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HYDROLYSIS PRODUCTS OF FLAVINS (ISOALLOXAZINES)

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Abstract: Hydrolysis of flavin (I) with Triton B gave the 4a-spirohydantoin (II) as the main product along with III, showing that a main nucleophilic attack of hydroxide ion was initiated on the 10a position of I.

Some oxidation reactions catalyzed by the flavins are assumed to proceed through covalent addition of anion of substrate to the isoalloxazine ring system.[1-3] In connection with study of the mechanism of flavin-catalyzed reaction, it is important to determine chemically which position of isoalloxazine is most susceptible to nucleophiles. In 1977, one of authors (F. Yoneda) reported that hydrolysis of 10-alkylisoalloxazine (for example, Id) with benzyltrimethylammonium hydroxide (Triton B) gave the 4a-spirohydantoin (IId), structure of which was presented tentatively, suggesting that the 10a position was susceptible to nucleophiles such as hydroxide ion.[4] On the other hand, Bruice et el. reported that hydrolysis of 10-arylisoalloxazine (Ia or Ib), in which the 10a position was sterically blocked, gave isoimidazolone[4,5-b]quinoxaline (IIIa or IIIb) through an initial attack of hydroxide ion on the 4 position.[5] Then, in order to clarify the above proposals chemically, the hydrolysis of Ia,[5] Ib[6], and 10-benzylisoalloxazine (Ic)[6] as well as Id[7] with Triton B were investigated. We describe here the results of hydrolysis reactions.

Treatment of I with 1.5 eq. of 40% methanolic Triton B in DMF at room temperature in the dark[8] gave the major product, 4a-spirohydantoin (II)[9,10] and the minor product (III)[11] in yields listed in Table 1. The structure elucidation of III rests upon their spectral data and comparison of these data with those of IIIa reported by Bruice et al.[5] The ^{13}C-NMR spectra of IIa-c showed the presence of three carbonyl carbons (δ ca. 164, 154, 150) and one quarternary sp^3 carbon (δ ca. 68). As can be seen from Table 1, the similarity of ^{13}C-NMR spectra of IIa-c to that of IId suggested that these compounds had the same skeleton. Then, IIb was treated with 1.5 eq. of Triton B in DMF at 130° in the dark to afford the quinoxalone (IVa)[13] and benzimidazole (V)[14] in 66.4 and 4.8% yields, respectively.[15] The structures of IVa and V were elucidated from their IR, ^{1}H-NMR and ^{13}C-NMR data. The structure of IVa was confirmed by identification with a synthetic sample prepared by condensation[16] of N-phenyl-o-phenylenediamine and diethyl ketomalonate, followed by aminolysis with methylamine. Further hydrolysis of IVa with Triton B in DMF at 125° resulted in the formation of V. Therefore, it would be reasonable to assume that the main product in hydrolysis of I is the 4a-spirohydantoin (II) containing a quinoxalone moiety in its molecule.

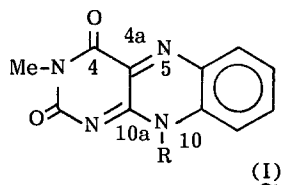
In order to confirm this assumption, IIb was subjected to an X-ray analysis. Thus, single crystals of IIb for X-ray analysis were prepared as d_6-DMSO solvate of IIb ($C_{17}H_{14}N_4O_3 \cdot C_2D_6OS$) by crystallization from chloroform containing a small amount of d_6-DMSO.

Table 1. Yields of hydrolysis products (II) and (III), and ^{13}C-NMR data of skeleton carbon of II

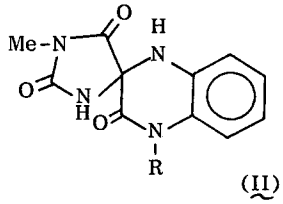
I	II	III	^{13}C-NMR data[i]				
a	54.4%	16.4%	164.6(s)	153.6(s)	150.0(s)	125.8(s)	119.1(s)
			118.3(d)	113.0(d)	108.4(d)	108.3(d)	68.0(s)
b	75.6	3.9	164.2	154.5	150.0	125.4	121.4
			118.2	112.9	109.9	108.5	68.0
c[ii]	12.7	14.3	164.7	155.2	149.9	126.0	118.9
			118.1	112.9	109.6	108.2	67.7
d	55.6	36.6	164.6	154.1	149.9	125.9	118.5
			117.9	113.1	108.7	108.4	67.5

i) For signals which are not given in this table, see the corresponding compound in reference 10.

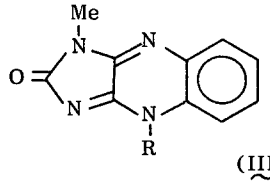
ii) In this case, a major product (16.2% yield) was an unidentified compound (A), data of which are given in reference 12.



(I)

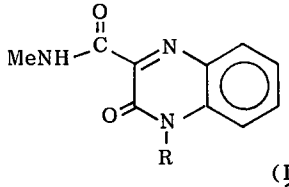


(II)



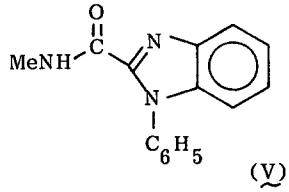
(III)

a R=2',6'-$(CH_3)_2C_6H_3$ b R=C_6H_5 c R=$CH_2C_6H_5$ d R=C_2H_5



(IV)

a R=C_6H_5
b R=C_2H_5



(V)

Scheme 1

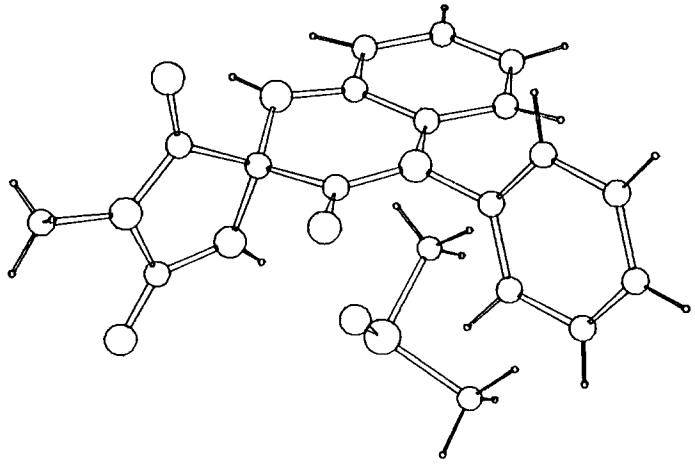


Fig. 1. Molecular Structure of IIb·d_6-DMSO solvate

The crystals were monoclinic, space group $P2_1/a$ with unit cell dimensions a=16.844(8), b= 10.806(3), c=10.720(4) Å, β=96.69(3)°, Z=4, and Dx=1.392 g/cm^3. A total of 2509 unique reflections having Fo>3σ(Fo) were measured on a Rigaku AFC-5 diffractometer using CuKα radiation. The structure was solved by the direct method using the program MULTAN 78[18] and refined by the full-matrix least-squares method to R=0.088.[19] The molecular structure so derived is depicted in Fig. 1, proving the above-mentioned assumption. The structures of the main hydrolysis products of Ia-d, therefore, can be definitely represented by formulas (IIa-d), respectively, showing that hydroxide ion initially attacks the 10a position of I to provide the corresponding 4a-spirohydantoins. In contrast with the report by Bruice et al.,[5] these results indicate that hydroxide ion attacks mainly the 10a position of isoalloxazine (I) regardless of the substituent of 10 position. Therefore, a nucleophilic addition of substrate to 10a as well as 4a or 5 position[1-3] of I should be considered in a flavin-catalyzed reaction.

Reactions of I with softer nucleophiles are under investigation.

References and Footnotes

1) C. Walsh, Acc. Chem. Res., 13, 148 (1980) and references cited therein.
2) T. C. Bruice, Acc. Chem. Res., 13, 256 (1980) and references cited therein.
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7) F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, J. Am. Chem. Soc., 98, 830 (1976).
8) Hydrolysis of Ib with methanolic KOH (1.4 eq.) in the dark at r. t. for 26 hr gave IIb (15.7%) and IIIb (1.5%) along with recovery of Ib (62.2%).
9) All new compounds gave satisfactory data of IR ($CHCl_3$), ^{1}H-NMR (200 MHz in d_6-DMSO), ^{13}C-NMR (50.10 MHz in d_6-DMSO) and high resolution MS.
10) IIa: mp 253-255°C. IR (Nujol) ν: 3300, 1775, 1725, 1715, 1685 cm^{-1}. ^{1}H-NMR δ: 1.92 (3H, s), 2.03(3H, s,), 2.91(3H, s), 5.98(1H, d, J=7.6 Hz), 6.63(1H, dd, J=7.6, 7.6 Hz), 6.86(1H, d, J=7.6 Hz), 6.95(1H, dd, J=7.6, 7.6 Hz), 7.20-7.40(3H, m), 7.63(1H, s), 9.50(1H, s). ^{13}C-NMR δ: 130.2(s), 129.9(s),128.3(s), 123.0(d), 122.9(d), 122.8(d), 18.4(q), 11.1(q), 10.8(q).
IIb: mp 227°C. IR (Nujol) ν: 3350, 3270, 1770, 1725, 1710, 1690 cm^{-1}. ^{1}H-NMR δ: 2.92 (3H, s), 6.13(1H, d, J=7.6 Hz), 6.66(1H, dd, J=7.6, 7.6 Hz), 6.85(1H, d, J=7.6 Hz), 6.95(1H, dd, J=7.6, 7.6 Hz), 7.25-7.63(5H, m), 9.44(1H, s). ^{13}C-NMR δ: 130.7, 124.3, 123.1, 122.9, 18.6.
IIc: mp 246-249°C. IR (Nujol) ν: 3300, 3225, 1780, 1735, 1650 cm^{-1}. ^{1}H-NMR δ: 2.92 (3H, s), 5.21(2H, s), 6.70(1H, dd, J=7.5, 7.5 Hz), 6.78(1H, d, J=7.5 Hz), 6.91(1H, d, J=7.5 Hz), 6.93(1H, dd, J=7.5, 7.5 Hz), 7.25-7.37(5H, m), 7.56(1H, s), 9.43(1H, s). ^{13}C-NMR δ: 130.2, 122.8, 121.3, 120.5, 39.0, 18.5.
IId: mp 241-243°C. IR (Nujol) ν: 3320, 3250, 1785, 1730, 1645 cm^{-1}. ^{1}H-NMR δ: 1.15 (3H, t, J=7.1 Hz), 2.89(3H, s), 3.96, 3.98(each, 1H, q, J=7.1 Hz), 6.77(1H, dd, J=

7.7, 1.4 Hz), 6.83(1H, ddd, J=7.7, 7.7, 1.4 Hz), 6.96(1H, ddd, J=7.7, 7.7, 1.4 Hz), 7.13(1H, dd, J=7.7, 1.4 Hz), 7.43(1H, s), 9.24(1H, s). ^{13}C-NMR δ: 30.9, 18.4, 6.39.

11) IIIa: IR ν: 1725, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.95(6H, s), 3.54(3H, s), 6.89(1H, dd, J=8.2, 1.5 Hz), 7.35(1H, ddd, J=8.2, 8.2, 1.5 Hz), 7.50(1H, ddd, 8.2, 8.2, 1.5 Hz), 7.26-7.41(3H, m), 7.99(1H, dd, J=8.2, 1.5 Hz).
IIIb: mp > 296°C. IR ν: 1730, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.53(3H, s), 7.09(1H, dd, J=7.2, 1.4 Hz), 7.36(1H, ddd, J=7.2, 7.2, 1.4 Hz), 7.49(1H, ddd, J=7.2, 7.2, 1.4 Hz), 7.41-7.68(5H, m), 7.97(1H, dd, J=7.2, 1.4 Hz).
IIIc: mp 286.5°C. IR ν: 1722, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.53(3H, s), 5.80(2H, s), 7.26-7.49(7H, m), 7.58(1H, m), 7.92(1H, m).
IIId: mp 223-224°C. IR ν: 1725, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.53(3H, t, J=7.2 Hz), 3.44(3H, s), 4.63(2H, q, J=7.2 Hz), 7.48(1H, ddd, J=7.7, 7.7, 1.7 Hz), 7.55(1H, ddd, 7.7, 7.7, 1.7 Hz), 7.62(1H, dd, 7.7, 1.7 Hz), 7.88(1H, dd, J=7.7, 1.7 Hz).

12) Compound(A): mp > 300°C. MS: m/z 318(M^+). IR (Nujol) ν: 1705, 1645 cm^{-1}. ^{1}H-NMR δ: 3.15(3H, s), 7.36(5H, m), 7.39-7.50(4H, m).

13) IVa: mp 251-253°C. IR ν: 3280, 1682, 1540 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.02(3H, d, J= 4.7 Hz, collapsed to singlet by addition of D_2O), 9.60(1H, br. s, disappeared by addition of D_2O). ^{13}C-NMR ($CDCl_3$) δ: 162.6, 155.1, 144.9, 135.1, 134.5, 132.9, 132.6, 132.1, 130.5, 130.0, 127.9, 125.1, 115.7, 26.7.

14) V: mp 161-164°C. IR ν: 3420, 1680, 1545 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.95(3H, d, J=5.1 Hz, collapsed to singlet by addition of D_2O), 7.68(1H, br. s, disappeared by addition of D_2O). ^{13}C-NMR ($CDCl_3$) δ: 159.2(s), 143.8(s), 141.1(s), 138.0(s), 136.7(s), 129.2(2xd), 128.9(d), 127.3(2xd), 125.1(d), 123.8(d), 120.5(d), 111.6(d), 25.9(q).

15) Treatment of IId with Triton B under the same condition as that of IIa gave IVb in 80.9% yield, which was identified with a synthetic sample prepared by N-ethylation of ethyl 2-hydroxyquinoxaline-3-carboxylate[17] with NaH-EtBr, followed by aminolysis with methylamine.
IVb: mp 156-158°C. IR ν: 3300, 1682, 1547 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42(3H, t, J=7.2 Hz), 3.09(3H, d, J=5.0 Hz, collapsed to singlet by addition of D_2O), 4.40(2H, q, J=7.2 Hz), 9.68(1H, br. s, disappeared by addition of D_2O). ^{13}C-NMR ($CDCl_3$) δ: 162.4(s), 154.8(s), 144.8(s), 133.0(s and d), 132.7(s and d), 124.5(d), 113.6(d), 38.0(t), 26.6(q), 12.4(q).

16) cf. J. W. Wellman and M. Tishler, J. Am. Chem. Soc., 69, 714 (1947).

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19) The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Roard, Cambridge CB2 1EW, UK.

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