Crystal and Molecular Structure of a Donor–Acceptor Complex involving Protonated Riboflavin, Quinol, and Bromide Ions

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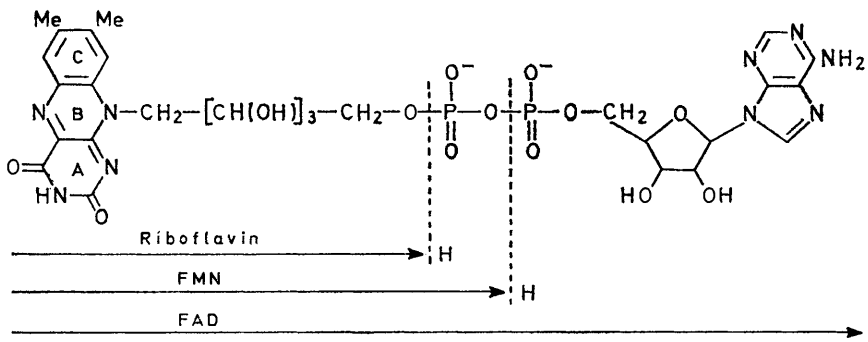
Black crystals of stoicheiometry riboflavin:quinol:hydrogen bromide:water 1:1:2:2 have been grown. The crystals are monoclinic, $a = 20{\cdot}55(5)$, $b = 13{\cdot}69(4)$, $c = 10{\cdot}18(4)$ Å, $\beta = 91{\cdot}2(1)°$, with four ' molecules ' of this composition in a cell of space group $P2_1$. The structure was solved from photographic data by Patterson and Fourier methods, and refined by least squares to R 0·145 for 2358 independent reflections. There are charge-transfer interactions between riboflavin molecules (as acceptors) and quinol molecules and bromide ions (as donors). The riboflavin molecules are probably in the semiquinone state and their interaction with the donors is not confined to a specific region of the isoalloxazine nuclei.

INVESTIGATIONS into the role of flavin co-enzymes have indicated the probability that charge-transfer states are involved in at least some aspects of their biological function.[1] Binding at active sites, stabilisation of its derivatives, and particular donor or acceptor species.[2] Extensive studies of the flavins, especially by Hemmerich and co-workers,[3] have done much to clarify the properties of these molecules in their different oxidation states and



radical states, and substrate interaction have all been associated with charge-transfer species at various times and there are a number of studies demonstrating the presence of such an interaction between isoalloxazine, or to demonstrate the existence of stable semiquinones under suitable conditions.

Suggestions that riboflavin, as FMN or FAD, could be involved in mitochondrial electron transport through a

[1] V. Massey and G. Palmer, *J. Biol. Chem.*, 1962, **237**, 2347.

[2] R. Foster, ' Organic Charge-Transfer Complexes,' Academic Press, London, 1969, p. 345.

[3] P. Hemmerich, G. Nagelschneider, and C. Veeger, *FEBS Letters*, 1970, **8**, 74.

charge-transfer interaction with ubiquinone [4] prompted us to attempt a structural analysis of a model for such a process. We hoped to verify the plausibility of the proposal, to seek information on the sections of the riboflavin molecule susceptible to charge transfer, and to investigate the environment of the CO(4)–N(5) region thought to be significant in flavin binding.[5] We report on a three-dimensional *X*-ray study of a riboflavin–quinol adduct.

EXPERIMENTAL

Unstable crystals of a riboflavin hydrobromide–quinol complex were grown from a solution of riboflavin and an excess of quinol in ethanol–12M-hydrobromic acid (1 : 1 v/v) as solvent. The badly formed black crystals were deposited from solutions set aside in the dark. They rapidly decomposed, as judged by *X*-ray diffraction photographs, on removal from their mother liquor and were therefore handled in quartz capillaries containing a small amount of solution. Elemental analysis indicated the stoicheiometry riboflavin : quinol : hydrogen bromide : water 1 : 1 : 2 : 2 (Found: C, 40·4; H, 4·6; N, 8·0. Calc. for $C_{23}H_{32}Br_2N_4O_{10}$: C, 40·35; H, 4·7; N, 8·2%).

Crystal Data.—$C_{23}H_{32}Br_2N_4O_{10}$, $M = 684·35$. Monoclinic, $a = 20·55(5)$, $b = 13·69(4)$, $c = 10·18(4)$ Å, $\beta = 91·2(1)°$ (σ taken from the spread of repeated film measurements), $U = 2864$ Å^3, $D_m = 1·58$ (by flotation of freshly prepared crystals), $Z = 4$, $D_c = 1·59$. Space group $P2_1$ or $P2_1/m$. Cu-K_α radiation $\lambda = 1·5418$ Å; μ(Cu-K_α) = 45·1 cm^{-1}.

All crystals showed marked changes in spot-shape and size over different regions of exploratory diffraction photographs and all showed some spot splitting. Numerous attempts to grow more perfect crystals failed and hence the best two available (<0·25 mm in longest dimension) were used in the collection of intensity data by Weissenberg film methods. This mode of recording allowed a visual assessment of all reflexions so that corrections for spot-shape and splitting could be made. This was done on a subjective basis except when clear division or elongation of spots allowed a more mechanical approach. Altogether data were collected for the *h*0—9*l* and *hk*0—7 layers and correlated by a least-squares method [6] to give 2358 independent observed reflexions. The correlation index was relatively high at 0·16, indicative of the trouble in obtaining the data and possible absorption effects which went uncorrected.

The Patterson synthesis, which indicated $P2_1$ as space group, was analysed for the positions of the four independent bromide ions then to be expected. Heavy-atom and subsequent electron density syntheses revealed the positions of the riboflavin and quinol molecules, failing only to reveal the water. Refinement was undertaken by a block-diagonal least-squares method, the data being weighted by the expression $w = [1 + (F_o - b)^2/a^2]^{-1}$ the control parameters

TABLE 1

Atomic co-ordinates and isotropic temperature factors

Atom	*x*/*a*	*y*/*b*	*z*/*c*	*B*/Å^2
Br(1)	0·0608(2)	0·0004(5)	0·0324(4)	
Br(2)	0·2213(2)	0·3088(5)	0·3557(4)	
Br(3)	0·4231(2)	0·2964(5)	0·0136(5)	
Br(4)	0·7289(3)	0·3111(7)	0·5167(5)	
O(11)	0·840(1)	0·279(2)	0·328(3)	4·0(6)
O(14)	1·093(1)	0·306(3)	0·522(3)	4·2(6)
C(11)	0·903(2)	0·286(5)	0·378(5)	4·5(9)
C(12)	0·916(2)	0·289(4)	0·510(4)	4·2(9)
C(13)	0·982(2)	0·291(4)	0·565(4)	3·9(9)
C(14)	1·031(2)	0·300(4)	0·473(4)	3·4(8)
C(15)	1·017(2)	0·299(5)	0·333(4)	3·7(9)
C(16)	0·956(2)	0·297(4)	0·289(4)	4·0(9)
N(101)	0·763(1)	0·044(2)	0·408(2)	0·7(5)
N(103)	0·714(1)	0·049(2)	0·619(3)	1·4(5)
N(105)	0·888(1)	0·051(2)	0·666(2)	0·9(5)
N(110)	0·877(1)	0·048(2)	0·397(2)	0·3(4)
O(102)	0·659(1)	0·039(2)	0·405(2)	2·4(4)
O(104)	0·774(1)	0·048(2)	0·800(2)	1·2(4)
O(114)	0·837(1)	−0·116(2)	0·256(2)	2·0(5)
O(115)	0·887(1)	0·042(2)	−0·011(2)	1·3(4)
O(116)	0·946(1)	−0·156(2)	−0·015(2)	2·6(5)
O(117)	0·773(1)	−0·219(2)	−0·012(3)	3·5(6)
C(102)	0·708(1)	0·040(3)	0·474(3)	1·2(6)
C(104)	0·773(2)	0·047(3)	0·680(3)	2·0(7)
C(104a)	0·834(2)	0·048(3)	0·607(3)	1·5(7)
C(105a)	0·942(1)	0·045(3)	0·599(3)	0·7(6)
C(106)	1·003(2)	0·045(4)	0·672(4)	3·1(9)
C(107)	1·060(1)	0·043(3)	0·597(3)	0·4(5)
C(108)	1·058(1)	0·048(3)	0·463(3)	1·1(6)
C(109)	0·996(2)	0·052(3)	0·390(3)	1·7(7)
C(109a)	0·938(1)	0·047(3)	0·458(3)	0·8(6)
C(110a)	0·825(1)	0·044(3)	0·468(3)	0·8(5)
C(111)	1·126(1)	0·041(3)	0·674(3)	0·9(5)
C(112)	1·121(2)	0·045(4)	0·384(4)	2·9(8)
C(113)	0·872(1)	0·053(3)	0·241(3)	1·7(6)
C(114)	0·881(2)	−0·048(3)	0·190(4)	2·0(7)
C(115)	0·860(1)	−0·049(3)	0·042(3)	1·4(6)
C(116)	0·877(1)	−0·140(3)	−0·037(3)	1·3(6)
C(117)	0·844(2)	−0·235(4)	0·008(5)	4·0(9)
O(21)	0·561(2)	0·265(3)	0·155(3)	5·3(8)
O(24)	0·769(2)	0·275(3)	−0·177(3)	5·1(8)
C(21)	0·612(2)	0·268(4)	0·068(5)	4·7(9)
C(22)	0·679(2)	0·263(4)	0·122(4)	3·4(9)
C(23)	0·732(3)	0·266(4)	0·039(5)	5·0(9)
C(24)	0·716(3)	0·273(4)	−0·094(5)	5·6(9)
C(25)	0·654(2)	0·281(4)	−0·145(4)	3·1(9)
C(26)	0·603(3)	0·279(5)	−0·072(5)	5·3(9)
N(201)	0·610(1)	0·030(2)	0·156(2)	0·8(5)
N(203)	0·663(1)	0·035(2)	−0·045(2)	1·3(5)
N(205)	0·488(1)	0·036(2)	−0·104(2)	0·9(5)
N(210)	0·499(1)	0·033(2)	0·172(2)	0·7(5)
O(202)	0·721(1)	0·029(2)	0·147(2)	1·7(4)
O(204)	0·603(1)	0·042(2)	−0·230(2)	1·7(4)
O(214)	0·549(1)	−0·133(2)	0·319(2)	2·1(5)
O(215)	0·464(1)	0·018(3)	0·578(3)	4·4(7)
O(216)	0·467(1)	−0·207(3)	0·573(3)	3·7(6)
O(217)	0·591(2)	−0·270(3)	0·682(4)	6·2(9)
C(202)	0·668(1)	0·030(3)	0·097(3)	0·7(6)
C(204)	0·602(2)	0·036(3)	−0·109(3)	1·6(7)
C(204a)	0·545(1)	0·033(3)	−0·037(3)	0·9(6)
C(205a)	0·433(2)	0·033(3)	−0·037(3)	1·6(7)
C(206)	0·374(1)	0·023(3)	−0·098(3)	1·1(6)
C(207)	0·315(2)	0·028(3)	−0·029(3)	2·1(7)
C(208)	0·317(2)	0·039(3)	0·117(3)	1·8(6)
C(209)	0·378(1)	0·038(3)	0·180(3)	1·5(6)
C(209a)	0·436(1)	0·038(3)	0·108(3)	0·6(6)
C(210a)	0·551(1)	0·031(3)	0·108(3)	1·4(6)
C(211)	0·253(2)	0·024(3)	−0·116(3)	2·1(7)
C(212)	0·258(2)	0·040(3)	0·194(4)	2·3(7)
C(213)	0·503(1)	0·044(3)	0·324(3)	1·1(6)
C(214)	0·496(2)	−0·060(3)	0·376(3)	1·5(7)
C(215)	0·515(2)	−0·042(4)	0·525(4)	2·9(9)
C(216)	0·520(2)	−0·137(4)	0·606(4)	3·0(8)
C(217)	0·583(2)	−0·191(4)	0·586(4)	3·6(8)
O(1)	0·696(2)	−0·216(4)	0·209(4)	1·8(9)
O(2)	0·337(2)	0·029(4)	0·537(4)	0·3(9)
O(3)	0·722(2)	−0·314(4)	0·404(4)	1·7(9)
O(4)	1·017(3)	−0·300(5)	0·050(6)	4·1(10)

[4] D. E. Fleischman and G. Tollin, *Biochim. Biophys. Acta*, 1965, **94**, 248.

[5] P. Hemmerich, F. Muller, and A. Ehrenberg, 'Oxidases and Related Redox Systems,' vol. 1, Wiley, New York, 1964, p. 157.

[6] A. D. Rae, *Acta Cryst.*, 1965, **19**, 683.

a and b being chosen to ensure as constant $\langle w(|F_o - F_c|)^2 \rangle$ as possible over F_o ranges. Form factors were taken from ref. 7. After the refinement of a model incorporating individual isotropic temperature parameters had converged at R 0·169 anisotropic motion was included for the bromide ions. A difference-Fourier map calculated after refinement again converged revealed four low-weight maxima which were taken to be the water molecules in sites of fractional occupancy. They were introduced into subsequent calculations as half-weighted atoms although the low temperature factors returned for three of them suggests that a slightly greater weighting would have been appropriate. Final convergence reduced R to 0·145, anisotropic temperature factors for all atoms bringing only an insignificant reduction to 0·140.

TABLE 2

Anisotropic thermal parameters ($\times 10^4$) for bromine

Atom	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
Br(1)	17·2(8)	76(4)	48(3)	−7(4)	−14(3)	24(7)
Br(2)	23·4(9)	49(3)	82(4)	12(4)	−24(3)	2(8)
Br(3)	30·3(12)	53(4)	97(4)	−3(5)	−20(4)	−16(9)
Br(4)	39·7(16)	119(6)	104(5)	11(7)	29(5)	−7(12)

The scattering factor of an atom is expressed by: $f = f_0 \exp[-(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl)]$.

TABLE 3

Bond lengths (Å)

O(11)–C(11)	1·38(6)	O(21)–C(21)	1·39(6)
O(14)–C(14)	1·38(6)	O(24)–C(24)	1·39(7)
C(11)–C(12)	1·36(7)	C(21)–C(22)	1·46(7)
C(11)–C(16)	1·44(7)	C(21)–C(26)	1·44(8)
C(12)–C(13)	1·46(7)	C(22)–C(23)	1·39(7)
C(13)–C(14)	1·39(7)	C(23)–C(24)	1·39(8)
C(14)–C(15)	1·44(7)	C(24)–C(25)	1·37(7)
C(15)–C(16)	1·32(7)	C(25)–C(26)	1·31(7)
N(101)–C(102)	1·33(4)	N(201)–C(202)	1·34(4)
N(101)–C(110a)	1·41(4)	N(201)–C(210a)	1·30(4)
N(103)–C(102)	1·49(4)	N(203)–C(202)	1·45(4)
N(103)–C(104)	1·35(5)	N(203)–C(204)	1·39(4)
N(105)–C(104a)	1·25(4)	N(205)–C(204a)	1·34(4)
N(105)–C(105a)	1·32(4)	N(205)–C(205a)	1·34(4)
N(110)–C(109a)	1·39(4)	N(210)–C(209a)	1·44(4)
N(110)–C(110a)	1·31(4)	N(210)–C(210a)	1·27(4)
N(110)–C(113)	1·59(4)	N(210)–C(213)	1·56(4)
O(102)–C(102)	1·22(4)	O(202)–C(202)	1·20(4)
O(104)–C(104)	1·22(4)	O(204)–C(204)	1·23(4)
O(114)–C(114)	1·46(5)	O(214)–C(214)	1·43(4)
O(115)–C(115)	1·47(4)	O(215)–C(215)	1·45(5)
O(116)–C(116)	1·44(4)	O(216)–C(216)	1·49(5)
O(117)–C(117)	1·49(6)	O(217)–C(217)	1·46(6)
C(104)–C(104a)	1·48(5)	C(204)–C(204a)	1·40(5)
C(104a)–C(110a)	1·42(5)	C(204a)–C(210a)	1·48(5)
C(105a)–C(106)	1·44(5)	C(205a)–C(206)	1·35(5)
C(105a)–C(109a)	1·44(4)	C(205a)–C(209a)	1·48(5)
C(106)–C(107)	1·41(5)	C(206)–C(207)	1·41(5)
C(107)–C(108)	1·37(4)	C(207)–C(208)	1·49(5)
C(107)–C(111)	1·56(4)	C(207)–C(211)	1·54(5)
C(108)–C(109)	1·47(5)	C(208)–C(209)	1·40(5)
C(108)–C(112)	1·54(5)	C(208)–C(212)	1·47(5)
C(109)–C(109a)	1·47(5)	C(209)–C(209a)	1·41(5)
C(113)–C(114)	1·50(5)	C(213)–C(214)	1·53(5)
C(114)–C(115)	1·56(5)	C(214)–C(215)	1·58(6)
C(115)–C(116)	1·52(5)	C(215)–C(216)	1·54(6)
C(116)–C(117)	1·54(6)	C(216)–C(217)	1·51(6)

Final atom positions and temperature parameters are listed in Tables 1 and 2, calculated bond lengths and angles in Tables 3 and 4. Structure factor data are listed in Supplementary Publication No. SUP 20682 (5 pp., 1 microfiche).*

* For details see Notice to Authors No. 7 in *J.C.S. Dalton*, 1972, Index issue.

The numbering system for the riboflavin and quinol molecules is shown in Figure 1.

TABLE 4

Bond angles (deg.)

O(11)–C(11)–C(12)	122(2)	O(21)–C(21)–C(22)	118(2)
O(11)–C(11)–C(16)	120(2)	O(21)–C(21)–C(26)	123(2)
C(12)–C(11)–C(16)	119(2)	C(22)–C(21)–C(26)	119(2)
C(11)–C(12)–C(13)	123(2)	C(21)–C(22)–C(23)	120(2)
C(12)–C(13)–C(14)	115(2)	C(22)–C(23)–C(24)	116(2)
O(14)–C(14)–C(13)	116(2)	O(24)–C(24)–C(23)	115(2)
O(14)–C(14)–C(15)	121(2)	O(24)–C(24)–C(25)	121(2)
C(13)–C(14)–C(15)	123(2)	C(23)–C(24)–C(25)	124(2)
C(14)–C(15)–C(16)	119(2)	C(24)–C(25)–C(26)	123(2)
C(11)–C(16)–C(15)	121(2)	C(21)–C(26)–C(25)	118(2)
C(102)–N(101)–C(110a)	124(2)	C(202)–N(201)–C(210a)	130(2)
C(102)–N(103)–C(104)	121(2)	C(202)–N(203)–C(204)	121(2)
C(104a)–N(105)–C(105a)	120(2)	C(204a)–N(205)–C(205a)	119(2)
C(109a)–N(110)–C(110a)	120(2)	C(209a)–N(210)–C(210a)	122(2)
C(109a)–N(110)–C(113)	119(2)	C(209a)–N(210)–C(213)	119(2)
C(110a)–N(110)–C(113)	121(2)	C(210a)–N(210)–C(213)	119(2)
N(101)–C(102)–N(103)	116(2)	N(201)–C(202)–N(203)	114(2)
N(101)–C(102)–O(102)	114(2)	N(201)–C(202)–O(202)	128(2)
N(103)–C(102)–O(102)	129(2)	N(203)–C(202)–O(202)	118(2)
N(103)–C(104)–O(104)	118(2)	N(203)–C(204)–O(204)	116(2)
N(103)–C(104)–C(104a)	122(2)	N(203)–C(204)–C(204a)	121(2)
O(104)–C(104)–C(104a)	120(2)	O(204)–C(204)–C(204a)	123(2)
N(105)–C(104a)–C(104)	121(2)	N(205)–C(204a)–C(204)	118(2)
N(105)–C(104a)–C(110a)	125(2)	N(205)–C(204a)–C(210a)	125(2)
C(104)–C(104a)–C(110a)	114(2)	C(204)–C(204a)–C(210a)	117(2)
N(105)–C(105a)–C(106)	118(2)	N(205)–C(205a)–C(206)	122(2)
N(105)–C(105a)–C(109a)	120(2)	N(205)–C(205a)–C(209a)	119(2)
C(106)–C(105a)–C(109a)	122(2)	C(206)–C(205a)–C(209a)	119(2)
C(105a)–C(106)–C(107)	117(2)	C(205a)–C(206)–C(207)	122(2)
C(106)–C(107)–C(108)	122(2)	C(206)–C(207)–C(208)	120(2)
C(106)–C(107)–C(111)	117(2)	C(206)–C(207)–C(211)	115(2)
C(108)–C(107)–C(111)	120(2)	C(208)–C(207)–C(211)	126(2)
C(107)–C(108)–C(109)	121(2)	C(207)–C(208)–C(209)	118(2)
C(107)–C(108)–C(112)	121(2)	C(207)–C(208)–C(212)	122(2)
C(109)–C(108)–C(112)	118(2)	C(209)–C(208)–C(212)	120(2)
C(108)–C(109)–C(109a)	119(2)	C(208)–C(209)–C(209a)	121(2)
N(110)–C(109a)–C(105a)	118(2)	N(210)–C(209a)–C(205a)	118(2)
N(110)–C(109a)–C(109)	123(2)	N(210)–C(209a)–C(209)	122(2)
C(105a)–C(109a)–C(109)	119(2)	C(205a)–C(209a)–C(209)	120(2)
N(101)–C(110a)–N(110)	121(2)	N(201)–C(210a)–N(210)	127(2)
N(101)–C(110a)–C(104a)	122(2)	N(201)–C(210a)–C(204a)	116(2)
N(110)–C(110a)–C(104a)	117(2)	N(210)–C(210a)–C(204a)	116(2)
N(110)–C(113)–C(114)	108(2)	N(210)–C(213)–C(214)	104(2)
O(114)–C(114)–C(113)	110(2)	O(214)–C(214)–C(213)	105(2)
O(114)–C(114)–C(115)	106(2)	O(214)–C(214)–C(215)	107(2)
C(113)–C(114)–C(115)	108(2)	C(213)–C(214)–C(215)	99(2)
O(115)–C(115)–C(114)	105(2)	O(215)–C(215)–C(214)	106(2)
O(115)–C(115)–C(116)	114(2)	O(215)–C(215)–C(216)	108(2)
C(114)–C(115)–C(116)	116(2)	C(214)–C(215)–C(216)	113(2)
O(116)–C(116)–C(115)	106(2)	O(216)–C(216)–C(215)	112(2)
O(116)–C(116)–C(117)	104(2)	O(216)–C(216)–C(217)	106(2)
C(115)–C(116)–C(117)	115(2)	C(215)–C(216)–C(217)	113(2)
O(117)–C(117)–C(116)	105(2)	O(217)–C(217)–C(216)	111(2)

DISCUSSION

The difficulties with the crystals have led to low accuracy in the final structural parameters and argument depending on exact values is not pursued. Bond lengths and angles are within 3σ (*ca.* 0·15 Å and 6°) of those expected and, within these limits, are in agreement with those found in the *X*-ray analysis of riboflavin hydrobromide.[8] The planes of best fit (Table 5) show that both quinol groups and the individual rings of each riboflavin are approximately planar but that the isoalloxazine nuclei as a whole are a little less so. It is

[7] 'International Tables for *X*-ray Crystallography,' vol. III, Kynoch Press, Birmingham, 1962.

[8] N. Tanaka, T. Ashida, Y. Sasada, and M. Kakudo, *Bull. Chem. Soc. Japan*, 1969, **42**, 1546.

possible that the same situation is present here as is found in riboflavin hydrobromide in which only the benzene ring is truly planar, the others being slightly

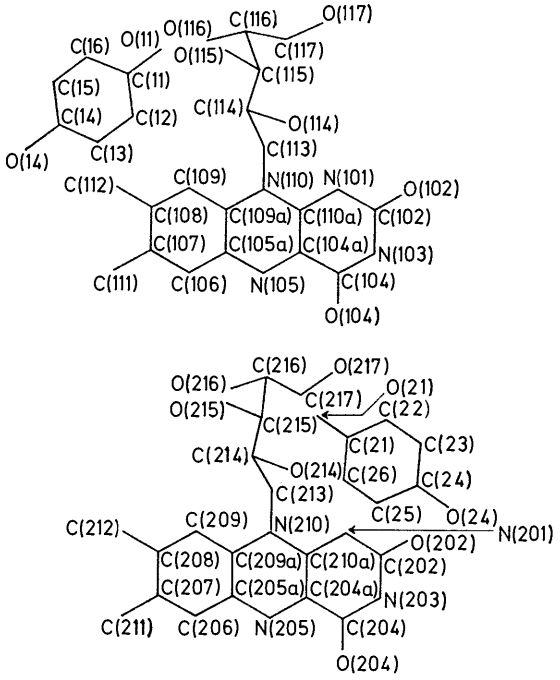


FIGURE 1 Atom numbering scheme

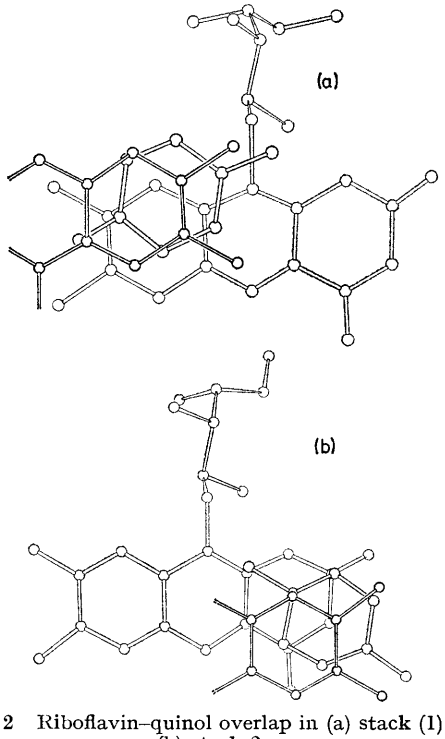


FIGURE 2 Riboflavin–quinol overlap in (a) stack (1) and (b) stack 2

puckered, but the errors outweigh the detection of any such effect.

Each riboflavin is associated with one particular quinol, the relative spatial arrangements being shown in Figure 2(a and b). These two molecules interleave in stacks (see also, the packing diagram of Figure 3). In stack (1) (in which every atom number begins with 1)

TABLE 5

Equations of planes of best fit referred to orthogonal axes and in the cosine form $AX + BY + CZ + D = 0$, where A, B, and C are the direction cosines, and $X = a + c \cdot \cos\beta$, $Y = b$, and $Z = c \cdot \sin\beta$. The displacements (Å) of relevant atoms from the plane are given in square brackets

Plane (1): quinol (1)

$$-0{\cdot}075X + 0{\cdot}997Y + 0{\cdot}021Z - 2{\cdot}619 = 0$$

[O(11) −0·02, O(14) 0·00, C(11) −0·01, C(12) 0·04, C(13) −0·02, C(14) 0·00, C(15) −0·02, C(16) 0·03, C(105a) 3·31, C(106) 3·38, C(107) 3·52, C(108) 3·49, C(109) 3·34, C(109a) 3·31, C(105a) * 3·29, C(106) * 3·37, C(107) * 3·45, C(108) * 3·53, C(109) * 3·52, C(109a) * 3·34]

* Atoms at 1·0 − x, 0·5 + y, 1·0 − z.

Plane (2): quinol (2)

$$0{\cdot}033X + 0{\cdot}996Y + 0{\cdot}081Z - 4{\cdot}146 = 0$$

[O(21) −0·01, O(24) −0·02, C(21) −0·01, C(22) 0·01, C(23) 0·01, C(24) −0·01, C(25) 0·01, C(26) 0·02, N(201) 3·18, N(203) 3·25, C(202) 3·19, C(204) 3·33, C(204a) 3·35, C(210a) 3·25, C(205a) * 3·54, C(206) * 3·49, C(207) * 3·55, C(208) * 3·57, C(209) * 3·48, C(209a) * 3·48]

* Atoms at 1·0 − x, 0·5 + y, − z.

Plane (3): riboflavin (1) ring system

$$-0{\cdot}004X + 1{\cdot}000Y - 0{\cdot}002Z - 0{\cdot}550 = 0$$

[N(101) −0·02, N(103) 0·04, N(105) 0·06, N(110) 0·02, C(102) −0·07, C(104) 0·01, C(104a) 0·02, C(105a) −0·03, C(106) −0·03, C(107) −0·06, C(108) 0·00, C(109) 0·07, C(109a) 0·00, C(110a) −0·03, O(102) −0·09, O(104) 0·02, C(111) −0·11, C(112) 0·00, C(113) 0·10]

Plane (4): N(101), N(103), C(102), C(104), C(104a), C(110a)

$$0{\cdot}011X - 0{\cdot}999Y + 0{\cdot}031Z + 0{\cdot}288 = 0$$

[N(101) −0·03, N(103) −0·04, C(102) 0·04, C(104) 0·02, C(104a) 0·00, C(110a) 0·01, O(102) 0·02, O(104) 0·05]

Plane (5): N(105), N(110), C(104a), C(105a), C(109a), C(110a)

$$0{\cdot}000X - 1{\cdot}000Y + 0{\cdot}013Z + 0{\cdot}570 = 0$$

[N(105) −0·04, N(110) −0·03, C(104a) 0·00, C(105a) 0·04, C(109a) −0·01, C(110a) 0·03, C(113) −0·12]

Plane (6): C(105a), C(106), C(107), C(108), C(109), C(109a)

$$0{\cdot}003X + 0{\cdot}999Y + 0{\cdot}032Z - 0{\cdot}873 = 0$$

[C(105a) 0·00, C(106) 0·03, C(107) −0·02, C(108) −0·01, C(109) 0·03, C(109a) −0·02, C(111) −0·03, C(112) −0·03]

Plane (7): riboflavin (2) ring system

$$0{\cdot}000X + 1{\cdot}000Y - 0{\cdot}015Z - 0{\cdot}441 = 0$$

[N(201) −0·06, N(203) 0·04, N(205) 0·06, N(110) −0·02, C(202) −0·05, C(204) 0·06, C(204a) 0·01, C(205a) 0·01, C(206) −0·12, C(207) −0·06, C(208) 0·07, C(209) 0·05, C(209a) 0·05, C(210a) −0·04]

Plane (8): N(201), N(203), C(202), C(204), C(204a), C(110a)

$$0{\cdot}002X - 0{\cdot}999Y - 0{\cdot}029Z + 0{\cdot}436 = 0$$

[N(201) −0·01, N(203) −0·01, C(202) 0·01, C(204) 0·00, C(204a) 0·01, C(210a) −0·01, O(202) 0·02, O(204) −0·06]

Plane (9): N(205), N(210), C(204a), C(205a), C(209a), C(210a)

$$0{\cdot}017X + 1{\cdot}000Y + 0{\cdot}003Z - 0{\cdot}637 = 0$$

[N(205) 0·02, N(210) 0·00, C(204a) 0·01, C(205a) −0·04, C(209a) 0·03, C(210a) −0·02, C(213) 0·15]

Plane (10): C(205a), C(206), C(207), C(208), C(209), C(209a)

$$0{\cdot}013X - 0{\cdot}997Y + 0{\cdot}074Z + 0{\cdot}318 = 0$$

[C(205a) −0·04, C(206) 0·04, C(207) 0·00, C(208) −0·04, C(209) 0·04, C(209a) 0·00, C(211) −0·03, C(212) −0·02]

the quinol lies over ring C [see Figure 2(a)] of the riboflavin, centred approximately over C(109), at a mean plane-to-plane distance of 3·39 Å. The mean distance to ring C of the riboflavin on the other side of the quinol is almost the same (3·41 Å) so that quinol and riboflavin are equally spaced. Their separation of *ca.* 3·4 Å cannot of itself be taken to indicate a charge-transfer interaction but the supposition is supported by the parallel stacking (see Table 5) and the mode of overlap (Figure 2) of the two species.[9] Similar stacking at 3·4 Å is seen, for example, in the tetramethyluric acid–pyrene complex.[10]

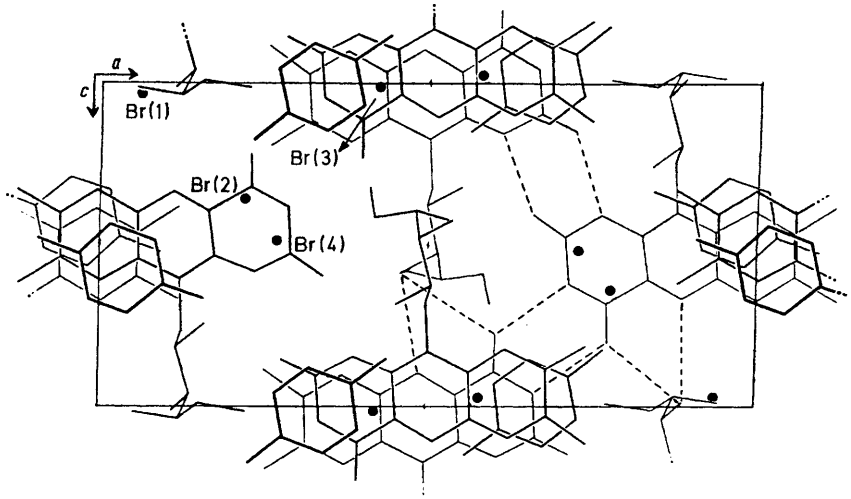


FIGURE 3 Projection of unit-cell contents. Some significant hydrogen bonds are shown by dotted lines

The other riboflavin–hydroquinone stack [stack (2)] is less ambiguous in proclaiming a charge-transfer interaction as here the association is clearly between a donor (*i.e.* quinol)–acceptor (riboflavin) pair. There is again interleaving but the quinol is now only 3·26 Å from ring A of the riboflavin on one side, but 3·52 Å from ring C of the isoalloxazine nucleus which approaches it from the other. Atom N(203) of ring A is approximately over the centre of the quinol. The plane-to-plane separation (3·26 Å) is good evidence for a charge-transfer complex so that, overall, one quinol–riboflavin stack [stack (1)] shows a continuous charge-transfer pathway and the other [stack (2)] discrete donor–acceptor pair interactions. The presence of these interactions accords with the deductions made for the 10-methylisoalloxazine–naphthalene-2,7-diol complex.[11]

The bromide atoms do not make close approaches to the flavin nuclei in the manner found in the crystal structure of riboflavin hydrobromide [8] and its naphthalene-2,7-diol adduct [11] in which a bromine is hydrogen bonded to N(3). In the present instance bromine atoms Br(1) and Br(3) are apparently hydrogen bonded to ribityl side-chains and Br(2), Br(3), and Br(4) to quinol molecules (see the observed intermolecular distances Table 6). In addition, however, Br(2), Br(3), and Br(4) are situated perpendicularly above and below regions of isoalloxazine rings (Figure 4). Distances from the mean riboflavin planes [Br(2)–ring A of riboflavin (1) 3·25, Br(3)–ring A of riboflavin (2) 3·23 Å, Br(3)–ring C of riboflavin (2) 3·23 Å, Br(3)–ring C of riboflavin (2) 3·61, Br(4)–ring A of riboflavin (1) 3·63 Å] and distances from nearest atoms [Br(2)–C(104) 3·29, Br(3)–C(204a) 3·32, Br(4)–N(103) 3·75 Å] suggest that both Br(2) and Br(3) interact with respective A rings of the two riboflavin molecules in a charge-transfer manner. The association is predominantly dimeric, although Br(3) is sandwiched (at 3·25 and 3·61 Å) between two riboflavin molecules in stack (2). The latter situation is somewhat reminiscent of graphite–bromine compounds where partial transfer of charge (to the bromine) is supposed. The interlayer graphite spacing (7·05 Å) [12] compares with the distance (6·78 Å) between the riboflavins.

TABLE 6

Intermolecular distances (Å)

Br(1) · · · O(116^{I})	3·22(3)	N(103) · · · O(204^{IV})	2·78(4)
Br(1) · · · O(4^{II})	3·27(6)	N(105) · · · O(115^{II})	3·29(3)
Br(2) · · · O(14^{I})	3·17(3)	N(201) · · · O(102)	2·70(3)
Br(2) · · · C(104^{III})	3·29(4)	N(203) · · · O(104^{V})	2·82(4)
Br(2) · · · O(3^{III})	3·16(5)	N(205) · · · O(215^{V})	3·27(4)
Br(3) · · · O(21)	3·18(3)	O(114) · · · O(1)	3·23(5)
Br(3) · · · C(204a^{II})	3·32(3)	O(115) · · · O(104^{V})	2·98(3)
Br(3) · · · O(217^{III})	3·25(4)	O(116) · · · O(4)	2·53(7)
Br(3) · · · O(1^{II})	3·30(5)	O(117) · · · O(1)	2·79(5)
Br(4) · · · O(11)	3·05(3)	O(214) · · · O(102)	3·12(3)
Br(4) · · · O(24^{IV})	3·25(3)	O(215) · · · O(204^{IV})	3·44(4)
Br(4) · · · N(103)	3·75(3)	O(215) · · · O(2)	2·65(6)
Br(4) · · · O(2^{III})	3·32(5)	O(1) · · · O(3^{III})	2·54(7)
N(101) · · · O(202)	2·78(3)		

Roman numerals as superscripts indicate the following equivalent positions relative to the reference molecule at *x*,*y*,*z*:

I $-1+x, y, z$	IV $x, y, 1+z$
II $1-x, 1/2+y, -z$	V $x, y, 1-z$
III $1-x, 1/2+y, 1-z$	

The flavin nuclei in each stack thus interact in some-

[9] S. C. Wallwork, *J. Chem. Soc.*, 1961, 494.
[10] F. De Santis, E. Giglio, A. M. Liquori, R. Puliti, and A. Ripamonti, *Nature*, 1961, **191**, 900.
[11] C. A. Langhoff and C. J. Fritchie, jun., *Chem. Comm.*, 1970, 20.
[12] W. T. Eeles and J. A. Turnball, *Proc. Roy. Soc.*, 1965, *A*, **283**, 179.

what different ways. In stack (1) this is to rings A and C, with bromine and quinol respectively, and the geometrical evidence clearly suggests the former is a donor–acceptor pair interaction, the latter an infinite stacking of alternate species. Only ring A interacts in stack (2), at least predominantly, associating with a quinol on one side and a bromide on the other. The arrangement in each stack is shown diagrammatically in Figure 4.

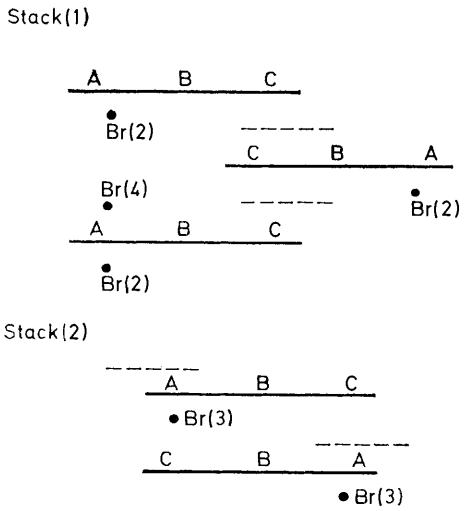


FIGURE 4 Diagrammatic view of donor–acceptor associations

The compound has crystallised as the dihydrobromide from 12M-hydrobromic acid–ethanol (1 : 1, v/v) although riboflavin itself crystallises as the monobromo salt from 6M-hydrobromic acid. Normally, in strong acid conditions, oxidised riboflavin exists as a cation protonated at N(1). Double protonation at N(1) and N(5) occurs in the semiquinone under these conditions to give, again, a singly charged species.[13] It could thus be argued that the formation of the dihydrobromide reflects increased basicity at N(5) through the attainment of a radical state by charge transfer from quinol and bromide donors. The very dark colour of the crystals is, of course, in agreement with the presence of the semiquinone form. If protonation does occur in the present instance the ability of N(5) to hold a hydrogen ion would be in line with one mode of reduction of riboflavin, *i.e.* proton transfer separate from, but perhaps in step with, electron transfer, and demonstrate the importance of charge transfer in some co-enzyme functions of this molecule.

The nature of the data has precluded the identification of hydrogen atoms but an examination of intermolecular approaches (Table 6) allows the prediction of likely hydrogen bonds and consequent deductions about the location of hydrogens. If the four water molecules be included, without occupancy considerations, there appear to be twenty-two hydrogen bonds with twenty-six appropriately bound hydrogens to account for them, seven on each riboflavin radical ion (FlH_3^+), two on each quinol and eight on the water molecules. The situation at N(1) and N(5) seems particularly clear for both riboflavins. Nitrogens N(101) and N(201) are bonded to carbonyl oxygens [O(202) 2·78, O(102) 2·70 Å] and must therefore be protonated. Atoms N(105) and N(205) are bonded to hydroxy-groups [O(115) 3·29, O(215) 3·27 Å] which are themselves bonded to carbonyl oxygens [O(104) and O(204)] again suggesting protonation of the nitrogens. That the oxygens cited as belonging to carbonyl groups are indeed so, is confirmed by the short C–O bond distances.

The hydrogen-bonding network seems to be normal so far as can be ascertained. Table 6 shows that both quinols bond to bromine atoms [Br(2), Br(3), and Br(4) twice], the distances involved (3·05—3·25 Å) being relatively short for hydrogen bonds to bromide ions and perhaps indicative of the electron-attracting power of both species in the bond arising from charge transfer to flavin nuclei. Each riboflavin is hydrogen bonded to two others by two sets of ' base pairs ', NH(3) · · · CO(4′) and NH(3′) · · · CO(4), and NH(1) · · · CO(2′) and NH(1′) · · · CO(2), which bind together the crystallographically independent molecules. These and some of the other hydrogen bonds are indicated on the crystal packing diagram (Figure 3) but no attempt has been made to represent the full network.

E.s.r. measurements reveal greatest spin density at N(5), N(10), C(6), and C(8) in riboflavin radicals,[14] so the lowest empty molecular orbital is primarily in this region. In the crystal structure of the 10-methylisoalloxazine–naphthalene-2,7-diol adduct [11] molecular overlap is seen to involve these atoms. Structural support for the presence of such a preferred region is not so clear in the present investigation. The riboflavin of stack (1) interacts with quinol in the vicinity of C(106), C(107), and C(108) on one side and close to C(108), C(109), and C(109a) on the other. That in stack (2) interacts with a quinol in the region of ring A, namely with C(202), N(203), and C(204). The bromide ions also interact with ring A of both riboflavins as mentioned earlier. It could be inferred therefore that any section of the flavin nucleus may be involved in a charge-transfer association, depending on the geometry of its partner, and that this is possibly a biological requirement for a molecule so widely concerned in oxidation–reduction reactions.

[2/2231 *Received, 25th September,* 1972]

[13] A. Ehrenberg and P. Hemmerich, in ' Biological Oxidations,' ed. T. P. Singer, Interscience, New York, 1968, p. 243.
[14] Ref. 13, p. 253.