Synthesis and Oxidation of 5,6-*O*-Isopropylidene-L-ascorbic Acid 3-Sulphate

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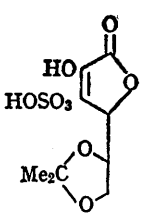
THAT ascorbic acid sulphate may be biologically important as a sulphating[1] or hydroxylating[2] agent is suggested in part by our observations on the chemical behaviour of 5,6-*O*-isopropylidene-L-ascorbic acid 3-sulphate (I) (IAAS).

The barium salt of IAAS is quite stable in aqueous solution, particularly in the absence of air. When treated with 6N-hydrochloric acid no precipitate of barium sulphate is formed until the solution is heated. At room temperature, on the other hand, anodic oxidation or the addition of such oxidizing agents as 30% hydrogen peroxide, ceric ammonium nitrate, bromine, or concentrated nitric acid cause an immediate appearance of cloudiness followed by precipitation of barium sulphate. Certain metal ions appear to enhance the appearance of the precipitate when IAAS is treated with hydrogen peroxide. The addition of the latter to the sodium salt of IAAS [prepared by passing the barium salt over IR 120(H^+) and neutralizing with two equivalents of sodium hydroxide] causes an immediate drop in pH.



(I)

The work of Todd, Clark, and others[3] on oxidative phosphorylation first led us to speculate that the 3-sulphate of ascorbic acid might act as a sulphate carrier as a consequence of oxidation. It is generally accepted that the biosynthesis of organic sulphates and sulphamates utilizes the

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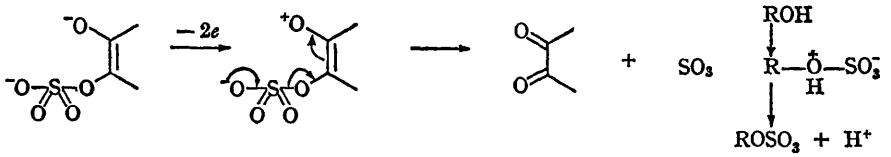
[1] W. van B. Robertson, *Biophys. J.*, 1964, **4**, 93; *Ann. New York Acad. Sci.*, 1961, **92**, 164.
[2] A. F. Wagner and K. Folkers, "Vitamins and Coenzymes," Interscience (Wiley, New York), 1964, Ch. 16.
[3] (a) V. M. Clark, D. W. Hutchinson, G. W. Kirby, and Sir Alexander Todd, *J. Chem. Soc.*, 1961, 715; (b) V. M. Clark, *Proc. Chem. Soc.*, 1964, 129.

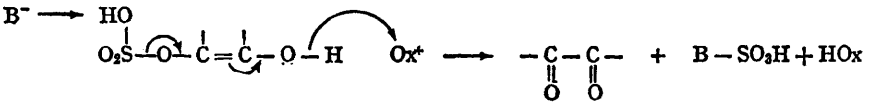
3′-phosphoadenosine-5′-phosphosulphate (PAPS) system,[4] but alternative dialysable co-factors have not been excluded by Dodgson.[5]

the barium salt. Direct sulphation of ascorbic acid failed to give a homogeneous product. Since the OH group at position 3 in ascorbic acid

Scheme (*a*)



Scheme (*b*)



It can be suggested that oxidative sulphation involves sulphur trioxide (Scheme *a*) or that the vinylogous persulphate transfers sulphate to a base under oxidizing conditions (Scheme *b*). The latter parallels the generalized P–XYZ system of Clark.[3b] If considered as a vinylogue of a mixed anhydride[6] of sulphuric and carbonic acid, IAAS would possess enhanced acylating activity.

In hydroxylations, on the other hand, ascorbic acid or IAAS might be considered as acting as vinylogues of hydrogen peroxide or peroxymonosulphuric acid, respectively. Both could give the free OH radical in the presence of Fe^{II}.

IAAS was best prepared by sulphating 5,6-*O*-isopropylidene-L-ascorbic acid[7] with pyridine–sulphur trioxide in dimethylformamide[8] to give is generally considered to be the most acidic,[6] IAAS is presumed to be the 3-sulphate. Preliminary polarographic analyses at pH 4·5 with the rotating platinum electrode comparing the $E_{\frac{1}{2}}$ (S.C.E.) of ascorbic acid (327 mv), its isopropylidene derivatives (326 mv) and IAAS (333 mv) show that the latter is slightly more difficult to oxidize. Whether one or two electrons are involved in what appears to be an irreversible oxidative process cannot be determined with the data at hand. Unlike its behaviour with ascorbic acid, the dye 2,6-dichlorophenol indophenol, cannot be used to titrate IAAS.

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[4] F. Lipman and P. W. Robbins, *J. Biol. Chem.*, 1957, **229**, 837; see also: *Science*, 1958, **128**, 575.
[5] K. S. Dodgson, *Colloquia*, 1959, **13**, 28; Fourth International Congress in Biochemistry, Vienna, 1958.
[6] C. S. Vestling and M. C. Rebstock, *J. Biol. Chem.*, 1944, **152**, 585.
[7] L. L. Salomon, *Experientia*, 1963, **19**, 619.
[8] K. B. Guiseley and P. M. Ruoff, *J. Org. Chem.*, 1962, **27**, 1479; 1961, **26**, 1248.