological-oxidative, sulfating agent as well. To evaluate these claims, a labelled compound having a high specific-activity is required and, in this connection, we now report the preparation of very pure L-ascorbic acid 2-sulfate-^{35}S of sufficient specific activity for biological evaluation.

Experimental details are unavailable for such a synthesis (>500 mCi/mmol). While the preparation of tracer levels (6 μCi/mmol) of ascorbic acid 2-sulfate-^{35}S has been reported[6], we have found that these procedures[6,7], as recently reported[1,8], are unsatisfactory since they generate an amorphous product that contains considerable amounts of inorganic sulfate. A modified procedure, reported[1] to give a 75% yield of ascorbic acid 2-sulfate, involves the addition of one or more equivalents of a tertiary amine prior to addition of the pyridine–sulfur trioxide complex in *N,N*-dimethylformamide to 5,6-*O*-isopropylidene ascorbic acid (**1**). The presence of a tertiary amine is believed to inhibit the hydrolysis of the 5,6-blocking group during sulfation (water may be an impurity in the pyridine–sulfur trioxide reagent) which in turn prevents sulfation at the 6-hydroxyl group. The same investigators[1] described a synthesis of ascorbic acid 2-sulfate directly from L-ascorbic acid by use of 1.5 equiv. of trimethylamine–sulfur trioxide, in a solution at pH 9.5–10.5 and 70° for 0.5 h. Within this optimal pH range, the hydroxyl group at C-2 was stoichiometrically converted to the sulfate ester with about 3% of another compound, thought to be the 2,6-disulfate, being formed.

For our purposes, this direct sulfation of L-ascorbate was not amenable to a synthesis of the 2-sulfate with high specific-activity since there were indications[9] that a high-level radioactive $^{35}SO_3$–trimethylamine complex would not be stable. Subsequently, we discovered that the discrete formation of a pyridine(or trialkylamine)–sulfur trioxide complex was unnecessary and that the sulfation could be carried out on **1** more efficiently (in 74% yield after recrystallization) with sulfur trioxide

in *N,N*-dimethylformamide. The presence of the pyridine complex apparently promotes considerable side-reactions, perhaps the hydrolysis of the 5,6-*O*-isopropylidene blocking-group mentioned above. Since high specific-activity sulfur trioxide-^{35}S is commercially available[10] and can be easily transferred *in vacuo* into a slurry of the protected ascorbate in *N,N*-dimethylformamide, this synthetic route was elected. Subsequent[6] removal of the protective isopropylidene group was effected by passage through a column of Dowex-50 (H^+) ion-exchange resin, a method[11] which seems to minimize contamination of the product with inorganic sulfate[7]. The resulting ascorbic acid 2-sulfate-^{35}S was crystallized as the dipotassium salt (46% yield) and, on another occasion, as the barium salt (33% yield); the insolubility of barium sulfate in water makes the purification in this latter case much easier.



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Although this method is particularly attractive for a small-scale radioactive synthesis, we have found it is also suitable for mass synthesis (~400 g). The sulfation of glycosaminoglycans by ascorbic acid 2-sulfate-^{35}S prepared in this manner has been studied and the results have been reported[12].

EXPERIMENTAL

General. — Melting points were determined with a Thomas–Hoover capillary apparatus and are uncorrected. Spectra were recorded on standard instruments by the staff of the Physical Chemistry Department and microanalyses were performed by the Microchemical Laboratory, both of Hoffmann-La Roche, Inc. Radiochemical purity was determined on thin-layer chromatograms with a Packard Model 7201 Radiochromatogram Scanner System and radioactivity was measured by the liquid scintillation technique with a Packard Tricarb Model 2010 spectrometer.

Dipotassium L-*ascorbate 2-sulfate dihydrate* (**3**). — A 50-ml round-bottomed flask containing a slurry of 5,6-*O*-isopropylidene ascorbic acid[13] (**1**, 388 mg, 1.8 mmol) and dry *N,N*-dimethylformamide (4 ml) was connected to a glass vacuum-line. After the mixture was frozen with liquid nitrogen, the flask was evacuated to 1 mtorr, and sulfur trioxide (201 mg, 2.5 mmol) was introduced by vacuum transfer. The mixture was warmed to −15°, stirred for 30 min at this temperature, and the resulting clear solution (reaction completed) diluted with water (16 ml). The aqueous solution was

absorbed onto Dowex 50 ion-exchange resin (AG 50W-X8, 75 ml wet-volume, H^+, 100–200 mesh) packed in water in a 28 cm × 1.8 cm column. The 5,6-*O*-isopropylidene-L-ascorbic acid sulfate (**2**) was kept on the column for 45 min. Elution with water (8-ml fractions collected) afforded ascorbic acid sulfate (**3**) contained in fractions 2 through 5. These fractions were combined and brought to pH 7.7 with 10% aqueous potassium hydroxide. Evaporation of the solvent *in vacuo* at 40° gave an off-white solid (855 mg) which was redissolved in water (4.75 ml). Addition of *N*,*N*-dimethylformamide (6.10 ml) precipitated potassium sulfate and other impurities. Separation of the mother liquor and concentration (to ~0.5 ml) *in vacuo* gave 600 mg of a solid. Crystallization from water (0.54 ml) and methanol (0.36 ml) (the solution was allowed to cool overnight at 5°) afforded 486 mg (74% yield) of white crystals. Recrystallization, as just described, from 0.43 ml of water and 0.29 ml of methanol afforded 300 mg (45% yield) of dipotassium L-ascorbate 2-sulfate as white prisms.

Anal. Calc. for $C_6H_6K_2O_9S \cdot 2H_2O$: C, 19.56; H, 2.74; S, 8.70; H_2O, 9.77. Found: C, 19.54; H, 2.59; S, 8.51; H_2O, 10.03.

Dipotassium L-ascorbate 2-sulfate-^{35}S dihydrate. — By following the procedure just described, **1** (137 mg, 0.64 mmol) was treated with sulfur trioxide-^{35}S (66 mg, 0.82 mmol, 630 mCi, specific activity 770 mCi/mmol)[10]. The yield of ^{35}S-labelled ascorbic acid 2-sulfate was 107 mg (213 mCi, ^{35}S being counted with ^{14}C as a secondary standard[14]; 46% chemical yield; 34% radiochemical yield; specific activity, 1.99 mCi/mg, 730 mCi/mmol). The radiochemical purity was >98% as determined by t.l.c. on cellulose F (Merck; 4:3:3, v/v, ethyl acetate–acetic acid–water, R_F 0.3).

Barium L-ascorbate 2-sulfate-^{35}S dihydrate. — By following the procedure just described, **1** (187.4 mg, 0.87 mmol) was treated with sulfur trioxide-^{35}S (60 mg, 0.75 mmol, 600 mCi, specific activity 800 mCi/mmol)[10]. After hydrolysis of the blocking group on the ion-exchange column, the collected fractions were combined and stirred for 1 h with barium carbonate (300 mg, 1.52 mmol). The insoluble materials were removed by filtration and the mother liquid was concentrated *in vacuo*. The resulting oil was dissolved in water (3.5 ml), the insoluble material removed by centrifugation, and the compound precipitated with an equal volume of methanol to afford 107 mg (33%) of barium L-ascorbate 2-sulfate-^{35}S dihydrate. Recrystallization from 1:1 water–methanol yielded 94 mg of the compound (146 mCi, ^{35}S being counted with ^{14}C as a secondary standard[14]; specific activity, 1.55 mCi/mg, 658 mCi/mmol). The radiochemical purity was >98% as determined by t.l.c. on cellulose F (Merck; 4:3:3, v/v, ethylacetate–acetic acid–water, R_F 0.3).

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