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Cis–Trans Isomerism in the Pyridyl Analogs of Azobenzene. A Kinetic and Molecular Orbital Analysis

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Abstract: Rate data have been obtained for cis–trans isomerizations of phenylazopyridines and symmetrical azopyridines in *n*-heptane. Activation energies ranged from 21 to 22.6 kcal mol^{-1}, with the 2- and 4-substituted compounds showing lower activation energies. Although the 2-substituted dyes had low E_a terms, the ΔS^* terms were considerably more negative than those of other compounds in the series. The slower isomerization rates for these compounds are attributed to coulombic interactions between the pyridyl nonbonded electrons and the azo electrons in the activated complex. The 2- and 4-substituted compounds showed a high sensitivity to acid catalysis. An activation energy of 9 kcal mol^{-1} was associated with the acid-catalyzed isomerization of *cis*-4-phenylazopyridine. CNDO/2 calculations were performed on the cis and trans configurations of these dyes and for the transition states. Inversion of one of the azo nitrogens was calculated to be overwhelmingly favored over rotation. Analysis of the results of the MO calculations showed facile inversion to be aided by electron withdrawal by the ring attached to the rehybridizing nitrogen which is manifested by a high C–N_{azo} π density and by a lowered electron density at the p orbital bearing the nonbonded electrons.

Since Hartley first studied the cis–trans isomerization of aromatic azo dyes,[1] a number of additional studies have been conducted.[2] Although both a rotational and an inversional transition state have been suggested for the mode of isomerization, the in-plane inversion mechanism in which one of the azo nitrogens is sp-hybridized in the transition state has found greater favor. Recently, however, the possibility of a rotational activated complex has been suggested[3a] for the isomerization of dipolar compounds substituted with para donor and para′-acceptor groups.[3]

The most remarkable observation for cis–trans isomerizations of para-substituted azobenzenes is nonlinearity in the Hammett plots for these compounds.[2c,f] Talaty and Fargo reported that the rates of isomerization of all para-substituted azobenzenes were greater than those of the parent compound.[2c]

The present study was designed to determine (1) activation parameters for the isomerization of the phenylazopyridines and azopyridines, (2) calculated activation parameters for isomerization, (3) the nature of the activated complex for these reactions, and (4) the electronic requirements for facile isomerization.

Experimental Section

Phenylazopyridines. These were prepared by the method of Campbell, *et al.*,[4] or by the modification of Brown.[5] In a typical procedure, 50 ml of 50% NaOH and 30 ml of pyridine were heated with the aminopyridine (8.0 g, 94 mmol) to 80°. Freshly sublimed nitrosobenzene (12.0 g, 110 mmol) was then added over a 45-min period. After an additional 30-min period, the reaction mixture was cooled and extracted with several portions of benzene. The solutions were dried, reduced *in vacuo*, and chromatographed on alumina. Fractions homogenous to tlc were combined and recrystallized from hexane to constant melting points with the following results: 2-phenylazopyridine (2-PAPy), mp 33–34° (lit.[4] 32–34°); 3-phenylazopyridine (3-PAPy), mp 52° (lit.[4] 52–53°); 4-phenylazopyridine (4-PAPy), 99–99.5° (lit.[4,6] 98–99°).

Azopyridines were synthesized by oxidation of the aminopyridines with NaOCl.[4,7] A cold aqueous solution of the aminopyridine (10 g in 200 ml) was added to 600 ml of 10% NaOCl. The reaction was carried out at 5–10° with a 45-min addition period. Extraction with ether, followed by drying over molecular sieves, reduction of solvent volume, and chromatography gave the azopyridines. Recrystallization from hexane gave crystals melting at 86–87° (lit.[4] 87°) for 2,2′-azopyridine (2,2′-APy); 140–140.5° (lit.[4] 140°) for 3,3′-azopyridine (3,3′-APy); 106.5–107.5° (lit.[4] 107.5–108°) for 4,4′-azopyridine (4,4′-APy).

Photolyses. The cis isomers of the compounds under investigation were generated by irradiation of hydrocarbon solutions of the dyes[8] by a Hanovia high-pressure, mercury-vapor lamp (No. 679A) suspended in an immersion well. The radiation was filtered by means of a Pyrex sleeve. Irradiation times were of the order of 30 min for 5-g samples of the dye.

***cis*-2,2′-APy and *cis*-3,3′-APy** were isolated by the procedures described by Campbell, *et al.*;[4] *cis*-2,2′-APy, mp 85° (lit.[4] 87°); *cis*-3,3′-APy, mp 80° (lit.[4] 82°).

***cis*-3-PAPy** was isolated by alumina column chromatography of irradiated solutions of the trans isomer by varying the eluent from benzene to ether. Recrystallization from hexane gave crystals melting 54–55°.

***cis*-4-PAPy and *cis*-2-PAPy.** A solution of 10 g of *trans*-4-PAPy in 200 ml of cyclohexane was irradiated for 30 min, followed by extraction with 500 ml of a pH 12 buffer. The aqueous extract was

Table I. Electronic Spectral Properties of the Phenylazopyridines and Azopyridines[a]

Compd		λ_1		λ_2	λ_3
		λ^b (ϵ)	f^c	λ (ϵ)	λ (ϵ)
AB	t[d]	448 (413)	0.0101	315 (22,500)	229 (15,100)
	c	437 (1300)	0.0238	270 (5080)	246 (12,000)
2-PAPy	t	456 (303)	0.0063	310 (18,600)	222 (12,800)
	c	437 (908)	0.0174	269 (5860)	240 (8800)
3-PAPy	t	449 (384)	0.0079	317 (21,700)	224 (12,000)
	c	440 (1100)	0.0202	271 (5570)	241 (10,200)
4-PAPy	t	453 (320)	0.0067	309 (19,700)	228 (9360)
	c	433 (970)	0.0154	*e*	343 (10,440)
2,2′-APy	t	466 (231)	0.0047	306 (15,000)	216 (16,000)
	c	448 (1070)	0.0189	266 (7600)	228 (8380)
3,3′-APy	t	450 (340)	0.0071	317 (20,100)	219 (12,500)
	c	441 (935)	0.0169	280 (6150)	238 (9260)
4,4′-APy	t	460 (230)	0.0048	286 (17,700)	215 (7500)
	c	431 (623)	0.0120	*e*	241 (9550)

[a] Solvent was *n*-heptane. [b] Wavelength in nanometers of the major absorption maxima. [c] Oscillator strength was estimated by $f = 4.6 \times 10^{-9}(\epsilon_{max})(\Delta\nu)^{1}/_{2}$, where $(\Delta\nu)^{1}/_{2}$ is the band half-width in wave numbers. [d] Trans isomer is designated as t; c designates cis isomer. [e] Appears as a shoulder to the λ_3 band.

Table II. Nuclear Magnetic Resonance Spectral Properties of the Phenylazopyridines and Azopyridines[a]

Compd	Proton[b]	Chemical shift,[c] δ	
		Trans	Cis
AB	Ortho	7.88	6.82
	Meta, para	7.37	7.17
2-PAPy	α_m	8.67 (1)[d]	8.30
	γ_m, β_o	7.72 (2)	7.40
	Ortho	8.05 (2)	6.6–7.2 (7)[e]
	β_p, meta, para	7.4 (4)	
2,2′-APy	α_m	8.80	8.18
	γ_m		7.37
	β_o	7.93	7.05
	β_p	7.47	7.72
3-PAPy	α_α	9.31	8.13
	α_β	8.76	8.35
	γ_o	8.1	7.15
	β_m	7.3	7.2
	Ortho	8.0	6.8
	Meta, para	7.5	7.15
3,3′-APy	α_o	9.23	8.10
	α_p	8.60	8.40
	γ_o	8.06	7.2
	β_m	7.20	7.2
4-PAPy	α_m	8.70	8.40
	β_o	7.65	6.61
	Ortho	7.80	6.75
	Meta, para	7.45	7.15
4,4′-APy	α_m	8.79	8.46
	β_α	7.65	6.60

[a] Spectra were taken of $CHCl_3$ solutions of the dyes with Varian Model HA-60 and T-60 spectrometers. [b] Phenyl protons are designated ortho, meta, and para with respect to the azo nitrogen; α, β, and γ represent positions on the pyridyl rings; subscripts designate position with respect to the azo nitrogen. [c] Values cited represent shift (in parts per million) from TMS measured at peak center. [d] Integrated values; assignments are tentative. [e] Considerable overlap of resonances observed [values expected in analogy to the other cis dyes in the series are ortho 7.2 (2); meta, para 7.1 (3); β_o 6.7 (1); and β_p 7.1 (1)]. [f] For information about the spectra of the trans isomers, see G. Ciacometti and G. Rigatti, *Nucl. Magn. Resonance Chem., Proc. Symp.*, 173 (1964).

washed with 20 ml of hexane which was added to the cyclohexane solution for further extraction. The aqueous solution of the cis dye was then extracted twice with 125-ml portions of cyclohexane which were treated with 100 μl of Et_3N and immediately frozen. The entire extraction procedure was repeated, and the cyclohexane extracts were lyophilized. Upon removal of the solvent, the yellow powder was dissolved in hexane at room temperature and recrystallized at −20°. The melting point determined by quick-plunge techniques was 57.5–58.5°. With the same procedure, *cis*-2-PAPy was isolated, mp 63°.

***cis*-4,4′-APy** was isolated by column chromatography on alumina from irradiated solutions of the trans isomer. As soon as resolution became apparent, the bands were cut away from each other. Extraction of the second band followed by removal of solvent and recrystallization fom hexane gave crystals melting at 84°. In an alternate isolation procedure, the irradiated solution of the trans isomer in benzene was extracted with 200 ml of a pH 12 buffer. The aqueous extract was extracted with three 100-ml portions of benzene. The last two were combined, treated with Et_3N, and lyophilized.

The identity and homogenity of the cis isomers were established by uv (Table I), nmr (Table II), and tlc analyses. The cis and trans isomers were analyzed similarly. The loss in intensity of the cis spectra accompanied by a concomitant increase in the intensity of the trans spectra was observed as the sample aged. Clearly defined and reproducible isosbestic points were observed in the uv spectra of the various cis–trans mixtures. Isomerization of the cis compound always yielded the trans isomer as checked by nmr, tlc, uv, and by melting point determination.

***cis*-Azobenzene**, which was used as a reference for the kinetic studies, was obtained from the trans isomer by photolysis, followed by chromatography,[9] and recrystallization until homogeneous by tlc.

Solvents for Kinetic Measurements. Spectra-analyzed *n*-heptane was refluxed and then distilled from Na under a nitrogen atmosphere. Acetonitrile was purified from technical grade material with the basic method described by O'Donnell, *et al.*[10] Final distillations under nitrogen were from P_2O_5 or molecular sieves. 2-Propanol (<0.05% H_2O from Matheson Coleman and Bell) was dried by two procedures. In the first, the alcohol was refluxed overnight with Ca followed by distillation. In the second case, the alcohol was allowed to stand over molecular sieves for an extended period. Methanol was pretreated by distillation from molecular sieves. The alcohol was then refluxed and distilled from Mg. Di-*n*-butylamine was allowed to stand overnight in the presence of KOH pellets. It was then refluxed and distilled from molecular sieves.

Buffers were prepared from reagent grade materials using distilled, deionized water.[11] The pH's of the solutions were determined with an Instrumentation Laboratory, Inc., Model 245 deltamatic pH meter with scale expander. Variation of the pH of the buffer with temperature was taken into account. The buffers were prepared to an ionic strength of 0.07 *M*.

Kinetic Measurements. The rates of isomerization were measured spectrophotometrically on a Cary Model 15 recording spectrophotometer by following the increase of absorbance at the π–π^* maxima of the trans isomer (near 315 nm) simultaneously with the decrease in absorbance in the 240–245 nm region (maxima for the cis isomers, minima for the trans isomers). With carefully determined values of the extinction coefficients of the cis and trans isomers at the two analytical wavelengths and with the absorbance readings at these two wavelengths, the concentration of both the cis and trans isomer can be determined for each time sampling.[12] A program was designed to calculate the ln ([cis] + [trans]/[cis]) terms and then calculate the rate constant from the weighted least-squares fitting of the first-order kinetic parameters.[13,14] The rate constants were checked using the absorbances in the 310–315 nm region in conjunction with the limiting absorbance values by least-squares analysis of $\ln [A_\infty/(A_\infty - A_t)]$ *vs.* time data.

The kinetic studies were conducted in flasks equipped with purging trees and sidearms for aliquot withdrawal by syringe. The flasks were painted black to protect the kinetic solutions from light. The kinetic baths were provided with opaque polyethylene-canopied dark boxes to assure the elimination of stray light. Care was taken at all times to protect the kinetic solutions from light or moisture. Many of the kinetic analyses were conducted under N_2, although no effect due to oxygen was observed. The constant temperature baths were regulated by Haake Model 51 circulators and by an American Instrument Co. constant-temperature bath equipped with a refrigeration unit.

Activation Parameters. Activation parameters were calculated with the relation $k = A \exp(-E_a/RT)$.[15] The values of ΔG^* and S^* were obtained through the relationship $E_a = \Delta H^* + RT$ and $\Delta S^* = [\ln A - 1.00 - \ln (kT/h)]R$. The estimated standard devia-

Table III. Activation Parameters for the Cis–Trans Isomerization of Azobenzene and Its Pyridyl Analogs in *n*-Heptane

Compd	E_a, kcal/mol	Log A (sec^{-1})	ΔG,[a] kcal/mol	ΔS*,[a] eu
AB	22.5	10.9	25.31	−11.0
2-PAPy	21.6	10.1	25.51	−14.5
3-PAPy	22.2	10.6	25.38	−12.0
4-PAPy	21.9	11.1	24.41	−9.9
2,2′-APy	21	9	26.2	−20
3,3′-APy	22.6	10.9	25.38	−10.7
4,4′-APy	21.4	10.1	25.30	−14.5

[a] Values of ΔG* and ΔS* were calculated at 40°, the middle of the experimental temperature range.

tions for the activation energies were ±2%. The temperature range for the kinetic studies was typically from 20 to 50°.

Results of the Kinetic Analyses

General. The activation parameters for the cis–trans isomerization of the phenylazopyridines and azopyridines are given in Table III. The cis isomers of the 2- and 4-pyridyl analogs of azobenzene were quite sensitive to catalysis by Lewis acids, notably traces of water in the solvents. The sensitivity of these dyes to catalysis is underscored by literature reports of the acid sensitivity of dyes with amino or hydroxy functional groups.[1,2e,16] The greater basicity of the pyridyl nitrogens of the phenylazopyridines and azopyridines would thereby render these compounds quite susceptible to adventitious catalysis. 2,2′-APy showed the greatest sensitivity in this regard. Glassware treated with acid cleaning solution, with subsequent base treatment and drying, caused anomalously fast isomerization rates for 2,2′-APy. The rates were considerably slower when experiments were conducted in glassware not subjected to this cleaning procedure. To check the eventuality that catalysis was occurring to some extent for the other dyes, presumably because of free hydroxy groups on the surface of the glassware, rate constants were checked with polyethylene containers serving as the kinetic flasks. Catalysis due to acid-cleaned glassware was demonstrated in the case of 2,2′-APy and 2-PAPy.

The order of the activation energies for the series of the 3- and 4-substituted pyridyl analogs of azobenzene parallels the results obtained by Schulte-Frohlinde[2d] for the similarly substituted nitroazobenzenes.

Ortho Effects. Assuming similar electronic distributions of 2-PAPy and 4-PAPy, it would be expected that these compounds would have similar kinetic behavior; however, this is not the case for these compounds or for 2,2′- and 4,4′-APy.

The ΔS* values (−14.5 eu for 2-PAPy and −20 eu for 2,2′-APy) may be an indication of rather severe coulombic interactions between the pyridyl nonbonded electrons and the azo electronic system in the activated complex, and that the difference in the degree of steric restriction for the cis isomer compared with the activated complex is significantly greater for the 2-substituted compounds than for the other dyes of the series. That the nonbonded electron interactions in the 2-substituted compounds are related to the large negative ΔS* values is supported by the comparative observations about the spectral properties of the dyes of this series. Both the 2-substituted compounds show an 8–9 nm bathochromic shift in the ultraviolet of the π–π* band of the trans isomers when the solvent is changed from heptane to methanol. The other dyes of the series, as well as many other aromatic dyes, show only modest (typically <2 nm) changes in the maxima position with this change in solvent.[17] The bathochromic shift is consistent with the premise that this steric interaction posed by the nonbonded pyridyl electrons causes a significant deviation from planarity in the trans isomer. When *trans*-2-PAPy and -2,2′-APy are solvated by methanol, hydrogen bonding to the azo and pyridyl nonbonded electrons probably reduces the repulsive interaction and thus the dihedral angle described by the aromatic π system and the azo π electrons. The same coulombic interaction is evident in the n–π* bands in the visible region of the spectra of the cis isomers. The n–π* band of the 2-substituted compounds are bathochromic with respect to the corresponding 4-isomer (Δ = 4 nm for 2-PAPy *vs.* 4-PAPy and Δ = 17 nm for 2,2′-APy *vs.* 4,4′-APy). This observation is consistent with the supposition that the coulombic interaction between the nonbonded electrons and the azo π system and/or with the adjacent ring increases the C—N=N bond angle. This brings the nonbonded azo electrons into closer proximity, thereby destabilizing the higher nonbonded MO level. This argument for the bathochromic shifts finds precedence and theoretical support in the work of Baird, *et al.*[18]

Table IV. Activation Parameters for the Isomerization of 3- and 4-Phenylazopyridine in Selected Solvents

Solvent	E_a, kcal/mol	Log A, sec^{-1}	ΔG*,[a] kcal/mol	ΔS*,[a] eu
	3-Phenylazopyridine			
n-Heptane	22.2	10.6	25.38	−12.0
Cyclohexane	22.2	10.6	25.36	−12.1
2-Propanol	22.1	10.2	25.83	−13.8
Methanol	22.8	10.4	26.16	−12.9
Acetonitrile	23.6	11.0	26.18	+10.1
	4-Phenylazopyridine			
n-Heptane	21.9	11.1	24.41	−9.9
Di-*n*-butylamine	23.8	12.6	24.28	−3.2
2-Propanol	20.2	10.7	23.33	−10.3
Methanol	20.7	11.0	23.33	−10.3
Acetonitrile	23.0	11.7	24.53	−6.9
Aqueous buffers[b]	22.6	11.7	24.14	−6.9
H^+ catalysis[b]	9.0			

[a] Calculated at 40°. [b] The solvents were aqueous buffers, pH 9–12, with μ = 0.067 *M*. Rate constants were obtained from the slopes and the *y* intercepts of the plots of $k_s + k_H[H^+]$ *vs.* H^+ (see text and ref 20 and 21).

It would be expected that the electronic distribution for the 2- and 4-substituted pyridyl dyes is similar, and that the activation energies for these compounds should also be closely related. Nonetheless, the 2-substituted dyes isomerize considerably slower than the corresponding 4-substituted compounds, and this must be taken as an indication that transition state steric interactions for the former compounds are a major contributing factor.

Although there is a scarcity of experimental activation parameters for the uncatalyzed isomerization of ortho-substituted compounds, the results of Gegiou, *et al.*, indicate that ortho substituents, such as in the case of 2,2′-, 4,4′-, and 6,6′-hexamethylazobenzene, do use steric restrictions in the activated complex.[16a,19]

Solvent Effects. The solvent effects observed in the isomerization of *cis*-3-phenylazopyridine (Table IV) are similar to those reported by Halpern, *et al.*, for the isomerization of azobenzene,[2a] the only notable difference being the consistently lower preexponential factors observed for 3-PAPy. In contrast, *cis*-4-PAPy showed atypical solvent-rate dependency. Although the rates of isomerization in the polar-aprotic solvent acetonitrile (ϵ 38) were slower than those in heptane (ϵ 1.9), fast isomerization rates were observed in the protic solvents. Rates of isomerization in methanol (ϵ 32.7) dried by different procedures or in methanol containing triethylamine or $NaOCH_3$ were comparable, yet considerably faster than those obtained in acetonitrile or heptane. Isomerization rates in 2-propanol were reproducible for the

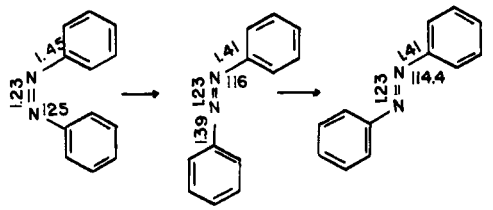

Figure 1. Bond angles and lengths used for CNDO/2 calculations of the energetics for the pyridyl analogs of azobenzene.

solvent dried by two procedures, yet they were still considerably faster than those observed in the aprotic solvents. Although the faster rates may be partially due to traces of water in the solvents, faster rates were also observed in the strongly basic solvent, di-*n*-butylamine, in comparison with the aprotic solvents.

To check the assumption that the isomerization of *cis*-4-PAPy was catalyzed by hydrogen bonding solvents, studies were conducted at 30.2 and 20.0° in a series of aqueous buffers. Resolution of the rate processes was accomplished with the plot of $k_{net} = k_s + k_H[H^+]$ *vs.* $[H^+]$.[20] The activation energy for the catalyzed isomerization was 9 kcal mol^{-1}.[21] Since the value of k obtained from the slopes of these plots represents a combined rate constant, the 9 kcal mol^{-1} activation energy would thereby be a conservative estimate of the barrier to isomerization of the protonated cis molecule.

Catalysis by hydrogen bonding solvents becomes reasonable with the assumption that the electronic effect of this type of solvation would be transmitted to the azo linkage in the same way but to a lesser extent than in the case of the molecule protonated at the pyridyl nitrogen.

Results of the CNDO/2 Calculations

General. In the CNDO/2 investigation of the azobenzene isomerization reported by Ljunggren and Wettermark,[22] inversion was favored over rotation by nearly 40 kcal mol^{-1}. However, the CNDO/2 calculations, due in part to the neglect of differential overlap assumption, failed to show the cis compound to be the less stable isomer. Another disquieting result of the CNDO/2 analysis was the prediction that n-π alignment of the ring π systems to the azo nonbonded electrons (ϕ = 90, 90°)[23] was favored over π-π alignment. Evidence to date is compelling that azobenzene is neither n-π aligned nor totally π-π aligned. In the crystalline state (ref 24), two forms are observed, one of which has ϕ = 17°. The solution uv spectra of the dye are conspicuously similar to the spectra of the crystalline dye suspended in KBr matrices (ref 25). Raman studies (ref 26) also indicate the nonplanarity of the trans isomer; however, comparison of the azobenzene spectrum with that of *N*-benzylideneaniline (which is partially n-π aligned in solution) indicates that the deviation from planarity is not a large one (ref 27).

In our studies,[28] it was found that if the C—N_{azo} bond length (CN) and the C—N=N bond angle (O) near that reported in the literature were used for the cis isomer, then the CNDO/2 predicted relative energies of the cis and trans isomers, and the activated complex became more realistic in comparison with the experimental values. The results of the calculations for inversional isomerization barrier given in Table V are based on the bond angles and lengths shown in Figure 1. For the phenyl rings, bond lengths of 1.397 Å and bond angles of 120° were used. For the pyridyl rings, the bond angles and lengths were those given by Bak and coworkers.[29] The bond angles and lengths for the activated complex and the trans isomer were modeled after those of Ljunggren and Wettermark.[22] With these parameters, the activated complex of azobenzene is calculated to be 23.0 and 19.2 kcal mol^{-1} above the cis and trans isomers, respectively. These values were obtained with ϕ = 90, 90°. For azobenzene, the n-π-aligned conformation (ϕ = 90, 90°) is calculated as the most stable conformation for the cis and trans isomers for the activated complex.

Table V. Relative Energies for the Inversional Isomerization Activated Complexes of the Pyridyl Analogs of Azobenzene[a]

Case	Compd	Substituent[b]	Densities[c] N*	Densities[c] N	Densities[c] C*	ΔE[d]
1	4-PAPy-H$^+$	Pyridyl	5.0861	4.9422	3.7333	12.5
2	4,4′-APy	Pyridyl	5.0993	5.0351	3.7931	21.3
3	4-PAPy	Pyridyl	5.1022	5.0335	3.7917	21.4
4	2-PAPy	Pyridyl	5.1120	5.0289	3.7376	21.5
5	2,2′-APy	Pyridyl	5.1031	5.0423	3.7390	21.7
6	4-PAPy	Phenyl	5.0960	5.0444	3.8226	22.7
7	2-PAPy	Phenyl	5.0949	5.0515	3.8254	22.9
8	3-PAPy	Phenyl	5.1016	5.0361	3.8238	22.9
9	AB	Phenyl	5.0967	5.0451	3.8190	23.0
10	3,3′-APy	Pyridyl	5.0934	5.0417	3.8491	24.1
11	3-PAPy	Pyridyl	5.1024	5.0370	3.8557	24.2

[a] Results of the CNDO/2 analysis of the isomerization. Energy values are based on activated complexes with ϕ = 90, 90°. [b] Substituent on the inverting nitrogen. [c] Valence electron densities; N* is the inverting nitrogen, N is the other azo nitrogen, and C* is the ring carbon bonded to N*. [d] Energy difference in kcal/mol between cis isomer and activated complex.

In the calculation of the total energies of the cis isomers, the values of CN, NN, and O were held constant, and the conformational energy was checked at ϕ = 90, 90° and ϕ = 45, 45°.[30] The conformation with ϕ = 90, 90° was favored except in the cases of 2,2′-APy, 2-PAPy, and 3-PAPy, for which the twisted forms (ϕ = 45, 45°) were favored by 0.8, 0.2, and 0.1 kcal mol^{-1}, respectively. In each case, the most favored conformation for ϕ = 45, 45° was the one in which the net molecular dipole moment was minimized. This is an agreement with the dipole measurements and estimates of geometry given by Campbell, *et al.*,[4] and Bullock and coworkers.[31]

The Rotational Activated Complex. Five rotational activated complexes were examined by CNDO/2 calculations in order to determine the magnitude of the energy barrier. 4-PAPy, 4-phenylazopyridine, protonated at the pyridyl nitrogen (4-PAPy-H$^+$) and 4,4′-APy were examined with the expectation that a low azo π density (with respect to that calculated for azobenzene) would allow rotation to be competitive with inversion. The CNDO/2 charge distribution for 4-PAPy-H$^+$ is marked by a large charge difference between the two azo nitrogens, quite similar to the molecular orbital results given by Kroner and Bock for the dipolar compound, 4-dimethylamino-4′-nitroazobenzene.[32]

The rotational energy barriers for the pyridyl azo compounds were: (1) 4-PAPy-H$^+$, π-π, π-π aligned, ΔE = 40.2 kcal mol^{-1}; n-π aligned, ΔE = 56.4 kcal mol^{-1}; (2) 4,4′-APy, π-π, π-π aligned, ΔE = 53.4 kcal mol^{-1}; n-π aligned, ΔE = 5.72 kcal mol^{-1}; and (3) 4-PAPy, π-π, π-π aligned, ΔE = 53.6 kcal mol^{-1}.[33] Again the CNDO/2 calculations predict substantial barriers to rotation. This is especially significant in consideration of the similarity of the electronic distribution of 4-PAPy-H$^+$ and the donor-acceptor substituted dyes.[3] These compounds and 4-PAPy-H$^+$ all have very low empirical activation energies; however, the barrier to isomerization by rotation calculated by the CNDO/2 method indicates that some other mechanism must be operating.

The Inversion Activated Complex. The lowest energy barrier to isomerization (ΔE = 12.5 kcal mol^{-1}) calculated for the dyes in this study was that of 4-PAPy-H$^+$ for inversion occurring at the nitrogen attached to the pyridyl ring. In all cases investigated, n-π alignment of the aromatic ring at-

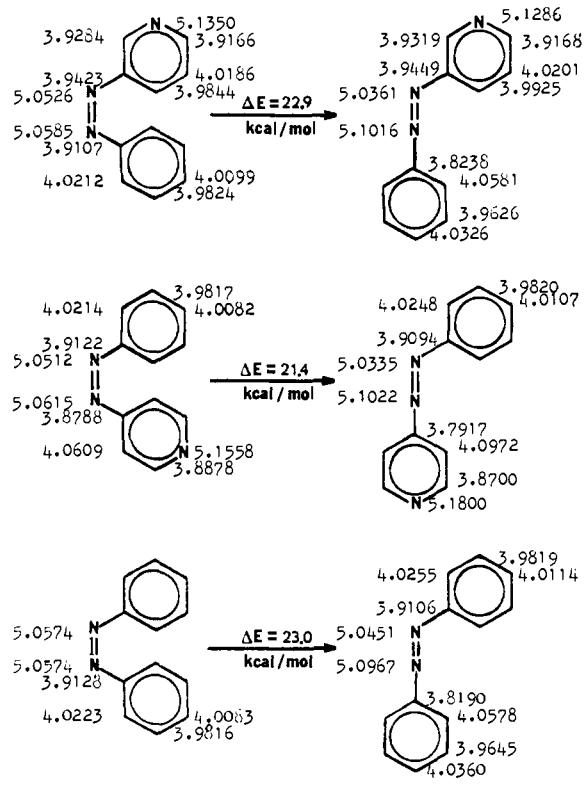

Figure 2. CNDO/2 valence electron densities of selected cis dyes and their linear activated complexes.

tached to the rehybridizing nitrogen (N*) was substantially favored over π–π alignment, whereas n–π alignment of the ring attached to the nitrogen not involved in the isomerization (N) was typically favored by only 1–2 kcal mol^{-1} over the π–π alignment. The results listed in Table V parallel the experimental results, with the 2- and 4-substituted compounds calculated as having lower barriers to inversion than the 3-substituted compounds.

Figure 2 shows examples of the electronic distribution for the cis isomers and activated complexes for 3-PAPy, 4-PAPy, and azobenzene (AB). Comparison of the calculated energy barriers with the electron densities at the various atomic orbitals showed the isomerization to be aided by electron withdrawal by the ring adjacent to the rehybridizing nitrogen; consequently, it appears that the p orbital bearing the azo nonbonded electrons is energetically favored to have a low electron density (Figure 3). The delocalization of these electrons, as gauged by the π density (C=N$_{azo}$) to the adjacent ring (Figure 4), is linearly related to the calculated energy. This observation is in keeping with the premise that the activation energy for the isomerization is related to the rehybridization energy in which the nonbonded electrons are forced into a less electronegative orbital in the activated complex. The cis–trans conversion should be favored by electron delocalization, particularly when the π system of the adjacent ring is electronegative with respect to that of azobenzene. These observations are in agreement with the correlations noted for the benzylideneaniline isomerizations in which inversion is favored when electron-withdrawing substituents are present on the aniline portion of the molecule.[34]

Discussion

The molecular orbital calculations predict that, in the case of the unsymmetrically substituted azo compounds, two distinct inversion routes, differing by 1–2 kcal mol^{-1}, are possible[35] (see Figure 5). The empirical activation energies obtained for the cis–trans isomerizations of unsymmetrically substituted compounds represent activation parameters associated with the two rate processes described by:

$$k_{net} = k_1 + k_2 = A_1 e^{-E_{a1}/RT} + A_2 e^{E_{a2}/RT}$$

However, the amount of curvature in the Arrhenius plots would be quite small providing that the differences in the activation energies for the two processes are of the order of those predicted by the CNDO/2 calculations. The empirical activation energies would be confined to the range E_{a1}–

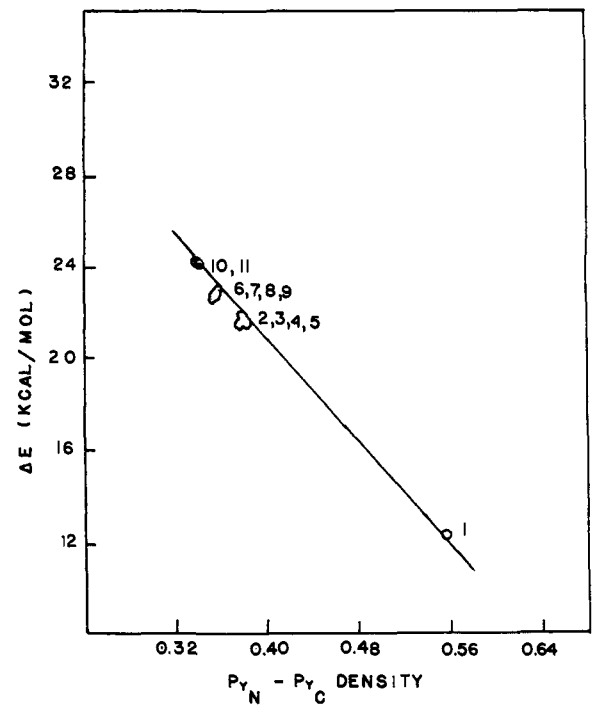

Figure 3. Comparison of CNDO/2 activation energies with π densities between the nonbonded electrons of the inverting azo nitrogen and the p orbital of the adjacent ring carbon. (See Table V for definition of numbering.)

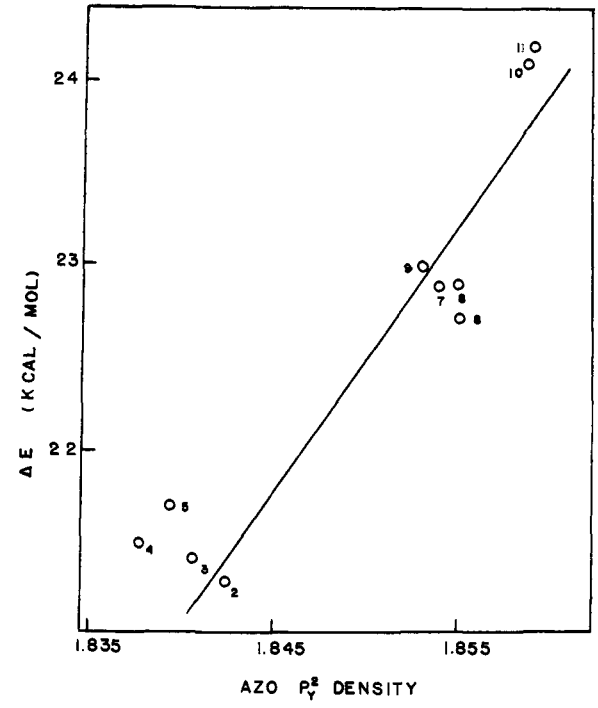

Figure 4. Comparison of CNDO/2 activation energies with the inverting azo nitrogen nonbonded electron densities. (See Table V for definition of numbering.)

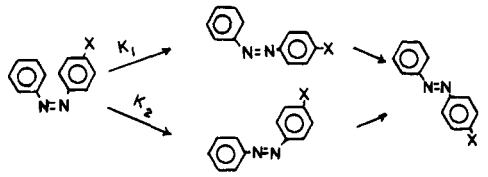

Figure 5.

E_{a2}, and the values determined for the preexponential term A would be between A_1 and A_2 or greater than either. For the symmetrically substituted dyes, the empirical A value would be twice that for the inversion of only one nitrogen. Thus, with the estimation that the preexponential factor associated with the inversion of an azo nitrogen bearing a phenyl, 3- and 4-pyridyl rings should be $10^{10.6}$, and that it should be in the order of 10^{10} for the 2-pyridyl ring, the following hybrid values of the activation energies and preexponential factors and calculated for the phenylazopyridines, based on the CNDO/2 energies given in Table V: 2-PAPy, E_a = 21.9 kcal mol^{-1}, $A = 10^{10.4}$ sec^{-1}; 3-PAPy, E_a = 23.0 kcal mol^{-1}, $A = 10^{10.7}$; and 4-PAPy, E_a = 21.5 kcal mol^{-1}, $A = 10^{10.8}$. The amount of curvature predicted for 2-PAPy is the largest, increasing by 100 cal mol^{-1} over the temperature range 20–65°.[36] The calculated variation in the activation energies for 3-PAPy and 4-PAPy was 20 and 40 cal mol^{-1} (over the same temperature range), respectively.

The range in the experimental activation energies for the compounds of this series is small with exception to the large difference in the activation energies for the isomerizations of neutral and protonated 4-phenylazopyridine. In general, however, it appears that the 2- and 4-substituted pyridyl analogs of azobenzene show lower activation energies than the 3-substituted compounds, which agrees quantitatively with the results of the MO calculations. Both experiment and CNDO/2 results are in accord in terms of the substantially lower activation energy for the acid-catalyzed isomerization of 4-PAPy.

Any quantitative comparison of the experimental and calculated activation parameters would require consideration of the effect of solvation. The solvent effects are quite difficult to assess; however, some qualitative assertions can be made. The solvent effect of *n*-heptane on the activation energy and preexponential factor would be expected to be the greatest when the difference in the dipole moments of the cis molecule and the activated complex is large.[2a,b,37] *n*-Heptane should facilitate the isomerization in cases where the cis molecule has the larger dipole moment. The CNDO/2 calculations show decreases in the dipole moment during isomerization in the order of 1.5 D for azobenzene, 2-PAP, and 2,2′-APy, a 0.6-D decrease for 3-PAPy, and modest increases for 4-PAPy, 3,3′-APy, and 4,4′-APy. Thus the effect of heptane as the solvent for the isomerization of these compounds would be to accelerate the isomerization of AB, 2-PAPy, 2,2′-APy, and 3-PAPy.

Although chemical intuition, MO calculations, and analogies drawn from the benzylideneaniline isomerizations predict that electron-withdrawing substituents should aid the isomerization of aromatic azo dyes, the faster rates (relative to azobenzene) observed with dyes substituted with electron-releasing groups represent the crux of the anomalous nature of the substituent effects on the isomerization. Bowie and Lewis found a linear relationship between the Hammett σ value and the ease of cleavage of the XPh–N=NPh bond upon electron impact for a series of substituted azobenzenes;[38] however, linearity in the Hammett plots of the azobenzene isomerizations is not observed. In fact, the plots seem to be composed of two different lines that intersect at $\alpha = 0.0$.[39]

Recent experimental evidence obtained from the study of the isomerization of various *N*-phenylimines[40] has been interpreted as indicating that inversion is the preferred route of isomerization for dyes with electron-withdrawing substituents, and that torsion about the C–N bond may be occurring when the substituent is electron releasing. Although the V-shaped Hammett plots obtained by Herkstroeter[40b] are compelling evidence of such a change in mechanism, the Hammett plots for the isomerization of *N*-benzylideneanilines do not show such behavior.

An alternate explanation for the V-shaped Hammett plots for azobenzene isomerizations emerges from the consideration of the CNDO/2 calculations of this study. That is, electron-donating groups may lower the activation energy for the inversion process when attached to the nitrogen *not* undergoing inversion, if the inversion of the azo nitrogen is facilitated by electron withdrawal of the adjacent ring. This hypothesis is plausible since azobenzenes substituted with either electron-donating or electron-attracting substituents[2d] have activation energies near 21 kcal mol^{-1}, but when both types of substituents are present in the 4 and 4′ positions of the same molecule, the activation energy is as much 7 kcal/mol lower than that of the unsubstituted compounds.[3] Although this study did not include kinetic or MO analyses of systems bearing electron-releasing substituents, the faster rates found for these compounds can be qualitatively explained by examination of the activated-complex electron distributions of Figure 2 and the data of Figures 3 and 4. A major resonance contributor could be activated complex I. Both 4-nitroazobenzene and 4-phenylazopyridine have resonance structures that can stabilize the activated complex. It also seems likely that electron-releasing substituents exert a stabilizing effect by reduction of the (+) character at the azo nitrogen not undergoing inversion.

Clearly, much is yet left to be understood about the cis–trans isomerization about carbon–nitrogen or nitrogen–nitrogen double bonds. Further experimental findings, more sophisticated molecular orbital analyses, as well as complete analyses of solvent–solute interactions will be required to complete the understanding of these isomerizations.

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$$cis + H^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} cis\text{-}H^+ \xrightarrow{k_3} trans\text{-}H^+$$

in which $k_H[H^+]$ is $k_1k_3[H^+]/(k_2 + k_3)$. The first-order rate constant describes the process cis → trans (k_s) in the aqueous system.
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Assignment of the Configuration of the Steroid Spin Label, 3-Doxyl-5α-cholestane

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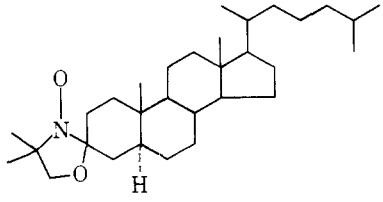
Abstract: There are two possible isomers of the useful steroid spin label, 3-doxyl-5α-cholestane [CA 18353-76-9]. In order to determine which of these isomers is present in the recrystallized synthetic product, the spin label was trapped in a thiourea inclusion crystal. An esr spectral analysis unambiguously identifies the included spin label as 3(e)-doxyl-5α-cholestane, the isomer with the C–N bond in the equatorial position with respect to the steroid A ring. By computer subtraction of the corresponding solution spectrum, it is estimated that at least 95% of the recrystallized synthetic product is the equatorial isomer. No axial isomer was detected in the recrystallized synthetic product or the inclusion crystals.

In 1967 Keana, Keana, and Beetham[1] introduced what has become one of the widely used spin labels for studying biological membranes. The molecule is 3-doxyl-5α-cholestane (**1**), the 4′,4′-dimethyloxazolidine-*N*-oxyl derivative of 5α-cholestan-3-one.



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Important information concerning the orientation, anisotropic motion, and diffusion of membrane lipids is being obtained using this steroid and closely related derivatives.[2-8] An analysis of the resulting electron spin resonance line shapes requires a knowledge of the stereochemistry of **1**. Given the stereochemistry of the parent compound, 5α-cholestan-3-one, the oxazolidine ring formation leads to two possible isomers as shown in Figure 1. The purpose of this study is to identify which of these two isomers predominates in the crystalline product used in the spin-labeling experiments.

We approach this problem by trapping **1** in the tubular cavities of a thiourea inclusion crystal. The basic structure of the thiourea inclusion crystal, which is independent of the guest molecule, has hexagonal cavities 7 Å in diameter extending along the sixfold symmetry axis. The guest molecule **1** can only orient in the tubular cavities with its long axis (z_2) parallel to the needle axis of the crystal. With this knowledge of the orientation and an analysis of the esr spectral anisotropy, it is possible to unambiguously identify the isomer of **1** present in the crystal.

Experimental Section

3-Doxyl-5α-cholestane was synthesized from 5α-cholestan-3-one (Steraloids, Inc.) by the procedure of Keana, *et al.*,[1] and recrystallized from ethanol (mp 160–161° uncor). Single crystals of the 3-doxyl-5α-cholestane–thiourea inclusion compound were grown as follows. The spin label (8 mg) was dissolved in 6 ml of ethanol saturated with thiourea (Mallinckrodt); this solution was saturated with D(+)-camphor (U. S. P. grade, Aldrich Chemical Co.); a small excess of camphor was then added and the solution warmed on a hot plate to dissolve the excess; the warmed solution was al-