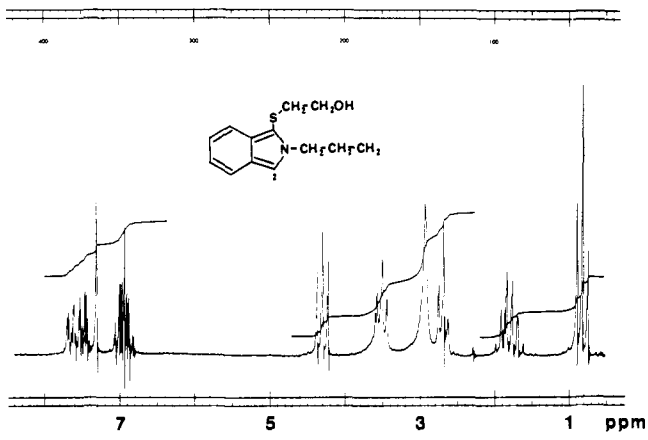
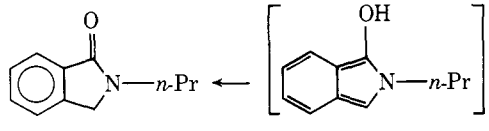


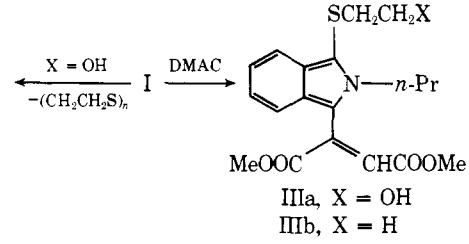
Figure 1. The 100-MHz proton NMR spectrum of Ia in CD_3CN.

However, no 1-alkylthio-substituted isoindoles have been reported, even though many isoindoles have been prepared.[5] For this reason, and to obtain further proof of structure I, two derivatives were prepared.

The MERC adduct Ia undergoes a spontaneous, albeit slow, apparently intramolecular sulfur to oxygen rearrangement to give an ethylene sulfide polymer and the 2,3-dihydro-1*H*-isoindol-1-one, II. The polymer was identified by infrared spectrum, mass spectral analysis, sulfur analysis (calcd for $(CH_2CH_2S)_n$ = 53.34%, obsd = 52.41%), and comparison with authentic polymer. II was isolated in 78% yield as a low melting solid (mp 33.1-34.7 °C[6] after four recrystallizations). The structure was assigned on the basis of the exact mass (obsd = 175.0995, calcd for $C_{11}H_{13}NO$ = 175.0996), infrared spectrum ($\nu_{C=O}$ (film) 1670 cm^{-1}),[7] and proton NMR spectrum in $CDCl_3$ (δ 7.8-7.3 (m, 4 H), 4.33 (s, 2 H), 3.49 (t, J = 7 Hz, 2 H), 1.65 (t of quart, $J = J'$ = 7 Hz, 2 H), 0.88 (t, J' = 7 Hz, 3 H)). The proposed mechanism for the formation of II is supported by the fact that the ET adduct Ib does not give II under the same conditions.



II



IIIa, X = OH
IIIb, X = H

Diels-Alder adducts have been formed from isoindoles, but, in most cases, a 1:1 substitution adduct (α to nitrogen) is formed before a 2:1 Diels-Alder-like product is obtained.[5,8] With Ia or Ib dimethyl acetylenedicarboxylate gives a red-black 1:1 adduct, suggesting a substitution product with extended conjugation. The ET adduct Ib yielded a crystalline product (34% yield; analytical sample (C, H, N, S) mp 73.0-73.5 °C[6]) which was assigned the structure IIIb on the basis of the proton NMR spectrum ($(CDCl_3)$ δ 7.7-6.8 (m, 5 H) 4.26 (broad, 2 H), 3.73 (s, 3 H), 3.47 (s, 3H) 2.72 (q, J = 7 Hz, 2 H), 1.73 (t of q, $J = J'$ = 7 Hz, 2 H), 1.12 and 0.82 (two t, J = 7 Hz, 6 H)). As expected, the vinyl proton appears as a singlet (7.18 ppm). The N-CH_2 signal is severely broadened due to restricted rotation caused by the two bulky α-substituents.[3] The spatial relationship of the two carbomethoxy groups is not known but stereochemical considerations indicate that the Z configuration should be preferentially formed.

In conclusion the structure of the fluorescent product in the reaction of OPTA and a thiol with primary amines has been determined. It should now be possible to utilize the special structural features of this adduct (I) in future fluorescence studies. Of additional interest is that these thio-substituted isoindoles appear to be among the smallest compounds yet described for the fluorescent detection of amino acids.[1d] Finally, this reaction provides an easy entry into the isoindole ring system. Details of these results, and studies on the mechanism of formation of I, will appear elsewhere.[3]

Acknowledgments. We would like to thank Dr. Peter Roller for determining the exact masses, Noel Whittaker for obtaining in the various mass spectra, and Dr. Herman Yeh for running the 100-MHz NMR spectrum and helpful discussions.

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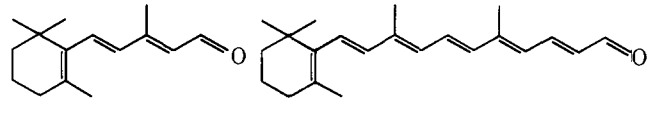
Emission Spectroscopy and State Ordering of Retinals[1]

Sir:

There has been considerable concern over the past several years regarding the fluorescence and state order in polyenes, including the visual pigment models, retinals, and their Schiff bases.[2-9] Fluorescence has been observed for the retinals and a dependence of ϕ_F on the excitation wavelength noted.[3,6] Reasons for the latter have been offered.[3,6] It has been proposed that the $^1A_g(\pi,\pi^*)$ state is lowest in polyenes in general, including the retinals.[5,6,8] Other recent works have indicated the possibility of a $^1(n,\pi^*)$ state being lowest[4,7,9] or essentially degenerate[3] with a lowest $^1(\pi,\pi^*)$.

In this communication we wish to report what we believe is firm evidence that a state principally of $^1(n,\pi^*)$ character is generally the lowest excited singlet state in non-hydrogen-bonding solvents, at least for the all-trans and 13-cis isomers. This is supported by spectral data on homologues and analogues of retinals such as I and II.



I II

Table I. Spectral Data for All-Trans Retinal in Various Solvents[a]

	3MP	EPA	3MP-Ph	EtOH[c]	MeOH[c]	TFE[c]	Ch_2Cl_2-Ph
λ_F^{max}[d]	520	540	600	600	620	650	680
λ_{exc}^{max}	395	410	420	395	395	420	420
λ_{abs}^{max}	385	385	420	385	385	413	418
T, K	77	77	77	213	213	233	183
solvent cond.[b]	R	R	R	F	F	F	F

[a] In nanometers, all values corrected for instrumental contributions. [b] R = rigid and F = fluid. [c] Relative intensity of fluorescence is TFE (3):MeOH (1.2):EtOH (1). [d] From a plot of photons/second/nanometer vs. nanometer, corrected for photomultiplier response.

All spectral studies were on samples purified by high-pressure liquid chromatography. The 3-methylpentane (3MP) was purified as given before.[3] EPA, ethanol (EtOH), methanol (MeOH), trifluoroethanol (TFE), and phenol (Ph) were of reagent grade or higher. None showed fluorescence upon excitation in the region of interest. Especially dried retinal and other solutes as well as 3MP were obtained. The solutes were pumped under high vacuum (10^{-5} Torr) for 5 h. Purified 3MP was treated with metallic sodium or 3A molecular sieves in vacuo and distilled onto the dried solute in an emission cell in vacuo.

It is first necessary to point out that we have strong evidence for the formation of dimers (n-mers) based both on absorption and emission data; for example, the relative ϕ_F of all-trans retinal is markedly dependent on concentration and wavelength of excitation (the latter varies with concentration). Emission from dimers appears from about 5×10^{-5} M with λ_F^{max} 550 nm.

In order to support the claim that fluorescence occurs only from the H-bonded species in retinal, we give the following evidence. First, if all-trans retinal is prepared under dry conditions with dry 3MP (as given above) at $1\text{-}2 \times 10^{-5}$ M, no fluorescence, $\phi_F \leq 10^{-4}$, is observed at any temperature down to 77 K. However, if water is added to the dried sample, fluorescence is observed, λ_F^{max} 520 nm. In a parallel experiment, if the retinal and 3MP are not dried carefully, then fluorescence λ_F^{max} 520 nm, can again be seen. When water is added to this undried sample, fluorescence is enhanced, but not shifted, λ_F^{max} 520 nm. Second, if phenol (10^{-3} M) is added to the dry experiment, fluorescence is observed at 77 K and above (ten times greater than that of undried experiments in 3MP). Third, retinal in the highly polar but non-hydrogen-bonding solvents dichloromethane to 183 K and acetonitrile to 230 K shows no fluorescence. Fourth, addition of phenol to dichloromethane solutions results in observation of fluorescence at temperatures as high as 253 K. This fluorescence increases in intensity monotonically to 183 K. In this experiment with dichloromethane (or 3MP), if the concentration of phenol is relatively high (10^{-3}-10^{-2} M), there is almost no dependence of ϕ_F on the wavelength of excitation (also nearly true in TFE). Fifth, fluorescence is also observed in EPA (77 K), EtOH (243 K), MeOH (243 K), TFE (295 K), and at lower temperatures. The fluorescence intensities in EPA (77 K) and TFE (233 K) are 3- and 10-12-fold greater, respectively, than in 3MP (undried). The data is summarized in Table I. Figure 1 gives representative spectra for the 3MP-Ph system. Similar results have been obtained for 13-cis retinal, although in fewer solvents.

Support for a contention that the above observations can be explained in terms of changes in state orderings can be obtained by looking at the behavior of some of the homologues of the retinals. For example, in the 9-cis isomer compound I, the $^1(n,\pi^*)$ can be discerned to be lowest in absorption; moreover, the dry, pure 9-cis isomer of compound I does not emit in 3MP. On the contrary, for compound II in 3MP, ϕ_F is 0.05 at 77 K and 6×10^{-3} at 298 K and ϕ_F is essentially independent of

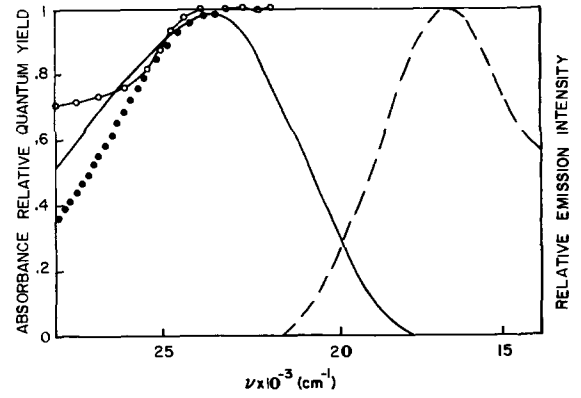


Figure 1. Fluorescence (- - -), absorption (–), excitation (•••), and relative quantum yield (-O-O-O-) of all-trans retinal plus phenol (10^{-3} M) in 3MP at 77 K.

wavelength. These results and the fact that the natural radiative lifetime of the fluorescing state of compound II is 19 ns (in 3MP at 77 K) are consistent with this state being principally of $^1(\pi,\pi^*)$ character.

We believe the foregoing provides strong evidence that for all-trans and 13-cis retinals, a state principally of $^1(n,\pi^*)$ character is generally the lowest excited singlet state in non-hydrogen-bonding solvents. We note this assignment is consistent with the state assignment and the fluorescent properties of various aromatic carbonyl compounds.[10-12] Furthermore, it appears that any fluorescence observed so far originates from H-bonded species. We believe this indicates that generally the lowest excited singlet state of the H-bonded retinals considered is principally of $^1(\pi,\pi^*)$ character—the natural lifetimes of all-trans retinal in (undried) 3MP and in 3MP with 10^{-3} M phenol are <20 and 17 ns, respectively. Because of these state changes, the degree that ϕ_F deviates from a constant as the excitation wavelength changes depends principally on the fraction of retinal that is H-bonded (as in EPA, EtOH, MeOH). In those cases where strong H bonding is expected as for phenol, the essential absence of wavelength dependence is consistent with a high degree of H bonding as would be anticipated for such a compound (similarly for TFE).

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Electronic Isotopic Substitution in Flow and Stopped Flow Nuclear Magnetic Resonance Studies of Chemical Reactions

Sir:

The techniques of flow[1-4] and stopped-flow[5,6] high resolution NMR have recently been developed and applied to the study of chemical reactions.[1-17] One of the most powerful techniques in the investigation of chemical reactions (especially rearrangements) by conventional methods has been the use of chemical isotopic substitution which enables the fate of specific atoms in the reactant molecule(s) to be traced in the transformation to product.[18] We present here a technique which enables this type of experiment to be performed for fast reactions in flowing systems using high resolution NMR without recourse to chemical substitution.

The principle of the technique is illustrated in Figures 1 and 2. In the normal continuous-flow NMR experiment, the two reactant solutions are equilibrated separately in the magnetic field, then flowed together, and the spectrum of the flowing, chemically reacting solution is recorded.[2,3] In the present experiment, the two flows are differentiated from each other by inverting or saturating some or all of the resonances of the reactant molecules in one of the two streams prior to mixing. Thus, the fate of this molecule may be traced in the products as in a chemical isotopic substitution experiment. One possible way of doing this is by use of adiabatic rapid passage (A.R.P.) techniques as illustrated in Figure 1. After magnetization, reactant stream A is passed in a continuous flow through a saturating field at the correct resonance frequency (ARP in Figure 1) before it reaches the mixing chamber. The frequency at the center of the main field is 100 MHz, that at the ARP coil ~70 MHz. In the usual ARP experiment with a stationary sample, the main magnetic field is very quickly swept through resonance.[19] In the present experiment with a flowing sample, the same net effect is achieved because of the large field gradient. Thus the molecules experience a large change in the magnetic field as they move toward the center of the gap.[20] The net effect of the ARP experiment is to completely invert the populations of the nuclear spin levels; subsequent measurement of their spectrum in the usual way at the center of the field will yield inverted signals. In this way, nuclei have been "labeled" without changing the chemical reactivity. The decay of the polarization induced by ARP back to Boltzmann equilibrium will take about three spin-lattice relaxation times; the technique will thus be best suited for the investigation of reactions which are relatively fast and will yield the initial positions of the reactant nuclei in the product(s).

The principle of the technique is illustrated in Figure 2. This shows spectra obtained from the mixing of a solution (B) of naphthalene in acetone with a solution (A) of phenol in methanol. Both solutions were equilibrated in the field and the phenol solution (A) passed through the ARP coil before entering the mixing chamber. Figure 2A shows the flow spectrum of the mixture recorded at a flow rate of 32 ml/min with the ARP power off. The small loss in resolution is due to the nonspinning flow tube and the effect of the flow itself.[1,2,20] In Figure 2B the flow is again 32 ml/min, but the ARP power is now on. The peaks due to the phenol protons have almost disappeared. Decreasing the ARP power causes an increase in these absorptions. In Figure 2C the same power level has been used but the flow rate has been increased to 58 ml/min. The degree of conversion is ~ 70%, the limiting situation for the experiment. The location of these nuclei in any subsequent reaction which might occur can now be traced.

Figure 2B illustrates another possible use of this technique; the removal of interfering peaks from a spectrum. Thus, for a given flow rate, the power may be adjusted to eliminate any given signal to zero intensity. This may even be done with solvent absorptions by inverting the solvent signal in one reactant stream and using this to cancel the positive absorption from the solvent peak in the other reactant stream giving zero

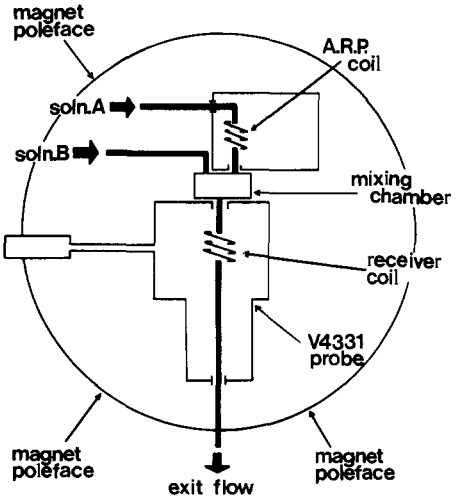

Figure 1. Schematic diagram of the equipment used to give the signal inversions described in the text.

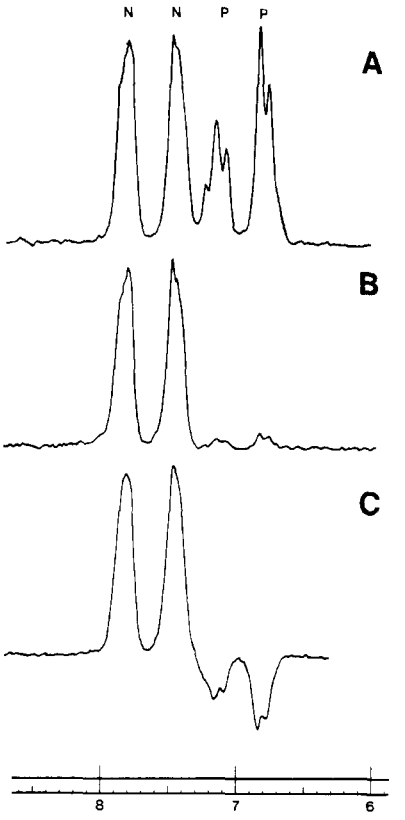

Figure 2. Flow NMR spectra of the aromatic absorptions of a mixture of phenol and naphthalene, the phenol solution passing through the ARP coil prior to mixing: (A) flow rate 32 ml/min, ARP power off; (B) flow rate 32 ml/min, ARP power on; (C) flow rate 58 ml/min, ARP power as in B.