Photochemical Reactions of 5-Azido-8-methoxypsoralen

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Introduction

Psoralens have been used as photoactive drugs in the treatment of numerous skin diseases and in the recently developed extracorporeal photochemotherapy.[1] Owing to their ability to intercalate into nucleic acids and photoreact with pyrimidines, psoralens have also been used as structural probes for nucleic acids and are now recognized as virucidal agents especially against enveloped viruses like the herpes virus or the human immunodeficiency virus type-1 (HIV-1).[2] Studies have consistently shown that the current Psoralen-UVA (PUVA) treatment leads to side effects including skin cancer.[3] To enhance the photobinding properties and reduce side effects, a wide range of structural modifications of psoralens have been attempted.[4] The extensive investigations on psoralens in the past have been exclusively concentrated on the photoinduced cycloaddition reactions with pyrimidine bases and the sensitization reactions with oxygen.

Investigation of the action mechanism for PUVA treatment at cell level revealed that psoralens could be found throughout a cell: in the nucleus, cytoplasm, and cell membrane.[5] The efficiency of the photochemical treatment may be related to an induced immunologic reaction against the photoaltered cells.[6] In addition, specific actions of psoralens to the cell surface that are *not* directly induced by DNA binding have also been reported.[7] In Laskin's work, photoinduced action of psoralens to the skin cell surface containing cytoplasmic receptors was investigated. It was found that the binding of psoralens to these receptors was specific, saturable, and reversible. Treatment of cells with psoralens followed by UV light leads to specific cell surface alterations, in particular, phosphorylation of the receptor for epidermal growth factor (EGF). The well studied photocycloaddition reactions of psoralen may be irrelevant to or ineffective in the photobinding to these receptors. To examine or enhance the photobinding of psoralen with these receptors, a commonly used photoaffinity labeling reagent, an azido or diazo derivative of psoralen, would be effective.

Coumarins, the parent compounds of psoralens, have been subjected to a similar extensive investigation of their photocycloaddition reactivities. The use of an azido functional group to introduce a nitrene intermediate for photoaffinity labeling has also been reported.[8] In our recent study, the photochemical reactions of 6-azidocoumarin in the presence of nucleophiles were investigated. It was found that, unlike 7-azido-4-methylcoumarin which gives 7-amino-4-methylcoumarin as the major product from its triplet nitrene intermediate, 6-azidocoumarin gives predominately nucleophilic addition products from its dehydroazepine intermediate.[9] Because of its ability to form a covalent linkage with a nucleophile, 6-azidocoumairn may have potential as a photoaffinity label. In a recent work reported by Platz *et al*. spectroscopic evidence showed that 5-azido-8-methoxypsoralen gave a triplet ground state nitrene upon irradiation.[10] In our study, the photochemical properties of 5-azido-8-methoxypsoralen in the presence of alcohols and water were investigated to assess potential applications in photoaffinity labeling and in PUVA treatment. The possibility of using a nitrene intermediate instead of the double bonds to form a covalent linkage between a psoralen moiety and its surrounding molecules was explored.

Experimental Section

General. All photolyses were conducted in Shell vials placed in a Rayonet photoreactor equipped with 16 tubes emitting 350 nm light (GE T5F8-BLB). All reagents were obtained from Aldrich and used without further purification. 5-Nitro-8-methoxypsoralen was synthesized as described by Maksyutina *et al*.[11] 5-Amino-8-methoxypsoralen was synthesized according to a procedure by Mahdavi *et al*.[12]

Preparation of 5-Azido-8-methoxypsoralen. To a stirred solution of 5-amino-8-methoxypsoralen (1.20 g, 5.2 mmol) in acetic acid was added 5 mL of concentrated sulfuric acid dropwise. The mixture was cooled to 0−5 °C with ice−water throughout the reaction. A solution of sodium nitrite (0.50 g, 7.2 mmol) in 5 mL of water was added dropwise over a 45 min period, and the mixture was stirred for an additional 30 min. Urea (0.35 g, 5.8 mmol) was added gradually, and the mixture was stirred for 10 min. A slurry of sodium azide (0.50 g, 7.7 mmol) and sodium acetate (10 g, 122 mmol) in 20 mL of water was slowly added to the reaction mixture. The mixture was then stirred for another 30 min and filtered. The precipitate was washed with cold water and dried under vacuum. The product was purified by column chromatography using methylene chloride to obtain 5-azido-8-methoxypsoralen in 96% yield (1.28 g, 5.0 mmol). Mp 135−137 °C. ^{1}H NMR ($CDCl_3$): δ 4.24 (3 H, s), 6.31 (1 H, d, J = 9.8 Hz), 7.18 (1 H, d, J = 2.0 Hz), 7.73 (1 H, d, J = 2.0 Hz), 8.05 (1 H, d, J = 9.8 Hz). ^{13}C NMR ($CDCl_3$): δ

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(1) (a) Lerner, A. B.; Denton, C. R.; Fitzpatrick, T. B. *J. Inrest. Dermatol.* **1953**, *20*, 299. (b) Parrish, J. A.; Fitzpatrick, T. B.; Tananbaum, L.; Pathak, M. A. *N. Engl. J. Med.* **1974**, *291*, 1207. (c) Edelson, R. L.; Berger, C. L; Gasparro, F. P.; Jegasothy, B; Heald, P.; Wintroub, B.; Vonderheid, E.; Knobler, R.; Wolff, K; Plewig, G.; McKiernan, G.; Christensen, I.; Oster, M.; Honigsmann, H.; Wilford, H.; Kokoschka, E.; Rehle, T.; Perez, M.; Stingl, G.; Laroche, L. *New Engl. J. Med.* **1987**, *316*, 297. (e) Edelson, R. L. *J. Photochem. Photobiol. B: Biol.* **1991**, *10*, 165.

(2) (a) Cimino, G. D.; Gamper, H. B.; Isaacs, S. T.; Hearst, J. E. *Annu. Rev. Biochem.* **1985,** *54*, 1151. (b) North, J.; Neyndorff, H.; Levy, J. G. *J. Photochem. Photobiol. B: Biol.* **1993**, *17*, 99.

(3) Wolff, K. *Br. J. Dermatol.* **1990**, *122*, 117.

(4) (a) Rodighiero, G.; Dall'Acqua, F.; Pathak, M. A. Photobiological Properties of mono-Furocoumarin Photochemistry and Its Main Biological Implications Functional Furocoumarin Derivatives. In *Topics in Photomedicine*; Smith, I., Ed.; Plenum Publishing: New York, 1984; pp 319−398. (b) Jakobs, A. E.; Piette. J. *J. Med. Chem.* **1995**, *38*, 869. (c) Song, P. S.; Tapley, K. J., Jr. *Photochem. Photobiol.* **1979**, *29*, 1177. (d) Gasparro, F. P. *Psoralen DNA Photobiology*; CRC Press: Boca Raton, 1988; Vols. I and II.

(5) Moreno, G.; Salet, C.; Kohen, C.; Kohen, E. *Biochem. Biophys. Acta* **1982**, *721*, 109.

(6) (a) Perez, M.; Edelson, R.; Laroche, L.; Berger, C. *J. Invest. Dermatol.* **1989**, *92*, 669. (b) Gasparro, F. P.; Dall'Amico, R.; O'Malley, M.; Heald, P. W.; Edelson, R. L. *Photochem. Photobiol.* **1990**, *52*, 315.

(7) (a) Heindel, N. D.; Van-Dongen, J. M. A. J.; Sachais, B. S.; Phillips, J. H.; Gallo, M. A.; Laskin, J. D. *J. Pharmaceut. Sci.* **1991**, *80*, 686. (b) Laskin, J. D. *J. Am. Coll. Toxicol.* **1990**, *8*, 797.

(8) Kanaoka, Y.; Kobayashi, A.; Sato, E.; Nakayama, H.; Veno, T.; Muno, D.; Sekine, T. *Chem. Pharm. Bull.* **1984**, *32*, 3926.

(9) Feng, K.; Mahdavi, F.; Partch, R. E.; Li, Y. *Photochem. Photobiol.*, **1995**, *62*, 813.

(10) Chen, T.; Michalak, J.; Platz, M. S. *Photochem. Photobiol.* **1995**, *61*, 600.

(11) Maksyutina, N. P.; Kolesnikov, D. G. *Zhur. Obshchei Khim.* **1961**, *31*, 1386.

(12) Mahdavi, F.; Bruton, T. C.; Li, Y. *J. Org. Chem.* **1993**, *58*, 744.

0022-3263/96/1961-0398$12.00/0

63.0, 103.00, 108.48, 118.66, 121.93, 130.12, 138.97, 143.50, 144.00, 146.24, 148.44, 159.77. FT-IR (KBr) ν (cm^{-1}): 2117 (N_3), 1733 (C=O). HRMS (m/z): calcd for $C_{12}H_7N_3O_4$: 257.0437, found: 257.0429.

Photolysis of 5-Azido-8-methoxypsoralen in the Presence of Methanol. A solution of 5-azido-8-methoxypsoralen (50 mL, 5×10^{-3} M) in methanol was irradiated for 10 min. The reaction mixture was concentrated under vacuum to give a solid. The mixture was separated via a silica gel column, using a solution of chloroform and ethyl acetate (v/v = 1:1) as eluent. The fraction of $R_f = 0.3$ was collected, and the solvent was removed to give 5-imino-8,8-dimethoxy-5,8-dihydropsoralen (**3**) in 97% yield. Mp 154−156 °C. ^{1}H NMR ($CDCl_3$): δ 3.45 (6 H, s), 6.46 (1 H, d, J = 9.8 Hz), 6.82 (1 H, d, J = 1.8 Hz), 7.63 (1 H, d, J = 1.8 Hz), 8.31 (1 H, d, J = 9.8 Hz), 9.90 (1 H, br). ^{13}C NMR ($CDCl_3$): δ 55.37, 92.52, 105.09, 114.16, 115.70, 119.85, 139.51, 145.03, 149.65, 156.51, 159.02, 159.32. FT-IR (KBr) ν (cm^{-1}): 3280 (N−H), 1732 (C=O). HRMS (m/z) calcd for $C_{13}H_{11}NO_5$: 261.0637, found: 261.0633.

Photolysis of 5-Azido-8-methoxypsoralen in the Presence of Ethanol. The same procedure was performed as described in previous section to give 5-imino-8-ethoxy-8-methoxy-5,8-dihydropsoralen (**4**) in 84% yield. Mp 116−118 °C. ^{1}H NMR ($CDCl_3$): δ 1.25 (3 H, t, J = 6.9 Hz), 3.44 (3 H, s), 3.65 (2 H, q, J = 6.9 Hz), 6.45 (1 H, d, J = 9.7 Hz), 6.78 (1 H, d, J = 2.0 Hz), 7.60 (1 H, d, J = 2.0 Hz), 8.30 (1 H, d, J = 9.7 Hz), 9.82 (1 H, br). ^{13}C NMR ($CDCl_3$): δ 15.0, 54.40, 61.11, 92.76, 105.52, 114.56, 116.33, 120.26, 140.15, 145.46, 150.76, 157.27, 160.00. FT-IR (KBr) ν (cm^{-1}): 3280 (N−H), 1732 (C=O). HRMS (m/z) calcd for $C_{14}H_{13}NO_5$: 275.0794, found: 275.0789.

Photolysis of 5-Azido-8-methoxypsoralen in the Presence of 2-Propanol. The same procedure was conducted as described in the previous section to give 5-imino-8-isopropoxy-8-methoxy-5,8-dihydropsoralen (**5**) in 44% yield, mp 128−130 °C, and 5a-amino-5,5-diisopropoxy-8-methoxy-5,5a-dihydropsoralen (**6**) in 19% yield, mp 76 °C (dec).

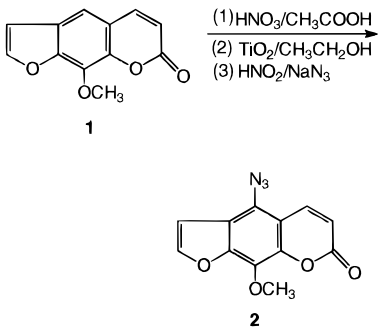
5. ^{1}H NMR ($CDCl_3$): δ 1.11 (6 H, q, J = 6.1 Hz), 3.40 (3 H, s), 4.14 (1 H, m), 6.45 (1 H, d, J = 9.7 Hz), 6.79(1 H, d, J = 1.9 Hz), 7.61 (1 H, d, J = 1.9 Hz), 8.28 (1 H, d, J = 9.7 Hz), 9.90 (1 H, br). ^{13}C NMR ($CDCl_3$): δ 23.81, 53.00, 68.80, 92.79, 105.86, 114.24, 116.34, 120.38, 139.97, 145.40, 151.04, 157.31, 160.02, 160.57. FT-IR (KBr) ν (cm^{-1}): 3280 (N−H), 1732 (C=O). HRMS (m/z) calcd for $C_{15}H_{15}NO_5$: 289.0950, found: 289.0947. **6**. ^{1}H NMR ($CDCl_3$): δ 1.28 (12 H, d, J = 6.6 Hz), 3.39 (2 H, br), 3.91 (3 H, s), 4.18 (2 H, m), 4.94 (1 H, s), 5.74 (1 H, s), 6.10 (1 H, d, J = 9.7 Hz), 7.65 (1 H, d, J = 9.7 Hz). FT-IR (KBr) ν (cm^{-1}): 3378 (NH_2), 1726 (C=O). HRMS (m/z) calcd for $C_{18}H_{23}NO_6$: 349.1525, found: 349.1527.

Photolysis of 5-Azido-8-methoxypsoralen in the Presence of Water. A solution (50 mL) of 5-azido-8-methoxypsoralen (5×10^{-3} M) and water (4 M) in acetonitrile was irradiated for 10 min. The solution was concentrated under vacuum to give a solid. The mixture was separated via a silica gel column, using a solution of chloroform and ethyl acetate (v/v = 1:2) as eluent. The fraction of $R_f = 0.3$ was collected, and the solvent was removed to give 5-imino-5,8-dihydropsoralen-8-one (**7**) in 93%. Mp 235−236 °C. ^{1}H NMR (CF_3COOD): δ 6.92 (1 H, d, J = 9.7 Hz), 7.10 (1 H, d, J = 0.9 Hz), 7.96 (1 H, d, J = 0.9 Hz), 8.26 (1 H, d, J = 9.7 Hz). ^{13}C NMR (CF_3COOD): δ 116.61, 118.50, 128.32, 137.84, 148.44, 150.00, 151.81, 156.91,160.00, 164.76, 176.05. FT-IR (KBr) ν (cm^{-1}): 3251 (N−H), 1745 (C=O, ester), 1661 (C=O, ketone). HRMS (m/z) calcd for $C_{11}H_5NO_4$: 215.0219, found: 215.0218.

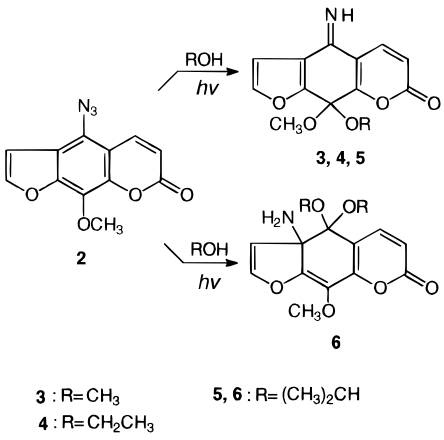
Results and Discussion

5-Azido-8-methoxypsoralen was prepared from 8-methoxypsoralen via nitration, reduction, and diazotization (Scheme 1). The photolyses of 5-azido-8-methoxypsoralen in the presence of alcohols were carried out under 350 nm light. HPLC and GC-MS were used to monitor the reaction. After 10 min irradiation in methanol, HPLC indicated that nearly 90% of the starting material, 5-azido-8-methoxypsoralen, had reacted and one product was formed. An addition product, **3**, that contains an 8-methoxypsoralenyl nitrene and a methanol moiety was isolated in a high yield. For the photolysis of 5-azido-8-methoxypsoralen in the presence of ethanol, a similar addition product, **4**, was isolated. In the presence of 2-propanol, however, two products, **5** and **6**, were obtained after the separation. NMR, FT-IR, and MS analyses indicate that product **5** has a structure similar to the products **3** and **4**. Product **6** has a structure containing one 8-methoxypsoralenyl nitrene and two molecules of 2-propanol. When the reactions were carried out in the presence of oxygen, the same results were obtained for all solvent systems, indicating that all products originated from a singlet nitrene.

Scheme 1



Scheme 2

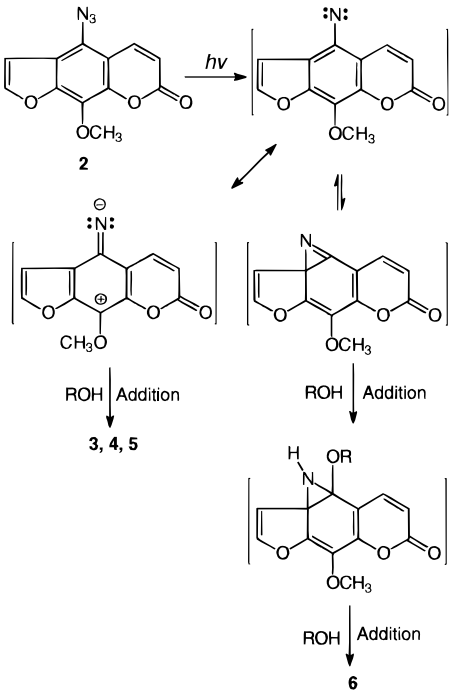


The yields of these addition products were significantly affected by the size of the alcohols. When the photolysis was carried out in the presence of methanol and ethanol, the azide gave products **3** and **4** in high yields, respectively (97% and 84%). In the presence of 2-propanol, however, the yields of products **5** and **6** were 44% and 19%, respectively. On the basis of the structures of these isolated products, alcohol acting as a nucleophile attacked the para position to the nitrene giving an intermediate, which, on protonation, formed **3**, **4**, or **5**. Because of steric hindrance, the addition rate is affected by the size of the alcohol. When the alcohol is 2-propanol, the addition becomes so slow that some of the nitrene forms an azirine intermediate that reacts with 2-propanol twice to give product **6** (Scheme 2).

Photolysis of an aryl azide generally gives a singlet nitrene that either undergoes intersystem crossing to yield its triplet state or rearrange to its singlet isomers. A monocyclic aryl nitrene isomerizes to a dehydroazepine intermediate, whereas a polycyclic aryl nitrene preferentially isomerizes to an azirine.[13] The isomeric intermediates, dehydroazepines or azirines, may react with nucleophilic species such as amines, alcohols, thiols,

(13) Li, Y.; Kirby, J. P.; George, M. W.; Poliakoff, M.; Schuster, G. B. *J. Am. Chem. Soc.* **1988**, *110*, 8092.

Scheme 3



halide ions, or water to give insertion products, 2-substituted 3*H*-azepines or 2-substituted anilines, respectively.[14,15] The primary product for a triplet aryl nitrene, however, would lead to an aniline generated by sequential hydrogen abstractions from the solvent molecules. The addition products formed in the photolysis of 5-azido-8-methoxypsoralen are from the nitrene intermediate directly. The adducts formed in the photoreaction of 6-azidocoumarin in the presence of amines are, however, through a dehydroazepine intermediate. The reason for the difference may be due to the stability of the singlet nitrene and the difficulty in expanding the benzene ring in psoralen to a dehydroazepine intermediate. It has been reported that an alkoxy group can significantly stabilize the singlet state of an electron deficient species such as carbenes.[16]

When the photolysis was carried out in water, a ketone product was isolated from the reaction mixture. According to the formation mechanism of the products, **3**−**5** (Scheme 3), the product **7** is clearly the result of an elimination of an alcohol from an initial nucleophilic addition product by water (Scheme 4). A competition experiment demonstrated that the relative reactivity of water versus methanol is ca. 1:20, which is consistent with their differences in nucelophilicity.

The UV spectrum of 5-azido-8-methoxypsoralen has four strong absorption bands, which correspond to the absorption of an azido group (352 nm), psoralen frame (314, 276 nm) and a carbonyl group (238 nm), respectively. While addition products show no absorption band that is due to the azido group, the bands that correspond to the carbon−carbon double bonds in the psoralen frame remain (Table 1). These results indicate that further irradiation with lower wavelengths may excite the double bonds to form cylcoaddition products with suitable partners in the environment. The additional reactivity may

Scheme 4

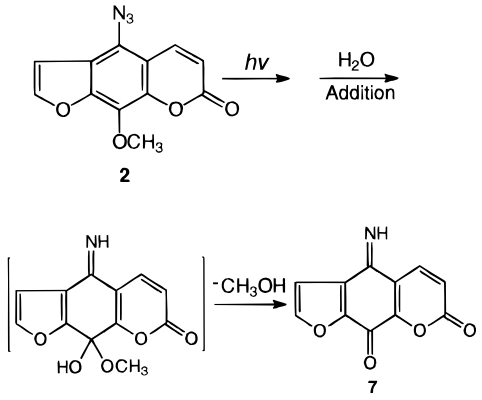


Table 1. UV and Fluorescence of Products 2−7

	UV (1×10^{-4} M)		fluorescence		
	λ	ϵ	λ_{em}	λ_{max}	ϵ^{rel}_{max}
azide **2**	238	2.28×10^5	314	390	1.0
	276	2.24×10^5			
	314	2.04×10^5			
	352	0.41×10^5			
product **3**	206	1.76×10^5	258	375	15.7
	258	1.35×10^5			
	280	8.2×10^4			
product **4**	206	1.77×10^5	258	355	8.1
	258	1.41×10^5			
	280	8.5×10^4			
product **5**	206	1.68×10^5	258	350	6.7
	258	1.19×10^5			
	280	8.5×10^4			
product **6**	222	2.19×10^5	356	400	9.5
	274	1.53×10^5			
	322	1.32×10^5			
	356	8.2×10^4			
product **7**	212	1.93×10^5	288	375	27.0
	282	1.64×10^5			
	400	5.1×10^4			

be useful to form multiple linkages with the substrate such as cross linking of interstrand DNA. All of the irradiation products of 5-azido-8-methoxypsoralen, **3**−**7**, give weak fluorescence, only 10 times stronger than their azido precursor (Table 1). The use of the fluorescence method to trace the location of the labeling, therefore, may be difficult.

Conclusion

For most aryl azides, the addition products are derived from their singlet isomers of nitrenes, dehydroazepines. In the case of 5-azido-8-methoxypsoralen, the addition occurred at the psoralen ring with the singlet nitrene intermediate due to steric restrictions on ring expansion and stability of the singlet nitrene provided by the electron-donating group. The ability to form covalent bonding indicates the potential usefulness of this azide in the investigation of psoralen compounds with biological systems and in PUVA treatments.

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Supporting Information Available: ^{1}H NMR spectra of new compounds in lieu of combustion analysis (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(14) Iddon, B.; Meth-Cohn, O.; Scriven, E. F. V.; Suschitzky, H.; Gallagher, P. T. *Angew. Chem.* **1979,** *91*, 965.
(15) Colman, R.; Suschitzky, E. F. V.; Thomas, D. R. *Chem. Ind.* **1981**, 249.
(16) Chuang, C.; Lapin, S.; Schrock, A.; Schuster, G. B. *J. Am. Chem. Soc.* **1985**, *107*, 4238.