TABLE I

BENZO[b]THIOPHENES[a]

Compd	Yield,[b] %	Bp, °C (mm)	Deriv	Mp, °C	Empirical formula
4a	68	. . .[c]	Picrate	148–149	C_8H_6S
4b[d]	61	79–84 (4)	Picrate	140–144	C_9H_8S
4c	70	88–91 (5)			$C_{10}H_{10}S$
4d	64	106–109 (4)			$C_{11}H_{12}S$
4f[e]	42	110–115 (4)	Picrate	118–122	$C_{10}H_{10}S$
4g	44	116–122 (4)	Picrate	102–104	$C_{11}H_{12}S$
4h	90[f]	110–115 (0.05)[g]	Semicarbazone	176–178	$C_{11}H_{10}OS$

[a] Satisfactory analytical data (0.3 for C, H, N) for benzo[b]thiophenes **4c** and **4d** and the indicated derivatives of **4b**, **4f**, **4g**, and **4h** are reported. [b] Isolated material, purity >95% (nmr) and based on thiophene component. [c] Steam distilled out of reaction mixture. [d] A. V. Sunthankar and B. D. Tilak, *Proc. Indian Acad. Sci., Sect. A*, **32**, 390 (1950); *Chem. Abstr.*, **47**, 12346*d* (1953). [e] M. Pailer and E. Romberger, *Monatsh. Chem.*, **91**, 1070 (1960). [f] Yield based on purified **3h**. [g] Nmr δ 2.5–3.3 (m, 4, aliphatic H), 6.80–7.57 (m, 5, aromatic H), 9.50 (t, 1, CHO); ir $\nu_{C=O}$ 1720 cm^{-1}.

overall yields. Attempts to effect such a transformation on **3e** gave complex mixtures of rearranged materials. Furthermore, variation of the Grignard component made possible the preparation of disubstituted systems. For example **1b**, upon reaction with **2b** and **e**, gave products **3f** and **g**, which, in turn, furnished **4f** and **g**.

Efforts aimed at extending the method led us to prepare benzo[b]thiophenes bearing ready-made functionalized side chains at C_4 and C_7. To this end, thiophene **2f** was treated with 2 equiv of **1a**, whereupon the product (**3h**) was cleanly converted in refluxing acid into aldehyde **4h**. Similarly, the 4-substituted isomer (**5**) was obtained by cyclization of the adduct of 2 equiv of **1a** and ethyl thiophene-3-carboxylate.

Experimental Section

General.—Melting points were determined on a Mettler apparatus and are uncorrected. Nmr data (Varian A-60, TMS as internal standard) were consistent with assigned structures. The intermediate crude oils **3a–g** were characterized solely by means of nmr and were converted as is into the products offered in Table I. Microanalyses were performed in our laboratories by Messrs. P. van den Bosch and H. Eding.

Starting Materials.—The required thienyl ketones were made by $SnCl_4$-promoted acylation of thiophenes as described for **2b**:[3] ethyl 2-thienyl ketone,[4] bp 102–104° (13 mm); isopropyl 2-thienyl ketone,[5] bp 104–106° (13 mm); and *tert*-butyl 2-thienyl ketone, bp 78–81° (3 mm), yield 61% (*Anal.* Calcd for $C_9H_{12}OS$: C, 64.25; H, 7.19. Found: C, 64.23; H, 7.25.) [nmr δ 1.31 (s, 9, *tert*-butyl). 6.94, (m, 1, H_4 thienyl proton), 7.39 (d, 1, H_5 thienyl proton), 7.62, (d, 1, H_3 thienyl proton)]. Ethyl thiophene-3-carboxylate was prepared from 3-bromothiophene.[6a,b] 2-(2-Bromopropyl)-1,3-dioxolane was prepared from crotonaldehyde, ethylene glycol, and HBr[7] and was then converted into **1b** using conditions described for **1a**.[2]

The preparation of the benzo[b]thiophenes is illustrated by the synthesis of **4d**.

7-Isopropylbenzo[b]thiophene (4d).—To a solution of **1a**, prepared from 1.6 g (0.065 g-atom) of Mg and 12.3 g (0.065 mol) of 2-(2-bromoethyl)-1,3-dioxolane in 50 ml of THF,[2] was added dropwise a solution of 5.1 g (0.036 mol) of **2d** in 20 ml of Et_2O. After 2 hr the mixture was poured onto 10% NH_4Cl solution, which was extracted with Et_2O. The organic phase, upon drying (Na_2SO_4) and solvent removal left 8.3 g (90%) of oily **3d**: nmr δ 0.87 (2d, 6, *i*-C_3H_7), 2.93 (s, 1, OH), 3.80 (m, 4, dioxolane protons). It was added slowly to 150 ml of refluxing 10% H_2SO_4. After 1 hr, the product was extracted into Et_2O, which was then scrubbed ($NaHCO_3$), dried, and evaporated to leave an oily residue. Fractionation thereof afforded 3.6 g of **4d** (64% based on **2d**), bp 106–109° (4 mm).

1,5-Di(1,3-dioxolan-2-yl)-3-hydroxy-3-(2-thienyl)pentane (3h).—This compound, mp 48–49° [$(i\text{-}Pr)_2O$], was prepared in 80% yield by treating **2f** with 2 equiv of **1a** as described above, nmr δ 3.50 (s, 1, OH). *Anal.* Calcd for $C_{15}H_{22}O_5S$: C, 57.30; H, 7.05. Found: C, 57.32; H, 7.03.

3-(4-Benzo[b]thienyl)propionaldehyde (5).—This compound, prepared from ethyl thiophene-3-carboxylate and 2 equiv of **1a**, followed by acid treatment of the resulting oil, was obtained in 40% yield, bp 126–128° (0.01 mm): nmr δ 9.58 (t, 1, CHO); ir $\nu_{C=O}$ 1720 cm^{-1}. The semicarbazone was prepared in alcohol and melted at 186–187°. *Anal.* Calcd for $C_{12}H_{13}N_3OS$: C, 58.28; H, 5.30; N, 16.93. Found: C, 57.91; H, 5.40; N, 16.93.

Registry No.—**1a**, 37610-80-3; **1b**, 37610-86-9; **2a**, 98-03-3; **2b**, 88-15-3; **2c**, 13679-75-9; **2d**, 36448-60-9; **2e**, 20409-48-7; **2f**, 2810-04-0; **3a**, 37610-85-8; **3b**, 37610-87-0; **3c**, 37610-88-1; **3d**, 37610-89-2; **3f**, 37610-90-5; **3g**, 37610-91-6; **3h**, 37610-92-7; **4a**, 95-15-8; **4a** picrate, 4500-67-8; **4b**, 14315-15-2; **4b** picrate, 37610-95-0; **4c**, 16587-42-1; **4d**, 37610-97-2; **4f**, 37610-98-3; **4f** picrate, 37610-99-4; **4g**, 37611-00-0; **4g** picrate, 37611-01-1; **4h**, 37611-02-2; **4h** semicarbazone, 37611-03-3; **5**, 37611-04-4; **5** semicarbazone, 37614-49-6.

Acknowledgment.—The authors wish to thank Professor C. Koningsberger for continued interest and encouragement and Mr. J. Lohmeyer for capable experimental assistance.

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Reactivity of First-Singlet Excited Xanthene Laser Dyes in Solution

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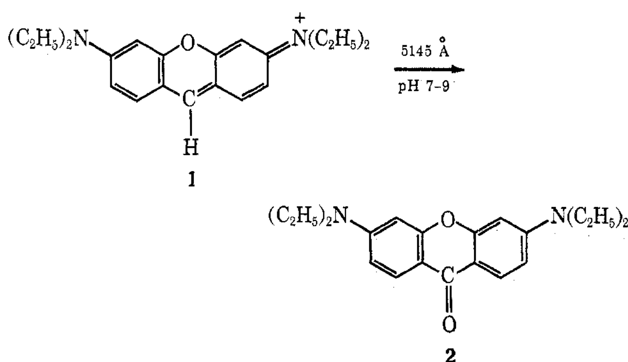
The useful lifetimes of xanthene laser dyes are limited primarily by an apparently irreversible photochemical reaction.[1] We report quantum efficiencies and photoproduct absorption spectra for this reaction.

(1) E. P. Ippen, C. V. Shank, and A. Dienes, *J. Quantum Electron.*, *QE*-**7**, 178 (1971).

The photochemical reaction was produced by irradiation of 4-ml samples of 2×10^{-4} *F* dye solution with 5145 Å excitation from a 1-W (cw) argon ion laser. At this wavelength, selective first-singlet excitation occurs for the fluorescent monomer form of the dye, producing excited states much lower in energy than those occurring in ultraviolet photochemistry. These excited states undergo chemical conversion as measured by fluorescent monomer absorbance decrease with quantum efficiencies in the range 10^{-6}–10^{-5}, under conditions of practical use—open solutions of dye in either ethanol or water containing a detergent.[1] We have observed a complication in this approach: the photoproducts arising from primary photobleaching enhance dye aggregation, which also reduces the fluorescent monomer concentration. Aggregation was more pronounced in water than in ethanol, was favored by basic conditions, and caused sharply increased rates of monomer decrease after 2–4-hr irradiation time.[2]

For Pyronin B (**1**), two photoproducts were observed, depending upon pH; these are not interconvertible by pH adjustment. At pH 7–9, a photoproduct with absorption bands centered at 332 and 382 nm was identified as the 9-xanthone (**2**) by comparison



with the spectrum of an authentic sample.[3] At pH 5.3, the absorption band of **1** at 552 nm decreases, and no absorption bands are observed at 332 and 382 nm. The pH 5.3 photoproduct absorbs in the 300–320-nm region. We find such absorption for the 9-xanthydrol, which may be formed from **1** by titration with base.[4] Neither the 9-xanthydrol nor the photoproduct air oxidize to **2**, even in refluxing ethanol. The reactivity of first-singlet excited Pyronin B in solutions containing oxygen and hydroxyl donors then appears to be similar in kind, but different in degree, to ground-state reactivity. On the other hand, the dependence of photoproduct structure on solution pH is not predicted from studies on the ultraviolet photochemistry of xanthenes.[5]

The photoproduct of Rhodamine 6G shows strong absorption below 250 nm, a moderately strong absorption band at 260–270 nm, and an inflection point near 300 nm. The 9-hydrols of similar dye molecules have been studied, and possess absorption bands in the following regions: 232–238, 265–276, and 302–317 nm.[6,7] We infer that the 9-hydrol is the photoproduct observed at all pH values. (The C_9 carbon atom in Rhodamine 6G and disodium Fluorescein is tertiary, and ketone formation cannot occur *via* 5145 Å excitation.) The photoproduct of disodium Fluorescein shows only the trailing edge of a strong absorption band from 190 to 235 nm. Photobleaching of disodium Fluorescein occurs in the presence or absence of oxygen,[8] involves formation of an excited dye–solvent complex,[9] and leads to long-lived OH adducts in the presence of moisture.[10] It is therefore plausible that the 9-hydrol is the photoproduct of disodium Fluorescein also.

The photoproduct spectra indicated that, at the C_9-bridgehead position, conversion to an alcohol, and also oxidation to a ketone if secondary, took place. We therefore expected the quantum efficiencies to be comparable among the three dyes but strongly pH dependent, as is observed (Table I). For Pyronin B, the lowest bleaching rate occurs in acid solution, which may be explained by extensive reversion of the 9-xanthydrol to **1** under acid conditions.[4] The rate of formation of xanthone **2** increases with pH, up to the pH at which **1** is chemically converted to 9-xanthydrol. Disodium Fluorescein, which can be studied only in basic media, shows a definite but small pH effect. For all three dyes, an upper limit of *ca.* 10^{-5} in quantum efficiency is observed, and the nature of the pH dependence does not suggest a bimolecular, diffusion-controlled hydroxide attack. Rhodamine 6G, for example, shows sharp minima at pH 7 (ethanol) and 9 (water). From these results, given in Table I, we con-

Table I
The pH Dependence of Photobleaching in Some Xanthene Dyes

Dye system	pH	Initial fluorescent monomer concn $\times 10^{-4}$ M^a	Quantum efficiency $\times 10^{-6}$
Pyronin B in ethanol (2×10^{-4} *F*)	5.3 (initial)	0.65	2.0
	7.1	0.86	4.9
	8.9	1.4	8.1
	9 and above	Chemical bleaching	
Disodium Fluorescein in ethanol (2×10^{-4} *F*)	7 and below	Chemical bleaching	
	8.9 (initial)	0.70	11
	11.2	1.2	13
	13.3	2.0	13
Rhodamine 6G in ethanol (2×10^{-4} *F*)	5.5 (initial)	1.6	8.1
	6.8	1.5	1.4
	9.3	2.0	11
	10.8	0.3	5.4
Rhodamine 6G in water containing 1.5% Triton X-100 (2×10^{-4} *F*)	5.5 (initial)	1.3	6.8
	7.1	1.4	6.8
	9.1	1.3	2.0
	11.1	1.0	7.3

[a] Based on $\epsilon_{552} = 8.3 \times 10^4$ (Pyronin B); $\epsilon_{497} = 9.7 \times 10^4$ (disodium Fluorescein); $\epsilon_{525} = 9.5 \times 10^4$ (Rhodamine 6G).

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clude that reaction occurs between the C_9 position in the excited dye molecule and oxygen-containing species in the immediate solvation sphere, the chemical nature of the latter being strongly predetermined by pH. The minima for Rhodamine 6G then are construed to represent the transition between acidic and basic solvation spheres, either of which favors hydrol formation. That hydrol may be formed in either acidic or basic media has been confirmed previously.[11]

Registry No.—1, 2150-48-3; disodium Fluorescein, 518-47-8; Rhodamine 6G, 989-38-8.

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Preparation of Uniformly ^{14}C-Labeled *p*-Hydroxybenzoic Acid[1]

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It was found that *p*-hydroxybenzoic acid (HBA) is converted to the benzoquinone nucleus of coenzyme Q (CoQ) in *Rhodospirillum rubrum*.[2-4] The conversion of HBA to CoQ also occurs in the rat.[2b,5] After these initial studies in 1963–1964, the biosynthetic significance of HBA was studied in several laboratories, and the following additional citations are representative and pertinent to interests in the availability of uniformly labeled [^{14}C]-*p*-hydroxybenzoic acid.

The complete sequence of biosynthesis from HBA to CoQ was elucidated for *R. rubrum*, and it was projected that the same sequence or a very closely related sequence would exist in mammalian tissue according to Friis, *et al.*[6]

Rudney[7] described studies on the biosynthesis of CoQ and polyprenylphenols in cell-free preparations of *R. rubrum*, *E. coli*, and rat tissue, and reported that the first two enzymatic systems in the biosynthetic pathway from HBA to CoQ were characterized. Trumpower, *et al.*,[8] utilized labeled precursors of CoQ, including HBA, in studies on liver slices and identified 5-demethoxy coenzyme Q_9 as an intermediate in the biosynthesis of CoQ_9 in the rat. Momose and Rudney[9] reported on the biosynthesis of 3-polyprenyl-4-hydroxybenzoate in the inner membrane of mitochondria from HBA and isopentenyl pyrophosphate.

Whistance, *et al.*,[10-12] have reported on the biosynthesis of CoQ in yeasts, gram-negative bacteria, and animals, and utilized tracer techniques with labeled HBA.

Nilsson, *et al.*,[13-15] utilized labeled HBA in the determination of precursors, in the biosynthesis of CoQ in genetically dystrophic mice, and in the biosynthesis of CoQ_{10} in beating cell cultures from heart tissue.

The preparation of [^{14}C]-*p*-hydroxybenzoic acid by the alkaline fusion of [^{14}C]tyrosine has been described.[2,16] However, this preparation from relatively expensive [^{14}C]tyrosine has given erratic and disappointing yields in our experience. Consequently, the procedure which has evolved from our many preparations of uniform ^{14}C-labeled HBA is described. The ready availability of [^{14}C]-HBA is essential to continuing studies on the biosynthetic conversion of HBA to CoQ in various systems, including normal and diseased tissues from experimental animals and humans.

Experimental Section

The fusion was carried out in a nickel crucible with a handle which was wrapped with asbestos for easy handling. The crucible was 44 mm deep, 44 mm in top diameter, 25 mm in bottom diameter, and 25 ml in capacity.

Uniformly labeled [^{14}C]-L-tyrosine (100 μCi) with a specific activity of 507 mCi/mmol was purchased from the Amersham-Searle Corp., Chicago, Ill. This tyrosine was received in an aqueous solution containing 2% ethanol. The solution was pipetted from its container into the crucible. The container and cap were washed thoroughly with 0.01 *N* HCl, and the washings were added to the crucible. The solution was evaporated by a warm-water bath under a stream of nitrogen. The inside surface of the crucible was well washed with 0.01 *N* HCl and the solution was evaporated. This washing was repeated about three times, and each time with a diminished volume to assure that the tyrosine was concentrated in one area of the bottom of the crucible. Approximately 150 mg of NaOH and 150 mg of KOH were finely crushed together and immediately added to the crucible in the region of the tyrosine. The NaOH and KOH were melted by placing the crucible in a Wood's Metal bath at 270°. The melted alkali was swirled around the crucible to encompass all the tyrosine. The temperature of Wood's Metal bath dropped about 15° on initial contact with the crucible and slowly climbed to 270°. After 10 min, the crucible was removed from the bath and allowed to cool. The melted residue solidified. Slowly, 1 ml of 10 *N* H_2SO_4 was added to dissolve the residue, and the solution was transferred to a small separatory funnel. The crucible was washed twice with 1 ml of water and the washings were poured into the separatory funnel.

The reaction mixture was extracted with 10 ml of ether. The extract was transferred into another small separatory funnel and extracted with 1 ml of H_2O. The extraction of the reaction mixture with ether followed by a water extraction of the ether was repeated ten times. The combined ether extract, 100 ml, was evaporated under vacuum. The residue was purified by thin layer chromatography on 1 mm silica gel G plates. The mobile phase for development was absolute methanol. Pure HBA was used as reference material. The area corresponding to the R_f value of HBA was removed and eluted four times with 50 ml of absolute methanol each time; this extraction was necessary to

(1) Coenzyme Q. CLVII.

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