Refractive Indices of *l*-Ascorbic Acid

E. G. Cox[1] states that *l*-ascorbic acid is optically negative with $\alpha = 1{\cdot}462$, $\beta = 1{\cdot}68$, and $\gamma > 1{\cdot}70$. We have examined a material isolated from peppers after the method of A. Szent-Györgyi[2] by Dr. A. G. Grollmann, of the Johns Hopkins Medical School. This substance gives the characteristic absorption spectrum of *l*-ascorbic acid with a maximum coefficient[3] at 2650 A.; the melting point is 188°, and the analysis (Mrs. M. S. Sherman) C, 40·80 per cent, H, 4·78 per cent (calculated for $C_6H_8O_6$; C, 40·89 per cent, H, 4·58 per cent). The compound as crystallised from methyl alcohol or acetone has $\alpha = 1{\cdot}465$, $\beta = 1{\cdot}600 \pm 0{\cdot}006$, and $\gamma = 1{\cdot}747$ for λ 5780 A. The optical sign is either positive or negative within the limit of experimental error, as is verified by the lack of curvature of the isogyre in a centred optic axis interference figure. No evidence was found for structural polymorphism, but the possibility was not rigorously eliminated. These constants, which are for crystals of the type shown in Szent-Györgyi's Fig. 2a[2], are published since they are of value in identification of ascorbic acid and since they substantiate Cox's deduction of a plane configuration for the molecule, which is in accord with the accepted furanose ring structure.

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[1] NATURE, **130**, 205, Aug. 6, 1932.
[2] *Biochem. J.*, **22**, 1387; 1928.
[3] Note R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds and F. Smith, *J. Chem. Soc.*, 1270; 1933.

Uroflavin, Maltoflavin and Redox-Potentials of Lyochromes

BESIDES hepatoflavin, the isolation of which was described in these columns recently[1], two further members of the lyochrome series have been obtained in a highly purified, though not definitely pure and crystalline state: uroflavin from normal human urine* and maltoflavin from malted barley. The process of preparation is very similar to the procedure adopted for the isolation of hepatoflavin.

Uroflavin as well as maltoflavin exhibit much the same properties as the lyochromes previously described. The yellow-red solutions show a strong green fluorescence. Whereas earlier observations with nickel oxide glass filters suggested that the fluorescence of lyochromes is mainly due to ultra-violet light, it was found by the use of a quartz monochromator that visible light of the blue-violet region and not ultra-violet radiation is responsible for the fluorescence. Both lyochromes yield chloroform soluble 'lumi-flavins' on strong irradiation in alkaline solution. Finally, both pigments lose their colour and fluorescence on reduction and regain these characteristics after reoxidation. The spectrographic examination, for which I am much indebted to Dr. E. R. Holiday, showed that maltoflavin and also uroflavin possess a sharp absorption band in the ultra-violet, the peaks of the band being at 255 mμ and at 281mμ respectively. In contrast to other lyochromes, there seems to be no specific absorption in the range of longer wave-lengths. The absorption curve of hepatoflavin shows two maxima, a sharp one at 258mμ and a flat one around 360mμ.

* Uroflavin is a component of the urochrome fraction, but not identical with urochrome *A* or *B* (cf. [2]).

The potentiometric study of the three lyochromes proves that they represent perfectly stable oxidation-reduction systems. Even in low concentration they impart stable and fairly reproducible potentials to noble metal electrodes within the range of a reasonable redox buffering capacity. As reductants hydrosulphite or palladium-hydrogen, and as oxidants ferricyanide or molecular oxygen, were used. The position of the normal potentials (E'_0, referring to the normal hydrogen electrode) was found as follows: Hepatoflavin: *p*H 5·88, $E'_0 = -0{\cdot}177$ v.; *p*H 7·3, $E'_0 = -0{\cdot}219$ v.; *p*H 8·62, $E'_0 = -0{\cdot}274$ v. Maltoflavin: *p*H 7·4, $E'_0 = -0{\cdot}216$ v. Uroflavin: *p*H 7·2, $E'_0 = -0{\cdot}217$ v. (phosphate buffer of isotonic strength was used throughout). The titration experiments were performed at room temperature (16°–19·5° on different days, constant within 1° during the experiments). The curves obtained so far indicate an electron

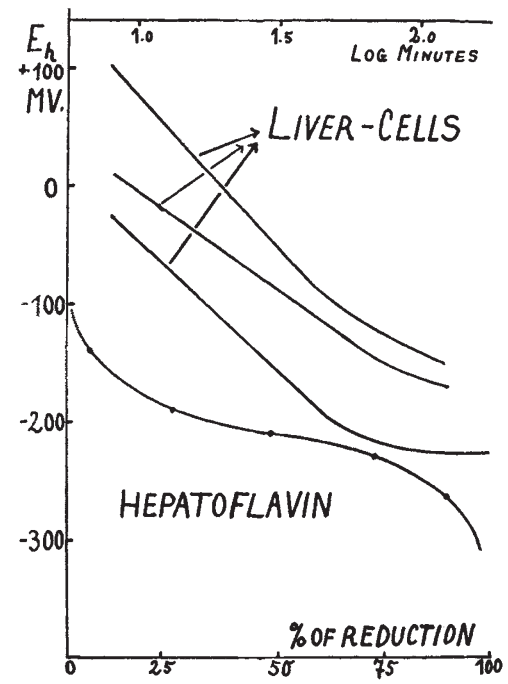

FIG. 1.

number of $n = 1$ rather than of $n = 2$. It should be mentioned that Bierich *et al.*[3], working with a product from mammalian tissues which is probably identical with lumi-flavin[1], report an E'_0 of $-0{\cdot}217$ v. at *p*H 7·2 and of $-0{\cdot}139$ v. at *p*H 5·39, but give index potentials corresponding to $n = 2$.

The physiological significance of the extremely negative position of the normal potentials of these widely distributed biological redox-systems awaits elucidation. Under normal aerobic conditions, if there is such a state as a uniform aerobic reduction potential (which would then be near to $r_H = 12$)[4], the cell flavin would be present entirely in the oxidised state. But around $r_H = 7$ ($E_h = -200$ mv.), which is considered to be the general anaerobic reduction potential of living cells, the cell flavin is exactly in its equilibrium range. This fact is illustrated in Fig. 1, which in its upper part shows an experiment of Clark *et al.*[5], in which the reduction potential of liver suspensions in phosphate buffer at *p*H 7·4 was observed, whilst in the lower part one of our curves