Inverse Electron Demand Diels-Alder Reactions of Psoralens. Synthesis and Mass Spectra of Novel Pyridazinocoumarins

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This paper is dedicated to Professor Raymond N. Castle

Diels-Alder reactions between the furan double bond of 8-methoxypsoralen and 1,2,4,5-tetrazine or 3,6-bistrifluoromethyl-1,2,4,5-tetrazine were accompanied by the release of diatomic nitrogen and the opening of the furan ring to leave a 6-pyridazinocoumarin. When 3,6-bis(methoxycarbonyl)-1,2,4,5-tetrazine was used as diene with 8-methoxy-, 5-methoxy- or 8-hydroxypsoralen as substrate a previously unknown heterocyclic framework was created. Formation of the fourth ring was accompanied by conversion of the furan ring to a pyrone, presumably by intramolecular transesterification with release of methanol. The characterization of the products of these reactions by exhaustive mass spectrometric analysis is discussed.

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Psoralens, especially 5- and 8-methoxypsoralen (5-MOP and 8-MOP), are commonly used for photochemotherapy of several skin diseases, including psoriasis, vitiligo, T-cell lymphoma and a number of autoimune disorders [1]. Their biological activity is generally attributed to their binding to cell DNA. When the initial complex between DNA and intercalated psoralen is irradiated with UV light, mono- and di-adducts are formed by [2 + 2] photocycloaddition reactions involving the 5,6 double bond of the DNA pyrimidine bases and the 3,4 and/or 4',5' double bonds of the psoralen [2].

In an effort to avoid the side effects resulting from the capacity of known psoralens to cross-link the DNA strands [3], a number of structurally modified psoralens have been synthesized, in most cases the modification affects the furan ring. In our laboratory, for example, good results have been obtained in recent years by fusing a fourth aromatic ring to the furan ring [4,5].

The heterocyclic azadiene Diels-Alder reaction is a well-tried tool in the synthesis of natural products [6,7]. The most widely used electron-deficient azadiene has been the symmetrically di-substituted 1,2,4,5-tetrazine, which has been effectively employed for the preparation of heterocyclic systems that are not easily constructed by other methods [8-11]. In this paper we report preliminary results on the Diels-Alder reactions of 1,2,4,5-tetrazines with psoralens. These are inverse electron demand reactions involving the LUMO of the diene and the HOMO of the psoralen. As expected, we found that the diene attached regioselectively to the furan ring, which has greater electron density than the pyrone ring.

As expected, cycloaddition of 1,2,4,5-tetrazine to 8-MOP was accompanied by the release of diatomic nitrogen and the opening of the furan ring [12] (Scheme 1), giving a 20% yield of compound **1**. Analogue **2** was obtained in 86% yield by reacting 8-MOP with 3,6-bistrifluoromethyl-1,2,4,5-tetrazine, which because of its volatility [13] was prepared by oxidizing the corresponding 1,2-dihydrotetrazine with 1 equivalent of 2,3-

Table 1

70 eV EI mass spectra of compounds **1** and **2**
[m/z values (relative abundances)]

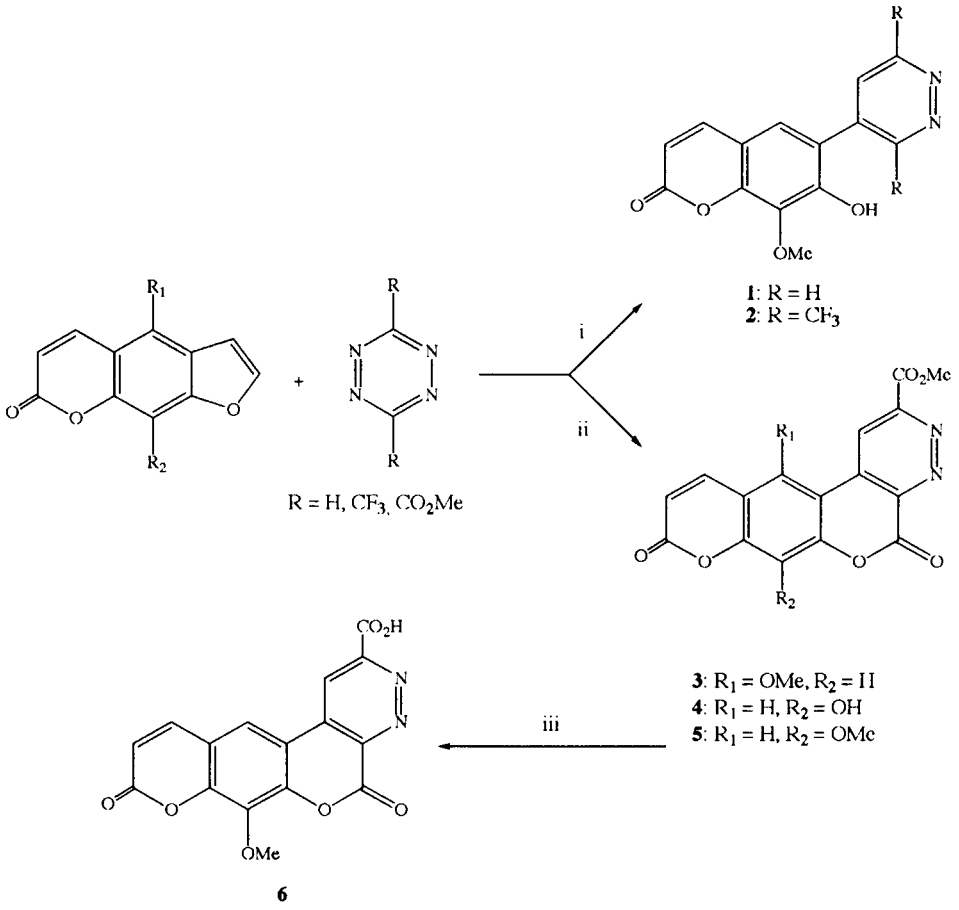
	Ionic Species	1	2
	$[M+H]^+$	271 (8)	407 (22)
	$M^{+\cdot}$	270 (100)	406 (100)
	$[M-CO]^{+\cdot}$	242 (5)	378 (6)
	$[M-CO_2]^{+\cdot}$	226 (6)	362 (2)
a	$[M-C_6F_6N_2]^{+\cdot}$	193 (2)	193 (8)
a'	$[a-CO]^{+\cdot}$	165 (1)	165 (2)
a"	$[a-CO_2]^{+\cdot}$	149 (4)	149 (3)
	$[M-C_3H_3O_2]^{+\cdot}$	199 (14)	335 (6)
b	$[M-HF]^{+\cdot}$	-	386 (12)
b'	$[b-CO]^{+\cdot}$	-	358 (18)
b"	$[b'-C_3H_3O_2]^{+\cdot}$	-	315 (14)
	$[C_{13}H_6F_3N_2O_2]^+$	-	279 (54)
	$[C_7H_7O_2]^{+\cdot}$	123 (1)	123 (5)
	$[C_4H_2N_2]^+$	78 (3)	-
	$[C_6F_6N_2]^+$	-	215 (1)

Table 2

70 eV EI mass spectra of compounds **3-5**
[m/z values (relative abundances)]

	Ionic species	3	4	5
	$[M+H]^+$	355 (9)	341 (4)	355 (16)
	$M^{+\cdot}$	354 (30)	340 (14)	354 (66)
	$[M-OCH_3]^+$	323(5)	-	323(2)
	$[M-CO]^{+\cdot}$	-	312 (6)	-
	$[M-CO_2]^{+\cdot}$	-	296 (13)	-
a	$[M-C_2H_2O_2]^{+\cdot}$	296 (100)	282 (100)	296 (100)
a'	$[a-CO]^{+\cdot}$	268 (13)	254 (31)	268 (39)
a"	$[a'-CO]^{+\cdot}$	240 (6)	226 (19)	240 (14)
b	$[M-C_3H_3O_2]^{+\cdot}$	283 (1)	269 (8)	283 (2)
b'	$[b-CO]^{+\cdot}$	255 (2)	241 (4)	255 (2)
b"	$[b'-CO_2]^{+\cdot}$	239 (3)	225 (3)	239 (4)
c	$[C_9H_2O_2]^{+\cdot}$	143 (3)	143 (0)	143 (4)
c'	$[c-CO]^{+\cdot}$	114 (7)	114 (25)	114 (9)
c"	$[c'-CO_2]^{+\cdot}$	98 (3)	98 (4)	98 (5)
d	$[C_5H_2N_2O_2]^{+\cdot}$	121 (2)	121 (1)	121 (1)
d'	$[d-CO]^{+\cdot}$	93 (3)	93 (2)	93 (1)
d"	$[d'-CO_2]^{+\cdot}$	77 (15)	77 (10)	77 (13)

Scheme 1



Reagents: (i) CH_2Cl_2, 100° in a sealed tube; (ii) reflux dioxane; (iii) 1) NaOH 2M, EtOH reflux 2) HCl 3M

dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane and was used in the reaction with 8-MOP without further purification.

When 3,6-bis(methoxycarbonyl)-1,2,4,5-tetrazine was used as diene with 5-MOP, 8-MOP or 8-hydroxypsoralen as substrate, the cycloaddition was accompanied by conversion of the psoralen furan ring to a pyrone ring (Scheme 1), possibly through loss of methanol upon intramolecular transesterification of intermediates analogous to compounds **1** and **2**. Compounds **3-5** were obtained in yields of 61-79%, and hydrolysis of **5** with 2*M* NaOH afforded the corresponding acid, **6**, in high yield. The new tetracycles **3-6** can be used as starting materials for developing a novel set of DNA-intercalating compounds.

In continuance of our previous work on the chemistry and mass spectrometry of coumarin compounds [14,15], we also determined the electron ionization (EI) behavior of the new coumarin derivatives. Compounds **1-5** all afforded well-defined 70 eV EI mass spectra. Both M^+ and $[M+H]^+$ peaks appear, the former being the base peak for **1** and **2**, and the other peaks are easily related to the structures of the neutral compounds. The fragmentation behaviors of compounds **1** and **2** (Table 1) are identical except that the spectrum of **2** shows peaks that appear to indicate the formation of a pyran ring. The spectra of compounds **3-5** (Table 2) show three main fragmentation pathways: path 1 begins with cleavage of the methoxycarbonyl group (M^+ to **a**), path 2 with opening/cleavage of the monofused pyranone (M^+ to **b**), and path 3 with opening/cleavage of the bis-fused pyranone and simultaneous loss of R_1 or R_2 (M^+ to **c**); all three paths continue with the loss of CO and/or CO_2 groups, as is expected for coumarins. Primary loss of CO or CO_2 groups was detected only for compound **4**, and primary loss of a methoxy group only for **3** and **5**.

EXPERIMENTAL

Melting points were determined in a Reichert Kofler thermopan apparatus and are uncorrected. The ir spectra (potassium bromide disc) were recorded in a Perkin-Elmer 1640FT spectrometer (ν in cm^{-1}). The ^1H and ^{13}C nmr spectra were recorded in a Bruker AMX 300 nmr spectrometer, using tetramethylsilane as internal standard (δ in ppm, J in Hz). Electron ionization (EI) mass spectra were obtained using a Micromass (altrincham, UK)

QMD 1000 mass spectrometer operating at 70 eV and 200 mA with an ion source temperature of 200°. The samples were introduced directly into the source and heated to 170°. Elemental analyses were performed by a Perkin-Elmer 240B microanalyser. Flash chromatography was performed using silica gel (Merck 60, 230-400 mesh).

7-Hydroxy-8-methoxy-6-(4-pyridazinyl)coumarin (**1**).

A solution of 8-methoxypsoralen (812 mg, 3.76 mmol) and 1,2,4,5-tetrazine (320 mg, 4.5 mmoles) [16] in dry dichloromethane (15 ml) was heated at 100° for 72 hours in a sealed tube. The precipitate was filtered out, washed with dichloromethane and purified by flash chromatography with 95:5 dichloromethane: methanol as eluent to obtain **1** as a yellow solid 203 mg (20%), m.p 224-226°; ir: ν 1165, 1201, 1354, 1410, 1458, 1570, 1605, 1728, 2945 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 10.8 (broad s, OH), 9.58 (d, 1H, H-2', J = 0.90), 9.28 (d, 1H, H-5', J = 5.40), 7.99 (d, 1H, H-4, J = 9.50), 7.90 (dd, 1H, H-6', J = 5.40, 0.90), 7.71 (s, 1H, H-5), 6.35 (d, 1H, H-3, J = 9.50), 3.91 (s, 3H, OCH_3); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ 159.81, 151.83, 151.70, 151.53, 148.74, 144.99, 135.47, 134.88, 126.11, 125.77, 124.90, 119.87, 113.00, 112.90, 61.66.

Anal. Calcd. for $C_{14}H_{10}N_2O_4$: C, 62.22; H, 3.73; N, 10.37. Found: C, 62.19; H, 3.75; N, 10.42.

7-Hydroxy-8-methoxy-6-[4-(3,6-bistrifluoromethyl)-pyridazinyl]coumarin (**2**).

To a solution of 1,2-dihydro-3,6-bistrifluoromethyl-1,2,4,5-tetrazine [13] (440 mg, 2 mmoles) in dichloromethane (15 ml) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (454 mg, 2 mmoles) and the mixture was stirred for 15 minutes at room temperature. The precipitate was filtered out and the filtrate was placed in a sealed ampoule with 8-methoxypsoralen (648 mg, 3 mmoles). The mixture was heated at 110° for 8 hours until the solution turned completely colorless. The solvent was removed under vacuum and the residue was purified by flash chromatography using 10:1 dichloromethane/ethyl acetate as eluent to give **2** as white crystals 697 mg (86%), m.p 203-205°; ir: ν 1407, 1421, 1458, 1576, 1605, 1731, 3186 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 10.8 (broad s, OH), 8.65 (s, 1H, H-6'), 8.00 (d, 1H, H-4, J = 9.50), 7.47 (s, 1H, H-5), 6.35 (d, 1H, H-3, J = 9.50), 3.89 (s, 3H, OCH_3).

Anal. Calcd. for $C_{16}H_8F_6N_2O_4$: C, 47.31; H, 1.98; F, 28.06; N, 6.90. Found: C, 47.30; H, 2.01; N, 6.94.

General Procedure for the Diels-Alder Reaction with 3,6-Bis-(methoxycarbonyl)-1,2,4,5-tetrazine.

Dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (198 mg, 1 mmol) [17] was added with stirring to a solution of the appropriate psoralen (1 mmol) in anhydrous dioxane (20 ml) at room temperature. The mixture was refluxed under anhydrous conditions until the deep red color of the tetrazine disappears. The precipitate was filtered out and washed with fresh dioxan and ethyl ether.

12-Methoxy-5,9-dioxo-5*H*,9*H*-6,8-dioxa-3,4-diazabenzo-[*a*]anthracene-2-carboxylic Acid Methyl Ester (**3**).

Compound **3** was obtained using the above procedure, 216 mg (61% yield) in 48 hours, mp 225-228° (dec); ir: ν 1060, 1082, 1277, 1405, 1577, 1600, 1619, 1727, 1772 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 9.18 (s, 1H, H-1), 8.24 (d, 1H, H-11, J = 9.85), 7.47 (s, 1H, H-7), 6.58 (d, 1H, H-10, J = 9.85), 4.06 (s, 3H, CO_2CH_3), 3.98 (s, 3H, OCH_3).

Anal. Calcd. for $C_{17}H_{10}N_2O_7$: C, 57.64; H, 2.84; N, 7.91. Found: 57.59; H, 2.88; N, 8.21.

7-Hydroxy-5,9-dioxo-5*H*,9*H*-6,8-dioxa-3,4-diazabenzo-[*a*]anthracene-2-carboxylic Acid Methyl Ester (**4**).

Compound **4** was obtained using the above procedure, 221 mg (65% yield) in 48 hours, mp 302-305° (dec), ir: ν 1071, 1127, 1144, 1170, 1127, 1144, 1170, 1246, 1445, 1615, 1630, 1728, 1760, 3292, 3326 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 8.95 (s, 1H, H-1), 8.42 (s, 1H, H-12), 8.04 (d, 1H, H-11, J = 9.60), 6.51 (d, 1H, H-10, J = 9.60), 4.08 (s, 3H, CO_2CH_3), 3.58 (s, 1H, OH).

Anal. Calcd. for $C_{16}H_8N_2O_7$: C, 56.48; H, 2.37; N, 8.23. Found: 56.42; H, 2.33; N, 8.26.

7-Methoxy-5,9-dioxo-5*H*,9*H*-6,8-dioxa-3,4-diazabenzo-[*a*]anthracene-2-carboxylic Acid Methyl Ester (**5**).

Compound **5** was obtained using the above procedure, 280 mg (79% yield) in 24 hours, mp 265-268° (dec); ir: ν 1135, 1170, 1415, 1605, 1627, 1756 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 9.05 (s, 1H, H-1), 8.85 (s, 1H, H-12), 8.05 (d, 1H, H-11, J = 9.60), 6.60 (d, 1H, H-10, J = 9.60), 4.10 (s, 3H, CO_2CH_3), 4.05 (s, 3H, OCH_3).

Anal. Calcd. for $C_{17}H_{10}N_2O_7$: C, 57.64; H, 2.84; N, 7.91. Found: 57.61; H, 2.87; N, 7.74.

7-Methoxy-5,9-dioxo-5*H*,9*H*-6,8-dioxa-3,4-diazabenzo-[*a*]anthracene-2-carboxylic Acid (**6**).

Addition of 2*M* sodium hydroxide (10 ml) to a suspension of ester **5** (100 mg, 0.3 mmole) in ethanol (10 ml) produced a homogeneous orange mixture. The orange mixture was refluxed for 30 minutes and then left to cool to room temperature, brought to *p*H 3 with 3*M* hydrochloric acid, and stirred for 20 minutes. The yellow precipitate was collected and washed with water, followed by methanol and diethyl ether, giving 85 mg (90%) of pure **6**, mp 200-202° (dec.); ir: ν 1087, 1199, 1254, 1278, 1309, 1424, 1458, 1582, 1610, 1715, 1760, 3000, 3500 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 8.92 (s, 1H, H-1), 8.60 (s, 1H, H-12), 7.85 (d, 1H, H-11, J = 9.55), 6.55 (d, 1H, H-10, J = 9.55), 3.87 (s, 3H, OCH_3).

Anal. Calcd. for $C_{16}H_8N_2O_7$: C, 56.48; H, 2.37; N, 8.23. Found: 56.45; H, 2.41; N, 8.20.

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