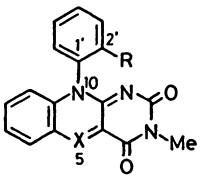
Novel Racemization of Atropisomeric Flavins and 5-Deazaflavins via Adduct Formation[1)]

Seiji SHINKAI,*, Hideki NAKAO, Toshiro YAMAGUCHI, and Osamu MANABE
Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852
(Received April 18, 1987)

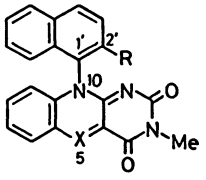
Synopsis. The molecular structures of covalent adducts derived from flavins and 5-deazaflavins are discussed on the basis of racemization of atropisomeric flavins and 5-deazaflavins. The 5-adducts with SO_3^{2-} and CN^- underwent facile racemization, indicating that the molecular structure changes from the "tense" planar state to the "relaxed" bent state. On the other hand, the 4a-adduct prepared by the photo-alkylation method did not racemize at room temperature. This indicates that the structure of the 4a-adduct is different from the bent 5-adduct. These results provide an information useful to discuss the reduced state of flavoproteins.

A number of papers have reported on the synthesis and the resolution of optical isomers caused by restricted rotation about a C–C single bond.[2-7)] The typical examples are sterically-hindered binaphthyls and biphenyls.[3-5)] In contrast, only a few precedents exist for such optical isomers including restricted rotation about a C–N single bond,[8-11)] and the characteristics arising from the C–N axis are not understood well. We recently synthesized several isoalloxazines (**1NR** and **2NR**) and 5-deazaisoalloxazines (**1CHR** and **2CHR**) with restricted rotation about the C(1′)–N(10) single bond.[12,13)] They were optically resolved by a liquid chromatographic method using a chiral packing column. It was found that these atropisomers did not recemize thermally (below 70 °C) or by visible light irradiation (at 30 °C) but the (5-deaza)isoalloxazines with a 2′-substituted phenyl group or a naphthyl group at N(10) racemized invariably when they were reduced to 1,5-dihydro forms.[13,14)] Detailed examination established that the facile rotation in the reduced state is due to conversion of the "planar" oxidized forms to the "bent" reduced forms by which the steric restriction is partically relaxed:[14)] that is, reduction changes the molecular geometry from the "tense" steric state to the "relaxed" steric state.



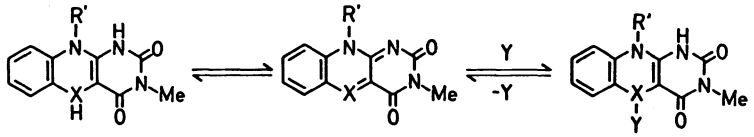
1XR

1NPh: X=N, R=phenyl
1CHPh: X=CH, R=phenyl



2XR

2NH: X=N, R=H
2NOMe: X=N, R=OMe
2CHOMe: X=CH, R=OMe



1,5-dihydro form — Y=CN^-, SO_3^{2-} (1)

It is known that flavins and 5-deazaflavins reversibly form covalent adducts (i.e., 5-adducts) with nucleophiles such as CN^- and SO_3^{2-} at 5-position.[14)] It would be of value to examine whether the racemization is also induced by the adduct formation. If they recemize, it provides unambiguous evidence for the bent structure. With this object in mind, we treated optically-pure **1XR** and **2XR** with the nucleophiles and checked whether recovered (5-deaza)isoalloxazines recemized.

Experimental

Materials. Preparations of 3-methyl-10-(2-phenylphenyl)isoalloxazine (**1NPh**), 3-methyl-10-(2-phenylphenyl)-5-deazaisoalloxazine (**1CHPh**), 3-methyl-10-(1-naphthyl)isoalloxazine (**2NH**), 3-methyl-10-(2-methoxy-1-naphthyl)isoalloxazine (**2NOMe**), and 3-methyl-10-(2-methoxy-1-naphthyl)-5-deazaisoalloxazine (**2CHOMe**) were described previously.[12,13)] The method of optical resolution was also described.[12,13)]

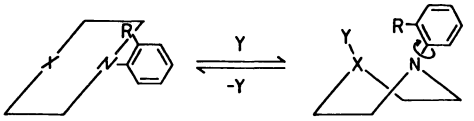
Reactions with KCN or K_2SO_3. The aqueous reactions of (5-deaza)isoalloxazines with KCN or K_2SO_3 were conducted aerobically in the quartz cuvette at 30 °C. The concentrations of the reactants are recorded in footnotes to Table 1. The progress of the reactions was followed spectrophotometrically by monitoring the disappearance of the absorption band at around 440 nm for isoalloxazines and 400 nm for 5-deazaisoalloxazines. The reaction of 5-deazaisoalloxazines and K_2SO_3 was finished immediately after mixing. Other reactions took 1—2 h. After completion of the reactions the solution (5 ml) was acidified with concd HCl (15 ml) and stirred for 1 h at room temperature. By this treatment the adducts decomposed to the oxidized forms, which were easily monitored by the recovery of the yellow solution color. Then, the solution was adjusted to pH 4 with 0.1 M Na_2HPO_4 (1 M=1 mol dm^{-3}) and extracted with chloroform. The chloroform solution was concentrated in vacuo and the residue (about 1 ml) was analyzed by HPLC using a chiral packing column (Sumipax OA-2000). The results are summarized in Table 1. We confirmed in a separate experiment that in the absence of the nucleophiles these (5-deaza)isoalloxazines do not racemize by the treatment described above.

Photo-Alkylation. (+)-**1NPh** (6.17×10^{-5} M) and ammonium phenylacetate (5.44×10^{-2} M) were dissolved in a mixed solvent of methanol–water (1.3:2.0 v/v). The solution pH was 7.1. The anaerobic solution in a Thunberg cuvette was photoirradiated by a 17-W flourescent lamp for 30 min at

room temperature. The absorption spectra taken every 5 min were very similar to those reported in Ref. 20 in which "pure" 4a-benzyl-4a,5-dihydroflavin resulted: That is, the absorption maximum (440 nm) decreased with photoirradiation with an isosbestic point (315 nm) giving rise to a new absorption maximum (350 nm). The solution was diluted with water and then extracted with chloroform. The chloroform layer was separated and dried over Na_2SO_4. The solution was concentrated in vacuo to dryness. The residue (dissolved in 0.5 ml chloroform) was subjected to HPLC analysis using a chiral packing column (Sumipax OA-2000) connected to a photo-diode array UV-VIS detector (Shimadzu SPD-M1A). In a separate study, we confirmed that photoirradiation in the absence of ammonium phenylacetate at room temperature does not induce racemization.

Results and Discussion

It is clearly seen from Table 1 that **1NPh**, **1CHPh**, and **2NH** racemize when they form the 5-adducts. It is firmly established by X-ray crystallographic studies that oxidized flavins are planar, whereas reduced 1,5-dihydroflavins are folded along a line through N(5) and N(10) like butterfly wings.[15,16] This is because the nitrogen atoms N(5) and N(10) have a high degree of tetrahedral sp^3 hybridization in the reduced state.[15,16] Although there exists no firm evidence for the molecular structure of 5-adducts, it has been expected that 5-adducts would be bent like 1,5-dihydro forms on the basis of the spectral similarity. Our finding that the 5-adducts derived from chiral (5-deaza)isoalloxazines with a 2′-phenylphenyl group or a naphthyl group easily racemize at room temperature clearly supports that the 5-adducts employ the bent structure which can relax the steric restriction (Eq. 2).



(2)

In contrast, the optical purities of 2NOMe and 2CHOMe are essentially unaffected by this treatment, indicating that racemization does not occur either in the 5-adduct state or in the transition state.[17] We have found that the redox treatment of these (5-deaza)isoalloxazines (Y=H in Eq. 2) does not change the optical purities.[12,13] This means that the steric bulkiness of the 2′-methoxy-1′-naphthyl group (but not of Y) is responsible for the retention of the optical purities. Therefore, the difference between the 2′-methoxy-1′-naphthyl group and the 2′-substituted phenyl group can be explained as follows: In the 2′-methoxy-1′-naphthyl group which would be comparable sterically with the 2′,6′-disubstituted phenyl group both 2′-methoxyl group and 8′-hydrogen provide steric hindrance to restrict the rotation, whereas in the 2′-substituted phenyl group the steric bulkiness is not enough to suppress the rotation.

The foregoing racemization trend is completely in accord with that observed for the redox treatment of the chiral (5-deaza)flavins.[12,13] Therefore, the 5-adducts should employ the bent structure with butterfly-like wings similar to the 1,5-dihydro forms.

Racemization may also take place via 4a-adducts (covalent adducts at 4a-position) if they employ the bent structure. The thermal nucleophilic attack usually occurs at 5-position of the isoalloxazine ring except a few cases.[18,19] It is known, on the other hand, that light-induced alkylation occurs at 4a-position.[20] By means of this photo-alkylation method we examined whether racemization takes place via 4a-adducts.

Accoding to Bruice et al.,[18,19] λ_{max} for 5-adducts characteristically appears between 296 and 330 nm and λ_{max} for 4a-adducts appears between 360 and 370 nm. In photo-alkylation of 6,7-dimethyl-10-(tetra-*O*-acetylribityl)isoalloxazine by phenylacetate, Walker et al.[20]

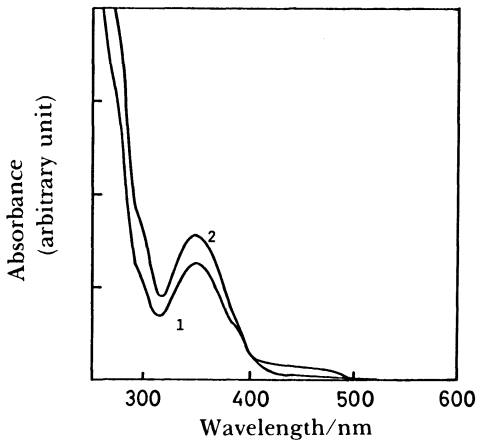
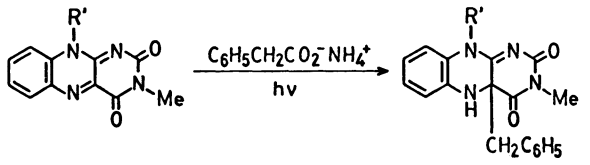


Fig. 1. Absorption spectra of the first peak (1) and the second peak (2) measured by a photo-diode array UV-VIS detector connected to a chiral peaking column.

Table 1. Racemization via Adduct Formation

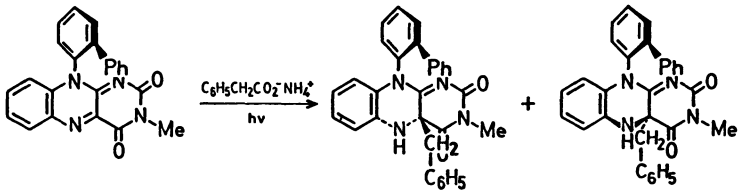
Flavin[a]	Optical purity of (+)-Isomer/e.e.%	Nucleophile[b]	Recovered flavin	
			(+)-Isomer/%	(−)-Isomer/%
1NPh	100	K_2SO_3	46.6	52.4
1CHPh	100	K_2SO_3	55.0	45.0
1CHPh	100	KCN	54.4	45.6
2NH	100	K_2SO_3	52.8	47.2
2NOMe	98.0	K_2SO_3	98.0	2.0
2CHOMe	100	K_2SO_3	100	0
2CHOMe	100	KCN	100	0

a) [1NPh]=6.20×10^{-5} M, [1CHPh]=4.74×10^{-5} M. The concentrations of other flavins were 5.00×10^{-5} M. b) [K_2SO_3]=0.752 M for isoalloxazines and 0.125 M for 5-deazaisoalloxazines, [KCN]=1.10×10^{-3} M. The reaction between 5-deazaisoalloxazines and KCN was not completely reversible because in the HPLC analysis we detected a small amount of some unknown products in addition to recovered 5-deazaisoalloxazines.



(3)

observed a new absorption maximum at 364 nm which was attributed to the 4a-adduct. In HPLC we observed two large peaks which gave the identical absorption spectra (λ_{max} 350 nm: Fig. 1). Therefore, these two peaks are attributed to the optically-isomeric 4a-adducts of 1NPh. The peak intensity ratio of the first vs. the second peak was 1.0:2.1. The ratio was affected neither by heating in chloroform for 2 h at 60 °C nor by keeping at room temperature in the dark for 3 days. These results suggest that the C(1')–N(10) axis in the 4a-adduct cannot racemize at room temperature and that the peak separation in HPLC is due to the chirality at 4a position. The absolute configuration of (+)-1NPh is not determined yet. If it could be drawn as in Eq. 4, it would result in two optical isomers, A and B. The attack from the 2'-phenyl side results in A while that from the 6'-hydrogen side results in B. Presumably, the attack from the sterically-crowded 2'-phenyl side is less advantageous. If so, the first weak peak (32%) is attributed to A and the second strong peak (68%) is attributed to B.



A B

(4)

Recently, Yoneda et al.[21] reported an X-ray crystallographic structure of 4a,5-dihydro-4a-hydroxy-3,10-dimethyl-5-deazaisoalloxazine, a 4a-adduct of 5-deazaisoalloxazine. The picture shows that N(10) with sp^2 nitrogen is almost planar while C(5) with sp^3 carbon is bent to the chair-like form, totally resulting in a half-chair conformation. On the other hand, almost nothing is known with certainty as to the 4a-adduct of isoalloxazine. If it is bent along the N(5)–N(10) like butterfly wings, the 4a-adduct of (+)-1NPh should racemize easily at room temperature. Thus, the present result suggests that N(10) in the 4a-adduct should be planar with sp^2 nitrogen which does not allow facile racemization.

According to Massey and Hemmerich,[22] a large number of flavoproteins may be classified into two groups from a viewpoint of their regiospecific reactivities, a 5-activated group and a 4a-activated group. The first group which uses the N(5) in the redox reactions is characterized by red semiquinone, high sulfite affinity, and *bent structure of reduced form*, whereas the second group which uses the C(4a) in the redox reactions is characterized by blue semiquinone, low sulfite affinity, and *almost planar structure of reduced form*. As demonsrated in the present study, the 5-adduct employs the bent structure while the 4a-adduct employs the almost planar N(10). The difference is well-correlated with the classification in flavoenzymes: that is, the reactions including N(5) take the bent intermediates while those including C(4a) take the planar intermediates not only in the flavoenzymes but also in the model systems. Therefore, the present results could provide a significant cross-link between enzymic reactions and model organic reactions.

References

1) Coenzyme Models. 49.
2) J. L. A. Webb, *J. Org. Chem.*, **18**, 1413 (1935).
3) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York (1962), p. 156.
4) R. Adams and H. C. Yuan, *Chem. Rev.*, **12**, 261 (1933).
5) M. Ōki, *Acc. Chem. Res.*, **17**, 154 (1984).
6) A. Luttrighaus and H. Gralheer, *Justus Liebigs Ann. Chem.*, **550**, 67 (1941).
7) T. H. Sidall and W. E. Stewart, *J. Org. Chem.*, **34**, 233 (1969).
8) W. H. Patterson and R. Adams, *J. Am. Chem. Soc.*, **55**, 1069 (1933).
9) C. Kashima and A. Katoh, *J. Chem. Soc., Perkin Trans. 1*, **1980**, 1599.
10) J. C. Jochims, H. von Voithenberg, and G. Wegner, *Chem. Ber.*, **111**, 1693, 2745 (1978).
11) M. Mintas, Z. Orhanovic, K. Jakopcic, H. Koller, G. Stühler, and A. Mannschreck, *Tetrahedron*, **41**, 229 (1985) and references cited therein.
12) S. Shinkai, H. Nakao, and O. Manabe, *Tetrahedron Lett.*, **26**, 5183 (1985).
13) S. Shinkai, H. Nakao, I. Kuwahara, M. Miyamoto, T. Yamaguchi, and O. Manabe, *J. Chem. Soc., Perkin Trans. 1*, in press.
14) P. Hemmerich, V. Massey, and H. Fenner, *FEBS Lett.*, **84**, 5 (1977).
15) L. Tauscher, S. Ghisla, and P. Hemmerich, *Helv. Chim. Acta*, **56**, 630 (1973) and references cited therein.
16) P. Kierkegaard, R. Norrestam, P. Werner, I. Csoregh, M. Glehn, R. Karlsson, M. Leijionmarch, O. Ronnquist, B. Sternland, O. Tillberg, and L. Torbjornsson, "Flavins and Flavoproteins," University Park Press, Baltimore (1971), p. 1.
17) M. Ōki, *Top. Stereochem.*, **14**, 1 (1983).
18) L. Hevesi and T. C. Bruice, *J. Am. Chem. Soc.*, **94**, 8277 (1972).
19) T. C. Bruice, L. Hevesi, and S. Shinkai, *Biochemistry*, **12**, 2083 (1973).
20) W. H. Walker, P. Hemmerich, and V. Massey, *Eur. J. Biochem.*, **13**, 258 (1970).
21) F. Yoneda, K. Tanaka, Y. Sakuma, H. Yoshino, and M. Takamoto, *Chem. Pharm. Bull.*, **32**, 3761 (1984).
22) V. Massey and P. Hemmerich, *Biochem. Soc. Trans.*, **8**, 246 (1980).