velops[5-7] contribute significantly to destabilization of the BS and RI transition states. That the steric impact of three-CH_3 groups is seemingly more effective in this regard than the contiguous tetrasubstitution arrangement in **9** is intriguing and may mean that geometric arrangements other than the accepted planar alternate form can lead to mechanical tub-to-tub inversion. Work is in progress to gain further insight into this question.

From the finding that **6** experiences ready reduction to **10** with K in ND_3, we see that the aromaticity of this dianion is adequate to offset the prevailing steric destabilization. The symmetry of **10** as revealed by its ^1H NMR spectrum (Figure 1) is consistent with a planar formulation. The polarographic half-wave potential for 2ϵ transfer to **6** ($E_{1/2} = -2.20$ V vs. SCE, anhydrous HMPA solution) can be related to the values obtained for the 1,2-me_2 (-1.95 V), 1,2,3,8-Me_4 (-2.43 V), and 1,2,3,4-Me_4 (-2.54 V) homologues.[1] In this instance, a direct quantitative correlation between the facility of reduction and anticipated ease of ring flattening is evident.

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References and Notes

(1) Part 3: L. A. Paquette and J. M. Photis, *J. Am. Chem. Soc.*, **98,** 4936 (1976).
(2) F. A. L. Anet and V. J. Basus, *J. Am. Chem. Soc.*, **95,** 4424 (1973), and the many relevant references cited therein.
(3) I. L. Karle, *J. Chem. Phys.*, **20,** 65 (1952); W. B. Person, G. C. Pimentel, and K. S. Pitzer, *J. Am. Chem. Soc.*, **74,** 3437 (1952); M. Traetteberg, *Acta Chem. Scand.*, **20,** 1724 (1966); G. Avitabile, P. Ganis, and V. Petraccone, *J. Phys. Chem.*, **73,** 2378 (1969); J. Bordner, R. G. Parker, and R. H. Stanford, Jr., *Acta Crystallogr., Sect. B*, **28,** 1069 (1972).
(4) J. B. Hendrickson, *J. Am. Chem. Soc.*, **89,** 7036, 7043, 7047 (1967); K. B. Wiberg, *ibid.*, **87,** 1070 (1965); M. Bixon and S. Lifson, *Tetrahedron*, **23,** 769 (1967); N. L. Allinger, J. A. Hirsch, M. Miller, J. J. Tyminski, and F. A. Van Catledge, *J. Am. Chem. Soc.*, **90,** 1199 (1968); F. A. L. Anet and J. Krane, *Tetrahedron Lett.*, 5029 (1973).
(5) F. A. L. Anet, A. J. R. Bourn, and Y. S. Lin, *J. Am. Chem. Soc.*, **86,** 3576 (1964); F. A. L. Anet and L. A. Bock, *ibid.*, **90,** 7130 (1968); P. Ganis, A. Musco, and P. A. Temussi, *J. Phys. Chem.*, **73,** 3201 (1969); D. Bryce-Smith, A. Gilbert, and J. Grzonka, *Angew. Chem., Int. Ed. Engl.*, **10,** 746 (1971).
(6) F. A. L. Anet, *J. Am. Chem. Soc.*, **84,** 671 (1962); D. E. Gwynn, G. M. Whitesides, and J. D. Roberts, *ibid.*, **87,** 2862 (1965); J. F. M. Oth, R. Merenyi, T. Martini, and G. Schröder, *Tetrahedron Lett.*, 3087 (1966); J. F. M. Oth, *Pure Appl. Chem.*, **25,** 582 (1971).
(7) N. L. Allinger, J. T. Sprague, and C. J. Finder, *Tetrahedron*, **29,** 2519 (1973); C. J. Finder, D. Chung, and N. L. Allinger, *Tetrahedron Lett.*, 4677 (1972).
(8) (a) K. Mislow and H. D. Perlmutter, *J. Am. Chem. Soc.*, **84,** 3591 (1962); (b) H. P. Figeys and A. Dralants, *Tetrahedron Lett.*, 3901 (1971); (c) G. W. Buchanan, *ibid.*, 665 (1972); (d) A. Rosdahl and J. Sandström, *ibid.*, 4187 (1972); (e) G. H. Senkler, Jr., D. Gust, P. X. Riccobono, and K. Mislow, *J. Am. Chem. Soc.*, **94,** 8626 (1972); (f) E. H. White, R. L. Stern, T. J. Lobl, S. H. Smallcombe, H. Maskill, and E. W. Friend, *Ibid.*, **98,** 3247 (1976).
(9) (a) L. A. Paquette, J. M. Photis, K. B. Gifkins, and J. Clardy, *J. Am. Chem. Soc.*, **97,** 3536 (1975); (b) L. A. Paquette, J. M. Photis, and G. D. Ewing, *ibid.*, **97,** 3538 (1975); (c) L. A. Paquette, *Tetrahedron*, **31,** 2855 (1975).
(10) J. J. Bloomfield and S. L. Lee, *J. Org. Chem.*, **32,** 3919 (1967).
(11) J. M. Photis and L. A. Paquette, *J. Am. Chem. Soc.*, **96,** 4715 (1974).
(12) Acceptable elemental analyses and consistent mass spectra were obtained for all key compounds described herein.
(13) L. A. Paquette, R. E. Wingard, Jr., and J. M. Photis, *J. Am. Chem. Soc.*, **96,** 5801 (1974).
(14) As a result of such prolonged heating, decomposition of the sample was noted when 200 °C was reached.
(15) Attempts to ascertain a more accurate estimate of the bond shift barrier by suitable methyl group labeling is in progress.
(16) That essentially complete resolution had been achieved was established by conversion of **2c** to its acid chloride (oxalyl chloride, C_6H_6, 0 °C, 30 min) and subsequent reaction with d-(+)-α-methylbenzylamine. The resulting amide, $[\alpha]^{25}{}_D$ +66.5° (*c* 18.8 EtOH), exhibits a single methyl ester absorption (δ 3.59) with no sign of the second signal which is present (ratio 1:1) when racemic **2c** is similarly treated (δ 3.59 and 3.66).
(17) University Fellow, 1974–1975.

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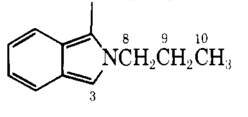
The Structure of the Fluorescent Adduct Formed in the Reaction of *o*-Phthalaldehyde and Thiols with Amines

Sir:

Several reagents are currently available for the fluorogenic detection of amino acids and proteins.[1,2] The reaction of these substances with *o*-phthalaldehyde (OPTA) and β-mercaptoethanol (MERC) is particularly attractive since the strongly fluorescent product allows smaller amounts of amino acids to be detected than is possible by other methods.[2] The full potential of the OPTA reaction has not yet been realized, however, nor can it be realized without a knowledge of the chemical structure of the adducts. In this communication we present evidence that the fluorescent OPTA reaction products are 1-alkylthio-2-alkyl-substituted isoindoles (e.g., I).



Ia, X = OH
Ib, X = H

Primary amines in general, in addition to amino acids and proteins,[2] react with OPTA and MERC to yield the same type of fluorescent products as indicated by thin layer chromatographic and uv and fluorescence spectral analysis.[3] Furthermore, the hydroxyl group of MERC is not essential for the reaction.[3] We thus chose *n*-propylamine and ethanethiol (ET), in addition to MERC, for our studies since they should present fewer chemical problems and simplify the task of structural elucidation. While the fluorescent adducts could not be isolated, solutions of the desired material could be readily prepared in at least 90% purity, when analyzed by NMR and thin layer chromatography. Mass spectral analysis of a solution containing the OPTA/MERC/*n*-propylamine product implicated a compound constructed from 1 equiv of each reactant minus 2 equiv of water. An exact mass determination gave $C_{13}H_{17}NOS$ as the only possible composition of the parent ion (obsd = 235.1033; calcd = 235.1031). Furthermore, the observed and calculated ions of the four major fragmentation peaks agreed to within 0.5 mmu.

The structure Ia was deduced from the infrared spectrum, which exhibited a broad OH band but no carbonyl or SH band, and from the proton NMR spectrum of the total reaction mixture (Figure 1). The large downfield shift of the N-CH_2 group and the integration of the signals at 6.8–7.8 ppm indicated that nitrogen had become part of a five proton aromatic ring system. A consideration of the structure of the starting materials and the negligible shift of the S-CH_2 group suggested attachment of the sulfur atom to what was most likely an isoindole ring system. Finally 2 equiv of water, predicted by the mass spectral analysis as being a side product of the reaction, were observed at 2.9 ppm (Figure 1). The NMR spectrum of the fluorescent ET adduct Ib yielded similar results and allowed a definitive assignment of the nonaromatic protons of Ia (C_8 at 4.30, C_{11} at 2.68, C_{12} at 3.50 ppm). Examination of the aromatic region in both spectra revealed a 2:1:2 proton pattern. The low field, two proton multiplet was assigned to the C_4 and C_7 protons. The signal at 7.31 ppm (1 H) is observed as a barely detectable doublet ($J \simeq 0.75$ Hz), as expected for the C_3 proton. No detailed proton NMR study of isoindoles has been reported but theoretical considerations predict a chemical shift pattern and assignment identical with that of Figure 1.[4]

The isoindole structure of I explains why secondary amines do not yield fluorescent products in the reaction with OPTA,[2]

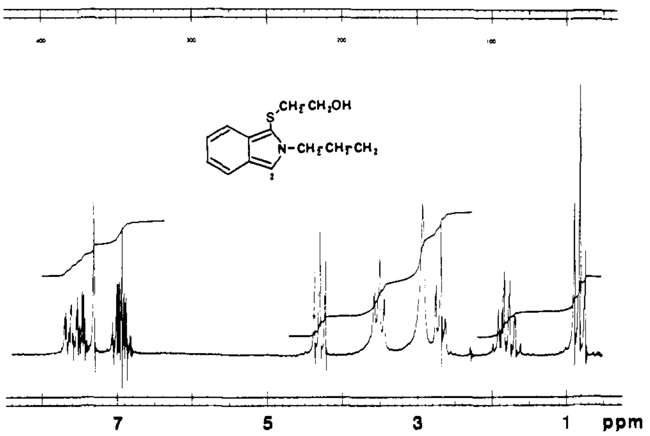
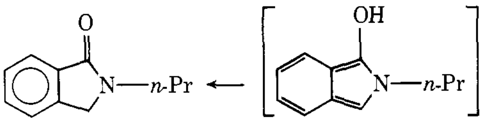


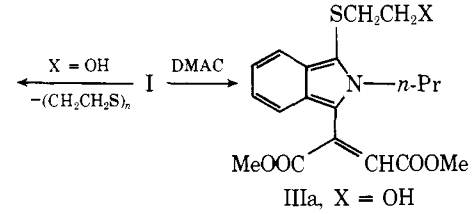
Figure 1. The 100-MHz proton NMR spectrum of Ia in CD_3CN.

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]

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References and Notes

(1) (a) H. J. Creech and R. N. Jones, *J. Am. Chem. Soc.*, **63**, 1661–1669 (1941); (b) G. Weber, *Biochem. J.*, **51**, 155–167 (1952); (c) R. F. Steiner, and H. Edelhoch, *Chem. Rev.*, 457–483 (1962); (d) P. B. Ghosh, and M. W. Whitehouse, *Biochem. J.*, **108**, 155–156 (1968); (e) E. N. Hudson, and G. Weber, *Biochemistry*, **12**, 4154–4161 (1973); (f) S. Udenfriend, S. Stein, P. Bohlen, W. Dairman, W. Leimgruber, and M. Weigele, *Science*, **178**, 871–872 (1972); (g) M. Weigele, S. De Bernardo, W. Leimgruber, R. Cleeland, and E. Grunberg, *Biochem. Biophys. Res. Commun.*, **54**, 899–906 (1973); (h) P. C. Leavis, and S. S. Lehrer, *Biochemistry*, **13**, 3042–3048 (1974); (i) W. H. Scouten, R. Lubcher, and W. Baughman, *Biochim. Biophys. Acta*, **336**, 421–426 (1974); (j) W. E. Harris and W. L. Stahl, *Biochim. Biophys. Acta*, **426**, 325–334 (1976); (k) C.-W. Wu, L. R. Yarbrough, and F. Y.-H. Wu, *Biochemistry*, **15**, 2863–2868 (1976).
(2) J. R. Benson and P. E. Hare, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 619–622 (1975).
(3) Manuscript in preparation.
(4) P. J. Black, R. D. Brown, and M. L. Heffernan, *Aust. J. Chem.*, **20**, 1305–1323 (1967).
(5) J. D. White and M. E. Mann, *Adv. Heterocycl. Chem.* **10**, 113–147 (1969).
(6) All melting points were determined on a Fisher-Johns hot stage and are uncorrected.
(7) I. Yamamoto, S. Yanagi, A. Mamba, and H. Gotoh, *J. Org. Chem.*, **39**, 3924–3929 (1974).
(8) R. Kreher and H. Hennige, *Tetrahedron Lett.*, 1911–1914 (1973).

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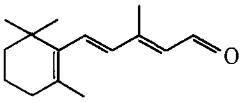
Emission Spectroscopy and State Ordering of Retinals[1]

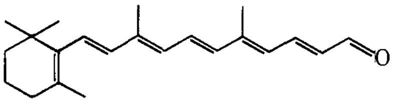
Sir:

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