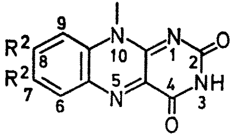
Crystal Structure of a Flavin–Naphthalenediol Molecular Complex

By CHARLES A. LANGHOFF and CHARLES J. FRITCHIE, JUN.
(*Richardson Chemistry Laboratories, Tulane University, New Orleans, Louisiana* 70118)

Summary The molecular complex 10-methylisoalloxazinium bromide:sesqui-naphthalene-2,7-diol monohydrate appears to be of charge-transfer nature and together with earlier work suggests that the flavin "chelate site," CO(4)–N(5), may be significant in flavin–protein binding, even in the oxidized state.



(Ia) R^1 = D-ribityl, R^2 = Me
(Ib) R^1 = Me, R^2 = D-ribityl

RIBOFLAVIN, (Ia), in the form of its 5′-phosphate (FMN) or the condensate of FMN with 5′-phosphatoadenosine (FAD), is an essential cofactor in the energy-conserving reactions of mitochondrial electron transport. Little is known about the nature of the active sites in flavoproteins except that metal atoms (Fe,Mo)[1] and sulphydryl groups[2] are often components, the former possibly occupying the "chelate site" CO(4)–N(5) of flavin semiquinone and that a π-complex, possibly of charge-transfer nature, may be formed[3–5] between the flavin and its normal reducing substrate, reduced pyridine nucleotide (DPNH). Only tyrosine[6] has been implicated among the specific protein components that may bind to the flavin; apparently the 5′-phosphate (and adenosine in FAD) is a more significant factor[7] in flavin–protein binding. Only recently[8] has the accurate geometry of the flavin nucleus and some indications of the relative binding activities of its functional groups become available.

To increase knowledge of the flavin nuclear geometry and more importantly to study the hydrogen-bonding and π-complexing properties of the flavin nucleus, we have begun studying molecular complexes of flavins with hydroxy-compounds.

Black lathlike crystals of 10-methylisoalloxazinium bromide: sesqui(naphthalene-2,7-diol) monohydrate, $C_{11}H_{10}O_2N_4{}^+Br^-\cdot1\frac{1}{2}C_{10}H_8O_2\cdot H_2O$, grow from a 47%-HBr solution of 10-methylisoalloxazine and an excess of the diol: triclinic; $a = 8\cdot42(1)$, $b = 10\cdot50(1)$, $c = 15\cdot26(1)$ Å, $\alpha = 98\cdot52(5)°$; $\beta = 109\cdot91(5)°$, $\gamma = 103\cdot29(5)°$, and $Z = 2$; space group $P\bar{1}$–C_i.[1] The structure was solved and refined by block diagonal least-squares procedures to $R = 5\cdot8\%$ with 1939 reflections of significant intensity measured on a Picker diffractometer and 1136 unobservably small reflections. The R-factor includes only 182 of the latter for which $|F_c|$ exceeds $|F_{\min}|$.

The distances within the flavin nucleus (Figure 1), and angles as well, agree with those previously found.[8] One naphthalenediol molecule lies above the flavin (Figure 2) over the phenylene and central rings, suggesting partial transfer of charge from the diol to the lowest unoccupied orbital of flavin. High-resolution e.s.r. measurements[9] reveal greatest spin density (proton hyperfine splitting) at N(5), C(8), N(10), and C(6); hence the lowest empty orbital in (oxidized) flavin is primarily in this region.

Since a bromide ion is hydrogen-bonded to NH(3) and a water molecule lies close to the molecular plane near the "chelate site" CO(4)–N(5) as before,[8a,8d] we suggest that NH(3) should be considered readily available as a hydrogen-bond donor and that CO(4) is a stronger receptor than CO(2), with the donor group likely to be fairly close to N(5). It seems likely that this "chelate site" will nearly always be occupied by a positive ion or dipole even in the oxidized form of the molecule and that a change in bond strength upon reduction may be a factor in configurational change likely to occur in enzymic function. In this structure flavins are hydrogen bonded through NH(1) and CO(2) in centrosymmetric "base pairs," with N–H · · · O = 1·87(1) Å. The remaining one-half diol in the empirical formula necessarily appears centrosymmetric in the crystal; its oxygen atoms are disordered over naphthalene positions 2, 3, 6, and 7, and it lies in a plane highly tilted with respect to the flavin plane. It is hydrogen bonded to the bromide ion and the water molecule.

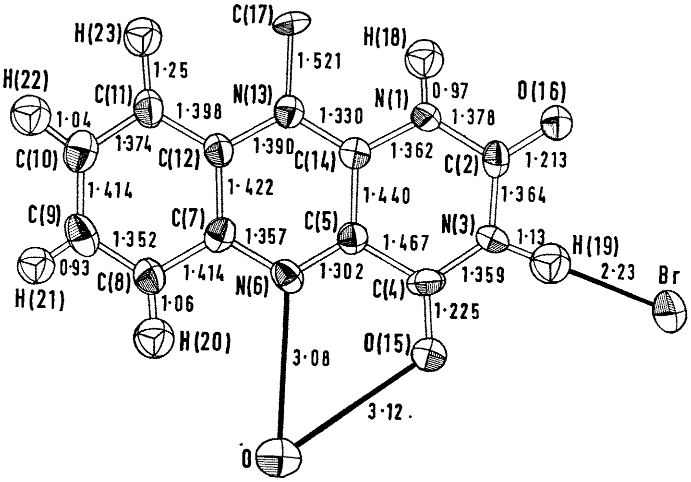


FIGURE 1. *Bond distances of the* N(1)-*protonated* 10-*methylisoalloxazinium cation. The mean* σ_{C-C} *or* σ_{C-N} *is* 0·007 Å.

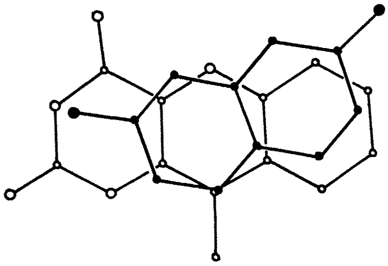


FIGURE 2. *Overlap of flavin cation and naphthalene-*2,7-*diol, viewed normal to mean plane of the flavin.*

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[4] (a) A. P. Nygaard and H. Theorell, *Acta Chem. Scand.*, 1955, **9**, 1587; (b) P. Strittmater, *J. Biol. Chem.*, 1961, **236**, 2329.

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[6] (a) C. J. Fritchie, jun., and B. L. Trus, *Chem. Comm.*, 1968, 1486; see also *Acta Cryst.*, 1969, **B25**, 1911. (b) N. Tanaka, T. Ashida, Y. Sasada, and M. Kakudo, *Bull. Chem. Soc. Japan*, 1967, **40**, 1739; see also *ibid.*, 1969, **42**, 1546; (c) P. Kierkegaard, R. Norrestam, P.-E. Werner, A. Ehrenberg, G. Eriksson, and F. Müller, *Chem. Comm.*, 1967, 288. (The standard deviations are probably rather high here because of the heavy iodine atom. Other structures presented by P. Kierkegaard at the VIII I.U.Cr. Congress in Stony Brook, New York, 1969, are more accurate and essentially agree with our results. We thank Prof. Kierkegaard for prior communication of his results); (d) an adenosine hydroxy-group also occupies this site in the intermolecular complex riboflavin·5′-bromo-5′-deoxyadenosine·3H$_2$O; D. Voet and A. Rich, American Crystallographic Association 1969, Spring Meeting Seattle, Paper L-4.

[9] Ref. 2, p. 239.