Condon ground state. Accordingly, the absorption is expected to be shifted to the blue and the fluorescence to the red, when the solvent is changed from isooctane to ether or acetonitrile (Figure 5b). The shifts will be larger in acetonitrile than in ether, since the former has a larger dielectric constant.

It should be noted that in both pyridazine and pyrimidine the magnitude of μ_e is much smaller than that of μ_g, so that the magnitude of the fluorescence shifts is far smaller than that of the absorption shift.

C. Hydrogen-Bonding Solvents. It is reasonable to assume that the diazine molecule has no proton-accepting power in the (n,π^*) excited state because of the removal of one of the lone-pair electrons. Thus, in the equilibrium excited state the solute molecule will behave as if it were in a nonhydrogen-bonding solvent, and there should be no stabilization due to hydrogen bonding. Also, in the Franck–Condon ground state, neither stabilization nor destabilization due to hydrogen bonding should occur, since the solvent orientation cannot be favorable to the formation of a hydrogen bond between solute and solvent molecules. Therefore, apart from dipole–dipole interactions, the $n \leftarrow \pi^*$ fluorescence frequency in a hydrogen-bonding solvent is expected to be unchanged from that in a nonhydrogen-bonding solvent (Figure 5c).

The additional effect of the dipole–dipole interaction is considered to be essentially the same as discussed in the preceding subsection. In other words, solvents of this sort are best regarded as having cooperative effects of hydrogen bonding and dipolar interaction on the absorption and only the dipolar effect on the fluorescence. Figure 4 can be well understood in terms of the above. Accordingly, it may be concluded that the hydrogen bond is virtually broken in the (n,π^*) singlet state for the diazines in methanol and in water.

It is to be noted that hydrogen bonding produces a sizable blue shift in the $n \rightarrow \pi^*$ absorption of pyrazine which has no net dipole moment. This is reasonable, because hydrogen bonding is essentially local.

Solvent Effects on the Photodimerization of Coumarin[1]

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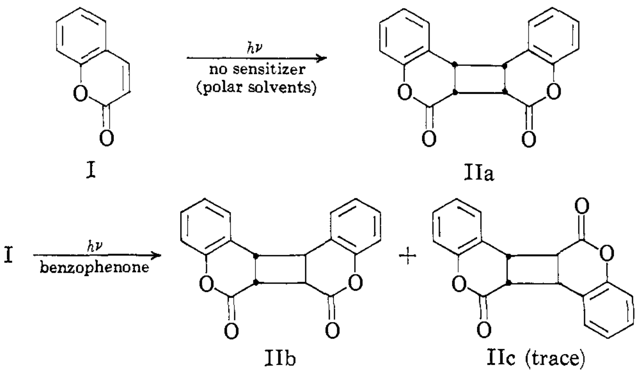
Contribution from the Department of Chemistry, Purdue University, Lafayette, Indiana. *Received June 9, 1966*

Abstract: The photodimerization of coumarin (I) has been studied in various solvents. The *anti* head-to-head dimer (IIb) is formed in nonpolar solvents to the virtual exclusion of the *syn* head-to-head dimer (IIa). The ratio of IIb to IIa decreases in polar solvents and is reversed in methanol. Conversion to IIb in a heavy-atom solvent (propyl bromide) is within experimental error of that found in *p*-dioxane and benzene. Photolysis in dilute solution favors the formation of IIb; the presence of piperylene quenches it. It is suggested that the *syn* dimer (IIa) is formed from a singlet excimer, whereas the *anti* dimer (IIb) arises *via* a monomeric triplet species.

The ability of heavy atoms (*i.e.*, atoms with high atomic numbers) to promote singlet–triplet radiationless transitions has been known for some time. While this phenomenon has been successfully utilized by spectroscopists,[2] there has as yet been no demonstration that a major change in the course of a photochemical reaction can be induced *via* this effect. The initial objective of the present work was to test for this possibility, using as our probe the photodimerization of coumarin (I).

At the time this research began,[3] it had been reported that: (a) direct irradiation of coumarin in polar solvents produced the head-to-head *syn* dimer IIa,[5] (b) irradiation of coumarin in the presence of a sensitizer (benzophenone) produced the head-to-head *anti* dimer IIb (together with trace amounts of IIc),[6] and (c) irradiation of coumarin in nonpolar solvents afforded neither dimeric product.[6,7]



These results were explained[7] by proposing that IIa is formed by the reaction of an excited singlet state of coumarin with a ground-state molecule, whereas IIb is produced by the attack of triplet coumarin on the

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(1) This work was presented in part at the 151st National Meeting of the American Chemical Society, Pittsburgh, Pa., March 1966.

(2) For recent discussions of the heavy-atom effect in spectroscopy, see: S. Siegel and H. S. Judeikis, *J. Chem. Phys.*, **42**, 3060 (1965); T. Medinger and F. Wilkinson, *Trans. Faraday Soc.*, **61**, 620 (1965); C. E. Thompson, *J. Opt. Soc. Am.*, **55**, 1184 (1965); S. P. McGlynn, T. Azumi, and M. Kasha, *J. Chem. Phys.*, **40**, 507 (1964).

(3) Although the solvents and analytical techniques employed differ, in the main, from those used by us, Schenck and co-workers[4] have recently independently observed solvent effects analogous to those which we have reported;[1] their results will be considered together with our own in the Discussion section of this paper.

(4) C. H. Krauch, S. Farid, and G. O. Schenck, *Chem. Ber.*, **99**, 625 (1966).

(5) R. Anet, *Can. J. Chem.*, **40**, 1249 (1962).

(6) G. O. Schenck, I. von Wilucki, and C. H. Krauch, *Chem. Ber.*, **95**, 1409 (1962).

(7) G. S. Hammond, C. A. Stout, and A. A. Lamola, *J. Am. Chem. Soc.*, **86**, 3103 (1964).

ground state. It was our hope that the photolysis of coumarin in heavy-atom solvents might provide a direct, nonsensitized route to the dimer IIb.

Results

The irradiation of coumarin was carried out in the heavy-atom solvents (ethyl iodide, propyl bromide, and butyl chloride); as a control, the photolysis in several other nonpolar solvents[8] (*p*-dioxane, benzene, and ethyl acetate) was also examined. Reaction conditions were analogous to those used by previous workers; *i.e.*, solutions 0.31 *M* in coumarin were irradiated for 68 hr using a 450-w Hanovia Type L mercury arc filtered by a tube of Pyrex glass. The results are presented in Table I.

Table I. Photolysis of Coumarin in Nonpolar Solvents

Solvent	% yield[a] of IIb[b]
Ethyl iodide	74
Propyl bromide	68
Butyl chloride	55
p-Dioxane	79
Benzene	79
Ethyl acetate	86

[a] The amount of recovered coumarin in these experiments varied from 46% (propyl bromide) to 93% (ethyl acetate). [b] The *syn* dimer IIa could not be isolated from any of these photolyses although our technique should have revealed anything in excess of 2% of this material.

It is evident from these data that, in contrast to the earlier reports, the photolysis of coumarin in nonpolar solvents affords dimeric product in good yield.[9] It is also clear that within these solvents, there is a dramatic preference for the formation of the *anti* dimer IIb.

In the light of these results, it became necessary to determine the relative efficiency of production of IIb in the halocarbon *vs.* nonhalocarbon solvents, in order to determine if a heavy-atom effect was operative. We examined propyl bromide, *p*-dioxane, and benzene, using a rotating turntable device designed to ensure identical irradiation conditions for the various samples.[10] Our results are shown in Table II. Considering the accuracy of these measurements (*ca.* ±1%) we must conclude that there is no firm evidence that the halocarbon solvent is unusually effective in producing *anti* dimer IIb. Further experiments on this point are in progress.

Table II. Comparative Conversions to IIb

Solvent	% conversion[9] to IIb
Propyl bromide	9
p-Dioxane	7
Benzene	7

By contrast with the data in Table II, the irradiation of coumarin in polar solvents gave quite different results. Acetonitrile, dimethylformamide, and methanol were used and the photolyses conducted as before; the data are presented in Table III.

Table III. Photolysis of Coumarin in Polar Solvents

Solvent	Products, % conversion[a,b] IIa	IIb
Acetonitrile	3	14
Dimethylformamide	3	4
Methanol	11 (11)[c]	0 (5)[c]

[a] Conversions are given here to facilitate presentation of the methanol data; recovered coumarin ranged between 70 and 80%. [b] These data are from experiments using a soft-glass filter; the use of Pyrex gave similar results. [c] Data in parentheses refer to an experiment analyzed by the method of Krauch, Farid, and Schenck.[4] This technique is particularly suitable for the detection of IIb formed in alcoholic solvents (see Experimental Section).

It is clear from these data that the preference in nonpolar solvents for formation of the *anti* dimer IIb is markedly reduced in solvents of high polarity [$\epsilon = 33$ (methanol) to 38 (DMF and acetonitrile)]; in fact, the situation becomes completely reversed in the protic solvent, methanol, where formation of the *syn* dimer IIa is now favored.

Since the earlier work presupposed the nonexistence of dimer formation in nonpolar solvents, two rather unusual observations reported by Hammond, Stout, and Lamola[7] were reexamined using our analytical techniques. The first of these was the study by these workers of the effect of high dilution (0.01 *M*) on the unsensitized photolysis of coumarin in benzene, in which case *anti* dimer IIb was isolated. Our data are presented in Table IV and indicate that there is, in fact, a marked dependence of product distribution on the concentration of coumarin, high dilution favoring formation of IIb.

Table IV. Dependence of Product Distribution on the Concentration of Coumarin in Methanol[a]

Concn, *M*	Products, % conversion[b] IIa	IIb
0.31	12	0
0.01	1	13

[a] A Pyrex filter was used in these experiments. [b] These experiments were carried out before publication of the method of Schenck and co-workers[4] for analysis of IIb; such analysis would undoubtedly raise the % conversion to IIb in both experiments by several per cent (*cf.* footnote *c*, Table III).

The second observation reported[7] was that irradiation of coumarin in dioxane containing a small amount of benzophenone (*ca.* 10%) produced IIb even though coumarin absorbed more than 98% of the incident light. This was taken to indicate that a double-energy-transfer mechanism was operative, with the first step being the trivial process-emission by coumarin and absorption by benzophenone. Following intersystem crossing to the benzophenone triplet, energy transfer would then lead to formation of coumarin in its triplet state. Our results are shown in Table V.

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(8) The dielectric constants of these solvents range from $\epsilon = 2$ (benzene, dioxane) to $\epsilon = 8$ (ethyl iodide, propyl bromide).

(9) The term *yield* is used herein to denote percentage of product based on the amount of coumarin which has reacted; *conversion* refers to the percentage of starting material converted to the product.

(10) These experiments were carried out using a soft-glass filter (transmission >300 mμ). Ethyl iodide was not used because it absorbs light in this region.

Table V. Irradiation of Coumarin in Dioxane Containing Varied Amounts of Benzophenone[a]

Mole % benzophenone (rel to coumarin)	% incident light absorbed by coumarin	% conversion to IIb
0	100	5
2	95–99[b]	50
10	. . .[c]	71

[a] Conducted with a rotating turntable using a Corex filter tube and a NiSO$_4$ filter solution. Test tubes were of Pyrex. [b] This range reflects the uncertainty in the annular space of an Hanovia immersion well and, therefore, the path length of the filter solution. [c] Not calculated.

It may be seen from these data that the conversion to IIb increases by a factor of 10 even while the benzophenone added absorbs only 1 to 5% of the incident light. Two interpretations of this result are possible. One is the double-energy-transfer mechanism mentioned above; the other is direct sensitization of coumarin by the small amount of benzophenone excited by the incident light. A choice cannot be made between these on the basis of the data at hand.

Some quantum yields might be of interest here though the uncertainties in their derivation require that they be considered only as very rough approximations.[11] In the absence of benzophenone, the quantum yield (ϕ) for formation of IIb can be estimated at *ca.* 7×10^{-4}. If the double-energy-transfer mechanism is operative, the over-all ϕ for formation of IIb with 2 mole % benzophenone must be ten times this number or *ca.* 7×10^{-3}. However, the benzophenone absorbs a maximum of 83% of the coumarin emission (based on its concentration, extinction coefficient at 366 mμ, and a path length of 2 cm); in fact, the effective path length must be somewhat less than 2 cm and undoubtedly not all of the light absorbed by coumarin is reemitted. Thus, the ϕ for dimerization of coumarin triplet to IIb in this run has a value of $>10^{-2}$ if this mechanism is operative. This, in turn, implies that excited coumarin in the absence of benzophenone undergoes intersystem crossing less than 8% of the time.

Alternatively, sensitization by benzophenone excited only by the incident light would require a ϕ for formation of IIb of *ca.* 2×10^{-1}. This leads to intersystem crossing in the pure coumarin of *ca.* 0.4%. Neither of these values for the efficiency of intersystem crossing are close to the high efficiency observed for coumarin phosphorescence at 77°K and lower concentration of substrate.[7]

Implied in the above discussion is the assumption that the formation of IIb occurs *via* triplet coumarin even in the absence of sensitizer. That this assumption is valid is suggested by the results of quenching experiments with piperylene (Table VI).

It may be seen that, in both the polar and nonpolar solvents, added piperylene inhibits the formation of *anti* dimer IIb. In the acetonitrile experiments, the ratio of IIb/IIa is reduced from 4.7 to 2. We take this data to indicate that IIb must, at least in part, be derived from a triplet species.[12]

Table VI. Irradiation of Coumarin in the Presence of Piperylene

Solvent	Mole % piperylene	% conversion to IIa	% conversion to IIb
Propyl bromide[a]	0	0	9
Propyl bromide[a]	5	0	4
Acetonitrile	0	3	14
Acetonitrile	5	5	10

[a] These runs were carried out under identical conditions on a rotating turntable.

Discussion

It is evident from this and other work[4] that polar solvents strongly favor the formation of the *syn* dimer IIa,[13] whereas nonpolar solvents lead almost exclusively to *anti* dimer IIb. (A third dimer, the *syn* head-to-tail cyclo adduct (IId), has recently been isolated[4] from the nonsensitized photolysis of coumarin and shows a solvent dependency analogous to IIa.) Furthermore, the formation of IIb is quenched by piperylene and favored by high dilution. By contrast, the formation of IIa is favored at low temperatures.[4,14] Solutions of coumarin in methyl alcohol obey Beer's law over the range of concentrations used in these studies.

We feel that these data may best be explained by proposing that IIa and IId are formed from coumarin excimers[15–17] *derived from the singlet excited state while IIc is formed from the monomeric coumarin triplet.* Such a postulate accounts for the observed stereochemistry of IIa, the dilution effect, the temperature dependency of the product distribution, and the solvent effect. Excimer formation is known to be favored at low temperatures and high concentrations. The complexes are felt to be sandwiched, parallel planar configurations[18] which would lead to the *syn* head-to-head dimer and, to the extent that rotation in the plane occurs, the *syn* head-to-tail dimer. Finally, it is quite reasonable that high solvent polarity might favor excimer formation[17] since there is probably appreciable charge transfer involved in the complex.[18] The low

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(11) The considerations used in deriving these numbers as well as the percentage of absorbed incident light (Table V) are described in the Experimental Section. The following arguments rest on the assumption that IIb is always formed from the same triplet state and at the same rate regardless of the route by which the triplet level is reached. Formation of IIb from the singlet state would lower the numbers derived for intersystem crossing.

(12) An alternative rationale is that the quenching action of piperylene is due to photoaddition and that both IIa and IIb are derived, in nonsensitized photolyses, from singlet-state coumarin. The results in acetonitrile would then require postulating two singlet-state species of different reactivity toward piperylene [excimer and monomer? (see Discussion)]. Though this possibility cannot be excluded by the present data, we favor the postulate of a triplet precursor for IIb; further experiments with other quenchers are in progress.

(13) Protic solvents seem especially effective in this since both dimethylformamide and acetonitrile, by comparison with methanol, show higher conversion to IIb than IIa even though their dielectric constant is greater than that of the alcohol.

(14) Interestingly, we find that irradiation of solid coumarin (deposited from dichloromethane) leads to a larger conversion to IIb than to IIa even though frozen solutions produce good yields of IIa.[4]

(15) B. Stevens and E. Hutton, *Nature*, **186**, 1045 (1960).

(16) It has been suggested that anthracene dimerizes *via* prior excimer formation: *cf.* A. Dammers-de Klerk, *Mol. Phys.*, **1**, 141 (1958).

(17) In unpublished studies (A. Lamola, private communication) Dalton, Hammond, and Lamola observed that the rate of dimerization of β-methoxynaphthalene is increased *ca.* tenfold in alcohol compared to benzene. Excimer fluorescence was found to occur and to be similarly influenced by solvent (tenfold increase in intensity in alcohol). They suggest that an excimer may be the precursor of the dimer and that the solvent effect is acting in the excimer-forming step.

(18) J. Ferguson, *J. Chem. Phys.*, **28**, 765 (1958); M. A. Slifkin, *Nature*, **200**, 766 (1963); J. Murrell and J. Tanaka, *Mol. Phys.*, **7**, 363 (1964); F. Smith, A. Armstrong, and S. McGlynn, *J. Chem. Phys.*, **44**, 442 (1966), and references cited therein.

observed quantum yields in the nonsensitized photolyses could be due to collisional self-quenching in nonpolar solvents and self-quenching *via* excimer formation in polar solvents. These suggestions are summarized in the following scheme.

$$C \xrightarrow{h\nu} C^{*(1)}$$

$$C^{*(1)} + C \xrightarrow[\text{solvents}]{\text{polar}} CC^{*} \text{ (excimer)}$$

$$CC^{*} \longrightarrow 2C \text{ (self-quenching)}$$

$$CC^{*} \longrightarrow \text{IIa} + \text{IId}$$

$$C^{*(1)} + C \xrightarrow[\text{solvents}]{\text{nonpolar}} 2C \text{ (self-quenching)}$$

$$C^{*(1)} \longrightarrow C^{*3}$$

$$C^{*(3)} \longrightarrow \text{IIb}$$

Solvent polarity has recently been shown to play an important role in photochemical systems where excited-state, inversion is possible.[19–21] This and other studies[1,4,17,22] now indicate that there are additional mechanisms whereby the polarity of the solvent may exert a profound effect on a photochemical reaction. Further studies on coumarin and related dimerizations are in progress in these laboratories.

Experimental Section

Materials. Coumarin and benzophenone (from Eastman) were used without further purification. Ethyl acetate (anhydrous), propyl bromide, and acetonitrile (Spectroquality) were from Matheson Coleman and Bell (MC and B) and were treated batchwise with Fisher Molecular Sieve, Type 4A, followed by distillation. Dimethylformamide (Dupont, Tech), ethyl iodide (Columbia), and methanol (Fisher, acetone free, absolute) were purified in the same fashion. *p*-Dioxane (MC and B, Spectroquality) was used directly or distilled from calcium hydride; benzene (Baker, Spectrograde) was used without further purification, whereas regent grade material was distilled from calcium hydride (all benzene used for chromatography was distilled from calcium hydride). Silica gel (60–200 mesh) was from Davison Chemical Co. Compound IIa (mp 276–277°) was prepared by irradiation of coumarin in absolute ethanol[5] and recrystallized from acetic acid or acetone.[4] Compound IIb (mp 181–182°) was prepared by irradiation of a mixture of coumarin and benzophenone in absolute ethanol[6] and recrystallized from benzene–heptane. Large samples were later prepared by direct photolysis in dioxane.

Spectra and Physical Data. Infrared spectra were obtained *via* chloroform or acetonitrile solutions from a Beckman IR-8 or *via* a KBr pellet from a Perkin-Elmer 221. Ultraviolet spectra were obtained from a Bausch and Lomb 505; Beer's law data from a Cary 14. Melting points were from a Fisher-Johns hot stage and are corrected. Microanalyses were by Dr. C. S. Yeh of this department.

Irradiation Procedure. Irradiations were carried out using an Hanovia 450-w mercury arc set in a quartz immersion well; all photolyses were conducted with a Pyrex or soft-glass filter insert and in a nitrogen atmosphere. Where rigorously identical irradiation conditions were desired (Tables II, V, and VI of the text), the immersion well was set at the center of a rotating turntable and the entire apparatus immersed in a water bath. The rack of the turntable takes 21-mm diameter test tubes with total available volumes of *ca.* 45 ml. Samples were either degassed three times *in vacuo* or flushed with nitrogen for 1 hr prior to irradiation. They were then sealed *in vacuo* and irradiated. Although the method of deoxygenation did not appear to have much consequence in the final results, the deoxygenating procedure was the same for all samples compared in any run.

Analytical Procedure. In a typical photolysis, 5.0 g of coumarin (0.034 mole) was dissolved in 110 ml of solvent and irradiated for 68 hr. The solvent was evaporated on a rotary evaporator and the residue taken up in 25 ml of dry benzene. The solution was filtered from the IIa which precipitated[23] (some of the nonpolar solvents gave small amounts of a complex of IIb and IId[4]), and an aliquot containing 500–600 mg of reaction mixture was chromatographed on 40 g of silica (predried for 24 hr at 110°); column dimensions were 11 mm × 1 m. The column was eluted with a mixture of 2.5% ethyl acetate in benzene (anhydrous conditions throughout these operations are essential). Taking *ca.* 33-ml fractions, dimer IIb was eluted in fractions 6, 7, and 8; coumarin in fractions 8–15. The column was then eluted free of remaining organic material using methyl alcohol. When necessary, fractions containing mixtures of IIb and coumarin were rechromatographed in an identical fashion. Fractions were analyzed by thin layer chromatography (1:1 benzene–ethyl acetate followed by treatment with I_2 vapor) to detect traces of IIb in coumarin and by infrared spectroscopy to detect traces of coumarin in IIb. The melting points and infrared spectra of IIa and IIb obtained in this fashion were identical with those of authentic samples.

The major variable in this procedure is the small amount of IIb which may be lost by hydrolysis on the column. That this remains fairly constant from chromatogram to chromatogram is indicated by the precision of the results obtained. For example, the results from a typical turntable photolysis are shown in Table VII.

Table VII. Isolation of IIa by Chromatography

Sample	Chromatography no.		
	1	2	3
Propyl bromide	9.0	8.2	9.0
Propyl bromide + piperylene	3.4	3.7	
p-Dioxane	6.9	7.1	
Benzene	7.5	6.2	

Our procedure was compared directly to that of Schenck's[4] in two cases. In one, we obtained a 14% conversion *vs.* 11% by the Schenck method; in the other, the data were 10% *vs.* 13%.

Irradiation of Coumarin in Methanol. The dihydroxy diester of IIb was prepared by refluxing a sample of the dimer in anhydrous methanol overnight (the *syn* dimer IIa is stable under these conditions). After removal of the solvent *in vacuo*, the residue was recrystallized three times from chloroform–carbon tetrachloride to give a sample, mp 176–178°, $\lambda_{\max}^{\mathrm{CH_3CN}}$ 2.95 and 5.77 μ, for analysis. *Anal.* Calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.37; H, 5.77. This material, when crystallized from benzene, melted at 181–187° and had a band in its infrared spectrum at 11.75 μ indicating the presence of solvent of crystallization.

When coumarin was photolyzed in methanol, a determined effort was made to find both IIb and its alcoholysis product, both by chromatography and by crystallization from benzene. Neither material could be isolated from reaction mixtures 0.3 *M* in coumarin but the photolysis of 600 mg of coumarin in 418 ml of methanol resulted in the deposition of 77 mg of the ester from benzene and the isolation of 13 mg of IIb through chromatography. Chromatography also provided 30 mg of material (mp 250–280°) which, upon recrystallization from acetic acid, gave a sample (8 mg) of IIa, mp 274–276°.

Because of the tendency for dimer IIb to be easily attacked by water or alcohol, the Schenck procedure,[4] in which the crude reaction residue is hydrolyzed completely and then selectively relactonized, is more desirable for working up photolyses in alcoholic solvents. Using this procedure, an experiment with 0.3 *M* coumarin in methanol gave 530 mg of IIa and 248 mg of IIb. Although we did not repeat our experiment in dilute solution, the effect of dilution in reversing product distribution appears un-

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(19) G. Porter and P. Suppan, *Trans. Faraday Soc.*, **61**, 1664 (1965).

(20) O. Chapman, Organic Photochemistry Symposium, Organic Chemistry Group, Chicago Section, American Chemical Society, April 1966.

(21) It is not impossible that excited-state inversion is a factor in the dimerization of coumarin and this possibility is under investigation.

(22) D. Schulte-Frohlinde, H. Blume, and H. Gusten, *J. Phys. Chem.*, **66**, 2486 (1962); R. Steinmetz, W. Hartmann, and G. Schenck, *Chem. Ber.*, **98**, 3854 (1965); P. Wagner, C. Stout, S. Searles, Jr., and G. Hammond, *J. Am. Chem. Soc.*, **88**, 1242 (1966).

(23) Approximately 15 mg of IIa is soluble in this volume of benzene; in two runs where a comparison was made, no additional IIa could be isolated by the Schenck procedure[4] over and above that obtained by such crystallization from benzene.

equivocal and is confirmed in the recent report by Krauch, Farid, and Schenck.[4]

Irradiation of Coumarin in Dioxane Containing Benzophenone. The procedure to be described here may be taken as typical of our rotating turntable experiments. Six Pyrex test tubes (21 mm) were charged with 2.20 g (15 mmoles) of coumarin and 45 ml of *p*-dioxane. The tubes were divided into sets of two, one set containing 0 mg, one set with 52 mg (0.28 mmole), and the third set with 260 mg (1.5 mmoles) of benzophenone. After degassing with N_2, the tubes were sealed and placed in a turntable which surrounded a Hanovia 450-w immersion well. The whole apparatus was immersed in a large water bath and the table made to rotate by a small motor. During the photolysis the temperature of the system reached *ca.* 32°. An aqueous solution of nickel sulfate (250 g of the hexahydrate in 500 ml of water) was pumped through the immersion well as a coolant and a cylindrical Corex filter placed inside the well.

The mercury lines emitted by the lamp (A) and the combined absorbances, in units of absorbance (log I_0/I), of the filter solution and the Pyrex glass are as follows: 2967 (0.37), 3025 (0.25), 3130 (0.16), 3340 (0.13), 3660 (1.30). (These numbers are for an annular space of 3.7 mm in the immersion well; this number may be as large as 5.6 mm according to the manufacturer's specifications.) Using the wattage of light emitted by the lamp at each wavelength (according to specifications), the absorbances given above, and the absorbances of coumarin and the various concentrations of benzophenone at 3660 A, one obtains a net absorption for coumarin of 95–99% of the light, depending on the dimensions of the annular space.

The quantum yields presented in the text were derived from the duration of the photolysis (96 hr) and the portion of light captured by each tube. The latter is a maximum of $^1/_9$ (the fraction of the total circumference of the table occupied per tube); we have arbitrarily reduced this by a factor of 2 to take into consideration reflection, lamp intensity fluctuation, etc. Thus, the ϕ's given in the text are based on $^1/_{18}$ of the photons theoretically being transmitted by the filter solution and Pyrex.

After completion of the irradiation, the tubes were opened and aliquots examined in the fashion described above.

Irradiation of Coumarin in the Presence of Piperylene. The irradiation in acetonitrile was conducted as described under "Analytical Procedure" (see above) except that the photolysis mixture contained 200 μl (2.0 mmoles) of distilled piperylene (Aldrich Chemical Co.). The photolysis in propyl bromide, conducted on the turntable, involved the use of 45 ml of solvent, 2.2 g (15 mmoles) of coumarin, and 75 μl (0.75 mmole) of piperylene. A control containing no piperylene was run simultaneously.

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The Conformation of Poly-L-tyrosine in Quinoline from Dielectric Dispersion Studies

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Contribution from the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa, and the Department of Chemistry, Columbia University, New York, New York. *Received June 23, 1966*

Abstract: The dipole moments, rotational relaxation times, and parameters expressing the polydispersity have been determined for poly-L-tyrosines of molecular weights 26,000–68,000 in dilute quinoline solution from measurements of dielectric dispersion and loss in the frequency range 1–100 kc. The molecular weight dependence of the dipole moment and relaxation time demonstrate that the molecular conformation is that of a rigid α helix over the temperature range 15–50°. The sense of the helix was determined by observing the effect of bromine substitution at the 3 position upon the dipole moment. An analysis of the accessible side-chain conformations showed that bromination should result in an increase in dipole moment for a right-handed helix and a decrease for a left-handed helix. An observed decrease of 1.0 ± 0.1 D. per residue was within the range predicted for the left-handed helix. This assignment of helix sense is opposite to that made by Fasman and co-workers on the basis of optical rotatory evidence and therefore appears to call for a reexamination of the theoretical basis for interpretation of the latter.

The purpose of the study reported here was to examine the dielectric dispersion behavior of poly-L-tyrosine in dilute solution, and from this to gain information on the dipole moment, molecular shape, and polydispersity of this material. It was further expected that conditions could thereby be discovered under which the polymer is in the form of a rigid helix, and that by studying the effect of brominating the tyrosine side chains at the 3 position (*ortho* to the hydroxyl group) the sense of the helix could be determined. It will be shown that the necessary conditions were met in quinoline solutions, and that the observed effect of bromination is consistent only with a *left-handed* sense of helix. To our knowledge this assignment of helix sense is the first to be made for a polypeptide in solution on an absolute basis, *i.e.*, as distinguished from those methods which are capable of determining only whether two polypeptides have the same or opposite helix sense.

The sense of the α helix has been one of the most elusive aspects of this structure. Molecular models reveal no obvious reason why one sense should be preferred over the other for any of the amino acids capable of taking on the structure, yet it is known from the effect of the structure on optical activity[2] that a preference exists. The theoretical interpretation of the optical rotation has apparently remained inconclusive as to the absolute configuration,[3] and in fact it appears that a determination of helix sense by some absolute

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(1) To whom inquiries should be addressed at Iowa State University.

(2) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).

(3) See G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965), for a summary of the current status of the application of the theory.