Excited States of Skin-Sensitizing Coumarins and Psoralens. Spectroscopic Studies[1]

William W. Mantulin[2] and Pill-Soon Song*

Contribution from the Department of Chemistry, Texas Tech University, Lubbock, Texas 79409. *Received March 6, 1973*

Abstract: Several skin-sensitizing psoralens and their model coumarins fluoresce weakly and phosphoresce relatively strongly. The excited states of some of these molecules have been investigated in rigid matrices at 12 and 77°K by means of luminescence and photoselection measurements. Coumarins and psoralens exhibit a relatively high phosphorescence to fluorescence ratio ($\Phi_P/\Phi_F > 1$); however, in coumarylpyrone (benzo[1,2-*b*:4,5-*b'*]dipyran-2,8-dione) and coumarins with strong charge transfer substituents, the ratio is significantly less than unity. In coumarylpyrone this is interpreted as being due to a decreased efficiency of the intersystem crossing from $S_1(\pi,\pi^*)$ to $T_1(\pi,\pi^*)$ as a consequence of the lowering in energy of the $^1(\pi,\pi^*)$ state below the $^3(n,\pi^*)$ state. For the series of molecules investigated, it was established that the 0–0 phosphorescence frequencies, $\bar{\nu}_{0-0}$, are essentially independent of substituents or the extent of conjugation. This result reinforces the model of energy localization in the $^3(\pi,\pi^*)$ state as proposed previously for coumarin and psoralen. The electronic structures of the excited states of selected compounds calculated by SCF–MO methods are consistent with this model. The MO data as well as the spectroscopic data may be correlated with the photobiological reactivity of these molecules toward DNA.

Coumarins, particularly psoralens, are known to photosensitize skin erythema and skin cancer in mice and guinea pigs, though skin cancer in man is not unequivocally established.[3,4] The skin-sensitizing potency of psoralens has been correlated with their photoreactivity toward pyrimidine bases of DNA *via* cycloaddition.[3,5–7] In an attempt to describe the electronic mechanism involved in the photocycloaddition of skin-sensitizing compounds to DNA bases, we have previously studied the excited states of coumarin as a model skin sensitizer.[7,8] The present paper summarizes more complete studies, many at high resolution, of the excited states of a series of coumarins and psoralens.

By far the most interesting molecule examined in this study is coumarylpyrone, benzo[1,2-*b*:4,5-*b'*]dipyran-2,8-dione. The impetus for considering this particular system (and its analog benzo[1,2-*b*:4,5-*b'*]dipyran-2,7-dione, which is yet to be synthesized) arose out of a previous work[7] concerned with the photocycloaddition of the excited coumarins to DNA bases. In this work it was suggested that the triplet state of coumarin and its derivatives undergoes cycloaddition (at the 3,4 double bond) with pyrimidine bases of DNA. Two such double bonds within the molecular framework as found in coumarylpyrone would theoretically provide two active sites with an opportunity to crosslink both strands of the DNA double helix. Figure 1 shows the structures and numbering systems for coumarin, psoralen, isopsoralen, and coumarylpyrone.

(1) Supported in part by the National Cancer Institute (CA13598-01), National Science Foundation (GB-21266A#1), and Robert A. Welch Foundation (D-182). Presented at the 164th National Meeting of the American Chemical Society, New York, N. Y., Aug 27–Sept 1, 1972.

(2) The Robert A. Welch Foundation postdoctoral fellow.

(3) L. Musajo, *Ann. Ist. Super. Sanita*, **5**, 376 (1969), and references therein.

(4) A. C. Giese, *Photophysiology*, **6**, 114 (1971).

(5) (a) L. Musajo, F. Bordin, G. Caporale, S. Marciani, and G. Rigatti, *Photochem. Photobiol.*, **6**, 711 (1967); (b) L. Musajo, F. Bordin, and R. Bevilacqua, *ibid.*, **6**, 927 (1967); (c) L. Musajo and G. Rodighiero, *ibid.*, 11, 27 (1970).

(6) (a) C. H. Krauch, D. M. Krämer, and A. Wacker, *ibid.*, **6**, 341 (1967); (b) D. M. Krämer and M. A. Pathak, *ibid.*, **12**, 333 (1970).

(7) P. S. Song, M. L. Harter, T. A. Moore, and W. C. Herndon, *ibid.*, **14**, 521 (1971).

(8) P. S. Song and W. H. Gordon, *J. Phys. Chem.*, **74**, 4234 (1970).

Experimental Section

Materials. Crystalline, puriss grade coumarin (1,2-benzopyrone) was obtained from Matheson Coleman and Bell. Recrystallization was carried out, but later it was found to be unnecessary, as the coumarin was free of luminescent impurities at a desired sensitivity setting of the detector unit. Furthermore, the phosphorescence decay from this sample was exponential. All other coumarins used were obtained from Aldrich and recrystallization, tlc, or zone refining was performed for some coumarins which contained luminescence impurities. 8-Methoxypsoralen (xanthotoxin) and esculin, pfs grade, Sigma, were used as received. All other psoralens used were generously supplied by Professors L. Musajo and G. Rodighiero (Padova). Coumarylpyrone was a gift from Dr. A. K. Das Gupta (East India Pharmaceutical) and was also synthesized in our laboratories.[9] Dihydrocoumarin (Chemical Procurement Lab. and Aldrich) was zone refined in a cold room (*ca.* 6°) over several days. A trace coumarin like impurity was further removed by hydrogenation under H_2 pressure > 1 atm and with an activated charcoal (10% Pd) catalyst. Purification of 9,10-diphenylanthracene (Aldrich) was achieved by successive recrystallizations from ethanol. The solvents ethanol (U. S. Industrial Co.) and diethyl ether (Matheson Coleman and Bell) were of spectroquality. Phosphoric acid (85%, Matheson Coleman and Bell) was used as received. 3-Methylpentane (Phillips Petroleum), pure grade, contained traces of emitting impurities, which were removed by frontal analysis chromatography (adsorptive filtration), employing a basic alumina column.

Methods. Low-resolution emission spectra were measured on a modified Aminco-Bowman spectrophotofluorometer.[8,10] High-resolution emission and excitation spectra were recorded on a special spectrophotofluorometer designed in this laboratory.[11] In this instrument the light from a 150-W Xenon arc lamp (Hanovia) is collected by an ellipsoidal mirror and is focused onto the entrance slit of a synchronous tandem set of 0.25-m Ebert monochromators (Jarrell-Ash 82–410, linear dispersion of 33 Å, 3-Å resolution). The sample emission is monitored at right angles to the exciting light by a 0.5-m Ebert spectrometer (Jarrell-Ash 82–000, linear dispersion of 16 Å, 0.2-Å first-order resolution) coupled to an EMI 9659 photomultiplier detector. The photomultiplier tube (S-20 response) is constantly kept at reduced temperature (−40°) by a thermoelectric cooling unit (Products for Research). The signal from the photocathode is measured by a photon counting technique (amplifier-discriminator Model 1120 and ratemeter Model 1105, both from SSR Instrument Co.) and is displayed on a Honeywell strip-chart recorder (Electronic 15). Recorded spectra were not corrected for the response characteristics of the instrument.

(9) J. Marx, P. K. Chui, and P. S. Song, submitted for publication.

(10) M. Sun, T. A. Moore, and P. S. Song, *J. Amer. Chem. Soc.*, **94**, 1730 (1972).

(11) T. A. Moore, Ph.D. Dissertation, Texas Tech University, 1973.

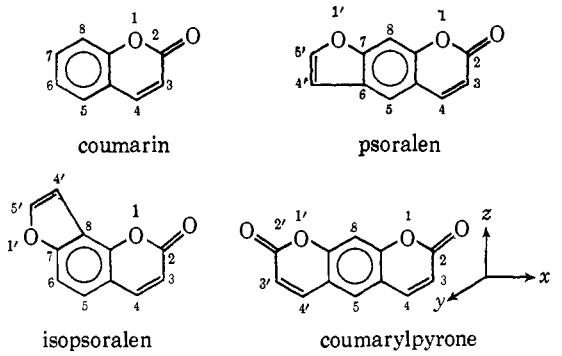


Figure 1. Structures and numbering systems with the corresponding coordinate assignment.

Modification of the sample compartment to accommodate a closed cycle helium refrigeration system (Cryogenic Technology) made it possible to measure luminescence spectra between room temperature and 12°K. The photoselection method, employing Polacoat uv sensitive polarizing and analyzing sheets, was used to determine polarized emission and excitation spectra. The polarized spectra were corrected by the Azumi-McGlynn formulation.[12] Phosphorescence lifetimes were obtained on an Aminco-Bowman spectrophotofluorometer in conjunction with a Textronix (564B-121N) oscilloscope, as described in a previous paper.[8] Corrected luminescence spectra, electronically compensated for instrumental bias as a function of wavelength, were measured on a Perkin-Elmer spectrophotofluorometer (Model MPF-3) and subsequently permitted determination of the ratio of the phosphorescence to the fluorescence quantum yields (Φ_P/Φ_F = area phosphorescence/area fluorescence).[13] This Perkin-Elmer instrument was also employed in the measurement of low-temperature (77°K) fluorescence quantum yields[13] relative to 9,10-diphenylanthracene [$\Phi_F(77°K) = 1 \pm 0.05$],[14-16] to an accuracy of about ±5%. Absorbance values at 77°K necessary to establish the quantum yields were obtained on a Cary 17 or 118 spectrometer. In quantum yield determinations absorbance was kept as low as practical, usually below 0.2 ($\lesssim 3 \times 10^{-5}$ *M*), in order to minimize errors due to the front surface imprisonment and inner-filter effects. Concentrations employed in measuring luminescence spectra were always less than 10^{-4} *M*. Fluorescence quantum yields of psoralens at 77°K were determined by assuming a Gaussian distribution in the region where a substantial fraction of the fluorescence emission is overlapped with the phosphorescence emission.

Molecular Orbital Computations. The Pariser-Parr-Pople SCF-MO-CI,[17,18] Pople-Nesbet unrestricted DODS,[19] and CNDO/II[20] calculations were carried out, using the same set of semiempirical integrals as previously described.[8]

Results

Figure 2 shows fluorescence, phosphorescence, and polarization spectra of coumarin in ethanol at 12°K (polarization spectra at 77°K). The results in ethanol and ether solution at 77 and at 12°K are almost identical, in contrast to the anomalous fluorescence and quenching of phosphorescence in dry isopentane or 3-methylpentane at 77°K.[8,21] The main features, including polarization characteristics of each vibronic intensity, at low resolution (77°K)[8] are confirmed at higher resolution and at 12°K. Although polarizations of the first two $\pi \rightarrow \pi^*$ bands are not mutually perpendicular,[22] the out-of-plane character of the 0-0 intensity and $^3(\pi,\pi^*)$ symmetry are consistent, because the polarization does not change its sign with respect to excitation into the third $\pi \rightarrow \pi^*$ band,[8] which has its polarization direction essentially perpendicular to the first absorption band.[22]

Figure 3 shows the high-resolution spectra of 8-methoxypsoralen which is one of the most potent skin

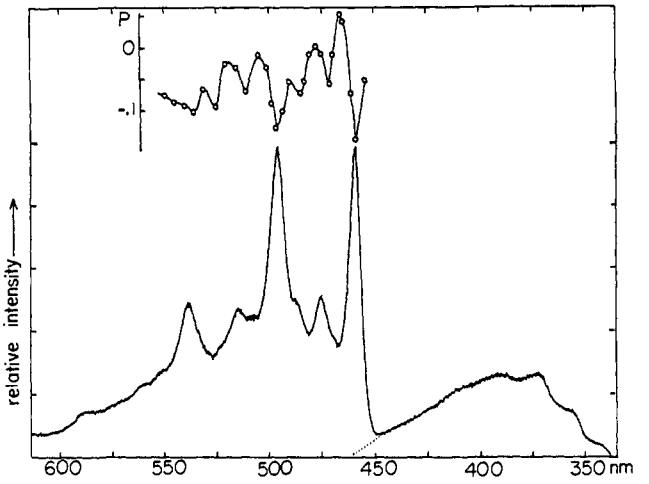


Figure 2. The fluorescence (right), phosphorescence (left), and polarized phosphorescence (—O—) spectra of coumarin in ethanol, obtained at a practical resolution of 1 Å. The excitation wavelength was 320 nm. The spectra were recorded at 12°K, except for the polarized phosphorescence spectrum which was recorded at 77°K. Cracking of the ethanol glass made it impractical to measure accurate polarization at 12°K.

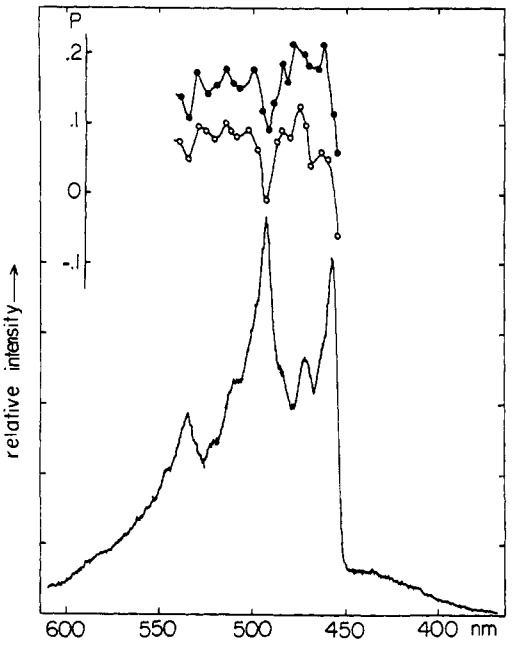


Figure 3. The fluorescence (right), phosphorescence (left), and polarized phosphorescence spectra of 8-methoxypsoralen (—●— with respect to the first $\pi \rightarrow \pi^*$ band at 345 nm and —O— with respect to the second $\pi \rightarrow \pi^*$ band at 310 nm) in ethanol at 77°K, recorded at a practical resolution of 2 Å. Essentially identical spectra (except for the polarization spectrum) were recorded in ether and at 12°K.

(12) T. Azumi and S. P. McGlynn, *J. Chem. Phys.*, 37, 2413 (1962).
(13) C. A. Parker, "Photoluminescence of Solutions," Elsevier, Amsterdam, 1968, pp 262, 279.
(14) E. C. Lim, J. D. Laposa, and J. M. H. Yu, *J. Mol. Spectrosc.*, 19, 412 (1966).
(15) W. W. Mantulin and J. R. Huber, *Photochem. Photobiol.*, 17, 139 (1973).
(16) M. A. Mahaney, W. W. Mantulin, and J. R. Huber, *J. Amer. Chem. Soc.*, in press.
(17) R. Pariser and R. G. Parr, *J. Chem. Phys.*, 21, 466, 767 (1953).
(18) J. A. Pople, *Trans. Faraday Soc.*, 49, 1375 (1953).
(19) J. A. Pople and R. K. Nesbet, *J. Chem. Phys.*, 22, 571 (1954).
(20) J. A. Pople and G. A. Segal, *ibid.*, 44, 3289 (1966).
(21) J. B. Gallivan, *Mol. Photochem.*, 2, 191 (1970).
(22) T. A. Moore, M. L. Harter, and P. S. Song, *J. Mol. Spectrosc.*, 40, 144 (1971).

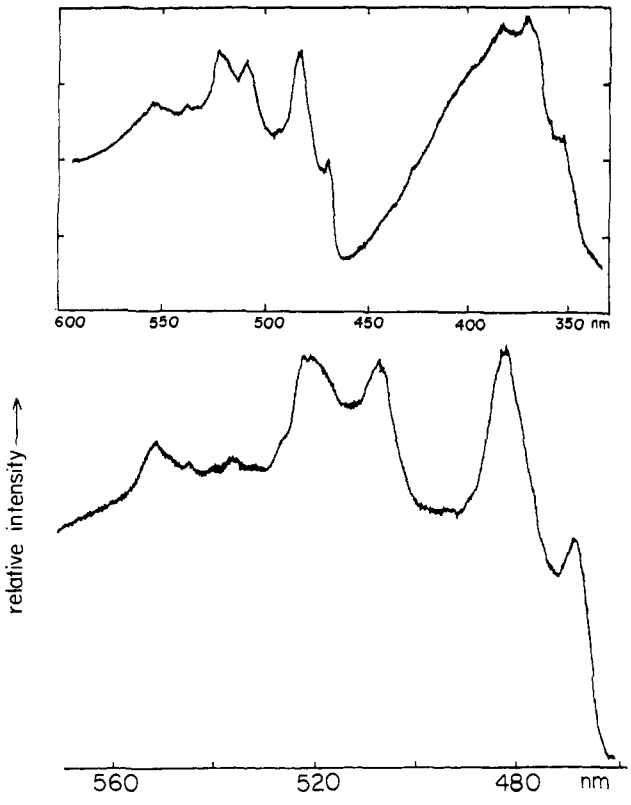


Figure 4. Fluorescence (top right) and phosphorescence (top left) spectra of 3-methylcoumarin in ethanol at 13°K, recorded at 1.5-Å resolution. The phosphorescence spectrum in an expanded scale is also presented at the bottom. The 0–0 phosphorescence polarization with respect to 325- and 275-nm excitation gave −0.17 and −0.1°, respectively.

sensitizers known. The phosphorescence spectra of 8-methoxypsoralen and coumarin at even higher resolution (0.4 Å) and at an expanded scale (figure not shown) revealed very close similarity, and can in fact be superimposed onto each other except for an additional weak intensity shoulder in the former. Although the general pattern of the polarization for various vibronic intensities of the phosphorescence spectrum is the same for both 8-methoxypsoralen and coumarin, more 0–0 in-plane intensity is evident in the phosphorescence emission of the former because polarization with respect to the first and second $\pi \rightarrow \pi^*$ transitions of mutually perpendicular (nearly) polarizations[22] is +0.05 and −0.06, respectively. Nevertheless, more than 50% of the 0–0 intensity is attributable to the out-of-plane polarization. Results similar to coumarin in regard to the 0–0 phosphorescence frequency and polarization are revealed in the case of isopsoralen, 8-methylpsoralen, and 4,5′,8-trimethylpsoralen (figures not shown). In all cases described above, the phosphorescence is considerably more intense than the fluorescence.

In contrast to coumarin and most of the psoralens with a more or less common 0–0 phosphorescence frequency (regardless of the extent of conjugation and substituents), some of the substituted coumarins and 5-substituted psoralens evidence a red shift of the 0–0 phosphorescence band. For example, stronger charge transfer substituents such as the hydroxyl group shift the 0–0 band considerably more than the methyl group, as shown by 7-hydroxycoumarin (figure not shown).

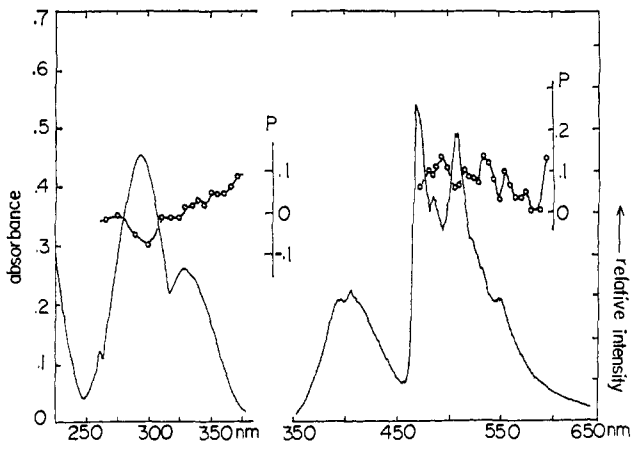


Figure 5. Absorption (left), fluorescence (middle), phosphorescence (right), and polarized phosphorescence excitation (—○—, λ_{em} 470 nm) and emission (—○—, λ_{ex} 358 nm) of coumarin-3-carboxylic acid in ethanol at 77°K, recorded at low resolution (2 nm for both fluorescence and phosphorescence).

Furthermore, in 7-hydroxycoumarin (umberiferone) the phosphorescence intensity is considerably weaker than the fluorescence. Similar results are displayed by 3-phenylcoumarin (figure not shown), 6,7-dihydroxycoumarin-6-glucoside (esculin, figure not shown), 5,7-dimethoxycoumarin (figure not shown), 3-methylcoumarin (Figure 4), 5-methoxypsoralen (figure not shown), and 5-hydroxypsoralen (bergaptol, figure not shown).

The charge transfer substituent at position 4 exerts a blue shift on the 0–0 phosphorescence band. Substituents at position 3 are, however, strongly bathochromic. Figure 5 illustrates spectra for coumarin-3-carboxylic acid. The polarization spectrum is again similar to all other compounds examined, except for the fact that the phosphorescence polarization with respect to the second $\pi \rightarrow \pi^*$ transition is more negative than with respect to the first transition. The ratio Φ_P/Φ_F is relatively high (2.82), however. 3-Acetyl-6-bromocoumarin showed predominant out-of-plane polarization of its phosphorescence. Phosphorescence is the dominant intensity. Its lifetime is only 0.013 sec, owing to the intramolecular heavy-atom effect. The 0–0 phosphorescence is red shifted to 497 nm, as both substituents enhance the delocalization of the triplet state.

Figures 6a and 6b show spectra for coumarylpyrone at high resolution. While its 0–0 phosphorescence frequency and lifetime are nearly identical with those of coumarin, the absorption and fluorescence bands are highly structured in contrast to most other coumarin and psoralen derivatives examined. In addition, fluorescence intensity is stronger than the phosphorescence. Nevertheless, alternating polarization characteristics and vibrational structure in the phosphorescence spectrum of coumarylpyrone are remarkably similar to those of the other coumarins and psoralens, although the polarization is highly positive. It is necessary to assign the absorption bands of coumarylpyrone in interpreting its strongly in-plane polarized phosphorescence (see Discussion).

The absorption spectrum of coumarylpyrone in ethanol is presented in Figure 6a. The first transition is intense, structured, and a mirror image of the fluores-

cence (with a Stoke's shift of approximately 315 cm^{-1} at 77°K). The polarized fluorescence excitation spectrum ($p \sim 0.35$) in conjunction with the results from a SCF-MO-CI P-P-P calculation (*cf.* Table I), assuming

Table I. Calculated $\pi \rightarrow \pi^*$ Transition Energies and Oscillator Strengths (f) for Coumarylpyrone by Means of the SCF-MO-CI P-P-P Method

Transition	Orbital origin	Transition energy, nm (cm^{-1}) Calcd	Obsd[a]	Oscillator strength, f[b]
$^1B_1 \leftarrow A_1$	$\Phi_{10} \leftarrow \Phi_9$	333.7 (29,969)	354.4 (28,210)	0.57 (1)
$^1A_1 \leftarrow A_1$	$\Phi_{11} \leftarrow \Phi_9$	290.6 (34,406)	297 (33,670)	0.00 (0.4)
$^1B_1 \leftarrow A_1$	$\Phi_{11} \leftarrow \Phi_8$	275.2 (36,342)	275 (36,364)	1.39 (1.6)
$^1A_1 \leftarrow A_1$	$\Phi_{10} \leftarrow \Phi_8$	257.6 (38,818)	Buried?	0.20
$^3A_1 \leftarrow A_1$	$\Phi_{11} \leftarrow \Phi_9$	509.5 (19,627)	461.5[c] (21,670)	0
$^3B_1 \leftarrow A_1$	$\Phi_{10} \leftarrow \Phi_9$	479.2 (20,869)		0

[a] Absorption maximum in ethanol at 77°K. [b] Numbers in parentheses are relative absorption peak heights. [c] The 0-0 phosphorescence band.

C_{2v} symmetry, confirm that the first transition is indeed long axis (x) polarized and leads to a $^1B_1(\pi,\pi^*)$ assignment. The calculation further predicts that the second transition results in a $^1A_1(\pi,\pi^*)$, which is polarized along the short molecular axis (z). This suggestion does not seem to be borne out by the polarization data (*cf.* Figure 6), unless the forbiddenness (*cf.* Table I) of this band is considered. The third transition, which is also long axis (x) polarized, combines with the first to contribute to a high-polarization value ($p \sim 0.3$) in the region of the second band. Furthermore, the fact that a weak band at approximately 290 nm is visible (*cf.* Figure 6a) strongly suggests that the $^1A_1$ state steals intensity by means of a vibronic interaction from allowed states. Being long axis polarized, the third band is also assigned to a $^1B_1(\pi,\pi^*)$ state. The results and interpretation of the absorption spectrum remain the same in diethyl ether solution.

High-resolution studies of the coumarin phosphorescence at 12 and 77°K have revealed three fundamental vibrations at 737, 1253, and 1596 cm^{-1} (*cf.* Table II). At 12°K a very weak vibration of 510 cm^{-1} is also resolved. The most negatively polarized and intense vibrational progression ($\Delta\bar{\nu} \sim 1596$ cm^{-1}), beginning at

Table II. Vibrational Modes Resolved from High-Resolution Phosphorescence Spectra in Ethanol at 12°K (±2°)

Compound	$\Delta\bar{\nu}$, ±30 cm^{-1} [a]
Coumarin	510,[d] 737, 1253, 1596
6-Methylcoumarin	754, 1280, 1592
3-Methylcoumarin[b]	584, 1093, 1588
Psoralen	360, 718, 1276, 1586
8-Methoxypsoralen	347, 716, 1284, 1572
5-Hydroxypsoralen	320, 641, 1266, 1579
4,5',8-Trimethylpsoralen[c]	528, ~1250, 1544
cis-Coumarylpyrone	130, 749, 1264, 1587

[a] The same vibrations are resolved in ether and ethanol at 77°K within experimental ranges of error introduced by underlying fluorescence tails. [b] Obtained at 14°K. [c] Obtained at 77°K. [d] Very weak.

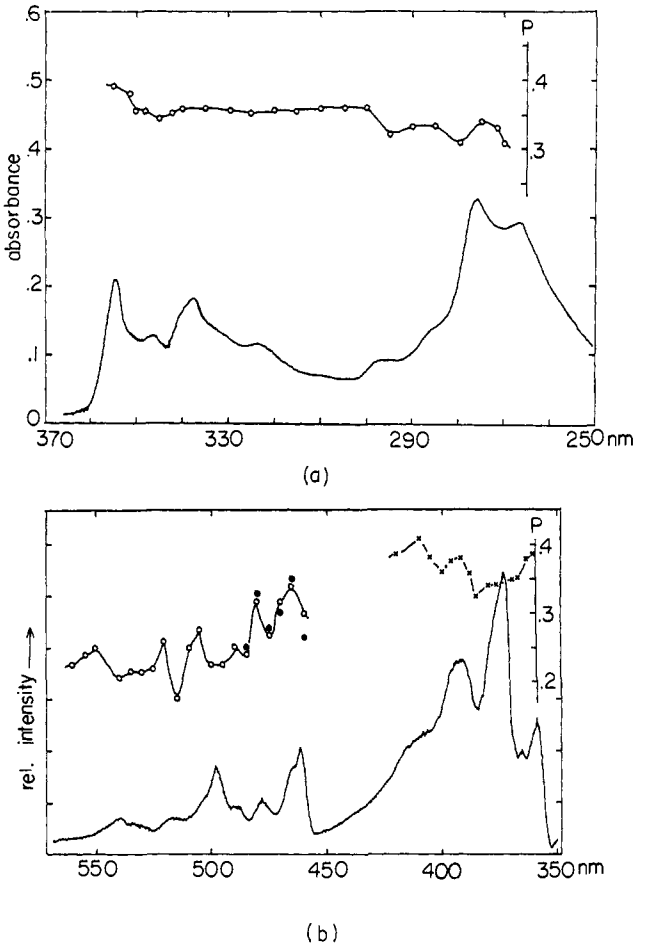


Figure 6. (a) Absorption and polarized fluorescence excitation (—○—, λ_{em} 360 nm) spectra of coumarylpyrone in ethanol at 77°K. (b) Fluorescence (right), polarized fluorescence (—×—, λ_{ex} 355 nm), phosphorescence (left), and polarized phosphorescence (—○—, λ_{ex} 355 nm; —●—, λ_{ex} 297 nm) spectra of coumarylpyrone in ethanol at 13°K, recorded at 1-Å resolution.

the 0-0 band, has been assigned to a C=C stretching mode in the pyrone moiety.[7,8] The polarization of the weak vibronic component ($\Delta\bar{\nu} \sim 737$ cm^{-1}) exhibits more in-plane character, relative to the former series. This vibrational mode has been assigned to a C-H out-of-plane vibration.[7,8] The weak component ($\Delta\bar{\nu} \sim 1253$ cm^{-1}) is probably associated with a benzenoid ring mode. The C-H out-of-plane mode ($\Delta\bar{\nu} \sim 737$ cm^{-1}) is apparently associated with the ethylenic double bond of the pyrone moiety. Thus, substitution of the 3,4 positions in coumarin and psoralen derivatives replaces this mode with a new vibrational mode of lower frequency, whereas substituents at other positions except for the 5 position of psoralens retain the 737 cm^{-1} mode of coumarin (*cf.* Table II). For example, the characteristic C-H out-of-plane mode disappears in 4-hydroxycoumarin (new mode ~553 cm^{-1}), 4-methyl-7-hydroxycoumarin (new mode ~528 cm^{-1}), 3-methylcoumarin (new mode ~584 cm^{-1}), coumarin-3-carboxylic acid (new mode ~568 cm^{-1}), and 3-acetyl-6-bromocoumarin (new modes ~319, ~600 cm^{-1}). It should be noted that 6-methylcoumarin does not reveal a new mode of $\Delta\nu$ ~500-600 cm^{-1}, in contrast to 3-methylcoumarin (*cf.* Table II).

The high-resolution phosphorescence spectrum of psoralen at 12°K remarkably resembles those of cou-

Table III. Absorption (λ^{A}_{max}), Fluorescence (λ^{F}_{max}), 0–0 Phosphorescence (λ^{P}_{0-0}), Phosphorescence Lifetime (τ_P in Sec), Fluorescence (Φ_F), and Phosphorescence Quantum Yields (Φ_P) and Polarization of 0–0 Phosphorescence Band in Degree (P_{0-0}) in Ethanol at 77°K

	First $\pi \rightarrow \pi^*$ λ^{A}_{max} ($\bar{\nu}_A$, cm^{-1})	λ^{F}_{max} ($\bar{\nu}_F$, cm^{-1})	λ^{P}_{0-0} ($\bar{\nu}_P$, cm^{-1})	τ_P, sec	Φ_P/Φ_F[a]	Φ_F	Φ_P	P_{0-0}[b]	Remark
Coumarin	313 (32,260)	380 (26,320)	458.5 (21,840)	0.45 (8)	5.83	0.009	0.055	−0.15	*c*
		390 (25,640)	459.8 (21,750)	0.41	5.50				
		379 (26,390)	447.5 (22,350)	1.30	2.70				*d*
Psoralen	330 (30,300)	409 (24,450)	456 (21,930)	0.66	7.1	0.019	0.13	0.11	
8-Methoxypsoralen	345 (28,990)	~440 (22,730)	456.5 (21,900)	0.72	13.05	0.013	0.17	0.06	
				0.79	16.9				*c*
Isopsoralen	330 (30,300)	401 (24,940)	452 (22,100)	0.77	8.26			0.03	
8-Methylpsoralen	332 (30,120)	415 (24,100)	456.5 (21,904)	0.86	10.1			~0.10	
4,5′,8-Trimethylpsoralen	337 (29,670)	416 (24,040)	446.5 (22,400)	1.22	5.98			0.05	
4-Methyl-7-hydroxycoumarin	327 (30,580)	375 (26,670)	453 (22,080)	1.43	≲0.05			−0.08	
6-Methylcoumarin	325 (30,770)	406.8 (24,580)	465.1 (21,500)	0.41	4.53	0.015	0.066	−0.09	
7-Hydroxycoumarin	333 (30,030)	375 (26,670)	469 (21,320)	0.83	≲0.01			−0.14	
3-Phenylcoumarin	325 (30,770)	410 (24,390)	547 (18,280)	*e*	≲0.05				*e*
5,7-Dimethoxycoumarin	330 (30,300)	378 (26,450)	472 (21,190)	0.85	≲0.05			−0.21	
6,7-Dihydroxycoumarin-6-glucoside	335 (29,850)	390 (25,640)	480 (20,830)	*e*	≲0.01			−0.12	
3-Methylcoumarin	310 (32,260)	375 (26,670)	469.1 (21,320)	0.34	1.08			−0.17	
5-Methoxypsoralen	335 (29,850)	427 (23,420)	472 (21,190)	1.21	11.85	0.019	0.22	−0.20	
5-Hydroxypsoralen	~345 (28,990)	442 (22,600)	466 (21,460)	1.34	10.2	0.023	0.24	−0.14	
Coumarin-3-carboxylic	335 (29,850)	405 (24,690)	470 (21,280)	0.44	2.82			0.06	
3-Acetyl-6-bromocoumarin	350 (28,570)	?	497 (20,120)	0.013	≲0.01			−0.15	
4-Hydroxycoumarin	317 (31,750)	335 (29,850)	420 (23,810)	1.4	2.4			−0.16	
Dicoumarol	321 (31,150)	338.5 (29,540)	436.3 (22,920)	1.1	0.75			0.06	
3,4-Dihydrocoumarin	275 (36,360)	313 (31,970)			~0				
o-Coumaric Acid	327 (30,580)	390 (25,640)			0				
p-Coumaric Acid	317 (31,550)	375 (26,660)			0				
Coumarylpyrone	354.4 (28,210)	373 (26,810)	461.5 (21,670)	0.44	0.55	0.20	0.11	0.30	
	354 (28,250)	371 (26,950)	462 21,640	0.44	0.36				*c*

[a] The phosphorescence fluorescence quantum yield ratios are good within ±10%, as fluorescence spectra of some of the compounds examined overlap heavily with phosphorescence spectra. [b] The 0–0 phosphorescence polarization with respect to the first $\pi \rightarrow \pi^*$ transition. [c] In ether. [d] In 85% phosphoric acid. [e] Too weak to measure accurately.

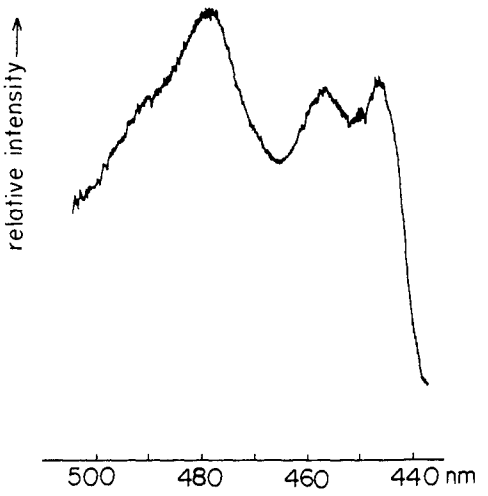


Figure 7. 0–0 phosphorescence band and vibrational progression of 4,5′,8-trimethylpsoralen in ethanol at 77°K, recorded at an expanded scale and 1-Å resolution.

marin (Figure 2) and other derivatives in vibronic features and frequencies. Resolved vibrational modes are compared in Table II. Figure 7 displays the high-resolution phosphorescence spectrum of 4,5′,8-trimethylpsoralen, clearly illustrating the disappearance of the C–H mode, which is replaced by the C–CH_3 out-of-plane mode ($\Delta\bar{\nu} \sim 528$ cm^{-1}). Finally, Table III lists pertinent spectroscopic data, including phosphorescence lifetimes of various coumarin and psoralen derivatives.

Discussion

Previously reported low-resolution studies, employing polarization methods,[7,8,21,22] dichroic spectra,[22] and SCF–MO–CI calculations,[8,22] established that for coumarin the lowest excited singlet state is of (π,π^*) character. Attempted measurements of fluorescence lifetimes of the coumarins were unsuccessful owing to the fact that fluorescence decay was faster than the time resolution (*ca.* 2 nsec) of the lifetime apparatus.[23] Since the radiative lifetime is 1.3×10^{-8} sec (from integration of the absorption band) in coumarin, the observed mean lifetime of the fluorescence is predicted to be 1.2×10^{-11} sec (from the fluorescence quantum yield in Table III). Such a short lifetime and broad bands are indicative of the efficient radiationless transitions from the $^1(\pi,\pi^*)$ state, and intersystem crossing appears to account for only part of the radiationless disposition of the excitation energy (*cf.* Table III). We suggest that broadness and short lifetime of absorption and fluorescence, exhibited by coumarin and its derivatives, are likely to be a consequence of the breakdown of the discrete Born–Oppenheimer state by a nearby $^1(n,\pi^*)$ state in a manner described by the theory of radiationless transitions.[24,25] The CNDO calculation of coumarin indeed predicts that the $^1(n,\pi^*)$ state is located only slightly above the lowest $^1(\pi,\pi^*)$ state but considerably below the second $^1(\pi,\pi^*)$ state. As expected from the foregoing discussion, the ratio

(23) We thank Dr. A. Halpern for allowing us to use his fluorescence lifetime apparatus.
(24) M. Bixon and J. Jortner, *J. Chem. Phys.*, **48**, 715 (1968).
(25) R. M. Hochstrasser, *Accounts Chem. Res.*, 1, 266 (1968).

Φ_P/Φ_F decreases from 5.83 at 77°K to 3.2 at 12°K in ethanol.

The lowest triplet state of coumarin can be unambiguously assigned to a $^3(\pi,\pi^*)$ type, on the basis of lifetime,[7,8,21] polarization,[7,8,21] MO calculations,[7,8] esr spectroscopy,[26,27] and triplet–triplet absorption.[28]

The quantum yield ratio of coumarin in phosphoric acid glass ($\Phi_P/\Phi_F = 2.7$) is less than that in other glasses (Table III) and the phosphorescence lifetime is longer. Considering the relative perturbations of such an environment on (n,π^*) and (π,π^*) states, it is likely that vibronic interactions between the phosphorescent $^3(\pi,\pi^*)$ state and the $^3(n,\pi^*)$ state as well as spin–orbit coupling between (n,π^*) and (π,π^*) states govern relative dispositions of the excitation energy and lifetimes of the excited states.

In general, psoralens tend to show more positive polarization of their 0–0 phosphorescence than coumarins, except for 5-hydroxy- and 5-methoxypsoralens (Table III). In part, the in-plane contribution of the 0–0 phosphorescence polarization of psoralens can be attributed to direct spin–orbit coupling between the ground and lowest triplet states on the basis of the significant differences in permanent dipole moments predicted between these states. For example, CNDO calculations predict dipole moments of 6.9 and 4.04 D[7] for the ground and triplet states of psoralen, respectively, and 3.25 and 0.79 D for the ground and triplet states of isopsoralen, respectively. On the other hand, 5-hydroxy- and 5-methoxypsoralens do not change their dipole moments significantly in the triplet states, as the predicted difference is only $\Delta\mu \sim 0.8$ D. 8-Methoxypsoralen is also predicted to show only a small change in dipole moment upon excitation to the triplet state ($\Delta\mu \sim 0.5$ D). Nevertheless, it shows considerable in-plane intensity in the phosphorescence as indicated by the polarization spectra in Figure 3. Furthermore, unlike other coumarins and psoralens, 8-methoxypsoralen shows a more positive polarization of the phosphorescence with respect to the first $\pi \rightarrow \pi^*$ transition than with respect to the second $\pi \rightarrow \pi^*$ transition (Figure 3). In this case, the two-center spin–orbit couplings by the CT substituent may account for the in-plane contribution to the phosphorescence polarization, since the first $\pi \rightarrow \pi^*$ transition is polarized nearly along the C–OCH_3 axis.[22] On the other hand, it is reasonable to assign the in-plane phosphorescence intensity in 5- and 8-substituted psoralens to the short axis (axis containing CT substituent) component. The predominantly in-plane polarized phosphorescence of coumarylpyrone (Figure 6 and Table III) requires a special treatment (later in this section).

The phosphorescent states of a series of coumarin and psoralen derivatives have several striking features in common with the parent compound coumarin: (1) the 0–0 phosphorescence band occurs in approximately the same energy region, except for specific exceptions; (2) the vibrational resolution follows the pattern of coumarin; and (3) the polarization of the vibronic bands relative to each other resembles the trend in coumarin. These triplet state characteristics are attributed to the partial localization of the triplet excitation in the C=C bond region of the pyrone moiety, as previously suggested.[7,8] The localized nature of the phosphorescent $^3(\pi,\pi^*)$ state of coumarin can also be deduced from its relatively large ZFS parameters, D ($\sim$0.12),[26,27] as compared with those of naphthalene which may be regarded as iso-π-electronic with coumarin. Furthermore, the D value for coumarin is significantly higher than that expected from the linear D *vs.* E_T correlation.[29] Since D is expected to decrease with the extension of the π-electron conjugation,[30] a relatively high D value for coumarin indicates a trend toward localization of the $^3(\pi,\pi^*)$ state.

CNDO, P–P–P, and DODS calculations for many of the compounds listed in Table III reconfirm the previously reported characteristic electronic structure of the $^3(\pi,\pi^*)$ state.[7,8,31,32] Namely, spin density accumulates at positions 3 and 4 and the 3–4 bond order decreases, thus lengthening the C=C bond in the triplet state. Table IV illustrates these features using coumarin, 7-hydroxycoumarin, psoralen, and coumarylpyrone. The C=C bond lengthening appears to be responsible for the presence of the C=C stretching mode in the phosphorescence of coumarins and psoralens. Furthermore, the C=O stretching frequency (1731 cm^{-1} in α-pyrone[33,34] and 1720 cm^{-1} in coumarin[35]), often prevalent in the spectra of aromatic ketones, is significant by its absence in the emission spectra of coumarins and psoralens. Bond lengthening and accumulated spin densities at the 3,4-C=C bond would account for the appearance for the C–H out-of-plane bending mode in the phosphorescence bands.

Table IV. The C=C Bond Distances of Selected Coumarins in Their Different Electronic States[a]

Compound	State	$R_{C_3-C_4}$	$R_{C_{4'}-C_{5'}}$
Psoralen	S_0	1.362	1.364
	S_1	1.387	1.380
	T_1	1.434	1.380
Coumarin	S_0	1.363	
	S_1	1.401	
	T_1	1.440	
7-Hydroxycoumarin	S_0	1.364	
	S_1	1.417	
	T_1	1.420	
Coumarylpyrone	S_0	1.363	
	$S_1(^1B_1)$	1.383	
	$T_1(^3A_1)$	1.404	
	$T_2(^3B_1)$	1.409	

[a] Calculated from the SCF π-bond orders (P_{C-C}) using the formula R_{C-C} (Å) = 1.517 − 0.18P_{C-C}.

Alternation of the 3,4-C=C bond either strongly shifts the 0–0 phosphorescence frequency (Figure 8) or almost completely erases the typical coumarin phosphorescence (Table III). For example, saturation of the 3,4-C=C bond of coumarin produces dihydro-

(26) D. R. Graber, M. W. Grimes, and A. Haug, *J. Chem. Phys.*, **50**, 1623 (1969).
(27) (a) B. S. Kirkiacharian, M. Prak, and C. Hélène, *C. R. Acad. Sci., Ser. C*, **266**, 1548 (1968); (b) B. S. Kirkiacharian, R. Santus, and C. Hélène, *Photochem. Photobiol.*, **16**, 455 (1972).
(28) B. R. Henry and R. V. Hunt, *J. Mol. Spectrosc.*, **39**, 466 (1971).
(29) J. S. Brinen and M. K. Orloff, *Chem. Phys. Lett.*, 1, 276 (1967).
(30) S. A. Boorstein and M. Gouterman, *J. Chem. Phys.*, **39**, 2443 (1963).
(31) N. K. Ray and V. K. Ahuja, *Photochem. Photobiol.*, in press.
(32) Details of these calculations are available upon request.
(33) L. J. Bellamy, "Advances in Infrared Group Frequencies," Methuen & Co., London, 1968, pp 27–29, 164.
(34) Y. Kamano, *Kagaku No Ryoiki*, **24**, 421 (1970).
(35) P. S. Song, unpublished ir results in chloroform.

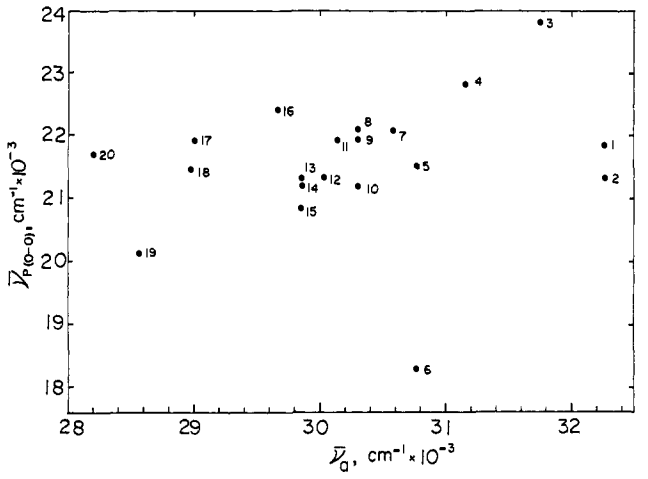
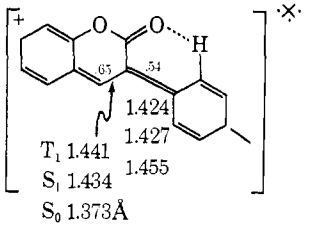


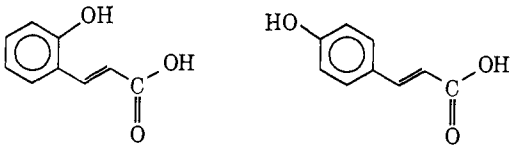
Figure 8. Plot of the 0–0 phosphorescence band ($\bar{\nu}_p$) against absorption maxima ($\bar{\nu}_a$ in cm^{-1}) in ethanol at 77°K: (1) coumarin, (2) 3-methylcoumarin, (3) 4-hydroxycoumarin, (4) dicoumarol, (5) 6-methylcoumarin, (6) 3-phenylcoumarin, (7) 4-methyl-7-hydroxycoumarin, (8) isopsoralen, (9) psoralen, (10) 5,7-dimethoxycoumarin; (11) 8-methylcoumarin, (12) 7-hydroxycoumarin, (13) coumarin-3-carboxylic acid, (14) 5-methoxypsoralen, (15) 6,7-dihydroxycoumarin-6-glucoside (esculin), (16) 4,5′,8-trimethylpsoralen, (17) 8-methoxypsoralen, (18) 5-hydroxypsoralen, (19) 3-acetyl-6-bromocoumarin, and (20) coumarylpyrone.

coumarin, whose emission resembles that of the phenols and not the coumarins. Extensive low-temperature zone refining and additional hydrogenation eliminated what appears to be the impurity coumarin phosphorescence from dihydrocoumarin. On the other hand, chromenes which retain the 3,4-C=C bond exhibit a phosphorescence similar to that of the coumarins.[36] Another illustration of the spectral perturbation of the double bond occurs in 3-phenylcoumarin. Its unusually low 0–0 phosphorescence frequency (Table III) indicates a significant delocalization of the $^3(\pi,\pi^*)$ state relative to other coumarins. Bond distances for the 3,4-C=C and -C—C bonds between coumarin and phenyl moieties, as calculated from mobile bond orders (SCF–MO–CI), increase and decrease upon excitation, respectively. There is also less tendency for spin localization at positions 3 and 4, relative to coumarin. Delocalization of the triplet state of 3-phenylcoumarin can, therefore, be represented by the following resonance structure.

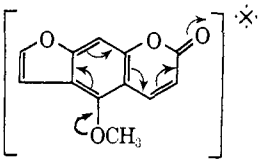


An open, hydrolysis form of coumarin, *o*-coumaric acid, as well as *p*-coumaric acid, emits no phosphorescence (Table III) because of an efficient $T_1 \rightsquigarrow S_0$ radiationless transition resulting from reduced rigidity in the localized ethylenic chromophore. It should be recalled that phosphorescence from ethylene itself has never been observed.

In general, absorption and fluorescence bands show



bathochromic behavior with extended conjugation of the coumarin π-electron system. In contrast, the 0–0 phosphorescence frequencies of most coumarins and psoralens, except for 3,4-substituted compounds, remain roughly invariant (Figure 8). It is significant that the 0–0 phosphorescence from the psoralens is more closely isoenergetic with the parent compound, coumarin, than with the substituted coumarins. 5-Hydroxy- and 5-methoxypsoralens deviate from this pattern. This deviation is particularly intriguing because 8-methoxypsoralen exhibits a 0–0 phosphorescence frequency which is essentially identical with that of coumarin or psoralen. Thus, the following CT resonance form may contribute to the delocalization of the $^3(\pi,\pi^*)$ state of the 5-substituted psoralens. Such a resonance delocalization is not possible in 8-methoxypsoralen.



This model is also predicted from spin density and bond distance data calculated by SCF–MO methods. The spin density at positions 3 and 4 of 5-methoxypsoralen is lower by 0.05 relative to 8-methoxypsoralen. The C–OCH_3 bond distance is calculated to be 0.01 Å shorter in 5-methoxypsoralen than in 8-methoxypsoralen. It is pertinent that strong red shifts of the phosphorescence of coumarins with CT substituents (5-OH, 6-OH, 6,7-OH, and 6,7-OCH_3) are accompanied by long phosphorescence lifetimes and small ZFS parameters, D^* (<0.1 cm^{-1}).[27]

The phosphorescence of coumarylpyrone strongly resembles that of coumarin in regard to vibrational progressions (*cf.* Table II) and the 0–0 phosphorescence frequency (*cf.* Table III and Figure 6b). The phosphorescence lifetime is also very similar. However, the polarization of the phosphorescence is very positive, accounting for about 85% of the phosphorescence in terms of in-plane (x) intensity. Although there are several possible causes for the dominant in-plane polarization of the $\pi \rightarrow \pi^*$ phosphorescence, the SCF–MO–CI P–P–P results shown in Table I provide evidence for only one. Namely, the lowest triplet state is predicted to be A_1 rather than B_1, in contrast to the singlet manifold. The C_{2v} symmetry of coumarylpyrone then permits spin–orbit coupling between $^1B_1$ and $^3A_1$ states, since the matrix element

$$\langle {}^1B_1 | R_y | {}^3A_1 \rangle \neq 0$$

where R_y is the effective rotation operator (90° rotation about the y axis) of the spin–orbit Hamiltonian. The positive polarization of the phosphorescence with respect to the second transition ($^1A_1$) at the 297-nm region can also be interpreted consistently by assuming that the forbidden $^1A_1 \leftarrow {}^1A_1$ transition steals its intensity from $^1B_1$ state(s) *via* a b_1 vibration, resulting in

(36) J. Kolc and R. S. Becker, *Photochem. Photobiol.*, 12, 383 (1970).

x polarization of this band and x polarization of the phosphorescence with respect to this band. In contrast to the case of coumarylpyrone, direct spin–orbit coupling is between $^3(\pi,\pi^*)$ and $^1(n,\pi^*)$ states and thus out-of-plane phosphorescence is dominant for coumarin.[8]

Finally, Figure 9 illustrates three possible Jablonski diagrams which accommodate the luminescence and lifetime data listed in Table III. Coumarylpyrone may be described by diagram b. These diagrams are presented only as a qualitative guide for interpreting the spectroscopic behavior of coumarins and psoralens, and more definite diagrams will not be possible until the locations of $^{1,3}(n,\pi)$ as well as upper $^3(\pi,\pi^*)$ states are experimentally determined.

Conclusions

(1) Relatively high-resolution studies and vibrational analysis of the phosphorescence of a series of coumarins and psoralens provide evidence that the $^3(\pi,\pi^*)$ states of most of these compounds are partially localized in the C=C bond of the pyrone moiety.

(2) The polarized phosphorescence spectra of psoralens were found to be similar to those of coumarins. However, polarization values (in degrees) of the former are generally more positive (*i.e.*, more in-plane component) with respect to $\pi \rightarrow \pi^*$ excitations than the latter. This probably reflects the contribution of unequal permanent dipole moments of S_0 and T_1 states of psoralens to the $T_1 \rightarrow S_0$ transition dipole *via* S_0–T_1 spin–orbit coupling. The spin–orbit coupling between the $^3A_1$ and $^1B_1$ states of coumarylpyrone (C_{2v}) can account for the dominant in-plane intensity of the 0–0 phosphorescence.

(3) Photobiological reactivities of coumarins and psoralens as skin sensitizers can be described in terms of the localized $^3(\pi,\pi^*)$ state, which is known to be the reactive state, for cycloaddition to the DNA pyrimidine bases. In this connection, coumarylpyrone possessing two reactive C=C bonds can be used as a crosslinking agent of a double helix DNA.

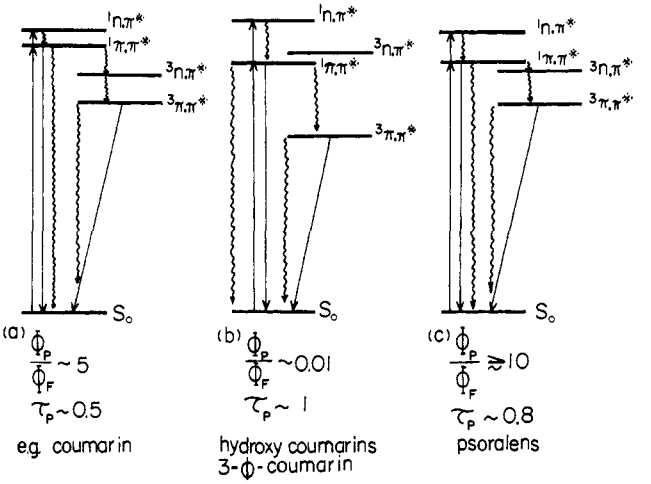


Figure 9. Approximate Jablonski diagrams. Wavy lines represent radiationless transitions which are assumed to be inversely proportional to the energy separation between the initial and final states.

Acknowledgments. Preliminary experiments were carried out by M. L. Harter. The Paul B. Elder Company has generously supplied us with 8-methoxypsoralen and 4,5′,8-trimethylpsoralen in the later stage of this work, when these compounds were needed. Thanks are also due the Texas Tech University Institute of Environmental Chemistry for a grant.

Ring Inversion in Dioxene. Comparison of the Barrier Heights by Nuclear Magnetic Resonance and Far-Infrared Measurements

R. H. Larkin and R. C. Lord*

Contribution from the Spectroscopy Laboratory and the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.
Received February 26, 1973

Abstract: The temperature dependence of the proton nmr spectrum of 1,4-dioxene has been measured from −150° to room temperature. Line-shape analysis yields a barrier to inversion of the twisted ring of 7.62 ± 0.15 kcal/mol. This number agrees within experimental error with the energy of the saddle point on the potential surface that represents the minimum required for inversion as determined by high-resolution far-infrared spectroscopy. From the angular dependence of the vicinal proton coupling constants, a twist angle of 39.3° was calculated. This compares with 48.54° estimated from the potential surface as mapped from the far-infrared data.

In recent years the barriers to inversion for a number of four-membered and five-membered ring compounds have been determined by high-resolution far-infrared spectroscopy.[1–4] In four-membered rings the problem can be treated with good accuracy as one dimensional because the vibrational modes of high fre-

(1) C. S. Blackwell and R. C. Lord, "Vibrational Spectra and Structure," J. R. Durig, Ed., Marcel Dekker, New York, N. Y., 1972, Chapter 1.

(2) J. Laane, "Vibrational Spectra and Structure," J. R. Durig, Ed., Marcel Dekker, New York, N. Y., 1972, Chapter 2.

(3) C. S. Blackwell, L. A. Carreira, J. R. Durig, J. M. Karriker, and R. C. Lord, *J. Chem. Phys.*, **56**, 1706 (1972).

(4) T. Ikeda and R. C. Lord, *J. Chem. Phys.*, **56**, 4450 (1972).