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PHOTOCHEMISTRY OF ELECTRON-TRANSPORT QUINONES–III.

CHARACTERIZATION OF *IN VITRO* PHOTOPRODUCTS OF THE PHOTOSYNTHETIC PLANT QUINONE, PLASTOQUINONE-9

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Abstract—Near-UV irradiation of the photosynthetic electron transport quinone, plastoquinone-9, in the presence of methanol and water, gave rise to mixtures of diastereoisomers of the dihydrobenzofurans **2a** and **2c**, respectively, as the major products. In both cases minor amounts of the plastochromenol-8 **4a** and the benzoxepin **6a** were isolated and characterized.

Electron transport quinones, substituted 1,4-benzo- and naphthoquinones, play a vital role in the respiratory and photosynthetic elements of biological systems.[1] These quinones, acting as electron acceptors, are reversibly reduced to hydroquinones[2,3] or possibly semiquinones[4,5] when functioning *in vivo*. This function is linked to the formation of adenosine triphosphate that occurs during respiration[1] and photosynthesis.[6]

Electron transport quinones are photolabile to near-UV and visible light; hence, for cells normally living under sunlight exposure such as leaf cells, algae, and bacteria there must be cellular mechanisms to protect these quinones and maintain their function. It is conceivable that a fraction of the quinones is converted to photoproducts either naturally or when one or more of the protective mechanisms malfunctions.

Selective destruction of quinones in bacterial membranes has been carried out by taking advantage of their photolability to near-UV light,[7,8] and such studies have proved valuable in pinpointing the role of quinones in respiration.

Electron transport quinones have been implicated in ageing,[9] heart disease,[10] muscular dystrophy,[11] and cancer;[12] therefore, a systematic study of their photochemistry is a subject of biological relevance. This paper[13] describes the characterization of *in vitro* photoproducts of plastoquinone-9, PQ-9 **1a**, the quinone found in thylakoid membranes of chloroplasts, (photosynthetic organelles of the plant), and in similar unencapsulated membranes embedded in some blue-green algal cells.

RESULTS

Irradiation of PQ-9 in mixtures of benzene and methanol. The near-UV ($\lambda \simeq 365$ nm) irradiation of solutions of PQ-9 dissolved in benzene–methanol mixtures gave rise to one major and many minor photoproducts. The major photoproduct, arising from the photo-addition of methanol to the quinone in 15 to 20% yield, was identified as a mixture of diastereoisomers of dihydrobenzofuran **2a**. This assignment of structure was based on the analysis of the diastereoisomers and their acetates by UV, IR, NMR, and mass spectrometry. The material **2a** exhibited λ_{max}^{EtOH} at 300 nm, shifted to 318 nm on addition of base. Acidification reversed the maximum to 300 nm. These spectral shifts were identical to those previously observed[13b] for the dihydrobenzofuran **3a**, a photo-adduct of methanol and plastoquinone-1 (PQ-1, **1b**). The NMR spectrum of **2a** had signals at δ 1·13s, 1·15s (CH_3C—O), 1·26s, 1·34s (—CH_2—$\underset{|}{C}$—O), 1·60bs (broad singlet), 1·68bs [(C=C—Me)$_8$], 1·9–2·2 m[6,7-Me and (—CH_2—)$_8$], 3·0–3·3m (3–H), 3·26s, 3·29s (OMe), 4·66 m (2–H), 5·12bs [(—CH=)$_8$], and 6·48s (4—H). These data are comparable to those observed for **3a** except for the additional resonances attributable to the octaprenyl side-chain of **2a**. That **2a** consisted of two diastereoisomers was inferred from the observation of methoxyl group resonances at δ 3·26 and 3·29. Methyl resonances at δ 1·13 and 1·15 and methylene resonance at δ 1·26 and 1·34 could also be attributed to the presence of two diastereoisomers. Comparison of the areas of the two methoxyl

signals indicated that the diastereoisomers were present in a 1·0 to 1·4 ratio.

Two diastereoisomeric acetates, **2bS** and **2bF** (Slower and Faster moving), were obtained by repetitive TLC from acetylated **2a**. The relative amount of the acetates, 1·5 to 1·0, respectively, was almost identical to that found by NMR (*vide supra*). The IR and UV spectra of **2bS** and **2bF** were identical (Experimental Section). The UV spectra, λ_{max}^{EtOH} 289 nm (ϵ 3400), 284 nm (ϵ 3200), were comparable to the one reported for **3b**,[13b] λ_{max}^{EtOH} 289 (ϵ 3100), 283 nm (ϵ 3000). Mass spectra provided further confirmation of structure for **2bS** and **2bF**; the molecular ion (M^+) of both appeared at *m/e* 822, the value expected for acetate of a methanol addition product to PQ-9 (M^+ at *m/e* 748). Another peak observed at *m/e* 217 was attributed to the fragmentation pattern shown in Diagram 1. The NMR spectrum of **2bS** consisted of

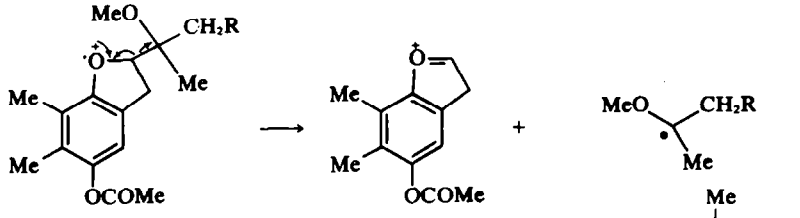
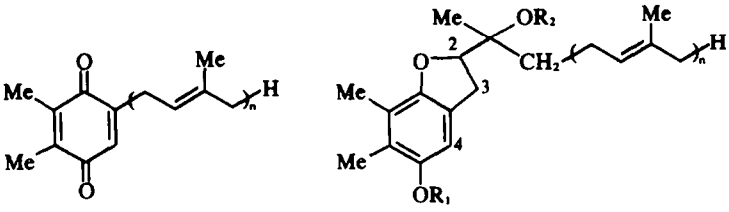


DIAGRAM 1.

signals at δ 1·15s (Me—$\overset{\vee}{C}$—O), 1·26s (—CH_2—$\overset{\vee}{C}$—O), 1·60bs, 1·68bs [(C=C—Me)$_8$], 2·01bs[6-Me and (—CH_2—)$_8$], 2·13s (7-Me), 2·28s (COMe), 3·12 d(J = 9·6 Hz, 3-H), 3·29s (OMe), 4·73t (J = 9·6 Hz, 2-H), 5·12bs [(—CH=)$_8$, and 6·66s (4aH). The spectrum of **2bF** was identical except for three features: the two proton doublet at δ 3·12 was replaced by an unresolved two proton multiplet between 3·0 and 3·4; the methoxyl singlet at 3·29 was replaced by a similar signal at 3·25; and the one proton triplet at 4·73 (J = 9·6 Hz) was replaced by a triplet with the same chemical shift but with J = 9·0 Hz. The unexpected equivalence of the 3-protons of **2bS** has been noted before[13b,14] for dihydrobenzofurans with similar substituents at the 2-position of the furan ring. This equivalence of the 3-protons is an accidental effect rather than an inherent feature of the NMR spectra of dihydrobenzofurans. Thus, the 2- and 3-protons of 2-methyl-2, 3-dihydrobenzofuran exhibit[15] the predicted ABX splitting pattern. The 3-protons of **2bF** are also non-equivalent since they give rise to an unresolved multiplet between δ 3·0 and 3·4, overlapping the singlet at 3·2 due to the methoxyl group. The conformational differences that render the 3-protons equivalent in **2bS** but non-equivalent in **2bF** cannot be simply rationalized. The 2-proton, however, still gives rise to a triplet at δ 4·73 (J = 9·0 Hz) in the spectrum of **2bF**, the HX signal of an ABX system having $J_{AX} = J_{BX}$.

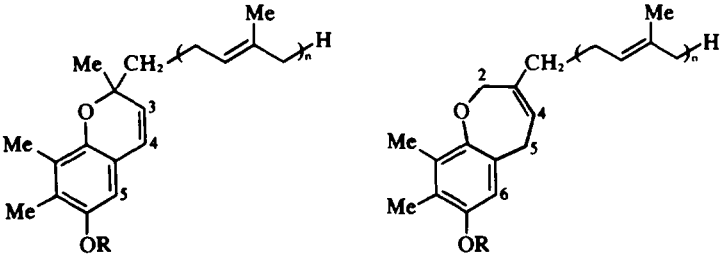
Taken together, these data provide unequivocal evidence that the major photoproduct produced following near-UV irradiation of PQ-9 in benzene–methanol mixtures was the dihydrobenzofuran **2a**. From this same mixture two minor photoproducts were separated and isolated as acetates, plastochromenol-8, **4a**, (3% yield[16]) and benzoxepin, **6a**, (5% yield). These assignments of structure were made on the basis of the following data: The faster running photoproduct had properties (UV, IR, R_f) identical with a synthetic sample of plastochromenol-8 acetate, **4b**, prepared by acetylation of the product isolated from the overnight cyclization of PQ-9 in pyridine. Confirmation of the structure of the synthetic chromenol acetate followed from its mass, UV, and NMR spectra. Thus, in the mass spectrum the molecular ion, M^+, was at *m/e* 790. Its UV spectrum (λ_{max}^{EtOH} 228, 236, 268, and 316 nm, sh 275 and 369) was similar to that of the chromenol acetate of PQ-9 prepared by Hemming *et al*[17] by absorption and elution of PQ-9 on to and from aluminum oxide. In the NMR spectra absorbances were observed at δ 1·36s (Me—$\overset{\vee}{C}$—O), 1·60bs, 1·68bs [(C=C—Me)$_8$], 2·01bs [(—CH_2—)$_8$ and 8-Me], 2·12s (7-Me), 2·28s (—COMe), 5·05–5·11m [(—CH=)$_8$], 5·52d (J = 10Hz, 3-H), 6·24d (J = 10 Hz, 4-H), and 6·49s (4-H). These data are similar to those[13b] observed for plastochromenol-0 acetate, **5**, except for the additional signals arising from the octa-prenyl side-chain.

Assignment of structure to the minor photoproduct isolated as the acetate with the lower R_f was based on its mass spectral analysis, and following its reduction with lithium aluminum hydride, the similarity of the product to benzoxepin **7**, a photoproduct of PQ-1.[13b] The molecular ion of the acetate was at *m/e* 790, and other peaks were observed in the mass spectrum at *m/e* 721, 653, 585, 517, 449, 371, 303 and 235, peaks consistent consecutive loss of eight isoprene units from the side chain. The reduced substance exhibited λ_{max}^{EtOH} at 284 nm (ϵ 2500). On addition of base λ_{max} shifted to 300 nm (ϵ 3200),



n = 9, **1a**
n = 1, **1b**

n = 8, **2a**: $R_1 = H$, $R_2 = Me$
2b: $R_1 = COMe$, $R_2 = Me$
2c: $R_1 = R_2 = H$
2d: $R_1 = COMe$, $R_2 = H$
n = 0, **3a**: $R_1 = H$, $R_2 = Me$
3b: $R_1 = COMe$, $R_2 = H$



n = 8, **4a**: R = H
4b: R = COMe
n = 0, **5**: R = COMe

n = 8, **6a**: R = H
6b: R = COMe
n = 0, **7**: R = H

DIAGRAM 2.

but this reverted to 284 nm after acidification. The spectrum and the shifts in λ_{max} were similar to those observed for 7.[13b] NMR analysis of the acetate revealed signals at δ 1·54bs [C=C—Me)$_8$], 1·68s (terminal trans-Me of side-chain), 2·01bs [$(CH_2—)_8$ and 9-Me], 2·21s (8-Me), 2·29s (COMe), 3·35bs (5-CH_2), 4·42 (2-CH_2), 5·12 [(C=C—H)$_8$], 5·56bs (4-H) and 6·60s (6-H). The similarity of these signals with those observed for the PQ-1 photoproduct, 7[13b] 3·27bs (5-CH_2), 4·32 (2-CH_2), 5·57bs (4-H), and 6·37 (6-H), supported the assignment **6a** given to the second minor photoproduct. The OAc and OH groups cause a significant difference in the chemical shifts (0·23 ppm) of the 6-proton in **6b** and 7.

Irradiation of PQ-9 in Aqueous t-Butyl Alcohol. The near-UV irradiation of PQ-9 in aqueous t-butyl alcohol gave rise to one major and many minor photoproducts. Two of the latter were converted to acetate derivatives and identified as plastochromenol-8, **4a**, and the benzoxepin, **6a**. They were isolated in yields of about 2 and 4%, respectively. The major photoproduct, isolated in only 7 to 10% yield,[16] was identified as a diastereoisomeric mixture of the dihydrobenzofurans **2c** on the basis of the following observations: Its UV spectrum exhibited λ_{max}^{EtOH} 300 nm which shifted to 318 nm upon addition of base; the original spectrum was restored upon acidification. The photoproduct was converted to an acetate derivative by reacting it at room temperature for 24 h with dry pyridine and acetic anhydride. The spectral properties of the acetate were λ_{max}^{EtOH} 280, 284 nm and ν_{max}^{film} 1765 (C=O), 3450 (OH) cm^{-1}. Lack of complete esterification pointed to a tertiary OH group. This was confirmed by conversion of the acetate by use of a diazomethane-fluoroboric acid mixture[18] at − 10°, to a mixture of two methyl ethers that were separable by TLC and that had R_f's, UV, and IR spectra identical to the stereoisomers **2bS** and **2bF**. These data left no doubt that the major photoproduct following irradiation of PQ-9 in aqueous t-butyl alcohol was a diastereoisomeric mixture of the dihydrobenzofurans **2c** resulting from the addition of water to PQ-9.

DISCUSSION

The photochemical reactions of PQ-9 in methanol-benzene mixtures and aqueous t-butyl alcohol were analogous to those of the model compound, PQ-1 previously studied.[13b] From the two respective reaction mixtures, the diastereoisomers **2c** and **2a** were isolated as the major photoproducts accompanied in each case by minor ones, the benzoxepin and chromenol derivatives of PQ-9. Similarly, irradiation of PQ-1 under a variety of conditions almost always gave rise to the benzoxepin and the chromenol as photoproducts, while irradiation in methanol or aqueous acetonitrile gave in addition the methoxy or hydroxy dihydrobenzofuran derivatives of PQ-1, respectively.

In the study with PQ-1[13b] formation of dihydrobenzofurans was attributed to solvent addition to

the zwitterionic species **8**. The latter, **8**, could have arisen from the triplet diradical **9** following intersystem crossing, **9** having been first engendered by attack of the electron-deficient oxygen atom of the $n\pi^*$ triplet state of the quinone on the olefinic double bond of the side-chain (Diagram 3). An alternative and perhaps more likely explanation stems from the work of Leary and Porter[14] who observed formation of the o-quinone methide **10** during flash photolysis of vitamin K_1, **11**. The addition of ROH (R = H or Me) to an analogous plastoquinone methide could account for the formation of the dihydrobenzofurans **2a** and **2c**. This idea is supported by recently reported work on the addition of methanol to photolytically generated o-benzoquinone methide[19] and the thermal ring closure of o-quinone methides to chromenes.[20] Plastochromenol-8 **4a**, was isolated from both irradiation mixtures suggesting that an o-quinone methide indeed may be formed upon irradiation of PQ-9.

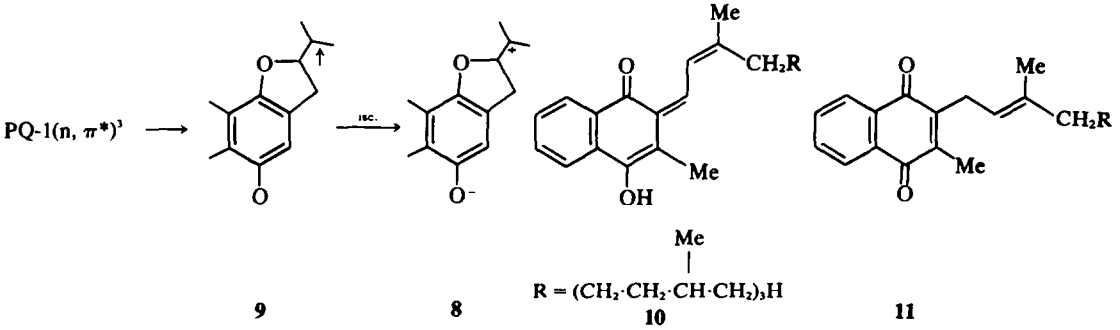


DIAGRAM 3.

The formation of the benzoxepin **6a** most likely involves a hydrogen abstraction reaction of the photoexcited quinone. In view of the number of possible sites for hydrogen abstraction from the long side-chain of PQ-9 it seems unlikely that this product arises from *inter*molecular hydrogen abstraction. However, examination of a molecular model of the quinone reveals that *intra*molecular hydrogen abstraction from the methyl group of the first isoprene unit *via* an 8-membered transition state[21] is a possible first step in the formation of **6a** (Diagram 4).

So far only two photoproducts of PQ-9 have been reported in cells: plastohydroquinone[3] and a partially characterized dimer[22] isolated from horsechestnut leaves. The dimer arose through the addition of the quinone ring of one molecule to one of the nine double bonds of the second molecule. However, the authors noted that it could have been an artifact of the isolation procedure.

In vitro photostudies with quinones have received considerable attention. The photoreduction of quinones by chlorophyll has been studied by numerous investigators[23-25] and several alternate mechanisms postulated. In other studies quinones have been used as suitable electron acceptors in the Hill reaction, i.e. the oxidative cleavage of water by

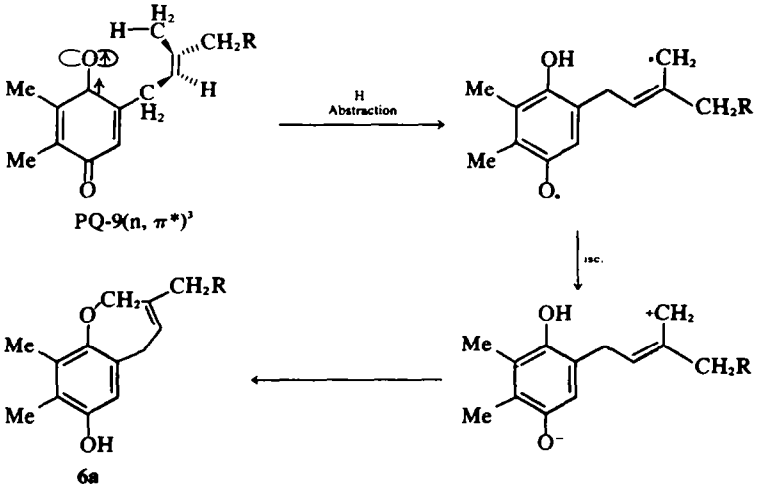


DIAGRAM 4.

illuminated chloroplasts. The isolation of **2c** suggests the possibility of still another interaction between PQ-9 and water within the cell: photoaddition.

The possible interaction of PQ-9 with other membrane substituents has been commented upon previously.[13b]

EXPERIMENTAL

PQ-9 exhibited the expected spectroscopic features (UV and NMR) and traveled as a single component by TLC in several solvent systems. Spectroscopic methanol was used without distillation, and other solvents were purified in the following ways: t-butyl alcohol was distilled once; benzene was distilled twice, the second time over P_2O_5; pyridine was distilled and stored over KOH pellets; acetic anhydride was distilled before use. All solvents for preparative TLC were redistilled. The preparation of silica gel plates has been described.[13a] All the work was carried out in red light. Solutions of PQ-9 in pyrex glassware were flushed with prepurified nitrogen (Matheson) for 20 min prior to and during the irradiation at room temperature. The solution was placed 3 cm from 5 General Electric 15W F15-T-8BLB "black lights" mounted in parallel. The NMR, IR, and UV instrumentation was that used before.[13b] Spectroscopic data that appear in the Results section are not reported in this section. NMR spectra were run in solutions of $CDCl_3$ unless otherwise indicated and signals are δ values.

Irradiation of PQ-9 in Benzene-Methanol Mixtures. In a typical experiment 50 mg of PQ-9 in a mixture of benzene (5 ml) and methanol (45 ml) was irradiated for 3 h. The solvent was evaporated at room temperature (water pump) and the residue chromatographed on six TLC plates with 4% ether in benzene as solvent. Examination of the plates under UV light revealed many minor, fluorescent quenching bands in addition to a PQ-9 band (R_f 0·9, 4 mg). Spraying the edge of the plates with dilute aqueous $KMnO_4$ revealed two major bands (R_f's 0·55 and 0·3). Elution of these with ether and distillation of the ether left brown oils A (8 mg) and B (13 mg), respectively. A and B were obtained as colorless oils following rechromatography on four plates with benzene and five plates with 4% acetone in benzene, respectively.

Acetylation of Fractions A and B. Samples of A and B were dissolved separately in dry pyridine (1 ml), and acetic anhydride (0·5 ml) was added. The solutions were flushed with nitrogen, stoppered, and left in the dark overnight at room temperature. The pyridine and acetic anhydride were then removed at $< 30°$ (oil pump); and the residual oils, chromatographed.

Isolation of **4b**, **6b**, **2bF** *and* **2bS** *from Acetylated A and B.* Acetylated A (7 mg) was repetitively (4 ×) chromatographed on four plates with hexane containing 3% ether and 27% benzene. This procedure separated A into 3 fractions: A_1 (~ 1 mg), A_2 (2 mg), and A_3 (2 mg), all colorless oils. A_1 was identified as plastochromenol-8 acetate, 2, 7, 8-trimethyl-2-(4, 8, 12, 16, 20, 24, 28, 32-octamethyl-3, 7, 11, 15, 19, 23, 27, 31-tritracontaoctaenyl)-2H-1-benzopyran-6-yl acetate **4b** by spectral and chromatographic comparison with authentic material synthesized by the procedure described below. The identification of A_3 as the benzoxepin acetate, 2, 5-dihydro-8, 9-dimethyl-3-(4, 8, 12, 16, 20, 24, 28, 32-octamethyl-3, 7, 11, 15, 19, 23, 27, 31-tritracontaoctaenyl)-1-benzoxepin-7-yl acetate, **6b**, is described in the Results section. The small amount of material available precluded a structure determination for A_2.

Acetylated B (12 mg) was resolved into two colorless oils by repetitive chromatography (3 ×) on six plates with hexane containing 3% ether and 47% benzene. These were identified as the two diastereoisomers of 2, 3 - dihydro - 2 - (1 - methoxy - 1, 5, 9, 13, 17, 21, 25, 29, 33 - nonamethyl - 4, 8, 12, 16, 20, 24, 28, 32 - tetratricontaoctaenyl) - 6, 7 - dimethyl - 5 - benzofuranyl acetate, **2bF** (4 mg) and **2bS** (6 mg) (ν_{max}^{film} 2920vs, 2350s, 1765vs, 1665w, 1600w, 1205vs, 1086s cm^{-1}; λ_{max}^{EtOH} 289 nm (ϵ 3400), 284 nm (3200).

Plastochromenol-8 acetate **4b**. PQ-9 (20 mg) was left overnight in dry pyridine (2 ml) in the dark under nitrogen at room temperature. The brown oil remaining following removal of the pyridine at $< 30°$ (oil pump) was chromatographed on two plates with 3% ether and 47% hexane in benzene as solvent. A strong fluorescent quenching band was observed together with many minor bands.

Elution of the main band with ether and removal of the solvent afforded a yellow oil which was immediately acetylated. Plastochromenol-8 acetate, **4b** (13 mg, corresponding to a 62% yield of plastochromenol-8 itself) was obtained as a colorless oil following chromatography (*vide supra*).

Irradiation of PQ-9 in Aqueous t-Butyl Alcohol. In a typical experiment the quinone (48 mg) was irradiated for 3 h in a mixture of t-butyl alcohol (85 ml) and water (60 ml). After the addition of 40 ml of water, the mixture was extracted 3 × with benzene (25 ml), and the benzene extract was dried over anhydrous Na_2SO_4. The brown oil remaining following evaporation of benzene at room temperature (water pump) was chromatographed on six plates with 4% ether in benzene. From the fraction with R_f 0·55 (7 mg) there was obtained by acetylation and further chromatography (*vide supra*) plastochromenol-8 acetate **4b** (~ 1 mg) and the benzoxepin acetate **6b** (2 mg). The material barely moving from the starting line afforded a brown oil, **2c**, which upon acetylation and rechromatography was isolated as the colorless oil **2d** (5 mg). Methylation of this acetate (*vide infra*) yielded **2bS** and **2bF** in the ratio 1·5 to 1·0.

Methylation of **2d**. A 1:1 fluoroboric acid–water mixture (0·02 ml) in ether (1 ml) was added to a solution of **2d** (9 mg) in ether (5 ml) at $-10°$. To this mixture held below 0° was added dropwise a cold (ethanol-free) ethereal solution of diazomethane until the yellow color persisted for a few minutes. After the mixture warmed to room temperature it was washed with dilute sodium bicarbonate (3 ml) and dried over Na_2SO_4. The colorless oil remaining, following removal of solvent (water pump), afforded **2d** (2 mg), **2bS** (3 mg), and **2bF** (2 mg) after chromatography (*vide supra*).

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