The Crystal Structure of 10-Methylisoalloxazine Hydrobromide Dihydrate: Hydrogen-bonding Properties of Protonated Flavin

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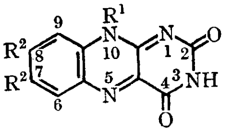
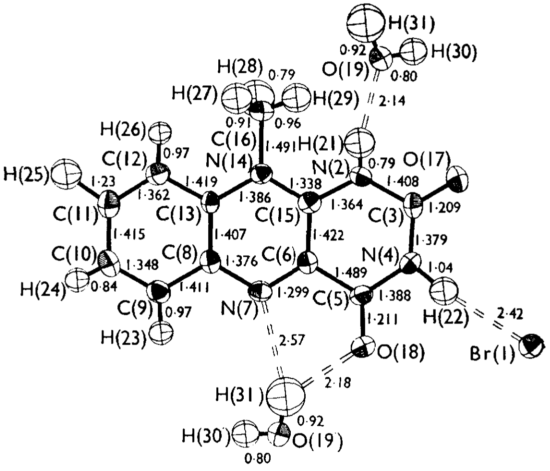
RIBOFLAVIN (I; R^1 = D-ribityl and R^2 = methyl) is the coenzyme of a large family of flavoproteins, catalysts in the energy-producing chain of oxidative phosphorylation.[1] It has been shown to be vicinal to the metal at the active site, generally iron, and to participate in the catalytic redox reactions. The mode of bonding of flavin to protein and substrate is not fully understood, although the flavin in its semiquinoid redox state apparently chelates to the metal of the protein through N(5) and CO(4) [numbering as in (I)]. In the oxidized state, the flavin is reported to have little affinity for metals except for some, such as silver(I) and copper(I), which are thought to have a certain electron-donor ability.[1] The hydrogen bonding found in 10-methylisoalloxazine hydrobromide dihydrate (I; R^1 = Me, R^2 = H) sheds further light on the relative basicities of the various potentially basic sites in the fully oxidized ring system.



(I)

Crystals of 10-methylisoalloxazine hydrobromide dihydrate were grown by evaporation of concentrated solutions of the flavin in 48% hydrogen bromide. The space group is $P2_1/c$ (monoclinic), with $a = 9{\cdot}42 \pm 0{\cdot}01$, $b = 11{\cdot}81 \pm 0{\cdot}01$, $c = 13{\cdot}64 \pm 0{\cdot}02$ Å and $\beta = 118{\cdot}27 \pm 0{\cdot}06°$. All reflections having $2\theta \leqslant 40°$ for Mo-K_α radiation were measured with an automatic Picker four-circle diffractometer in the 2θ scan mode.[2] Of these, 1686 were significantly above background and 668 unobserved. The observed reflections were processed in the usual way[3] to give structure factors, F_0, and their standard deviations. The structure was solved by using the bromide ion as a heavy atom, and has been refined by least-squares[4] to a current R value of 4·8%. All atoms were refined, hydrogen atoms isotropically and heavier atoms anisotropically. All of the hydrogen atoms, including the acidic one at N(2) (see Figure) and those on the water molecules, were first located on difference maps. The average standard deviation in a bond (except bonds to hydrogen atoms) is about 0·007 Å.

The Figure shows thermal ellipsoids of the heavy atoms, with spheres scaled to show the size of the isotropic vibration parameters, B, of the hydrogen atoms. The difference map clearly revealed H(21) at N(2), confirming earlier evidence[5,6] that N(2) is far more basic than N(7). Furthermore, this study as well as those of riboflavin hydrobromide hydrate[5] and of 1,3,10-trimethylalloxazinium iodide[6] indicates a fairly short bond at C(6)–N(7) and a long one at C(5)–C(6). All other ring distances are suggestive of extensive delocalization. The ring system is more nearly planar than that in reference 6, and is essentially bowed along the long axis.



FIGURE

Of special interest is the evidence regarding relative basicities of the various nitrogen and oxygen atoms provided by the fairly extensive hydrogen-bonding system, most of which is shown in the Figure. It should be realized that the orientation of water (19) [and symmetry-related (19′)] is partly fixed by a bond through H(30) to the second water molecule. Thus the close approach (not an hydrogen bond) of H(31′) to N(7) may be partly artifactual. However, it seems that in protonated flavin, O(18) is more basic than O(17) and that N(7) carries sufficient basicity to help stabilize the positive charge attracted primarily to O(18). The nitrogen atoms are more basic than the oxygen atoms (*i.e.* oxo-forms are preferred). Because both H(21) and H(22) are involved in hydrogen bonding, their relative attractiveness for negative charge cannot confidently be determined. The bond to bromide must be considered fairly strong (it is 0·7 Å shorter than the sum of van der Waals radii)[8] despite the fact that the simplest canonical resonance forms have the positive charge located primarily on N(2) and N(14).

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[1] For a review of metal–flavin interactions, see P. Hemmerich, F. Müller, and A. Ehrenberg, "Oxidases and Related Redox Systems", Wiley, New York, 1964, vol. 1, p. 157.

[2] T. Furnas, "Single Crystal Orienter Instruction Manual", *X*-Ray Department, General Electric Co., Milwaukee, Wisconsin, 1957.

[3] C. J. Fritchie, jun., *Inorg. Chem.*, 1967, **6**, 1199.

[4] E. W. Hughes, *J. Amer. Chem. Soc.*, 1941, **63**, 1737.

[5] K. H. Dudley, A. Ehrenberg, P. Hemmerich, and F. Müller, *Helv. Chim. Acta*, 1964, **47**, 1354.

[6] N. Tanaka, T. Ashida, Y. Sasada, and M. Kakudo, *Bull. Chem. Soc. Japan*, 1967, **40**, 1739.

[7] P. Kierkegaard, R. Norrestam, P. Werner, A. Ehrenberg, G. Eriksson, and F. Müller, *Chem. Comm.*, 1967, 288.

[8] L. Pauling "The Nature of the Chemical Bond", Cornell University Press, Ithaca, New York, 1948, p. 189.