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Conformational Change Accompanying the Ionization of Ascorbic Acid in Aqueous Solution*

by

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A change in sign of the Cotton effect is more commonly ascribed to a conformational equilibrium or less often to asymmetric solvation (1). A reversal in sign of the Cotton effect has now been observed to accompany ionization of a proton from the hydroxyl group attached to the number three carbon of L-ascorbic acid (I). This reversal is considered to be unusual since ascorbic acid contains a conformationally rigid five membered ring.

Materials and Methods.

The CD spectra were obtained with a Durrum-Jasco ORD/UV-5 optical rotatory dispersion recorder with a circular dichroism attachment. A 450-w Osram xenon arc lamp was used as a light source, and a standard camphor sulfonic acid solution was used as a calibration standard ($\Delta\epsilon$ at 289 mμ

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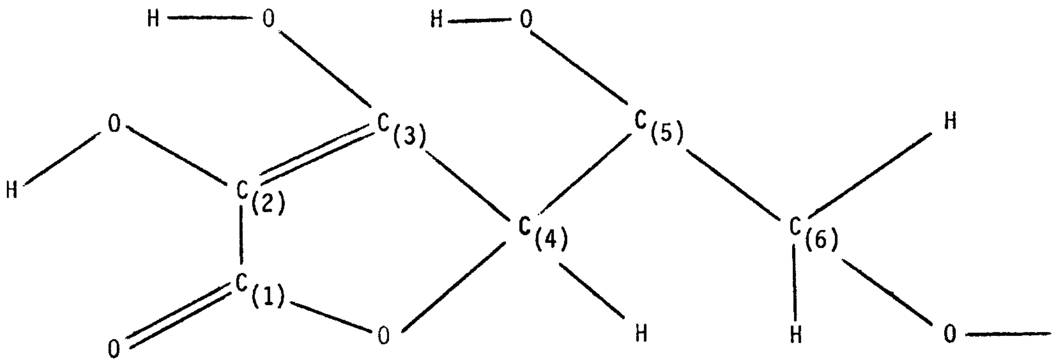
equal to 2.09). All solutions (0.002 M) were freshly prepared and examined in 1 mm path length cells at ambient temperatures on the ±0.002 ΔD or ±50 md scale. The nmr spectra were obtained with a Varian A-60A spectrometer under ambient conditions.

A sample of L-ascorbic acid (Sigma) was used without futher preparation. This sample was found to have a specific rotation at the sodium D line (588 mμ) in 0.1 M HCl of +20°.

Anal. Calcd for $C_6H_8O_6$: C, 40.92; H, 4.58. Found: C, 41.24; H, 4.52.

Results and Discussion

The pH dependence of the ORD and CD spectra were measured from 300 mμ down to the absorption limit (about 200 mμ), with particular attention given to the region between 225 and 275 mμ. It was in this wavelength region where it was found that a reversal in the sign of the Cotton effect accompanied the ionization of the acidic proton. Since the ORD results paralleled the CD results, attention was focused on the CD measurements for convience. The results obtained are illustrated in Fig. 1. It may be seen that the protonated form of ascorbic acid has a negative CD spectrum, whereas the ionized form has a positive CD spectrum.



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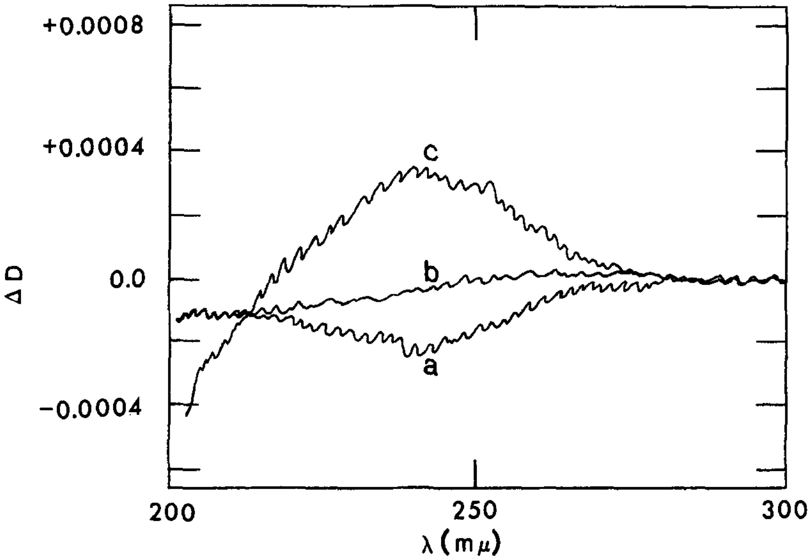

Fig. 1. Tracings of CD curves for various states of protonation of L-ascorbic acid: (a) 1.0 M HCl solution, (b) CD H_2O blank, and (c) pH 6.0, 0.1 M succinate-borate solution.

Various CD curves were obtained in the range of pH 2.0 to 6.0 in 0.1 M succinate-borate, and it was found that these spectra could be represente as the sum of two individual spectra with $\Delta\epsilon$ equal to -1.0 and +2.5 corresponding to the protonated and ionized forms of the acid, respectively. A similar procedure has been used previously to obtain the dissociation constants of tartaric acid with the aid of polarimetry (2).

The interpretation given for the reversal in the sign of the Cotton effect upon ionization is that the conformation of the five-membered ring is different in the protonated and ionized form. This view is supported by the x-ray results of Hvoslef which reveal that the enediol portion of the ring is planar in the protonated form (3), whereas in the monoanion form the lactone group is planar and the enediol group has lost its planarity (4). This view is supported by the nmr spectra of the two forms of ascorbic acid in D_2O in that the only difference between the two forms is a 26 c.p.s. shift to lower field for the methylene proton on carbon atom number four, which is parallel to the shifts observed by Sheinblatt (5) for the methylene protons of glycylglycine upon ionization of the carboxyl group.

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