insufficient to decompose before reaching the collector, a major portion of those formed from the 2-alkanols must have a different structure (presumably $CH_3CH{=}OH^+$) from those originating from 1-alkanols. Metastables corresponding to $C_2H_5O^+ \rightarrow H_3O^+$ are approximately 35% as abundant for 2-alkanols as for 1-alkanols, representing the maximum proportion of the $C_2H_5O^+$ ions that can have a common structure.[13] Also for 2-alkanols the collision-induced metastables for $C_2H_5O^+ \rightarrow CHO^+$ are not flat topped, as observed for the unimolecular metastables,[10] indicating that a different transition state is involved. The $C_2H_5O^+$ ion from isopropyl ether should also have the structure $CH_3CH{=}OH^+$;[14] its characteristics are consistent with less isomerization than for the $C_2H_5O^+$ of the 2-alkanols.[13]

The metastable characteristics of the $C_2H_5O^+$ ions from the ethoxy compounds are identical within experimental error to those of the 2-alkanols. However, pathways for direct formation of $CH_3CH{=}OH^+$ as well as $CH_3CH_2O^+$ are indicated,[11] so the extent of the isomerization $CH_3CH_2O^+ \rightarrow CH_3CH{=}OH^+$ before metastable decomposition is not clear. $C_2H_5O^+$ ions from CH_3OCH_2R compounds appear to represent a separate structure, consistent with our previous findings.[10]

Note that the collision-induced process will provide a lower probability for a rearrangement reaction only if a competitive pathway of higher frequency factor is operative; thus collision-induced spectra could resemble normal spectra, as found by Jennings.[9] The reduction or elimination of rearrangement processes in mass spectra has obvious important applications for structural studies;[2] for example, the data cited indicate improved usefulness for isotopic studies of aromatic compounds and for distinguishing $HOCH_2CH_2$- and $HOCH(CH_3)$- moieties in molecular structures.[15]

(13) Note that lowering the energy of the $C_2H_5O^+$ ions should lower the rate of isomerization before collision but should have little effect on the rate of collision-induced isomerization.

(14) F. W. McLafferty, *Anal. Chem.*, 29, 1782 (1957).

(15) The financial support of the National Institutes of Health is gratefully acknowledged (Grants GM 12755 and FR 00354).

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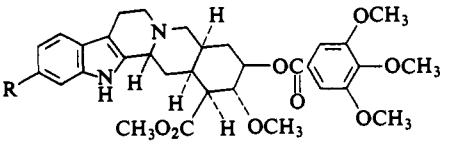
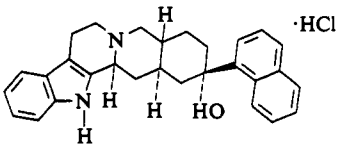
Induction of Fluorescence Polarization in Intramolecular Electronic Energy Transfer

Sir:

In a recent paper we reported[1] observation of 100% intramolecular singlet–singlet electronic energy transfer between the nonconjugated chromophores of the indole alkaloids reserpine (I), deserpidine (II), 17-naphthylyohimbol (III), and rescinnamine. A combination of



I, R = OCH_3
II, R = H



III

exchange and resonance mechanisms was postulated to explain this high efficiency. The average donor–acceptor separation (6–12 Å) was too great in all four cases to rationalize the transfer of all singlet excitation *via* the Förster mechanism, yet small enough to allow the possibility of physical overlap of donor and acceptor π orbital systems.

For the purpose of elucidating further the mechanism of this energy transfer as well as determining the presence of any preferred transition dipole–dipole orientations of donor and acceptor, we have studied the polarization of reserpine and 17-α-naphthylyohimbol fluorescence[2] as a function of solvent viscosity and exciting wavelength.[3,4] Solutions were invariably 6.7×10^{-4} *M* in each of five solvents:[5] propylene glycol, isopropyl alcohol, ethanol, methanol, and anhydrous ether. Measurements of the degree of polarization were obtained at the fluorescence maximum with excitation at 300 and 280 nm. A measurement at 77°K in ethanol glass was also recorded. The degree of polarization (P) was calculated from eq 1 where $I_\parallel$ and $I_\perp$ are the fluorescence intensities at mutually

$$P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp} \qquad (1)$$

parallel and perpendicular settings of Glan–Thompson prisms mounted at 45° angles to both excitation source and detector.[6]

Two factors should affect the polarization of the fluorescence of the acceptor moiety in the alkaloids studied. First is the rotational depolarization, the degree varying inversely with solvent viscosity. Movements of the acceptor moiety about flexible bonds would be expected to contribute more to this form of depolarization than rotation of the entire molecule, due to the large molecular volume of the alkaloids. Rotational depolarization may be eliminated by observing the polarization of emission of the compound at 77°K in a rigid glass.

The second factor is depolarization due to the energy-transfer process itself. Transfer due to a long-range

(1) R. D. Rauh, T. R. Evans, and P. A. Leermakers, *J. Am. Chem. Soc.*, **90**, 6897 (1968).

(2) Rescinnamine does not emit at room temperature, and hence is necessarily omitted from this study.

(3) P. P. Feofilov, "The Physical Basis of Polarized Emission," Consultants Bureau, New York, N. Y., 1961, pp 108–171.

(4) G. Weber, *Trans. Faraday Soc.*, **50**, 552 (1954).

(5) The trivial mechanism of energy transfer does not occur at this concentration; see ref 1.

(6) R. Shimada and L. Goodman, *J. Chem. Phys.*, 43, 3037 (1965).

Table I. Fluorescence Polarization[a] Induced by Energy Transfer as a Function of Solvent Viscosity in Reserpine

Compound	λ_{exclt}, nm	Solvent	η, cP	P_{obsd}	P_{mod}[b]	P_{ET}
Reserpine	280	Ethanol (glass)	$\sim 10^4$	0.077	0.138	0.009
		Propylene glycol	20	0.071	0.098	0.028
		Isopropyl alcohol	2.40	0.079	0.079	0.040
		Ethanol	1.20	0.140	0.079	0.100
		Methanol	0.50	0.100	0.039	0.080
		Ethyl ether	0.23	0.153	0.01	0.152
	300	Ethanol (glass)	$\sim 10^4$	0.040	0.077	0.020
		Propylene glycol	20	0.023	−0.034	0.033
		Isopropyl alcohol	2.40	0.021	−0.022	0.028
		Ethanol	1.20	0.028	−0.014	0.033
		Methanol	0.50	0.043	−0.010	0.046
		Ethyl ether	0.23	0.046	0.00	0.046

[a] Precision of polarization measurements was within ±5%. Precision of calculated values of P_{ET} will necessarily be somewhat poorer; however, scrutiny of the data reveals that error limits are within ±10% at the extreme. [b] Model is methyl 3,4,5-trimethoxybenzoate.

Table II. Fluorescence Polarization[a] Induced by Energy Transfer as a Function of Solvent Viscosity in 17-α-Naphthylyohimbol

Compound	λ_{exclt}, nm	Solvent	η, cP	P_{obsd}	P_{mod}[b]	P_{ET}
17-α-Naphthylyohimbol	280	Ethanol (glass)	$\sim 10^4$	0.20	0.36	0.028
		Propylene glycol	20	0.24	0.38	0.051
		Isopropyl alcohol	2.4	0.23	0.32	0.078
		Ethanol	1.2	0.26	0.35	0.083
		Methanol	0.50	0.20	0.36	0.020
		Ethyl ether	0.23	0.23	0.33	0.061
	300	Ethanol (glass)	$\sim 10^4$	0.20	0.36	0.078
		Propylene glycol	20	0.19	0.35	0.060
		Isopropyl alcohol	2.4	0.22	0.32	0.100
		Ethanol	1.2	0.19	0.33	0.066
		Methanol	0.50	0.17	0.34	0.046
		Ethyl ether	0.23	0.17	0.32	0.050

[a] Precision of polarization measurements was within ±5%. Precision of calculated values of P_{ET} will necessarily be somewhat poorer; however, scrutiny of the data reveals that error limits are within ±10% at the extreme. [b] Model is α-naphthylcarbinol.

dipole–dipole coupling may occur between chromophores whose emission and absorption transition dipoles are not perfectly aligned, although the probability of such transfer decreases to zero as the angle increases to 90°. However, energy transfer *via* the exchange mechanism, which operates at smaller donor–acceptor separations, does not depend upon *transition dipole* orientation, although exchange interaction is not totally independent of the relative orientation of the chromophores.

In the case of reserpine and 17-α-naphthylyohimbol, all emission is characteristic of the acceptor moieties. Hence, the polarization of fluorescence may be given by eq 2 where P_{obsd} is the observed polarization of acceptor

$$P_{obsd} = P_A\left(\frac{\varepsilon_A}{\varepsilon_A + \varepsilon_D}\right) + P_{ET} \qquad (2)$$

fluorescence, P_A is the polarization of fluorescence of an appropriate acceptor model (referred to in the tables as P_{mod}), ε_A and ε_D are the extinction coefficients of donor and acceptor chromophores at the wavelength of excitation, and P_{ET} is the degree of polarization associated with the energy-transfer process itself.[7,8] As appropriate acceptor models we chose methyl trimethoxybenzoate (reserpine) and α-naphthylcarbinol (for the yohimbol), in which viscosity effects on fluorescence polarization were essentially classical, *i.e.*, polarization decreased with decreasing solvent viscosity, especially in the reserpine case.

Table I contains the values of P_{ET} calculated from eq 2, as well as P_{obsd} and P_{mod} (acceptor model) for reserpine, and Table II contains these quantities for 17-α-naphthylyohimbol in the solvents mentioned. It is startling to note that, in the case of reserpine, a fairly general increase in P_{obsd} and a *virtually monotonous increase* in P_{ET} (as calculated from eq 2) are observed with *decreasing* solvent viscosity. One explanation for this behavior is that the energy-transfer process requires the donor and acceptor to explore and to find some preferred relative conformation.[9] This can happen more easily if the viscosity is low and if the donor emission lifetime is long enough relative to the time required for energy transfer. The values of P_{ET} for 17-α-naphthylyohimbol increase in going from a viscosity of about 10^4 to about 2 cP, then decrease between 2 and 0.23 cP (the lowest viscosity examined). Hence, even though perfect alignment of donor and acceptor emission and absorption dipoles is impossible in this case,[1] this also shows anticlassical viscosity effects, indicating some selection of donor–acceptor orientations.

(7) The value of P_{ET} depends upon: the angles between donor absorption and acceptor emission dipoles, the variety of donor–acceptor orientations giving rise to energy transfer, and the movement of the acceptor moiety during the lifetime of its excited singlet state. Equation 2 is thus somewhat arbitrary, but acceptable to our spectroscopic colleagues for these purposes.

(8) Since the absorption spectrum of the alkaloid is identical with that for a 1:1 molar mixture of the two components,[1] ε_A and ε_D are the same in the model compounds as they are in the composite alkaloid.

(9) A referee has suggested that "the increase in P_{obsd} with decreasing viscosity can be explained by postulating that the absorption at these wavelengths is due to two different vibronic transitions" and that "small changes in the relative strengths of these two dipoles can result from variations in solvent shifts of the two transitions." Although this explanation may account for some scatter, it is unlikely that the general trends can be explained by this mechanism since all of the solvents, excepting diethyl ether, are alcohols and should show similar effects on the vibronic levels.

In both cases, as soon as the energy-transfer process is complete, the acceptor is subject to fluorescence depolarization due to movements during the lifetime of its excited singlet state.[3] If we are to reason that solvents of low viscosity allow for a higher probability of preferred dipole–dipole orientations, we must also take into account this "classical" viscosity effect operating in the opposite direction. Hence, the value of P_{ET} might be expected to reach a maximum at some intermediate solvent viscosity. This is observed in the case of 17 α-naphthylyohimbol. Reserpine shows a general increase in P_{ET} in going toward less viscous solvents, but the results are somewhat erratic, perhaps due to the presence of favored conformations for different solvents, and the molecule's greater over-all flexibility. Nevertheless, we conclude that the results for both molecules indicate an enhancement of efficiency of energy transfer due to the attainment of favorable electronic transition dipole–dipole orientations of the donor and acceptor during the energy-transfer process.

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(10) Alfred P. Sloan Fellow.

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Nuclear Magnetic Resonance Study of Calcium-43

Sir:

Nuclear magnetic resonance methods have become an important means of investigating a variety of interactions that are biochemically significant. While proton resonance measurements offer the greatest sensitivity, nuclei such as the halogens offer the advantages of selectively amplifying certain interactions with sites associated with large molecules such as enzymes or proteins.[1] With the exception of sodium-23 measurements[2] little progress has been made in utilizing magnetic resonance of alkaline or alkaline earth cations for probing the environment of these ions in the presence of macromolecules. Because calcium ion has been critically implicated in a variety of life processes, a study of the calcium-43 isotope was undertaken to determine the suitability of nmr as a method of directly studying the calcium environment in systems of biological interest. This note reports measurements of calcium-43 relaxation times in aqueous adenosine triphosphate solutions which indicate that the calcium-43 relaxation times are such that nmr should be a powerful approach for the study of biologically significant calcium complexes.

Calcium-43 was purchased as the carbonate 31.68% enriched from the Union Carbide Corp. at Oak Ridge National Laboratories. Adenosine triphosphate was obtained as the disodium salt from Calbiochem; all other chemicals used were Baker and Adamson analyzed reagents. The calcium concentration in the solutions studied was 0.91 *M*. The ATP solutions were made by successive additions of the solid to the initial calcium solution; the pH was then adjusted by addition of sodium hydroxide. In adjusting the pH the molar concentration of the calcium decreases; however, the ATP concentration decreases by the same factor so that the ratio ATP/Ca remains the same.

The nmr spectrometer employed a Varian V-4210A variable-frequency radiofrequency unit crystal locked at 4.00 MHz, a Varian 12-in. magnet, flux stabilizer, slow sweep unit, and homogeneity control unit. A Princeton Applied Research Model JB-4 lock-in amplifier was used in conjunction with a Dynakit audioamplifier and a Hewlett-Packard Model 201CR audio oscillator to stabilize the base line. Spectra were recorded using a Sanborn Model 7700 recorder in order to provide a rapid response time. Relaxation times were measured using the method described by Sykes[3] and the errors shown are mean deviations from the mean of three or more separate measurements.

In strongly acidic solutions the calcium-43 T_2 is 1.2 ± 0.1 sec. A measurement of the calcium-43 relaxation time is shown in Figure 1 where no precautions were taken to exclude carbonate and T_2 is 0.85 ± 0.1 sec. The pH was adjusted to 6.4 and the relaxation time measured as a function of adenosine triphosphate concentration; the data are shown in Figure 2.

Calcium-43 has a nuclear spin of $^7/_2$ which implies that the nucleus may possess a quadrupole moment. With T_2 as long as 1 sec it is possible that there are other than quadrupolar contributions to T_2. In solid CaF_2, however, the quadrupole interaction is the dominant relaxation mechanism,[4] and for environments other than solvated calcium ion the quadrupolar mechanism should dominate. For a nucleus that is quadrupole relaxed, the transverse relaxation time in sec^{-1} is given by eq 1, where q is the

$$\frac{1}{T_2} = \frac{2\pi^2}{49}(e^2qQ)^2\tau_c \qquad (1)$$

electric field gradient at the nucleus with quadrupole moment Q, τ_c is the correlation time for the reorientation of the field gradient with respect to the applied field, and the asymmetry parameter has been neglected.[5]

The linear dependence of the relaxation time on the ATP concentration is explained by the exchange of the calcium free in solution with the calcium bound to the phosphate portion of the ATP molecule. The exchange rate as measured by temperature-jump techniques is greater than 2.5×10^5 sec^{-1}, and this rate is sufficiently

(1) T. R. Stengle and J. D. Baldeschwieler, *Proc. Natl. Acad. Sci. U. S.*, **55**, 1020 (1966).
(2) F. W. Cope, *J. Gen. Physiol.*, **50**, 1353 (1967).
(3) B. D. Sykes, *J. Am. Chem. Soc.*, 91, 949 (1969).
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(5) A. Abragam, "The Principles of Nuclear Magnetism," Oxford, The Clarendon Press, 1961, p 314.