Photoaddition Reaction of 5,7-Dimethoxycoumarin with Adenosine

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The photoreaction of 5,7-dimethoxycoumarin with adenosine has been carried out in a dry film state. The mixture of DMC and adenosine was irradiated with 350 nm UV light and two major products were isolated. The structure was determined by various spectroscopic measurements involving ^{13}C nuclear magnetic resonance and fast atom bombardment mass spectrometry. These addition products were produced by covalent bond formation between the pyrone ring at carbon 3 or 4 and the sugar ring moiety of adenosine at carbon 5'.

Introduction

Psoralens (furocoumarins) are a class of compounds found in a wide variety of plants and fungi and have been used since ancient times as dermal photosensitizing agents for the treatment of various skin pigmentation disorders.[1]

The reaction between psoralens and nucleic acids has been the subject of intense study.[2,3] The molecular mechanism of the psoralen (furocoumarin) photosensitization is believed to involve photochemical modification of DNA via covalent photocoupling of psoralens to pyrimidine bases, especially thymine, forming cyclobutane type monoadducts and diadducts leading to the DNA crosslinking.[4,5] However, the photoaddition of psoralen to poly [A] is quite efficient and one adenine base in E. coli t-RNA is involved in photoaddition of 8-methoxypsoralen to this t-RNA.[6,7] Decout et. al[8] investigated the intercalation between 8-alkoxypsoralen and adenine, indirectly by using the model compound in order to elucidate the role of adenine ring in the complexation process. The nature of this photoaddition reaction between the excited psoralen and purine bases have not been understood at all and the structure of the adducts was partly characterized[9,10] even though it is essential to understand the mechanism of the psoralen photosensitization. No derect chemical or structural proof for psoralen-purine base cross-adducts has been reported in the literature. The exact biological role of psoralen remains to be established and we investigated the photochemical reaction of 5,7-dimethoxycoumarin, a model compound of psoralen, with adenosine.

Materials and Methods

Materials. Adenosine (Ado; Sigma Chemical Co.) was found to be free of impurities and used without further purification. 5,7-Dimethoxycoumarin (DMC) was obtained from Aldrich Chemical Company and recrystallized from ethanol. Kiesel Gel GF_{254}(Merck) was used for silica gel thin layer chromatography. Pre-packed Lobar Lichroprep RP-8 (2.5 cm × 30 cm, 43-60 μm) was purchased from Merck for low pressure preparative column chromatography.

Solvents for HPLC were HPLC grade water, methanol (Merck), acetonitrile (Merck) and tetrahydrofuran (Merck). Extra pure methanol (Merck and Kanto Chemical Co.) and other common solvents were used without further purification.

Proton and carbon-13 NMR solvents of methanol-d_4 (99.5%), D_2O (99.95%), acetone-d_6 (100.0%), acetic acid-d_4 (99.5%) and pyridine-d_5 (100.0%) were purchased from Aldrich Chemical company.

Irradiation Apparatus. Irradiations were carried out in a Rayonet Photochemical Reactor (The Southern New England Ultra-Violet Company) Model RPR-208 or RPR-100 equipped with 350 nm fluorescent lamps. Two modules of RPR-208 were stacked together and arranged in a horizontal position, allowing the photolysis of sample in a solid film state.

Irradiation of DMC with Adenosine. 5,7-Dimethoxycoumarin (100 mg) and 1.3 g of adenosine (molar ratio 1:10) were dissolved in 800 m*l* methanol. The resulting solution was poured into Petri dishes and heated to dryness. The thin transparent substances obtained in the Petri dished were placed at 15 cm distance from the RUL-350 nm lamps, arranged in a horizontal position. After irradiation for 30 min, the dry substances were dissolved in methanol and the solvent was evaporated off to form a dry film again and irradiated. This process was repeated several times for a better yield of photoproducts.

Analysis of DMC-adenosine photoreaction mixtures. After irradiation of mixture of 5,7-dimethoxy-coumarin and adenosine in the solid state as a dry film, the reaction mixtures were dissolved in methanol and concentrated. The residues were filtered to remove the unreacted excess adenosine.

The photoreaction mixtures were analyzed by silica-gel GF_{254} thin layer chromatography utilizing chloroform-methanol (7:1, vol/vol) as a developing solvent and visualized by the Mineral lamp.

The concentrated solution of the crude photolysis mixtures was diluted with water. DMC, DMC dimer and photosplitted products were removed by extracting the water solution with chloroform.

The photolysis mixtures dissolved in methanol-water were also analyzed by a high performance liquid chromatography under the following conditions: column; μ-Bondapak C-18 (7.8 mm × 30 cm), solvent; water-methanol-tetrahydrofuran (300:200:6, vol/vol), flow rate; 2.5 m*l*/min, detector; UV (254 nm).

Preparation of DMC-adenosine photoadducts. The crude photolysis mixtures, which were dissolved in methanol-water, were applied to semi-preparative column chromatography and separated into adenosine and photoadduct fractions. Semi-preparative liquid chromatography was carried out with the assembled low pressure preparative liquid chromatography under the following conditions: col-

umn; LiChroprep RP-8 (2.5 cm × 30 cm; 40-63 μm) Lobar-prepacked column, solvent; water-methanol-tetrahydrofuran (300:200:6, vol/vol), flow rate; 5.0 ml/min, detector; UV (254 nm). The isolated photoproduct fractions were collected and purified by reverse phase HPLC under the following conditions: column; μ-Bondapak C_{18}(7.8 mm × 30 cm), solvent; I. water-methanol-tetrahydrofuran (300:200:6, vol/vol), II. water-acetonitrile (4:1, vol/vol), flow rate; 2.5 ml/min, detector; UV (254 nm).

After running the preparative scale liquid chromatography, the purity for each collected fraction was checked on the same liquid chromatography in analytical scale. Each fraction was frozen in a dry-acetone bath and was lyophilized to get the white, cotton-like substances.

High performance liquid chromatography. High performance liquid chromatography was performed on a Waters Associates Model 244 liquid chromatograph equipped with Model 6000A solvent delivery system, Model 440 UV absorbance detector (254 nm and 280 nm), and Model U6K universal injector. The reverse bonded phase column (μ-Bondapak C_{18}) was used for analysis and semi-preparative purpose.

Spectroscopic Measurements

Ultraviolet-visible spectra were recorded on a Cary 17 spectrometer. Infrared spectra were recorded in potassium bromide pellets on an Analect Instruments FX-6160 FT-IR spectrometer.

Mass Spectrometer. Electron impact (20 or 70 eV) ionization method (EI) mass spectra were obtained on a Hewlett Packard 5985 A GC/MS system and JEOL JMX-DX 300 with a source temperature from 150 to 250 °C.

FAB (fast atom bombardment)[11,12] mass spectra were recorded on a JEOL JMX-DX 300 double focusing equipped with a FAB ionization source. Argon was used as the bombarding gas and the operating pressure inside the ion source housing during FAB was 3×10^{-5} torr. The ion source is normally operated at gun voltage of 3 kV and an accelerating voltage of 3 kV. For FAB mass the sample is loaded in a glycerol-DMSO matrix. All data were collected, stored, and processed with JMA-DA 5000 data system.

^{1}H NMR. Proton NMR spectra were recorded in the Fourier transform mode on either Bruker AM-200-SY or a General Electric QE-300 spectrometer at 20°C. A spectral width of 1500 Hz was used in each spectrum and spectra were recorded in 99.5% methanol-d_4 or 100.0% acetone-d_6 containing a few drops of acetic acid-d_4. Typically 100-400 scans were accumulated according to the concentration of the samples. Chemical shifts are referenced to tetramethylsilane (TMS = 0.00 ppm) as an internal standard. Spectral assignments were made with the aid of extensive homonuclear decoupling experiments and COSY (correlated spectroscopy) method. 2-D COSY methods were run on a Bruker AM-200-SY as reported in the literature.[13,14]

^{13}C NMR. Carbon-13 NMR spectra were run on a Bruker AM-200-SY spectrometer (50 MHz) with wide band decoupler at spectral width 250 ppm and DEPT (Distortionless Enhancement of Polarization Transfer) method[15,16] and at 16 K or 32 K (for DEPT method) data points under internal lock signal of pyridine-d_5 solvent using tetramethylsilane as an internal standard (TMS = 0.00 ppm) at 20 °C. Typically 6,000-30,000 scans were accumulated depending on the concentration of the samples.

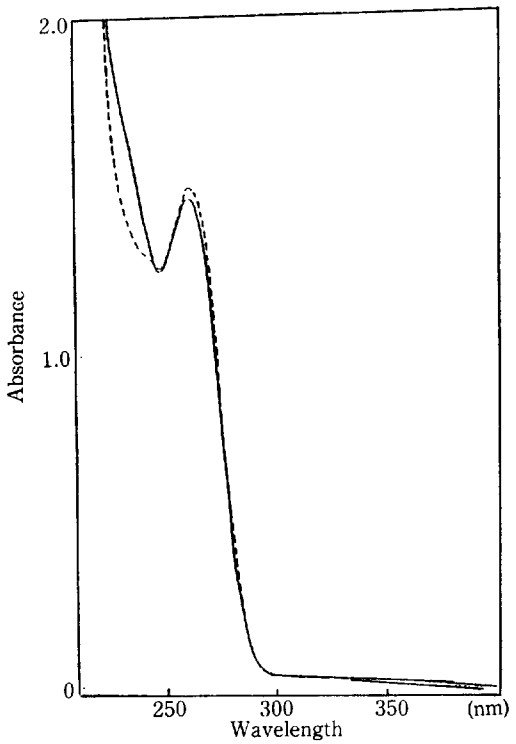

Figure 1. UV Spectra of Photoadduct I(--) and II (—) in water.

Results and Discussion

Photoproducts of DMC and adenosine formed on direct irradiation with 350 nm light in a solid state as a dry film were detected by silica gel TLC. A TLC analysis shows two major DMC-adenosine adducts at Rf's 0.30 and 0.35. These major photoadducts were isolated by Lobar LiChroprep PR-8 column chromatography and were further analyzed and separated by reverse-phase HPLC. In HPLC separation of photoadducts, water-acetonitrile (4:1, vol/vol) for photoadduct I and water-methanol-tetrahydrofuran (300:200:6, vol/vol) for photoadduct II were used as the eluent in an isocratic mode.

The separation yields of photoadduct I and II in dry film state reactions (5 irradiations) were less than 0.2 % as measured relative to adenosine.

The photoadducts were thermally labile and a rapid destruction occurs even at low temperature in solution. Photoadduct II is less stable than I, showing the melting points (decomposition temperature) of 219-221°C (for photoadduct I) and 205-207°C (for photoadduct II). Fluorescence of DMC was not quenched by adenosine and there is no evidence for the singlet exciplex formation between DMC and adenosine.

The absorption spectra (Figure 1) of the isolated photoadduct I and II are similar to each other and 325 nm band (λ_{max} of DMC) is absent. These UV spectra are exactly the sum of spectra of adenosine and 3,4-dihydro-DMC showing λ_{max} at 260nm which is λ_{max} of adenosine. It indicates that the 3,4-pyrone double bond of DMC is saturated and adenosine chromophore remains unchanged strongly suggesting that the photoadducts are not C_4-cycloaddition products. Generally, [2 + 2] photocycloadduct is photosplitted by short wavelength UV light but the UV absorption spectra didn't change to that of the starting materials on irradiation of photoadduct I and II with 254 nm UV light.

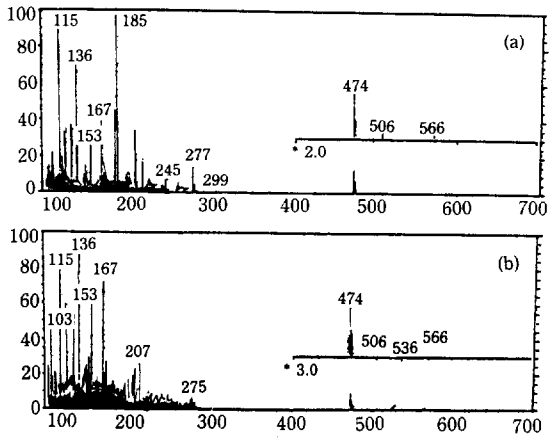

Figure 2. FAB Mass Spectra of (a) Photoadduct I and (b) Photoadduct II.

Table 1. Fragmentation Patterns of Electron Impact Mass Spectra of the DMC-adenosine Photoadducts

m/e	Fragment		m/e	Fragment
473	M^+(DMC + Adenosine)	(II)	284	$P^+ - 3H_2O$
454	$M^+ - H_2O$	(I)	207	DMC + H
446	$M^+ - HCN$	(II)	178	DMC − CO
429	$M^+ - CO_2$		163	DMC − (CO + CH_3)
338	(DMC + Rib) − H; P^+		135	Adenine
320	$P^+ - H_2O$	(I)	108	Adenine − HCN
302	$P^+ - 2H_2O$		81	Adenine − 2HCN

Two most characteristic features in IR spectra of photoadducts are the carbonyl stretching band of DMC moiety and the extremely broad hydroxyl stretching band of adenosine moiety.[17] The carbonyl stretching bands of photoadduct I and II shown at 1,773 cm^{-1} and 1,770 cm^{-1} can be assigned to the saturated lactone carbonyl stretching band. Conjugated carbonyl stretching vibration of DMC is shown at 1,710 cm^{-1}, but the carbonyl stretching bands of photoadducts are shifted to the higher frequencies due to the α,β-saturation of DMC moiety. The hydroxyl stretching band of adenosine moiety appears as a broad band at 3,600-3,000 cm^{-1}. However, the characteristic feature of the cyclobutane ring deformation band at 860 cm^{-1} is not observed in the infrared spectra of photoadduct I and II. It further supports the proposition that the photoadducts are not cycloaddition products but simple addition products and the pyrone double bond of DMC moiety is saturated.

Mass spectra of the photoadducts were determined by electron impact (EI) method and FAB (fast atom bombardment) method. Figure 2 shows FAB mass spectra of two photoadducts in the positive mode. This technique is particularily suitable for use with thermally labile and involatile samples. Abundant molecular ion ($M^+ + H$; 474) and quasi-molecular ion (M + Gly + H; 566) are shown with some fragment ions. The observed values correspond to the expected masses of a 1:1 DMC-adenosine adduct. Fragment ions corresponding to the DMC moiety (207) and adenine moiety (136) are also found.

Electron impact method is not appropriate due to the lability and the high polarity of the photoadducts but the analysis of the fragment ions provides the structural information. The fragmentation patterns of the photoadducts are similar to each other. In the mass spectra of photoadduct II (Table 1), a small molecular ion peak corresponding to a 1:1 DMC-adenosine adduct was observed at m/e 473. No such molecular ion peak was observed in the mass spectra of the photoadduct I. The highest observed mass occurs at m/e 455 which corresponds to the loss of H_2O from DMC-adenosine adduct in the spectra of photoadduct I. Additional fragmentation reactions include loss of the elements of HCN (m/e 446; photoadduct II) and CO_2 (m/e 429) from m/e 473. Fragmentations include the cleavage of the C-N glycosidic bond to yield the DMC-ribose fragment at m/e 338. The peaks of m/e 320 and 302 correspond to the elimination of H_2O and $2H_2O$ from m/e 338. The region below m/e 207 is

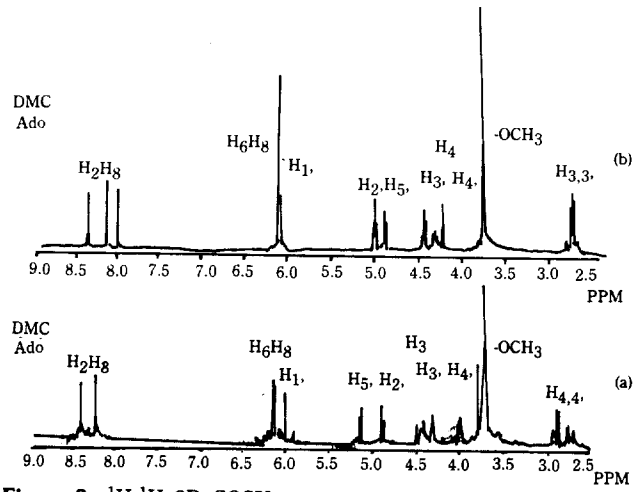

Figure 3. ^1H-^1H 2D COSY spectra of (a) Photoadduct I and (b) Photoadduct II in methanol-d_4.

Table 2. Proton Chemical Shift of DMC-adenosine Photoadducts*

Proton		Photoadduct I	Photoadduct II
5,7-OCH_3	(DMC)	3.71 s,	3.73 s,
		3.73 s	3.75 s
3-H	(DMC)	4.42 m	2.72 m
		($J_{3,4}$ = 10.2 Hz;	($J_{3,4}$ = 7.5 Hz;
		$J_{3,4'}$ = 4.2 Hz;	$J_{3',4}$ = 4.5 Hz;
		$J_{3,5'}$ = 7.7 Hz)	$J_{3,3'}$ = −15.0 Hz)
4-H	(DMC)	2.83 m	4.31 m
		($J_{4,3}$ = 10.2 Hz;	($J_{4,3}$ = 7.5 Hz;
		$J_{4',3}$ = 4.2 Hz;	$J_{4,3'}$ = 4.5 Hz;
		$J_{4,4'}$ = −17.1 Hz)	$J_{4,5'}$ = 5.7 Hz)
6,8-H	(DMC)	6.12 q	6.09 s
2,8-H	(Ad)	8.39 s	8.35 s
		8.21 s	8.13 s
1'-H	(Rib)	6.00 d	6.07 d
5'-H	(Rib)	5.14 dd	4.89 dd
		($J_{5',3}$ = 7.7 Hz)	($J_{5',4}$ = 5.7 Hz)
2'-H	(Rib)	4.88 dd	4.99 dd
3'-H	(Rib)	4.32 dd	4.43 dd
4'-H	(Rib)	4.00 dd	4.22 dd

*(a)Recorded in acetone-d_6 containing a few drops of acetic acid-d_4. (b) chemical shifts in ppm from tetramethylsilane as internal standard.

dominated by a set of fragment ions of DMC and adenosine. The base peak in the spectra of photoadduct I and II is observed at m/e 135.

DMC and its C_4-cycloadducts show the molecular ion peak of DMC at m/e 206 in the mass spectra.[18] However, in mass spectra of photoadduct I and II, a fragment of m/e 207 is shown due to the DMC + H fragment suggesting that photoadduct I and II are not C_4-cycloaddition products.

On the basis of these results with the UV spectra of the photoadducts, it is clear that photoadduct I and II are the DMC-adenosine 1:1 adduct and the data strongly suggest that addition reaction sites are ribose ring of adenosine and pyrone ring of DMC.

The proton NMR spectra (Figure 3) of the photoadducts support the involvement of the pyrone ring in the formation of DMC-adenosine photoadduct. The assignments were provided by the comparison of the chemical shift of pyrone ring protons with those of DMC, 3,4-dihydro-DMC[19] and analogous compounds reported in the literature.[20,21] The NMR data are tabulated in Table 2.

The presence of two methoxy groups was confirmed by signals at δ3.71, 3.73 ppm (for photoadduct I), and 3.73, 3.75 ppm (for photoadduct II) The proton NMR spectra of the photoadducts showed neither the pyrone ring vinyl protons of DMC at δ6.09 and 7.90 ppm (in chloroform-d) nor the 5'-methylenic protons of sugar ring at δ4.0 ppm. Signals of phenyl ring protons H(6) and H(8) are upfield-shifted with respect to the corresponding resonance signals of DMC. These observations are consistent with the formation of a covalent bond between the pyrone carbon-3 or 4 and the carbon-5' of ribose ring.

In the spectra of the photoadduct I, chemical shifts of adenine ring protons appear at δ 8.39 and 8.21 ppm and ribose-l' proton of sugar ring are distinct at δ6.00 ppm. Sugar ring 2'-H, 3'-H and 4'-H appear at δ4.88, 4.32 and 4.00 ppm, respectively. 5'-Methinic proton shows a significant downfield-shift to δ 5.14 ppm. The NMR peaks at δ4.42 ppm are due to 3-protons in the α,β-saturated DMC, but not same as those of cyclobutane ring protons. 4-Proton of DMC moiety is shown at δ 2.82 ppm. This is further confirmed by the observations of a characteristic AA' pattern and its X part in the spectra which are indicative of the presence of a methylene group and of a methinic proton at position 4 and 3, respectively. Homonuclear decoupling experiments show that there is an AA'X system at δ2.82 ppm ($^3J_{4,3}$ = 10.2 Hz and $^3J_{4',3}$ = 4.2 Hz). 4-Protons of DMC moiety would undergo geminal coupling ($^2J_{4,4'}$ = −17.1 Hz). Decoupling 3-proton (δ 4.42 ppm) results in an unperturbed AA' pattern and shows that 3-proton of DMC moiety undergoes scalar coupling with ribose 5'-proton ($^3J_{3,5'}$ = 7.7 Hz). It is now clear that reaction site is the carbon-3 of pyrone ring of DMC moiety of photoadduct II have undergone upfield shifts (relative to the parent DMC) to δ2.72 and 4.31 ppm, respectively, indicating that the 3,4 double bond have undergone photoreaction. The peak of ribose-1' H is characteristic at δ6.07 ppm. Chemical shifts of ribose 2'-H, 3'-H and 4'-H are respectively at δ4.99, 4.43 and 4.22 ppm in the spectra of photoadduct II. The peaks appeared at δ8.13 and 8.35 ppm are consistent with the presence of adenine residue. Specific decoupling experiments have also shown that there is a AA'X pattern. The methinic 4-proton of DMC moiety of the photoadduct II undergoes scalar coupling with ribose-5' proton ($^3J_{4,5'}$ = 5.7 Hz) and methinic proton also do with methylenic 3-protons ($^3J_{4,3}$ = 7.5 Hz and $^3J_{4,3'}$ = 4.5 Hz). The 3-protons of DMC moiety also undergo geminal coupling ($^2J_{3,3'}$ = −15.0 Hz). Therefore, this adduct indicates that the reaction sites of the photoadduct II are the carbon-4 of pyrone ring and ribose-5' carbon. These findings agree with those of the photoreaction of 8-MOP to 2'-deoxyadenosine.[22]

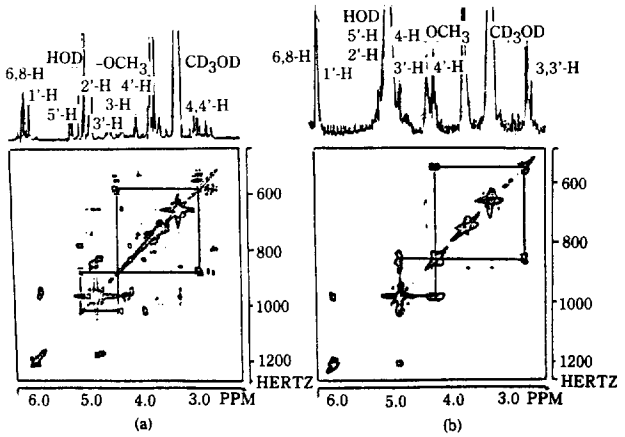

Figure 4. ^{1}H NMR Spectra of (a) Photoadduct I and (b) Photoadduct II.

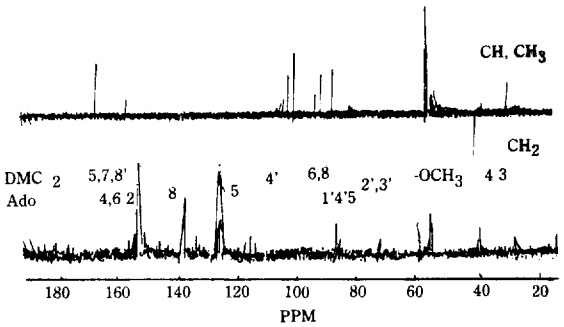

Figure 5. ^{13}C NMR Spectra of Photoadduct I in pyridine-d_5, upper; DEPT method, lower; wide band decoupling.

Proton-proton couplings in the photoadducts were studied by the two-dimensional Fourier transform method[13,14] and specific decoupling experiment in methanol-d_4. 2-D spectra of the photoadducts are shown in Figure 4. Cross peaks indicate couplings between protons in the photoadducts. If a cross peak shows up at coordinates (δ_A, δ_B) and (δ_B, δ_A), coupling is present between nuclei A and B. In 2-D spectra of photoadduct I, a cross peak shows up at coordinates (δ2.82 ppm, δ4.42 ppm) and (δ4.42 ppm, δ2.82 ppm), indicating the coupling is present between DMC-4 H (δ2.82 ppm) and DMC-3 H (δ4.42 ppm). A cross peak at coordinates (δ4.42 ppm, δ5.14 ppm) and (δ5.14 ppm, δ4.42 ppm) also indicates the coupling between DMC-3 H and ribose-5' H. The couplings between ribose-1' H and ribose-2' H ribose-2' H and ribose-3' H, ribose-3' H and ribose-4' H, and ribose-4' H and ribose-5' H are also confirmed by the corresponding cross peaks. 2-D spectra of photoadduct II exhibit the similar results. There is a cross peak between DMC-3 H (δ2.74 ppm) and DMC-4 H (δ4.25 pm), and between DMC-4 H and ribose-5' H. The other proton couplings were also confirmed by the corresponding cross peaks.

Further evidences for these structures have been obtain-

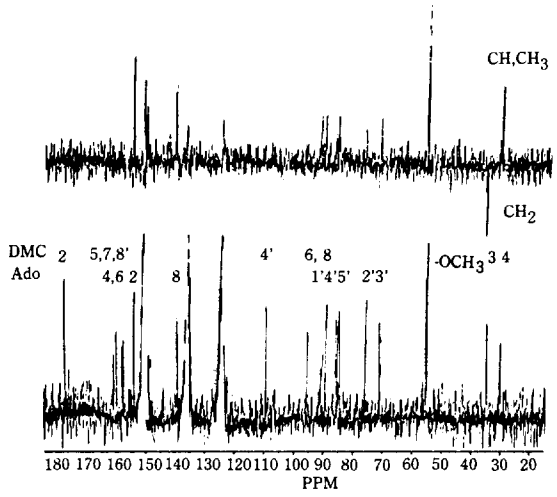

Figure 6. ^{13}C NMR Spectra of Photoadduct II in pyridine-d_5, upper; DEPT method, lower; wide band decoupling.

Table 3. ^{13}C Chemical Shift of DMC-adenosine Photoadducts[a]

Carbon		Chemical shift (ppm)[b] I	II
5,7-OCH_3	(DMC)	55.00	55.16
		55.29	55.60
2-C	(DMC)	177.97	177.54
3-C	(DMC)	33.33	35.03
4-C	(DMC)	35.96	30.44
6,8-C	(DMC)	90.65	90.77
	95.24	95.32	
4'-C	(DMC)	106.96	109.48
3'-C	(Rib)	72.45	70.81
2'-C	(Rib)	75.06	75.48
5'-C	(Rib)	82.59	84.96
4'-C	(Rib)	85.90	85.78
1'-C	(Rib)	89.31	89.19
5-C	(Ad)	121.18	
8-C	(Ad)	140.12	139.40
2-C	(Ad)	153.60	153.83
5-C	(DMC)	157.50	157.44
7-C	(DMC)	158.45	157.95
8'-C	(DMC)	160.44	159.78
4-C	(Ad)	160.88	160.92
6-C	(Ad)		

[a] Measured at 50 MHz. [b] Chemical shifts are expressed in downfield from TMS as an internal standard.

ed by the ^{13}C NMR data of both photoadduct I and II. The carbon-13 NMR spectra of the photoadducts were obtained utilizing proton noise decoupling and DEPT method in pyridine-d_5 with TMS internal standard (Figure 5 and 6). By a simple excitation sequence of DEPT method, the information about the number of adjacent protons is not reflected in residual signal splittings but in signal phases and intensities, methylene carbon signals appear as negative and those of methyl and methine carbons as positive signals. Quaternary

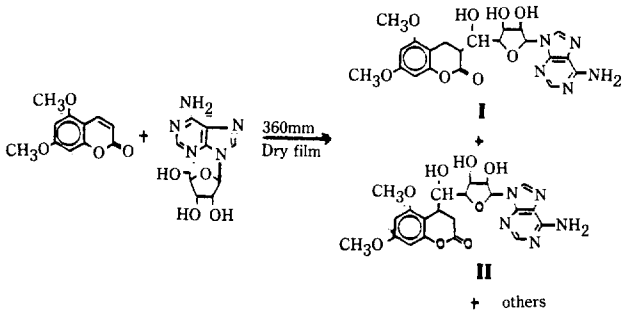

Figure 7. Photoreaction of 5,7-Dimethoxycoumarin and Adenosine.

carbon signals disappear. Carbon chemical shifts for the photoadducts are summarized in Table 3.

Assignments were made by analogy with carbon shifts for 3,4-dihydro-DMC, DEPT spectra of the photoadducts, and published carbon shifts for 5,7-dihydroxy-4-methyl-dihydrocoumarin.[20,23] The results agree with those of proton NMR spectra and give clear indication of carbon-carbon bonding site, carbon-3 or carbon-4 of DMC and ribose carbon-5' of adenosine.

In carbon-13 NMR spectra of the photoadduct I, methoxy carbons of DMC moiety appear at δ55.0 and 55.3 ppm. The presence of carbonyl carbon of the lactone ring was confirmed by signals at δ177.9 ppm. However, resonance signals of carbons on the pyrone double bond in DMC (δ138.7 and 110.8 ppm in chloroform-d) disappeared and so did that of carbon-5' (δ62.6 ppm in D_2O + DCl) of sugar ring. The resonance positions of the ribose carbons are almost unaffected by addition at the 5'-position. The ribose carbon-5' of the photoadduct exhibits a significant downfield shift with respect to those of adenosine and is characterized a methine carbon as shown in DEPT spectra. The presence of carbon-3 and carbon-4 of DMC moiety was confirmed by signals at δ 33.3 ppm and 36.0 ppm, respectively and the reaction site is determined as carbon-13 of DMC.

Carbon-13 NMR spectra of the photoadduct II are very similar to those of photoadduct I. Two methoxy carbons are shown in 55.2 and 55.6 ppm and carbonyl carbon of lactone ring in δ177.5 ppm. The methine carbon of ribose-5' carbon is shifted to the downfield. The carbon-3 and carbon-4 of DMC moiety appear at δ 35.0 and 30.4 ppm, respectively. Therefore, the reaction sites in the photoadduct II is the carbon-4 of DMC moiety and the ribose-5' carbon of adenosine moiety.

Conclusion

The results described in this study provide evidences for the detailed chemical and structural characterization of the photoadducts obtained from the photoreaction of 5,7-dimethoxycoumarin with adenosine in a dry film state. Fluorescence of DMC was not quenched by adenosine. Thers is no evidence for the singlet exciplex formation between DMC and adenosine. Two of the photoadducts of DMC with adenosine were isolated by preparative column chromatography and HPLC and were characterized by spectroscopic means such as UV, FT-IR, ^1H, ^{13}C NMR and mass spectrometry. The photoadducts of DMC with adenosine were not photosplitted by short wavelength UV light, and show λ_{max} at 260 nm which is the same as that of adenosine.

It indicates that adenosine chromophore remains unchanged, strongly suggesting that the photoadducts are not C_4-cycloaddition products but simple addition products in contrast to pyrimidine base adducts. Photobinding of DMC oc curs to adenosine through covalent bond formation between carbon-3 (for photoadduct I) and carbon-4 (for photoadduct II) of the pyrone ring of DMC and ribose carbon-5' of adenosine (Figure 7).

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References

1. T. B. Fitzpatrick and M. A. Pathak, *NCI monograph*, **66**, 3 (1984).
2. B. J. Parsons, *Photochem. Photobiol.*, **32**, 813 (1980).
3. P.-S. Song and K. J. Tapley, *Photochem. Photobiol.*, **29**, 1177 (1979).
4. D. Kanne, S. Kenneth, J. E. Hearst, and H. Rapoport, *J. Am. Chem. Soc.*, **104**, 6754 (1982).
5. J. E. Hearst, S. T. Isaacs, D. Kanne, H. Rapoport, and K. Straub, *Quart. Rev. Biophys.*, **17**, 1 (1984)
6. C. H. Krauch D. M. Krämer and A. Wacker, *Photochem. Photobiol.*, **6**, 341 (1967).
7. C.-N. Ou and P.-S. Song, *Biochemistry*, **17**, 1054 (1978).
8. J. Decout and J. Lhomme, *Photochem. Photobiol.*, **37**, 155 (1983).
9. S. C. Shim, H. K. Shim, and T. H. Cho, *Chem. Lett.*, **1986**, 19.
10. P. Vigny, M. Spiro, F. Gaboriau, Y. Le Beyec, S. Dellar Negra, J. Cadet, and L. Voituriez, *Int. J. Mass Spect. Ion Phys.*, **53**, 69 (1983).
11. M. Barber, R. S. Bordoli, R. D. sedgwick, and A. N. Tyler, *J. Chem. Soc. Chem. Comm.*, **1981**, 325.
12. M. Barber, R. S. Bordoli, G. J. Elliott, R. D. Sedgwick, and A. N. Tyler, *Anal. Chem.*, **54**, 645A (1982).
13. Ad Bax and R. Freeman, *J. Magn. Reson.*, **44**, 542 (1981).
14. Ad Bax, In "Two-dimensional Nuclear Magnetic Resonance in Liquids", P 69, Delft University Press, Delft, Holland (1984).
15. D. W. Brown, T. T. Nakashima, and D. L. Rabenstein, *J. Magn. Reson.*, **45**, 302 (1981).
16. C. Le Cocq and J.-Y. Lallemand, *J. Chem. Soc. Chem. Commun.*, **1981**, 150.
17. S. C. Shim and D. Y. Chi, *Chem. Lett.*, **1978**, 1229.
18. S. C. Shim, H. Y. Koh, and D. Y. Chi, *Photochem. Photobiol.*, **34**, 177 (1981).
19. S. C. Shim, Unpublished Results.
20. E. G. Crichton and P. G. Waterman, *Phytochemistry*, **17**, 1738 (1978).
21. B. S. Joshi and K. R. Ravindranath, *J. Chem. Soc., Perkin I*, **1977**, 433.
22. J. Cadet, L. Voituriez F. Gaboriau, and P. Vigny, in abstract of "9th International Congress on Photobiology and 12th Annual Meeting of the American Society for Photobiology," Philadelphia, pennsylvania, 1-6 July.
23. H. Duddeck and M. Kaiser, *Org. Magn. Reson.*, **20**, 55 (1982).

Asymmetric Reduction of Prochiral Ketones with Potassium 9-O-isopinocampheyloxy-9-boratabicyclo[3,3,1]nonane+

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Asymmetric reduction of a series of aliphatic ketones and representative other classes of ketones with potassium 9-*O*-isopinocampheyloxy-9-boratabicyclo[3,3,1]nonane (K 9-*O*-Ipc-9-BBNH) was studied. All the ketones examined were reduced smoothly to the corresponding alcohols in THF at-78°C. Thus, the reduction of 2-butanone, 3-methyl-2-butanone, 3,3-dimethyl-2-butanone, 2-octanone, and 4-phenyl-2-butanone provides 51% ee, 61% ee, 44% ee, 35% ee, and 33% ee of optical inductions, respectively. The reduction of other classes of ketones gave 52% ee for 2,2-dimethyl-cyclopentanone, 47% ee for acetophenone, 23% ee for 3-acetylpyridine, 50% ee for methyl benzoylformate, 4.8% ee for 2-chloroacetophenone, 30% ee for *trans*-4-phenyl-3-butene-2-one, and 2% ee for 4-phenyl-3-butyn-2-one. Thus, the reagent was found to be most useful in the asymmetric reduction of acyclic and cyclic aliphatic series of ketones.

One of the simplest and most useful methods for introduction of a chiral center in a molecule is the asymmetric reduction of prochiral ketones. Although this reaction has been studied extensively over the past several decades[2], it has only been the past few years that exceptional progress has been achieved[3]. Such the success has been accomplished particularly in alkyl aromatic ketones with chiral reducing agents, such as K-Glucoride[3b], diisopinocampheylchloroborane[3c], Binal-H[3d], borane-aminoalcohol[3e], Alpineborane[3f], modified lithium borohydride[3g], modified lithium aluminum hydride[3h]. For simple aliphatic ketones, however, success has been only limited[3e,3f,4]. Consequently, asymmetric reduction of such aliphatic ketones remains as a major challenge to organic chemists.

Recently, we have reported the preparation of new chiral dialkylmonoalkoxyborohydrides and their asymmetric reduction of acetophenone and 3-methyl-2-butanone[5]. During the study, we discovered an unexpected but highly in-