Coenzyme Models. Part 45.[1] Synthesis of Atropisomeric Flavins and their Novel Redox-induced Racemisation

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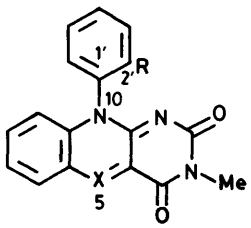
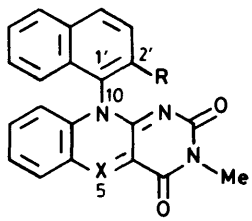
Seven (5-carba)isoalloxazines with restricted rotation about the C(1')–N(10) single bond were synthesized for the first time. They were optically resolved by a liquid chromatographic method. Six of these atropisomers (92.1—100% enantiomeric excess) (e.e.) did not racemise thermally (at 70 °C) or by visible light irradiation (at 30 °C). On the other hand, the (5-carba)isoalloxazines with a 2'-substituted phenyl group or a naphthyl group at N(10) racemised invariably when they were reduced to 1,5-dihydro forms [by four different reducing agents including photoreduction with ethylene diamine tetra-acetic acid (EDTA)]. The kinetic studies confirm that the racemisation process consists of slow, rate-determining reduction of chiral (5-carba)isoalloxazines followed by rapid rotation of the C(1')–N(10) single bond. The easy rotation in the reduced state is due to conversion of the 'planar' oxidised forms into the 'bent' reduced forms. These (5-carba)isoalloxazines are novel examples for atropisomers with a C–N axis, the rotation of which is induced only by the redox reaction.

In the past, a number of papers have reported on the synthesis and resolution of optical isomers caused by restricted rotation about a C–C single bond.[2–7] Typical examples are sterically hindered biphenyls and binaphthyls.[2,3] To the best of our knowledge, however, only a few precedents exist for such optical isomers which possess restricted rotation about a C–N single bond,[1,8–12] and the characteristics arising from the presence of the C–N axis are not understood well. The atropisomers with C–N axial chirality reported so far are aryl-substituted heterocyclic compounds such as 1-aryl-3-methoxy-2-methyl-4-pyridones and 1-aryl-4,6-dimethylpyrimidin-2(1*H*)-ones.[10,12] In some cases the atropisomers were optically resolved by the formation of the diastereoisomeric brucine salts,[8] but a liquid chromatographic (l.c.) method using optically active sorbents has not yet been applied successfully.[11,12]

In the course of our studies on flavin chemistry,[13,14] we noticed that the rotation of a phenyl group around the C(1')–N(10) single bond in 10-phenylisoalloxazine would be restricted when appropriate substituents are introduced onto the phenyl group. In fact, Bruice *et al.*[15,16] have already synthesized 10-(2',6'-dimethylphenyl)-3-methylisoalloxazine in which the rotation of the phenyl group is restricted by the 2'- and 6'-methyl groups. Although this molecule has no asymmetric centre, a new flavin-based atropisomer would be attained by eliminating one methyl group from 10-(2',6'-dimethylphenyl)-3-methylisoallozaxine. As for chiral flavin derivatives, there are only two examples in the literature: one possessed an asymmetric carbon at N(3)[17] and the other at N(10),[18] but there are no reported data about such atropisomeric flavins with respect to isomerisation about the C(1')–N(10) axis. We thus synthesized seven new isoalloxazines and (5-carba)isoalloxazines with restricted rotation about the C(1')–N(10) axis and succeeded in their optical resolution *via* an l.c. method. We have found (unexpectedly) that (5-carba)isoalloxazines with a 2'-substituted phenyl group or naphthyl group, the oxidised forms of which cannot be racemised at 70 °C, racemise easily when they are converted into the reduced forms. This redox-induced racemisation can be attributed to conversion of the 'planar' oxidised forms into the 'bent' reduced forms; this conversion reduces steric hindrance around the C(1')–N(10) single bond. This may be regarded as a new racemisation mode characteristic of atropisomers bearing an sp^2–sp^3 interconvertible nitrogen in the C–N axis.



(1XR)



(2XR)

(1NC$_2$Me) X = N, R = CO_2Me **(2NH)** X = N, R = H

(1NEt) X = N, R = Et **(2NOMe)** X = N, R = OMe

(1NPh) X = N, R = Ph **(2CHOMe)** X = CH, R = OMe

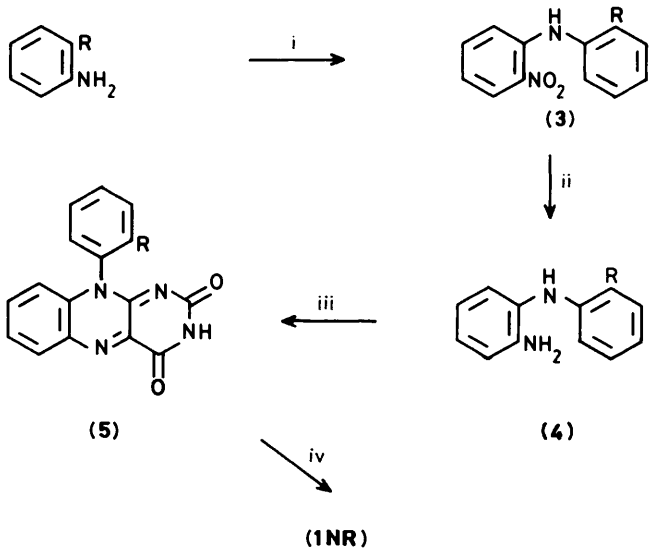
(1CHPH) X = CH, R = Ph

Experimental

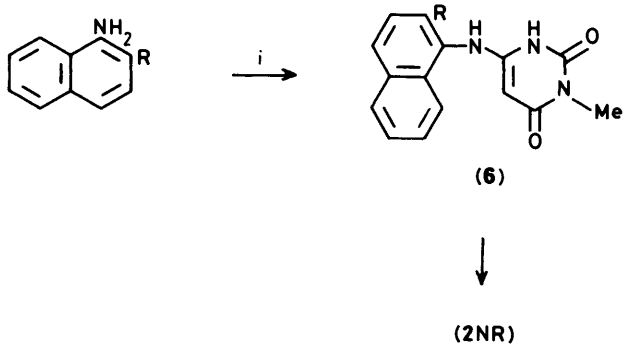
Materials.—Isoalloxazines were synthesized either by the reaction of *N*-substituted *o*-phenylenediamines with alloxane (Hemmerich's method)[19] or by the reaction of arylamines with 6-chlorouracils followed by condensation with nitrosobenzene (Yoneda's method).[20]

(**1NCO$_2$Me**), (**1NEt**), and (**1NPh**) were synthesized according to Hemmerich's method. We describe the synthesis of (**1NPh**) in detail and simply record the analytical data for other compounds.

Compound (**3**; R = Ph).—2-Aminobiphenyl (6.00 g, 35.5 mmol) and *o*-fluoronitrobenzene (2.01 g, 14.2 mmol) were treated at 190 °C for 25 h. The reaction mixture was dissolved in chloroform (100 ml), and the solution was washed with 4M-aq. HCl several times to remove remaining 2-aminobiphenyl. Finally, the chloroform solution was washed with water and then dried over Na_2SO_4 overnight. The solution was concentrated under reduced pressure, the residue being recrystallised from hexane: (yield, 47.6%), m.p. 74.5—76.0 °C; $\nu_{max.}$(KBr) 3 340 (νNH) and 1 570 and 1 340 cm^{-1} (νNO_2) (Found: C, 74.6; H, 4.8; N, 9.5. $C_{18}H_{14}N_2O_2$ requires C, 74.5; H, 4.9: N, 9.6%).



Reagents: i, *o*-$FC_6H_4NO_2$; ii, H_2, Pd–C; iii, alloxane; iv, MeI



Reagents: i, 6-chloro-3-methyluracil; ii, PhNO

Compound (**3**; R = Et).—The reaction of *o*-ethylaniline (8.60 g, 71.0 mmol) and *o*-fluoronitrobenzene (5.00 g, 35.4 mmol) was carried out in dimethyl sulphoxide (DMSO) at 118 °C for 28 h in the presence of potassium acetate (6.97 g, 71.0 mmol); work-up gave compound (**3**; R = Et) (yield, 54.6%), m.p. 53—54 °C (Found: C, 69.4; H, 5.8; N, 11.6. $C_{14}H_{14}N_2O_2$ requires C, 69.4; H, 5.8; N, 11.6%).

Compound (**3**; R = CO_2H).—The reaction of anthranilic acid (60.3 g, 0.44 mol) and *o*-fluoronitrobenzene (56.4 g, 0.40 mol) was carried out in DMSO at 130 °C for 16 h in the presence of sodium carbonate (33.9 g, 0.32 mol); work-up gave the acid (**3**; R = CO_2H) (yield, 62%), m.p. 218—219 °C (lit.,[21] 219 °C) (Found: C, 60.5; H, 3.9; N, 10.9. Calc. for $C_{13}H_{10}N_2O_4$: C, 60.3; H, 3.9; N, 10.8%).

Compound (**3**; R = CO_2Me).—A solution of the acid (**3**; R = CO_2H) (21.0 g, 81.4 mmol) in methanol (400 ml) was refluxed for 22 h in the presence of conc. H_2SO_4 (6 ml). After cooling, the orange precipitate was recovered by filtration and washed with cold methanol to afford the title ester (yield, 95%), m.p. 155—156 °C (lit.,[21] 155—157 °C); single spot on t.l.c.

Compound (**4**; R = Ph).—Compound (**3**; R = Ph) (1.00 g, 3.44 mmol) was reduced by catalytic hydrogenation using Pd–C (0.40 g) in methanol. After removal of the catalyst by filtration, the solution was concentrated under reduced pressure. The residue was recrystallised from hexane (yield, 81.4%), m.p. 86.0—87.5 °C; $\nu_{max.}$(KBr) 3 420 and 3 330 cm^{-1} (νNH_2), no band for νNO_2 (Found: C, 82.6; H, 6.1; N, 10.5. $C_{18}H_{16}N_2$ requires C, 83.0; H, 6.2; N, 10.7%).

Compound (**4**; R = Et).—Yield, 100%, m.p. 66—67 °C; single spot on t.l.c.; $\nu_{max.}$(KBr) 3 420 and 3 300 cm^{-1} (νNH_2), no νNO_2 band.

Compound (**4**; R = CO_2Me).—Ester (**3**; R = CO_2Me) was also reduced by catalytic hydrogenation. The product, (**4**; R = CO_2Me), which gave a single spot on t.l.c., was readily oxidised under aerobic conditions. We thus used the product for condensation with alloxane monohydrate without further purification.

Compound (**5**; R = Ph).—Compound (**4**; R = Ph) (0.30 g, 1.15 mmol) was treated with alloxane monohydrate (0.22 g, 1.37 mmol) in acetic acid (50 ml) in the presence of boric acid (90 mg, 1.46 mmol). The progress of the reaction was followed by t.l.c. The reaction was complete in 30 min at 60 °C. The solution was concentrated under reduced pressure. The yellow residue was well washed with hot ethanol and the title product gave a single spot on t.l.c. (yield, 54.5%), m.p. >300 °C; $\nu_{max.}$(KBr) 3 180 (νNH), and 1 710 and 1 680 cm^{-1} (νC=O) (Found: C, 71.7; H, 3.8; N, 15.1. $C_{22}H_{14}N_4O_2$ requires C, 72.1; H, 3.8; N, 15.3%).

Compound (**5**; R = Et).—Yield, 83.3%, m.p. 335 °C (decomp.); single spot on t.l.c. (Found: C, 67.9; H, 4.4; N, 17.6. $C_{18}H_{14}N_4O_2$ requires C, 67.7; H, 4.3; N, 17.7%).

Compound (**5**; R = CO_2Me).—Yield, 62.5%, m.p. >300 °C (Found: C, 62.0; H, 3.5; N, 15.9. $C_{18}H_{12}N_4O_4$ requires C, 62.1; H, 3.5; N, 16.1%).

Compound (**1NPh**).—Compound (**5**; R = Ph) (0.10 g, 0.273 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF) (60 ml), and the solution was treated with methyl iodide (0.45 g, 3.17 mmol) at 55 °C for 30 min in the presence of powdered K_2CO_3 (0.38 g). The progress of the reaction was followed by t.l.c. After cooling, the K_2CO_3 and KI were removed by filtration. The filtrate was diluted with chloroform (300 ml) and then washed with water several times. The solution was concentrated under reduced pressure at 50 °C. [When the DMF filtrate was directly concentrated under reduced pressure at 50 °C, a considerable amount of the required product (**1NPh**) was decomposed (probably owing to base-catalysed hydrolysis by a trace amount of water in the DMF).] The yellow residue was well washed with ethanol to give the title compound, which gave a single spot on t.l.c. (yield, 96.3%), m.p. 308—309 °C (decomp.); $\nu_{max.}$(KBr) no νNH band, 1 690 and 1 640 cm^{-1} (νC=O); $\delta_H(CDCl_3)$ 3.47 (3 H, s, NMe), 6.92 (1 H, d, 9-H), 8.19 (1 H, d, 6-H), and 7.3—7.7 (11 H, m, other ArH) (Found: C, 72.2; H, 4.4; N, 14.7%. $C_{23}H_{16}N_4O_2$ requires C, 72.6; H, 4.2; N, 14.7%).

Compound (**1NEt**).—Yield, 89%, m.p. 262—263 °C; $\nu_{max.}$(KBr) no νNH band, 1 710 and 1 660 cm^{-1} (νC=O); $\delta_H(CDCl_3)$ 1.09 (3 H, t, CH_2Me), 2.21 and 2.35 (2 H, 12 lines, CCH_2), 3.50 (3 H, s, NMe), 6.81 (1 H, d, 9-H), 8.36 (1 H, d, 6-H), and 7.1—7.7 (6 H, m, other ArH). The n.m.r. spectrum indicates that the methylene protons are not equivalent, giving a pair of 6-line multiplets (theoretically there should be 8 lines). Therefore, the rotation of the 2′-ethyl group should be fairly restricted (Found: C, 68.5; H, 4.9; N, 16.5. $C_{19}H_{16}N_4O_2$ requires C, 68.7; H, 4.9; N, 16.9%).

Compound (**1NCO$_2$Me**).—Yield, 87%, m.p. 295—297 °C; single spot on t.l.c. (Found: C, 62.8; H, 3.9; N, 15.4. $C_{19}H_{14}N_4O_4$ requires C, 63.0; H, 3.9; N, 15.5%).

Compounds (**2NH**) and (**2NOMe**) could not be synthesized by Hemmerich's method[19] because the condensation of compounds (**4**) with alloxane monohydrate gave only a trace amount of the required intermediates (**5**). We thus adopted Yoneda's method[20] to synthesize these isoalloxazines.

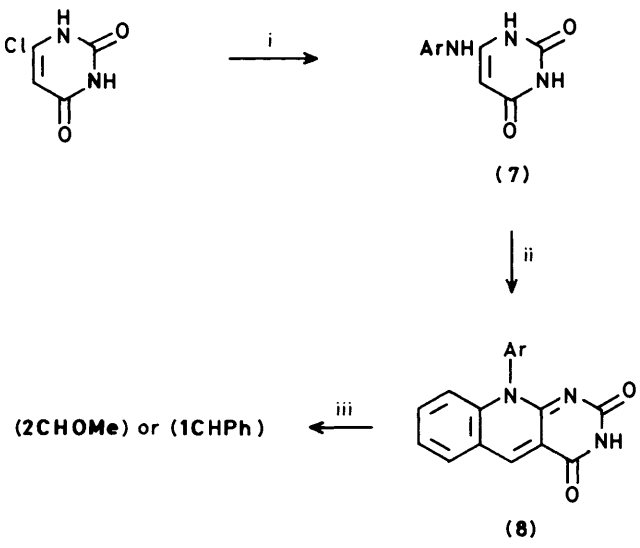
Compound (**6**; R = OMe).—6-Chloro-3-methyluracil (1.00 g, 6.23 mmol) and 1-amino-2-methoxynaphthalene (3.24 g, 18.7 mmol) were heated at 160—170 °C for 10 min. After cooling, the reaction mixture (suspension) was removed from the flask with diethyl ether. The product was recrystallised from ethanol with active charcoal (yield, 67%), m.p. 292—295 °C (Found: C, 64.4; H, 4.9; N, 14.1. $C_{16}H_{15}N_3O_3$ requires C, 64.6; H, 5.01; N, 14.1%).

Compound (**6**; R = H).—This compound was prepared by the reaction of 6-chloro-3-methyluracil (1.00 g, 6.23 mmol) and 1-aminonaphthalene (2.68 g, 18.7 mmol); yield 57%, m.p. 300—304 °C (decomp.)

Compound (**2NOMe**).—The uracil (**6**; R = OMe) (0.200 g, 0.732 mmol) and nitrosobenzene (0.240 g, 2.20 mmol) were refluxed for 20 min in acetic anhydride (2.0 ml)–acetic acid (0.5 ml). The solution was concentrated under reduced pressure, and the residue was recrystallised from ethanol (yield, 30.9%), m.p. >320 °C; single spot on t.l.c.; $\nu_{max.}$(KBr) 1 710 and 1 660 cm^{-1} (νC=O); δ_H([$^2H_6$]Me$_2$SO) 3.25 (3 H, s, NMe), 3.83 (3 H, s, OMe), 6.64 (1 H, d, 9-H), 7.80 (1 H, d, 4′-H), 8.10 (1 H, d, 8′-H), 8.29 (1 H, d, 3′-H), 8.33 (1 H, d, 6-H), and 7.4—7.7 (5 H, m, other ArH) (Found: C, 68.6; H, 4.2; N, 14.4. $C_{22}H_{16}N_4O_3$ requires C, 68.7; H, 4.2; N, 14.6%).

Compound (**2NH**).—This compound was prepared from the uracil (**6**; R = H) (0.60 g, 2.24 mmol) and nitrosobenzene (0.720 g, 6.72 mmol) according to the method described above; yield, 19%, m.p. 300—304 °C (decomp.); single spot on t.l.c. (Found: C, 71.0; H, 4.1; N, 15.5. $C_{21}H_{14}N_4O_2$ requires C, 71.2; H, 4.0; N, 15.8%).

Compounds (**1CHPh**) and (**2CHOMe**) were synthesized according to Scheme 1. The synthesis of 5-carbaisoalloxazines bearing simple 10-substituents has been reported by Yoneda *et al.*[22] We describe the synthesis of (**2CHOMe**) in detail and simply record the analytical data for (**1CHPh**).



Scheme 1. *Reagents:* i, $ArNH_2$; ii, *o*-ClC_6H_4CHO; iii, MeI

Compound (**7**; Ar = 2′-methoxy-1′-naphthyl).—6-Chlorouracil (1.47 g, 10.0 mmol) and 1-amino-2-methoxynaphthalene (4.14 g, 30.1 mmol) were heated at 170 °C for 10 min. After cooling, the reaction mixture (suspension) was removed from the flask with methanol. The product was washed with hot methanol and then recrystallised from acetic acid–water (2:1 v/v) with active charcoal (yield, 48.0%), m.p. >300 °C; single spot on t.l.c.; $\nu_{max.}$(KBr) 3 360 (νNH) and 1 700 cm^{-1} (νC=O) (Found: C, 63.3; H, 4.6; N, 14.8. $C_{15}H_{13}N_3O_3$ requires C, 63.4; H, 4.6; N, 14.8%).

Compound (**7**; Ar = biphenyl-2′-yl).—Yield, 74.9%, m.p. 314 °C (decomp.) (Found: C, 68.4; H, 4.6; N, 14.7. $C_{16}H_{13}N_3O_2$ requires C, 68.8; H, 4.7; N, 15.0%).

Compound (**8**; Ar = 2′-methoxy-1′-naphthyl).—6-[(2′-Methoxy-1′-naphthyl)amino]uracil (0.71 g, 2.51 mmol) and *o*-chlorobenzaldehyde (0.43 g, 3.06 mmol) were dissolved in DMF (20 ml) and the solution was heated at 160 °C for 8 h. DMF was removed by evaporation, and the residue (suspension) was removed from the flask with methanol. The yellow solid thus obtained was recrystallised from ethanol (yield, 76.3%), m.p. >330 °C; $\nu_{max.}$(KBr) 1 700 and 1 660 (νC=O) and 1 100 cm^{-1} (νC–O–C) (Found: C, 71.2; H, 4.0; N, 10.5. $C_{22}H_{15}N_3O_3$ requires C, 71.5; H, 4.1; N, 11.4%).*

Compound (**8**; Ar = biphenyl-2′-yl).—Yield, 78.3%, m.p. >320 °C; $\nu_{max.}$(KBr) 1 695 and 1 670 cm^{-1} (νC=O) (Found: C, 75.5; H, 4.1; N, 11.5. $C_{23}H_{15}N_3O_2$ requires C, 75.6; H, 4.1; N, 11.5%).

Compound (**2CHOMe**).—10-(2′-Methoxy-1′-naphthyl)-5-carbaisoalloxazine (0.37 g, 1.00 mmol) was methylated with methyl iodide (1.42 g, 10.0 mmol) in DMF (15 ml) in the presence of powdered potassium carbonate (1.38 g, 10.0 mmol). The reaction was continued for 21 h at 66 °C. The progress of the reaction was followed by t.l.c. After cooling, the solution was filtered to remove the insoluble salts and was then concentrated under reduced pressure. The viscous residue was diluted with water, the yellow precipitate being collected by filtration. This solid was recrystallised from ethanol (yield, 89.5%), m.p. >320 °C; single spot on t.l.c.; $\nu_{max.}$(KBr) 1 700 and 1 650 (νC=O) and 1 200 cm^{-1} (νC–O–C); δ_H($CDCl_3$) 3.43 (3 H, s, NMe), 3.83 (3 H, s, OMe), 6.5—8.2 (10 H, m, aromatic protons), and 9.08 (1 H, s, 5-H); M^+ 383 (Found: C, 71.5; H, 4.6; N, 9.8. $C_{23}H_{17}N_3O_3$ requires C, 72.0; H, 4.5; N, 10.9%).*

Compound (**1CHPh**).—Yield, 87.9%, m.p. >320 °C; single spot on t.l.c.; $\nu_{max.}$(KBr) 1 700 and 1 650 cm^{-1} (νC=O); δ_H($CDCl_3$) 3.42 (3 H, s, NMe), 6.7—7.9 (13 H, m, ArH), and 8.81 (1 H, s, 5-H) (Found: C, 75.6; H, 4.6; N, 10.8. $C_{24}H_{17}N_3O_2$ requires C, 76.0; H, 4.5; N, 11.1%).

Optical Resolution.—The racemic compounds (**1XR**) and (**2XR**) were optically resolved by an l.c. method using a chiral packing column (Sumipax OA-2000). The chromatograms for three isoalloxazines are illustrated in the Figure. The separation of (**2NH**) was complete, whereas that of (**2NOMe**) gave

* We repeated this recrystallisation several times but could not improve the elemental analysis data. This is probably due to inclusion of solvent molecules or other contaminants, which is characteristic of bulky molecules having an open space in the crystal lattice: for example, see M. Kaftory, K. Tanaka, and F. Toda, *J. Org. Chem.*, 1985, **50**, 2154 and references cited therein.

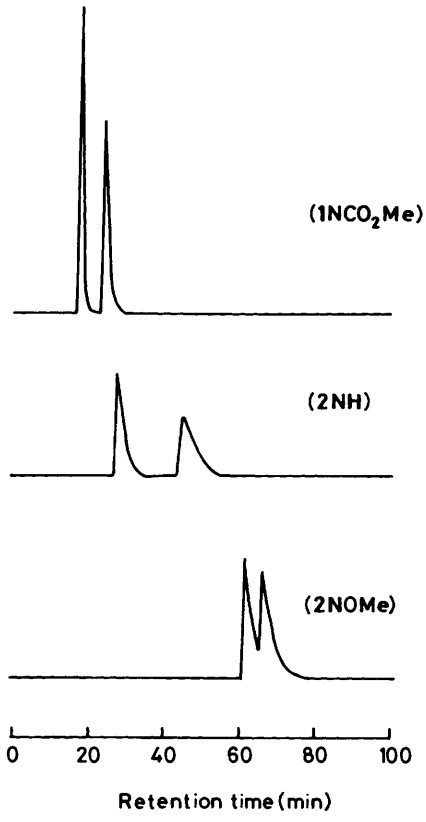


Figure. Chromatograms for optical resolution of compounds (**1NCO$_2$Me**), (**2NH**), and (**2NOMe**). Column, Sumipax OA-2000 (8φ × 30 cm); mobile phase, hexane–1,2-dichloroethane–ethanol 4:2:1 (v/v/v); detected at 254 nm. Flow rate: (**1NCO$_2$Me**) 1.0 ml min^{-1}, (**2NH**) 3.0 ml min^{-1}, (**2NOMe**) 1.5 ml min^{-1}

partially overlapped peaks. The chromatograms for other isoalloxazines and 5-carbaisoalloxazines were more or less similar to that of (**2NOMe**). We separated the eluant into three fractions and in every case obtained (+)-isomers from the first fraction and (−)-isomers from the last fraction.

Product Analysis.—Compounds (**1XR**) and (**2XR**) were first converted into the reduced forms, which were then reoxidised to the oxidised forms. The reduction was carried out in an anaerobic aqueous solution (pH 8.60 with 0.10M-borate) at 30 °C using a Thunberg cuvette, and the progress of the reduction was monitored spectrophotometrically. The reoxidation of reduced isoalloxazines was readily attained by introducing a dioxygen stream into the cuvette. Reduced 5-carbaisoalloxazines were reoxidised by potassium ferricyanide [$K_3Fe(CN)_6$] {[$K_3Fe(CN)_6$]: 5-carbaisoalloxazine, 4:1}. The solutions were subjected to h.p.l.c. analysis using the chiral packing column (Sumipax OA-2000).

Kinetic Measurements.—The rate constants for the reaction of isoalloxazines and 1-benzyl-1,4-dihydronicotinamide (BNAH) were determined in an aerobic aqueous solution (pH 9.5 with 0.01M-borate) by monitoring the disappearance of an absorption band of BNAH at 352 nm. The time-dependence of $O.D._{352}$ satisfied a first-order rate equation for up to three half-lives. As the reduced isoalloxazines are reoxidised immediately by dioxygen, the concentration of isoalloxazines can be regarded as being constant during the redox reaction.

The rate constants for racemisation were determined under the identical aerobic conditions by using a polarimeter apparatus (Nippon Bunko DIP-4). We confirmed that α_D is constant (for one day at 30 °C and 5 h at 70 °C) in the absence of BNAH. In the presence of BNAH, on the other hand, α_D decreased with reaction time and the time-dependence satisfied a first-order equation for up to two half-lives. Since the concentration of the oxidised form is apparently 'constant' under aerobic conditions, the decrease in α_D implied racemisation *via* the reduced forms.

Table 1. Optical resolution of isoalloxazines and 5-carbaisoalloxazines (100 mg) by h.p.l.c.[a]

	(+)-Isomer		(−)-Isomer	
Isoalloxazine	Recovery (mg)	e.e.[b] (%)	Recovery (mg)	e.e. (%)
(**1NCO$_2$Me**)	30	100	34	88.3
(**1NEt**)	31	92.1	30	87.1
(**1NPh**)	30	100	35	92.2
(**1CHPh**)	27	100	25	92.3
(**2NH**)	47	100	45	100
(**2NOMe**)	22	98.0	21	90.1
(**2CHOMe**)	28	100	20	91.6

[a] Stationary phase, Sumipax OA-2000; mobile phase, hexane–1,2-dichloroethane–ethanol 4:2:1 (v/v/v). [b] Enantiomeric excess (e.e.) = [(+) − (−)]/[(+) + (−)] for (+)-isomers.

Results and Discussion

Optical Resolution of Isoalloxazines and 5-Carbaisoalloxazines.—The results are summarised in Table 1.

It is seen from Table 1 that the (+)-isomers with e.e. higher than 90% are obtained from the first fraction. In cases where the peak separation was relatively good, we could recover 27—30 mg of the (+)-isomers with 100% e.e. from 100 mg of the racemic compounds.† On the other hand, the optical purities of the (−)-isomers were inferior to those of the (+)-isomers because of 'tailing' of the (+)-isomers.

In Table 2, we summarise absorption maxima and fluorescence maxima of racemic (5-carba)isoalloxazines and $[\alpha]_D$ of (+)-isomers. These chiral (5-carba)isoalloxazines have relatively large $[\alpha]_D$ values. In general, flavin derivatives are strongly fluorescent. Surprisingly, (**2NOMe**) and (**2CHOMe**) bearing the 2′-methoxy-1′-naphthyl group were non-fluorescent. This is probably due to intramolecular quenching of singlet (5-carba)isoalloxazines by the 2′-methoxy-1′-naphthyl group. This problem will be discussed elsewhere in more detail.

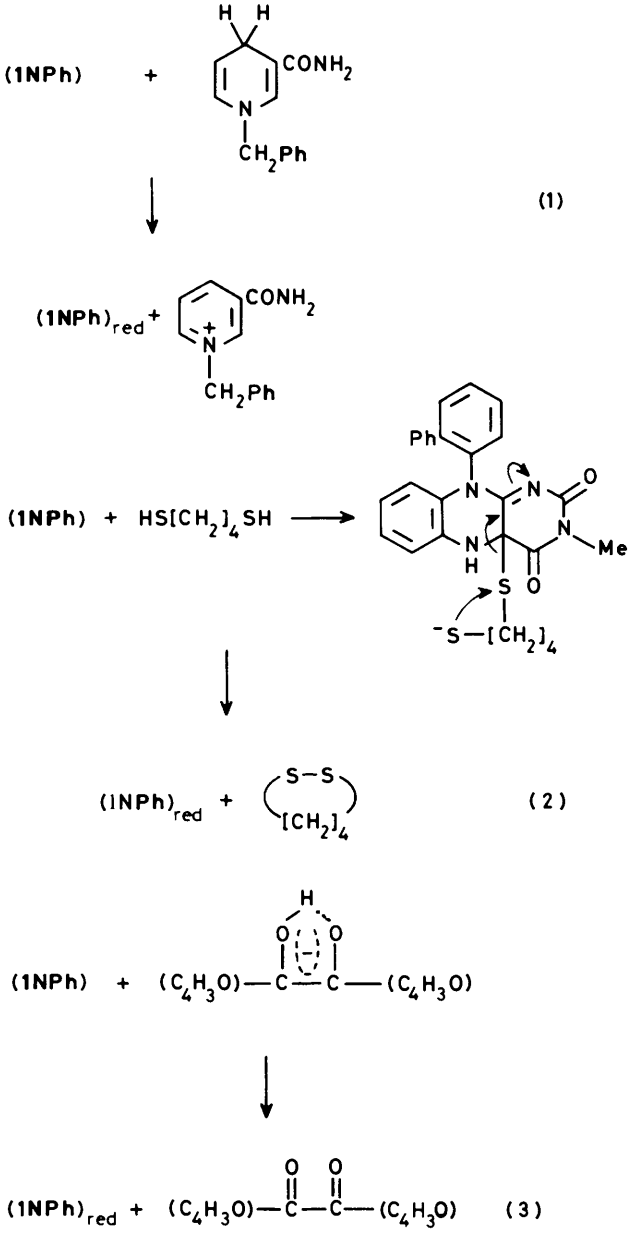
Product Analyses of Reoxidised (5-Carba)isoalloxazines.—First, we corroborated the fact that six chiral (5-carba)isoalloxazines [except (**1NCO$_2$Me**)] do not racemise at 70 °C;* the α_D value in butan-1-ol was constant for 5 h within experimental error (4%). After this treatment, the solutions were concentrated under reduced pressure at room temperature and subjected to h.p.l.c. analysis on the chiral packing column. The optical purities thus obtained were essentially identical with those of the starting (5-carba)isoalloxazines. Similarly, photoirradiation by a 17 W fluorescent lamp at room temperature did

* We found that the recovered isoalloxazine (**1NCO$_2$Me**) was sometimes optically pure and sometimes racemised. The origin of this curious, inconvenient behaviour is not yet understood. Compound (**1NCO$_2$Me**) racemised very slowly at 70 °C in butan-1-ol: k (first-order rate constant) = 1.35 × 10^{-6} s^{-1}.

Table 2. Spectroscopic properties of isoalloxazines and 5-carbaisoalloxazines

Isoalloxazine	Absorption spectrum[a] $\lambda_{max.}$ [$\varepsilon_{max.}$] (nm)		Fluorescence spectrum[a] $Em_{max.}$ [Excitation] (nm)	$[\alpha]_D^{25}$ (°)
(**1NCO$_2$Me**)	332 [8 050]	439 [9 330]	510 [450]	+470
(**1NEt**)	332 [7 080]	437 [8 480]	508 [450]	+216
(**1NPh**)	332 [6 000]	438 [7 020]	513 [450]	+575
(**1CHPh**)	317 [7 300]	398 [8 350]	466 [405]	+411
(**2NH**)	322 [4 370]	431 [5 420]	523 [450]	+432
(**2NOMe**)	326 [8 150]	434 [7 940]	non-fluorescent	+233
(**2CHOMe**)	319 [8 550]	401 [7 930]	non-fluorescent	+154

[a] 30 °C, Acetonitrile. [b] 25 °C, Methanol, *c* 0.014—0.016. The recorded values are corrected for 100% pure (+)-isomers.

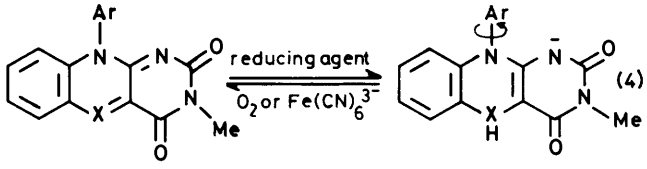


C_4H_3O = 2-furyl

not mediate racemisation of these (5-carba)isoalloxazines. This indicates that the 10-phenyl (or 10-naphthyl) groups cannot rotate even in the excited state.

Here, we reduced compound (**1NPh**) at 30 °C by four different methods: (i) BNAH reduction which occurs by a 'hydride equivalent' transfer [equation (1)],[14,23] (ii) butane-1,4-dithiol reduction which proceeds *via* a 4a-adduct intermediate [equation (2)],[24] (iii) carbanion reduction using 2,2′-furoin, which possibly occurs by successive electron transfers [equation (3)],[25,26] and (iv) EDTA photoreduction with a 17 W fluorescent lamp which proceeds *via* triplet isoalloxazines.[27]

In every case, the reactions were carried out in an anaerobic Thunberg cuvette and the progress of the reactions was monitored spectrophotometrically. After completion of reduction, a dioxygen stream was introduced into the cuvette in order to reoxidise reduced (**1NPh**) to (**1NPh**). The aqueous solutions were extracted with chloroform. Product (**1NPh**) was recovered quantitatively in the chloroform layers. H.p.l.c. analysis of the chloroform solutions (Table 3) established that recovered (**1NPh**) is racemised completely (within experimental error). As the oxidised form of (**1NPh**) does not racemise under identical reaction conditions, it is now clear that racemisation occurs only in the reduced form [equation (4)]. Redox-induced racemisation was also observed for the isoalloxazine (**1NEt**) and (**2NH**) (Table 3). Compound (**1CHPh**) was reduced by BNAH, the product was then reoxidised by potassium ferricyanide. H.p.l.c. analysis indicated that recovered (**1CHPh**) was also racemised.



Similar redox-treatments of compounds (**2NOMe**) and (**2CHOMe**) resulted in quite different results. The optical purities of the re-formed reactants were essentially unaffected by the redox-treatments, indicating that racemisation does not occur even in the reduced forms. Comparison with the data for (**2NH**) clearly establishes that the 2′-methoxy group in (**2NOMe**) plays a crucial role in inhibiting the rotation of the C(1′)–N(10) bond in the reduced form.

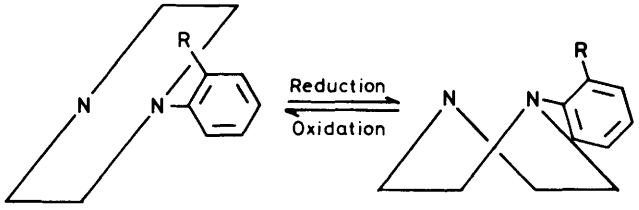
From a steric viewpoint, the naphthyl group would be regarded as an analogue of the 2-substituted phenyl group. Thus, the foregoing results are concisely summarised as follows: (i) chiral (5-carba)isoalloxazines with 2′-substituted phenyl groups at N(10) racemise in the reduced form, (ii) chiral (5-

Table 3. H.p.l.c. analysis of redox-treated isoalloxazines and 5-carbaisoalloxazines [(+)-isomers][a]

(5-Carba)isoalloxazine			Reducing agent		Recovered (5-carba)isoalloxazine	
	Concn. (mM)	e.e. (%)		Concn. (mM)	(+)-Isomer (%)	(−)-Isomer (%)
(1NCO$_2$Me)	0.0529	100	BNAH	0.506	50.1	49.9
(1NEt)	0.0510	92.1	BNAH	0.506	45.2	54.8
(1NEt)	0.0510	92.1	EDTA + *hν*	15.2	49.5	50.5
(1NPh)	0.0514	100	BNAH	0.501	50.6	49.4
(1NPh)	0.0514	100	butane-1,4-dithiol	0.499	49.5	50.5
(1NPh)	0.0514	100	2,2′-furoin	0.0997	49.7	50.3
(1NPh)	0.0514	100	EDTA + *hν*	1.53	49.5	50.5
(1CHPh)	0.0402	100	BNAH	37.9	51.6	48.4
(2NH)	0.0500	100	EDTA + *hν*	15.0	54.9	45.1
(2NH)	0.0500	100	BNAH	0.529	58.0	42.0
(2NOMe)	0.0495	98.0	BNAH	0.506	97.4	2.6
(2NOMe)	0.0495	98.0	EDTA + *hν*	15.2	97.0	3.0
(2CHOMe)	0.0510	100	BNAH	10.5	100	0

[a] pH 8.6 for BNAH, butane-1,4-dithiol, and 2,2′-furoin and pH 10.13 for photoreduction with EDTA.

carba)isoalloxazines with 2′-substituted naphthyl groups (and probably 2′,6′-disubstituted phenyl groups) at N(10) do not racemise even in the reduced form, and (iii) conclusions (i) and (ii) can apply for both isoalloxazines and 5-carbaisoalloxazines. It is unequivocally established on the basis of *X*-ray crystallographic studies that oxidised flavins are planar, whereas reduced flavins are folded along a line through N(5) and N(10) like butterfly wings.[28–30] The nitrogen atoms N(5) and N(10) in reduced flavins have a rather high degree of tetrahedral sp^3 hybridisation, whereas those in oxidised flavins are more or less close to planar sp^2 hybridisation.[29] Examination of CPK molecular models suggests that, as illustrated in Scheme 2, the



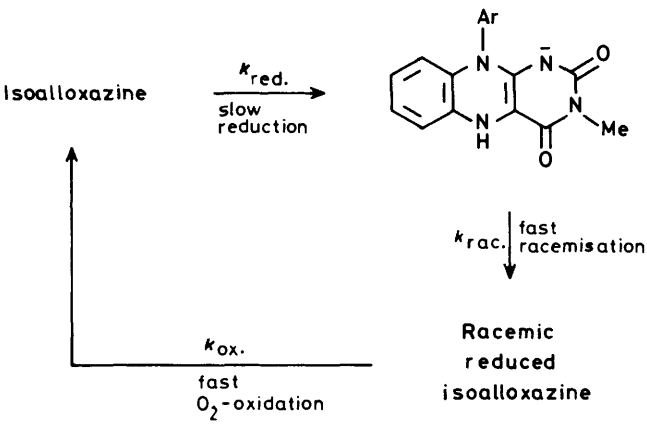
Scheme 2.

rotation of the 2′-substituted phenyl groups around the C(1′)–N(10) axis of oxidised (5-carba)isoalloxazines accompanies significant steric hindrance because of the collision between the (5-carba)isoalloxazine plane and the 2′-substituent, whereas it becomes possible in reduced (5-carba)isoalloxazines because of the reduced steric hindrance when N(10) is sp^3-hybridised. In (**2NOMe**) and (**2CHOMe**), either 2′-methoxy or 8′-hydrogen in the naphthalene ring collides with the bent 1,5-dihydro-(5-carba)isoalloxazine plane and therefore the rotation of the 2′-substituted naphthyl groups is sterically difficult.

An important conclusion can be drawn from the foregoing findings. In contrast to that of 1,5-dihydroflavins, the structure of 1,5-dihydro-5-carbaflavins is practically unknown.* The structure of 1,5-dihydro-5-carbaflavins includes a basic skeleton of 1,4-dihydronicotinamides and therefore may be close to the structure of 1,4-dihydronicotinamides. The geometry of 1,4-dihydronicotinamides has been determined by *X*-ray crystallography, which has shown the dihydropyridine ring to be planar.[31,32] It is also known, however, that when the dihydropyridine is included in the cyclic plane structure, it is somewhat distorted[33] or folded along a line through N(1) and C(4).[34] Hence, it seems rather difficult to speculate on the structure of 1,5-dihydro-5-carbaisoalloxazines on the basis of that of 1,4-dihydronicotinamides. As shown in Table 3, the redox treatment of chiral (**1CHPh**) afforded completely racemised (**1CHPh**). The result is rationalised only in terms of the rotation of the 10-phenyl group in the reduced form and provides direct evidence for the bent structure. Although we cannot rule out the possibility that the planar and the bent structure co-exist *via* an equilibrium, a significant amount of the bent structure should be present in 1,5-dihydro-5-carbaisoalloxazines.

Kinetic Examination of the Racemisation Process.—If racemisation occurs in the reduced state of isoalloxazines (Scheme 2), the racemisation process consists of the slow, rate-determining reduction of isoalloxazines followed by the fast rotation of the C(1′)–N(10) single bond (Scheme 3).

Reduced isoalloxazines are rapidly reoxidised by dioxygen ($k_{ox.}$ = 1.9 s^{-1}),[35,36] so that the concentration of oxidised isoalloxazines is apparently constant during aerobic reduction



Scheme 3.

* 4a,5-Dihydro-4a-hydroxy-3,10-dimethyl-5-carbaisoalloxazine has a bent structure along a line through C(5)–N(10); F. Yoneda, K. Tanaka, Y. Sakuma, H. Yoshino, and M. Takamoto, *Chem. Pharm. Bull.*, 1984, **32**, 3761.

1,5-Dihydro-5-carbaflavins are expected to be bent, according to CNDO calculations; F. Yoneda, personal communication.

Table 4. Pseudo-first-order rate constants (k_1') for racemisation and reduction of (+)-isoalloxazines[a]

Isoalloxazine	Concn. (mM)	BNAH (mM)	k_1' (racemisation) (s^{-1})	k_1' (reduction) (s^{-1})
(**1NEt**)	(0.251)	0.528	3.63×10^{-4}	3.35×10^{-4}
(**1NPh**)	(0.350)	0.762	8.42×10^{-5}	8.82×10^{-5}
(**2NH**)	(0.236)	0.528	1.05×10^{-4}	2.41×10^{-4}

[a] 30 °C. Aerobic, pH 9.5 with 0.01M-borate butter.

of isoalloxazines. This implies that in Scheme 3 the aerobic rate of reduction should be in accord with that of racemisation, because both rates reflect the same rate-determining step (*i.e.*, reduction). BNAH can reduce isoalloxazines but is not oxidised by dioxygen. In the reaction of BNAH and isoalloxazines, the aerobic rate of reduction can be estimated by the disappearance of the absorption band of BNAH (352 nm) while that of racemisation can be estimated by following the α_D change in the presence of BNAH. The pseudo-first-order rate constants (k_1') thus obtained are summarised in Table 4.

Examination of Table 4 reveals that both rate constants give good agreement for (**1NEt**) and (**1NPh**): that is, these reduced isoalloxazines can fully racemise before they are reoxidised (*i.e.*, $k_{rac.} > k_{ox.}$ in Scheme 3). In compound (**2NH**), the aerobic rate of reduction is greater by a factor of 2.3 than that of racemisation. This indicates that the racemisation step is partly competing with the O_2-oxidation step (probably, $k_{rac.} \approx k_{ox.}$). The difference suggests that the naphthyl group provides greater steric hindrance than the 2'-substituted phenyl group for the rotation of the C(1')–N(10) bond. Anyway, these results support the proposition that racemisation occurs in the reduced forms produced through the rate-determining reduction step.

Concluding Remarks.—We believe that the present paper demonstrates a new concept for atropisomers bearing a C–N axis. In conventional atropisomers such as biphenyls and binaphthyls, a chiral centre is constituted around a C–C single bond and the aromatic rings serve as simple sterically hindered 'planes'. On the other hand, the present (5-carba)isoalloxazines involve a C–N axis in the chiral centre and these 'planes' change their structures in response to redox treatments: that is, redox-induced interconversion between sp^2- and sp^3-hybridisation is the origin of the free rotation of the C–N axis. These findings are undoubtedly novel and may be regarded as characteristic of atropisomers bearing a C–N axis. Further characterisations and applications of these and related flavin derivatives are now under investigation. Of particular interest are (i) the application of these chiral isoalloxazines to asymmetric oxidation reactions, (ii) the asymmetric fluorescence quenching by chiral quenchers, and (iii) the oxidation of racemic reduced isoalloxazines in the presence of chiral compounds (or chiral aggregates), which may result in chiral oxidised isoalloxazines.

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References

1 Preliminary communication: S. Shinkai, H. Nakao, and O. Manabe, *Tetrahedron Lett.*, 1985, **26**, 5183; Part 44, S. Shinkai, H. Nakao, K. Ueda, O. Manabe, and M. Ohnishi, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 1632.
2 E. L. Eliel, 'Stereochemistry of Carbon Compounds McGraw-Hill, New York, 1962, p. 156.
3 R. Adams and H. C. Yuan, *Chem. Rev.*, 1933, **12**, 261.
4 J. L. A. Webb, *J. Org. Chem.*, 1953, **18**, 1413.
5 M. Nakamura and M. Ōki, *Tetrahedron Lett.*, 1974, 508; M. Ōki and G. Yamamoto, *Chem. Lett.*, 1972, 45; G. Yamamoto, M. Nakamura, and M. Ōki, *Bull. Chem. Soc. Jpn.*, 1975. **48**. 2592.
6 A. Luttrighaus and H. Gralheer, *Justus Liebigs Ann. Chem.*, 1941, **550**, 67.
7 T. H. Sidall and W. E. Stewart, *J. Org. Chem.*, 1969. **34**, 233.
8 L. H. Bock and R. Adams, *J. Am. Chem. Soc.*, 1931. **53**. 374. 3519; W. H. Patterson and R. Adams, *ibid.*, 1933, **55**, 1069.
9 J. C. Jochims, H. von Voithenberg, and G. Wegner. *Chem. Ber.*, 1978, **111**, 1693, 2745.
10 C. Kashima, A. Katoh, Y. Omote, and Y. Nakata. *Heterocycles*, 1978, **9**, 469; C. Kashima and A. Katoh, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1599.
11 A. Mannschreck, A. Eiglsperger, and G. Stühler, *Chem. Ber.*, 1982, **115**, 1568.
12 M. Mintas, Z. Orhanovic, K. Jakopcic, H. Koller, G. Stühler, and A. Mannschreck, *Tetrahedron*, 1985, **41**, 229 and references cited therein.
13 S. Shinkai, 'Enzyme Chemistry,' ed. C. J. Suckling, Champman & Hall, London, 1984, p. 40.
14 For comprehensive reviews of flavin chemistry, see F. Yoneda, *Yakugaku Zasshi*, 1984, **104**, 97; C. Walsh, *Acc. Chem. Res.*, 1980, **13**, 148; T. C. Bruice, *ibid.*, p. 256; V. Massey and P. Hemmerich, *Biochem. Soc. Trans.*, 1980, **8**, 246.
15 T. C. Bruice, L. Main, S. Smith, and P. Y. Bruice, *J. Am. Chem. Soc.*, 1971, **93**, 7327.
16 L. Hevesi and T. C. Bruice, *J. Am. Chem. Soc.*, 1972, **94**, 8277; S. B. Smith and T. C. Bruice, *ibid.*, 1975, **97**, 2875.
17 K. Tanaka, T. Okada, F. Yoneda, T. Nagamatsu, and K. Kuroda, *Tetrahedron Lett.*, 1984, **25**, 1741.
18 S. Shinkai, H. Nakao, T. Tsuno, and O. Manabe, *J. Chem. Soc., Chem. Commun.*, 1984, 849.
19 P. Hemmerich, B. Prijs, and H. Erlenmeyer, *Helv. Chim. Acta*, 1960, **43**, 372; P. Hemmerich, S. Fallab, and H. Erlenmeyer, *ibid.*, 1956, **39**, 1242.
20 F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, *J. Am. Chem. Soc.*, 1976, **98**, 830.
21 R. E. Strube, *U.S.P.* 3 291 790 (*Chem. Abstr.*, 1967, **66**, 55529).
22 F. Yoneda, K. Shinozuka, K. Tsukuda, and A. Koshiro, *J. Heterocycl. Chem.*, 1979, **16**, 1365.
23 M. F. Powell and T. C. Bruice, *J. Am. Chem. Soc.*, 1983, **105**, 1014 and references cited therein.
24 E. L. Loechler and T. C. Hollocher, *J. Am. Chem. Soc.*, 1975, **97**, 3235; 1980, **102**, 7312, 7322, 7328; I. Yokoe and T. C. Bruice, *ibid.*, 1975, **97**, 450.
25 S. Shinkai, T. Kunitake, and T. C. Bruice, *J. Am. Chem. Soc.*, 1974, **96**, 7140.
26 T. W. Chan and T. C. Bruice, *J. Am. Chem. Soc.*, 1977, **99**, 2389.
27 R. Traber, H. E. Kramer, and P. Hemmerich, *Biochemistry*, 1982, **21**, 1687 and references cited therein; V. Massey and G. Palmer, *ibid.*, 1966, **5**, 3181; A. J. W. G. Visser, F. Müller, and J. D. W. van Voorst, *Biochem. Biophys. Res. Commun.*, 1977. **77**, 1135.
28 L. Tauscher, S. Ghisla, and P. Hemmerich, *Helv. Chim. Acta*, 1973, **56**, 630 and references cited therein.
29 P. Kierkegaard, R. Norrestam, P. Werner, I. Csoregh, M. Glehn, R. Karlsson, M. Leijonmarch, O. Ronnquist, B. Sternland. O. Tillberg, and L. Torbjornsson, 'Flavins and Flavoproteins.' University Park Press, Baltimore, 1971, p. 1.
30 C. T. Moonen, J. Vervoort, and F. Müller, *Biochemistry*. 1984, **23**. 4859, 4868.
31 I. L. Karle, *Acta Crystallogr.*, 1961, **14**, 497.
32 H. Koyama, *Z. Kristallogr.*, 1963, **118**, 51; H. Koyama, J. M. M. Robinson, and F. Laves, *Nature*, 1963, **50**, 40.
33 F. Rob, H. J. van Ramesdonk, W. van Gerresheim, P. Bosma, J. J. Scheele, and J. W. Verhoeven, *J. Am. Chem. Soc.*, 1984. **106**, 3826.
34 P. M. T. de Kok and H. M. Buck, *J. Chem. Soc., Chem. Commun.*, 1985, 1009.
35 R. Spencer, J. Fisher, and C. Walsh. *Biochemistry*. 1977. **16**, 3586.
36 P. Hemmerich, P. Massey, and H. Fenner, *FEBS Lett.*, 1977. **84**, 5.

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