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Photochemical decomposition of midazolam. III — Isolation and identification of products in aqueous solutions

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Abstract: Midazolam, 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-*a*][1,4]benzodiazepine, decomposes photochemically in aqueous solution both under irradiation from a high-pressure mercury lamp and in normal daylight. The main decomposition product under the artificial radiation was 6-(8-chloro-1-methyl-4,5-dihydro-2,5,10*b*-triaza-benzo[*e*]-azulen-6-ylidene)-cyclohexa-2,4-dienone, which was not present in the solution exposed to daylight. 6-Chloro-2-methyl-4-(2-fluorophenyl)quinazoline was formed in both irradiation experiments and was the main decomposition product in normal daylight. Several minor products were formed in both solutions, the amounts depending on the pH of the solution. Only one decomposition product was formed in acidic solutions (pH < 2) irradiated with the high-pressure mercury lamp but numerous products were formed at higher pH.

Keywords: *Midazolam; benzodiazepine; photodecomposition, stability.*

Introduction

The 1,4-benzodiazepines include several photolabile drugs; the most frequently studied of these substances are chlordiazepoxide, diazepam and nitrazepam [1, 2]. Midazolam, 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5*a*][1,4]benzodiazepine (Fig. 1), an imidazobenzodiazepine derivative used as a hypnotic and in the induction of anaesthesia, decomposes in ethanolic solutions irradiated with a high-pressure mercury lamp [3, 4]. The main decomposition products are 6-chloro-2-methyl-4-(2-fluorophenyl)-quinazoline, N-desalkylflurazepam, and a solvent addition product, 7-chloro-2[(1-ethoxyethylimino)ethoxymethyl]-5-(2-fluorophenyl)-3H-1,4-benzodiazepine. Two benzophenone derivatives, 2-amino-5-chloro-2′-fluorobenzophenone and 2-acetamido-5-chloro-2′-fluorobenzophenone, and a demethylated quinazoline derivative, 6-chloro-4-(2-fluorophenyl)-quinazoline, are formed in minor amounts.

In the present part of the authors' study the photodecomposition of midazolam in irradiated aqueous solutions has been investigated and the influence of the pH of the medium and the source of irradiation on the nature and amounts of the photodecomposition products have been examined.

Experimental

Materials

Midazolam was kindly supplied by Hoffmann-La Roche (Basle, Switzerland). The identity and purity of the substance were verified by TLC and by UV, IR, ^{1}H-NMR and ^{13}C-NMR spectrometry. N-desalkylflurazepam, used as a reference, was obtained from Hoffmann-La Roche and used as such. The other reference substance, 6-chloro-2-methyl-4-(2-fluorophenyl)-quinazoline, was isolated in earlier experiments from an irradiated ethanolic solution of midazolam, and its structure was determined by IR, ^{1}H-NMR and ^{13}C-NMR spectrometry [3]. All other reagents and solvents were of analytical grade.

Apparatus

The artificial radiation source was a high-pressure mercury lamp, Hanau TQ 150. The pH of the solutions was measured with a Radiometer PHM83 autocal pH meter. The

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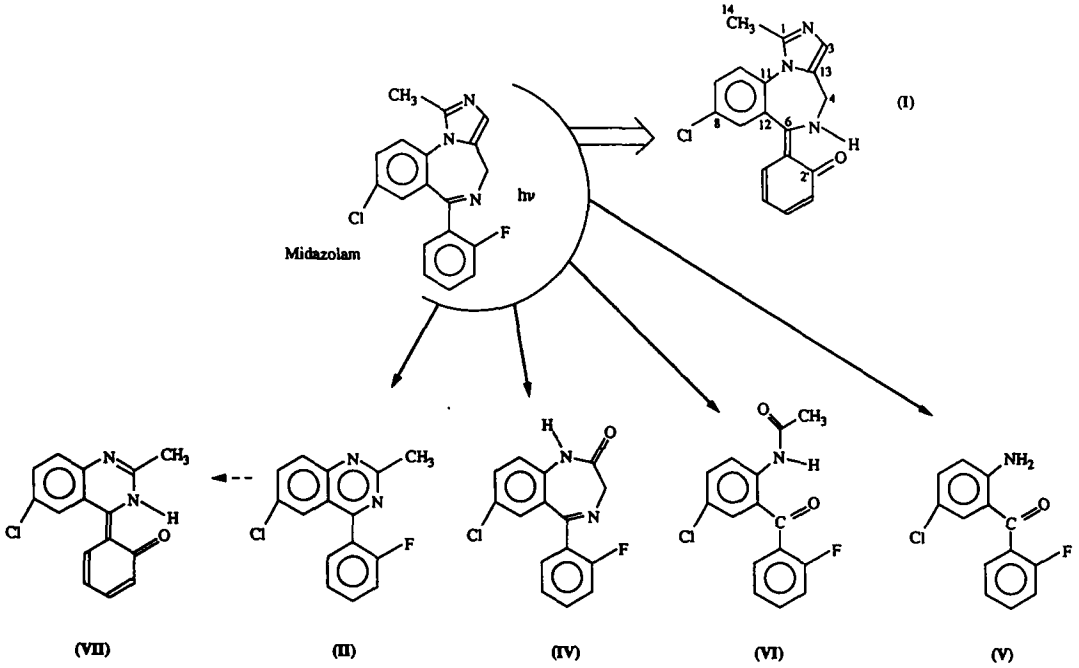

Figure 1
Photodecomposition of midazolam under irradiation from a high-pressure mercury lamp.

melting points were determined with an Electrothermal digital melting point apparatus and are uncorrected. The UV spectra were recorded using a Philips PU 8740 UV–vis spectrophotometer and the IR spectra were recorded with a Unicam SP 1000 infrared spectrometer (KBr disc). The ^{1}H-NMR, ^{13}C-NMR and HMBC spectra of compound I were run on a Varian Unity 500 FT spectrometer, HMBC with spectral widths of 5.5 kHz [F_2] and 26 kHz [F_1] and transformation size 2048 × 1024; 64 scans with 256 hypercomplex increments were recorded and processed by the method of Bax and Marion [5]. The ^{1}H-NMR and ^{13}C-NMR spectra of compound III and the two-dimensional COSY spectra of compound I were recorded on a Varian Gemini 200 FT spectrometer, COSY with spectral widths of 1.5 kHz [F_2] and 1.5 kHz [F_1] and transformation size 1024 × 1024; two-dimensional HETCOR spectra were recorded with spectral widths of 6.6 kHz [F_2] and 1.6 kHz [F_1] and transformation size 1024 × 512. A Jeol JMS-SX 102 mass spectrometer with direct inlet (electron energy 70 eV) was used to obtain the mass spectra. GC–MS spectra were recorded with a quadrupole mass spectrometer (HP 5970A) coupled to an HP 5890 gas chromatograph (ion source 70 eV). The analyses were carried out on a silica capillary column (OV-1) using a temperature program of 140–250°C 10°C at min^{-1}; temperature of the injector and detector was 280°C. TLC experiments were done on precoated 0.25 mm silica gel 60F_{254} aluminium sheets in unsaturated chambers and the spots were detected under UV light (254 nm). The solvent system was toluene–2-propanol (7:3, v/v).

Photodecomposition of midazolam

A 1.25 mM solution of midazolam was prepared in 0.1 M HCl and in buffers pH 2.5, pH 3.3, pH 4.1 and pH 4.9 (Britton–Robinson buffers prepared according to Brezina and Zuman [6]). A 10-ml aliquot of each solution was placed in a clear glass vial 1 cm from the mercury lamp. After 5 h irradiation the solutions were made alkaline with 1 M NaOH and extracted with dichloromethane. The dichloromethane extract was investigated by TLC.

For preparative purposes a 7.5 mM solution of midazolam was prepared in buffer pH 1.8 and a 5 mM solution in buffer pH 3.3. The solutions were transferred to 1-l beakers on a magnetic stirrer and the high pressure mercury lamp was immersed in the solution. The irradiation time was about 25 h.

In the study of the effect of daylight a 3 mM solution of midazolam was prepared in buffer pH 3.9 in a 500-ml Erlenmeyer flask. The flask was placed on a laboratory windowsill with southern exposure for 1.5 years (two summers

and one winter). From time to time the flask was shaken.

Isolation of the main decomposition products

Compounds I, II and III were isolated by flash chromatography [7]. The solution irradiated with the high-pressure mercury lamp was made alkaline with 1 M NaOH and extracted with dichloromethane. The extract was evaporated to dryness; the residue was dissolved in a small amount of the eluent and transferred to a silica gel column (Sorbsil C-60, length 26 cm, i.d. 2 cm). The column was eluted with toluene–2-propanol (7:3, v/v) to obtain a fraction of compound I from irradiated solution pH 1.8 and crude fractions of compounds I and II from irradiated solution pH 3.3. Final purification of compound I was done with a new column, using the same eluent, and the compound was crystallized from diethyl ether. Compound II was purified by flash chromatography, with toluene–2-propanol (8:2, v/v) as the eluent.

A precipitate was formed in the solution exposed to daylight and was filtered off. After TLC evaluation the precipitate was subjected to flash chromatography for the purification of compound II, with toluene–2-propanol (8:2, v/v) used as eluent. After TLC evaluation also the chloroform extract of the filtrate was subjected to flash chromatography for the isolation of compounds II and III; the eluent was toluene–2-propanol (7:3, v/v).

GC–MS analysis

GC–MS spectra allowed the identification of some minor decomposition products, which could not be isolated by flash chromatography. Flash fractions that contained two or more compounds were evaporated to dryness, after which the residue was dissolved in chloroform and injected in the GC–MS instrument.

Photodecomposition products

Compound I. 6-(8-Chloro-1-methyl-4,5-dihydro-2,5,10*b*-triaza-benzo[*e*]azulen-6-ylidene)-cyclohexa-2,4-dienone, yellow crystals from diethyl ether, mol.wt. 323.1, m.p. 146°C. HRMS: $C_{18}H_{14}ON_3Cl$ calculated 323.0827, found 323.0826. UVλ_{max}(EtOH): 330 nm (ϵ = 3600). IRν_{max}: 3470, 3360, 3265, 3050, 2980, 2830, 1600, 1480, 1410, 1310, 1205, 1010, 830, 820, 755, 645 cm^{-1}. ^1H- and ^{13}C-

Table 1
^1H- and ^{13}C-NMR data of compound I, solvent $CDCl_3$, TMS internal standard (s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets)

Number of H or C	δ ^1H (ppm) (multiplicity)	$^3J_{H,H}$ (Hz)	$^2J_{H,H}$ (Hz)	δ ^{13}C (ppm)
1				144.1
2				
3	6.93(s)			124.1
4	5.00(d)		13.3	44.3
	4.08(d)		13.3	
5	13.85(s)			
6				170.5
7	7.54(d)	$J_{7,9}$ = 2.4		131.4
8				132.3
9	7.64(dd)	$J_{9,7}$ = 2.4		131.4
		$J_{9,10}$ = 8.9		
10	7.41(d)	$J_{10,9}$ = 8.6		126.3
11				134.6
12				128.7
13				134.0
14	2.54(s)			14.8
1′				118.7
2′				162.1
3′	7.00(dd)	$J_{3',4'}$ = 8.3		118.3
		$J_{3',5'}$ = 1.2		
4′	7.32(dt)	$J_{4',3'}$ = 8.3		132.8
		$J_{4',5'}$ = 7.1		
		$J_{4',6'}$ = 1.8		
5′	6.78(dt)	$J_{5',3'}$ = 1.2		118.1
		$J_{5',4'}$ = 7.1		
		$J_{5',6'}$ = 8.0		
6′	7.13(dd)	$J_{6',4'}$ = 1.5		131.1
		$J_{6',5'}$ = 8.0		

NMR data in Table 1. MS *m/z* (% rel.int.): 325 (32, M + 2), 323 (88, M), 310 (33), 309 (20), 308 (100, M-15), 295 (10, M-28).

Compound II. 6-Chloro-2-methyl-4-(2-fluorophenyl)-quinazoline, white to yellowish crystals, mol.wt. 272.7, m.p. 126°C. TLC, UV, IR and GC–MS data in accordance with earlier investigations [3].

Compound III. 6-Chloro-2-methyl-4(1H)-quinazolinone, almost white crystals, mol.wt. 194.0. HRMS: $C_9H_7ON_2Cl$ calculated 194.0246, found 194.0246. ^{1}H-NMR ($CDCl_3$, TMS internal standard) δ: 11.40 (broad s, 1 H, NH), 8.26 (d, 1H, H-5, $J_{5,7}$ = 2.6 Hz), 7.74 (dd, 1H, H-7, $J_{7,5}$ = 2.5 Hz, $J_{7,8}$ = 8.6 Hz), 7.65 (d, 1H, H-8, $J_{8,7}$ = 8.8 Hz), 2.61 (s, 3H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$, TMS internal standard) δ: 162.8 (s, C-4), 153.4 (s, C-2), 148.2 (s, C-9), 135.6 (d, C-7), 132.7 (s, C-6), 129.0 (d, C-8), 125.8 (d, C-5), 121.8 (s, C-10), 22.5 (q, CH_3) ppm. MS *m/z* (% rel.int.): 196 (33, M + 2), 195 (12), 194 (100, M), 179 (7), 153 (19), 124 (8), 75 (7), 58 (12), 49 (45), 45 (10).

Compound IV. N-Desalkylflurazepam, 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one. TLC and GC–MS data were in accordance with those reported earlier [3].

Compounds V and VI. 2-Amino-5-chloro-2′-fluorobenzophenone and 2-acetamido-5-chloro-2′-fluorobenzophenone showed the same fragmentation patterns in GC–MS as earlier [3].

Compound VII. 6-(6-Chloro-2-methyl-3H-quinazolin-4-ylidene)-cyclohexa-2,4-dienone. GC–MS *m/z* (% rel.int.): 272 (23), 271 (41), 270 (64), 269 (100), 235 (58), 139 (23), 136 (23), 117 (52), 111 (29), 110 (58), 100 (23), 75 (52), 63 (23), 42 (23).

Results and Discussion

As in ethanolic solution, midazolam was also photolabile in aqueous solution. Figure 2 shows that the pH of the irradiated solution had a great effect on the photodecomposition. In 0.1 M HCl, midazolam was decomposed to just one product, which appeared as a yellow spot on the TLC plate in normal daylight (black spot in the photograph). When the pH of the irradiated solution was increased, numerous decomposition products were formed.

In order to obtain enough of the decomposition products for their isolation, larger amounts of solutions were irradiated: midazolam in buffer pH 1.8 for the isolation of the yellow decomposition product (compound I) and midazolam in buffer pH 3.3 for the isolation and identification of other decomposition products (compounds II, IV, V, VI and VII). Flash chromatography, which is convenient, fast and inexpensive, was used in the isolation.

Compound I was the main decomposition product in all solutions exposed to artificial radiation. The bright yellow of the compound suggested a structural element with a conjugated carbonyl function. The high-resolution mass spectrum of the compound indicated a molecular formula containing one oxygen atom and the MS spectrum showed a molecular peak at 323 *m/z*, which differed by only two mass units from that of midazolam [8]. In addition there was a typical chlorine satellite peak at 325 *m/z*. The base peak of the spectrum was at 308 *m/z* (M-15) suggesting the loss of a CH_3 group from the molecule. Together these findings indicated that the methyl group in the imidazole ring and the chlorine-substituted benzene ring were intact in the decomposition product. One prominent fragment ion in the mass spectrum was at 295 *m/z* (M-28, M-CO) suggesting a structure with a carbonyl or a phenolic OH group.

The IR spectrum of compound I showed three separate bands in the 3500–3200 cm^{-1} region, which could indicate the presence of a NH and/or an OH group with hydrogen bonding. The absorption at 1600 cm^{-1} could indicate a carbonyl-moiety conjugated to other double bonds.

The ^{1}H-NMR spectrum of compound I showed a clear three-proton pattern in the region 7.4–7.7 ppm due to the three protons in the chlorine-substituted aromatic ring in the benzodiazepine moiety. These protons were first assigned according to their couplings to each other. Four other aromatic protons formed another coupling pattern, which was verified by a homonuclear correlation spectrum (COSY, Fig. 3).

Failure to find in one-dimensional ^{1}H- and ^{13}C-NMR spectra couplings of protons or

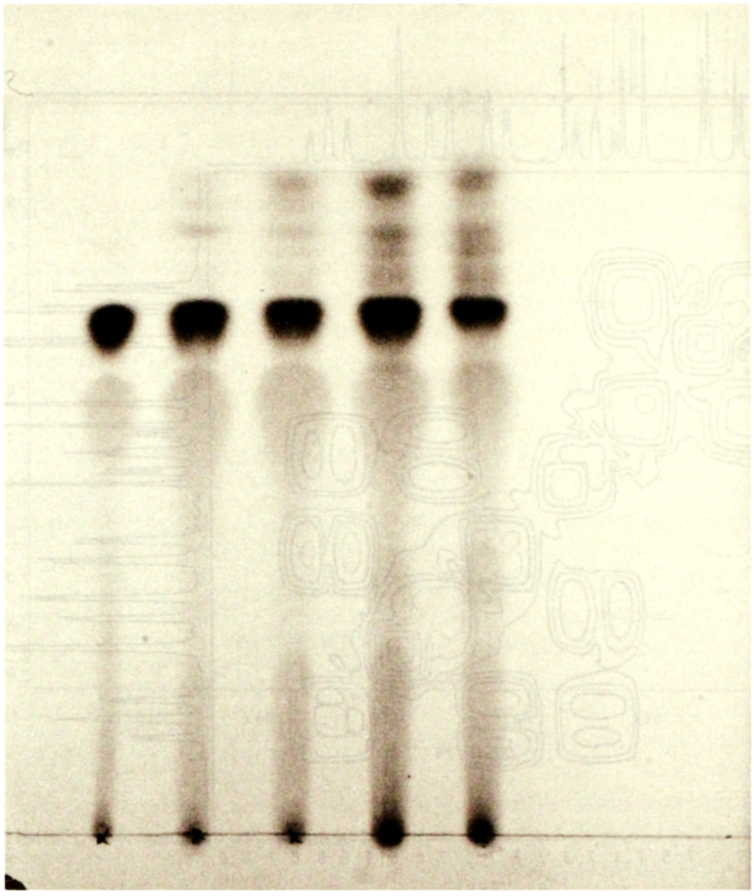

Figure 2
TLC evaluation of dichloromethane extracts of solutions of midazolam in 0.1 M HCl and buffers pH 2.5, 3.3, 4.1 and 4.9 (left to right) irradiated with a high-pressure mercury lamp.

carbon atoms to the fluorine atom led to the conclusion that the fluorine substituent was replaced in the ring by an OH group or a carbonyl moiety.

The heteronuclear correlation (HETCOR) spectrum, which associates the signals from directly bonded ^{13}C and ^{1}H, was used to assign the carbon atoms carrying one or more protons. The eight quaternary carbons of the compound were assigned by the heteronuclear multiple bond coherence technique (HMBC), which reveals the $^{3}J_{CH}$ and possibly the $^{2}J_{CH}$ couplings (Fig. 4). The spectrum showed the quaternary carbon at 170.5 ppm to have one $^{3}J_{CH}$ coupling to CH_2 protons in the benzodiazepine ring and a second $^{3}J_{CH}$ coupling to the H-6′ proton. The carbon at 170.5 ppm must thus be carbon C-6, although no $^{3}J_{CH}$ coupling of this carbon to proton H-7 was observed. The quaternary carbon at 162.1 ppm showed three-bond CH-couplings to protons in 6′ and 4′ positions, which allowed it to be identified as C-2′. The HMBC-NMR spectrum also confirmed the assignment of the protons of the aromatic rings by ^{1}H-NMR.

From the spectral data given above it was clear that the only change in the parent molecule structure was the loss of the fluorine atom at C-2′ and its replacement by an OH group or a C=O group. Replacement by a C=O group would further lead to the rearrangement of the double bonds in the six-membered ring system and to the appearance of a NH proton in the seven-membered ring. To decide between the OH and C=O groups, a ^{1}H-NMR spectrum was run in extra dry dimethylsulphoxide-d_6 to reveal the coupling of the possible exchangeable NH proton. In this spectrum, the resonance of one of the seven-ring CH_2 protons was a doublet of doublets, which indicated a geminal coupling of the proton and further 3.5 Hz coupling to the adjacent NH proton. The substituent at C-2′ is thus a carbonyl group (Fig. 1). Further

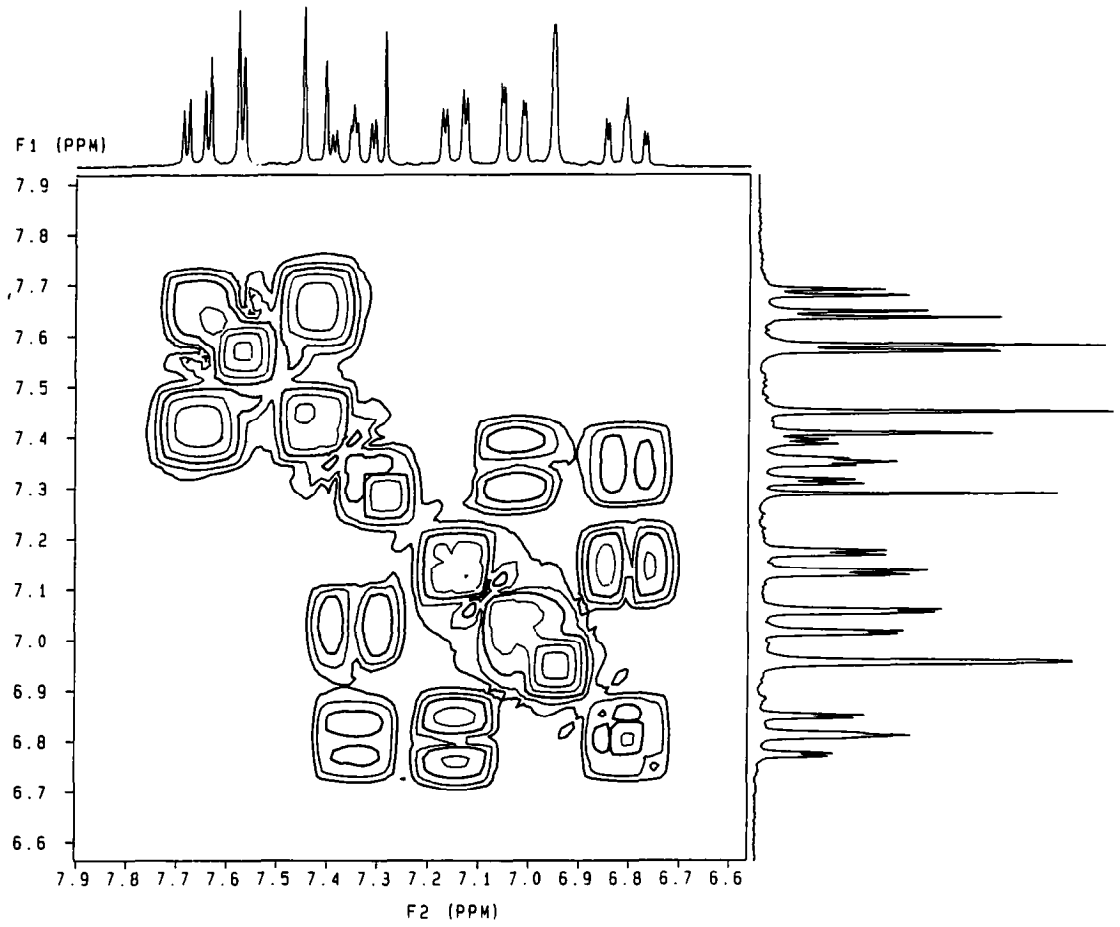

Figure 3
Aromatic region of the two-dimensional 200 MHz COSY NMR spectrum of compound I.

evidence of this structure element was obtained from the UV spectrum of the compound. Compared to the UV spectrum of midazolam, the spectrum of the decomposition product showed a new maximum at 330 nm due to the conjugated carbonyl function (Fig. 5).

Compound II (Fig. 1) was the main decomposition product in the solution (pH 3.9) exposed to daylight and it precipitated in the solution during the experiment. The same product was present in considerable amount in buffer pH 3.3 irradiated with the high-pressure mercury lamp. The compound was isolated in both experiments and its structure was verified to be 6-chloro-2-methyl-4-(2-fluorophenyl)-quinazoline by comparing the TLC, IR, UV and GC–MS data with data of the reference compound isolated earlier [3].

A novel spot was detected in TLC of the chloroform extract of the solution exposed to daylight. The high resolution mass spectrum of the isolated compound corresponded to the molecular formula $C_9H_7ON_2Cl$. The ^{1}H-NMR spectrum, in turn, showed three aromatic protons, a singlet of three protons at 2.6 ppm and a broad one-proton signal at 11.4 ppm, which could indicate an amide proton. The ^{13}C-NMR and DEPT spectra revealed the existence of five quaternary carbons, three CH carbons and a methyl carbon. From these it was concluded that the seven-membered ring had contracted to a six-membered ring and the fluorine-substituted benzene ring had been cleaved off. On the basis of spectral data, compound III was assumed to be 6-chloro-2-methyl-4(1H)-quinazolinone (Fig. 6).

N-Desalkylflurazepam (compound IV) and the benzophenone derivatives, 2-amino-5-chloro-2′-fluorobenzophenone (compound V) and 2-acetamido-5-chloro-2′-fluorobenzophenone (compound VI) (Fig. 1), were present in small amounts in both the artificially irradiated solution and the solution exposed to daylight. The compounds were identified by comparing the GC–MS data with those re-

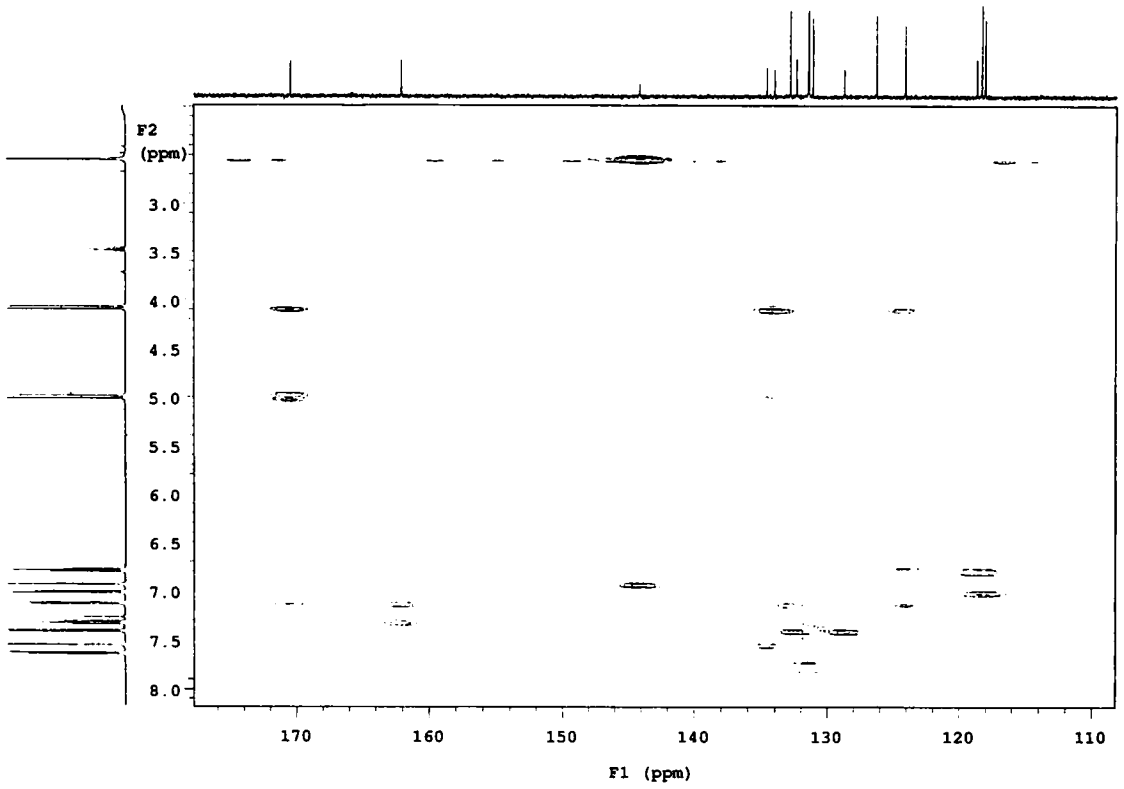

Figure 4
Two-dimensional HMBC-NMR spectrum of compound I.

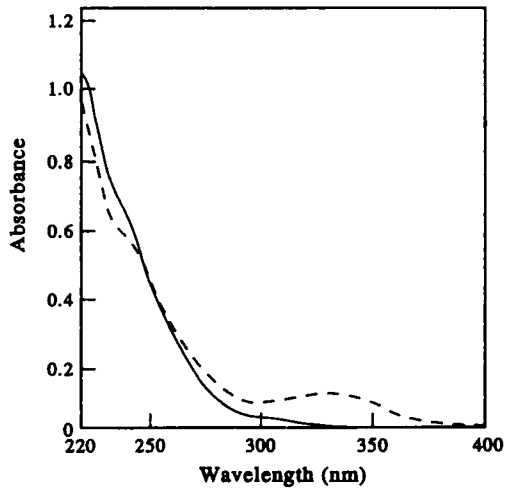

Figure 5
The UV spectra of compound I (dashed line) and midazolam (straight line) in ethanol.

corded earlier [3]. In addition to these compounds, the structure of one further decomposition product, compound VII, was deduced from the GC–MS spectrum. The fragmentation pattern of compound VII, present in the solution irradiated with the high-pressure

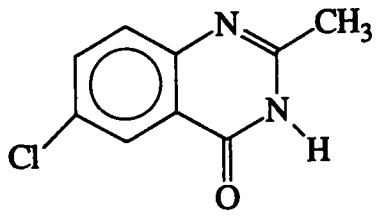

Figure 6
Structure of compound III, 6-chloro-2-methyl-4H(1H)-quinazolinone.

mercury lamp, was similar to that of compound II, 6-chloro-2-methyl-4-(2-fluorophenyl)-quinazoline. The molecular peak at 270 *m/z* differed from that of compound II by only two mass units. A chlorine satellite and a prominent M-1 peak were observed, and a fragment ion at *m/z* M-35 indicated the loss of the chlorine atom, just as in the MS of compound II. As well, some of the same fragments (136, 111, 110, 75) were observed. The molecular weight and the fragmentation together led to the conclusion that compound VII was 6-(6-chloro-2-methyl-3H-quinazolin-4-ylidene)-

cyclohexa-2,4-dienone (Fig. 1), probably formed as a decomposition product from the quinazoline (compound II), in the same way that compound I was formed from midazolam.

Four products detected in the photodecomposition of midazolam in ethanolic solutions were also detected in aqueous solution: a quinazoline derivative, N-desalkylflurazepam and two benzophenone derivatives. In addition, two new photodecomposition products, 6-(8-chloro-1-methyl-4,5-dihydro-2,5,10*b*-triaza-benzo[*e*]azulen-6-ylidene)-cyclohexa-2,4-dienone (compound I) and 6-(6-chloro-2-methyl-3H-quinazolin-4-ylidene)-cyclohexa-2,4-dienone (compound VII), were formed in aqueous solutions irradiated with a high-pressure mercury lamp. These two decomposition products contain one common feature: the loss of the fluorine atom and its replacement by a carbonyl group. Numerous reports have been published on photoinduced dehalogenation with loss of chlorine, bromine or iodine, but it appears that the loss of fluorine in photodecomposition has not been reported earlier.

One new photodecomposition product, 6-chloro-2-methyl-4(1H)-quinazolinone (compound III), was formed in the solution exposed to daylight. This decomposition product was not present in the solutions exposed to artificial radiation, which can be due to the slight difference in the pH of the solutions (pH 3.9 and pH 3.3) or to the radiation source, which remains to be studied.

It has been reported for some benzodiazepines that the wavelength of the radiation does not determine the character but only the concentration of the products [9, 10]. The photodecomposition of midazolam was independent of the irradiation source in ethanolic solution [3], but not in aqueous solution. The two radiation sources led to the formation of diverse photodecomposition products; the major decomposition product formed under irradiation of a high-pressure mercury lamp was not observed in solutions exposed to normal daylight. As well as the light source and solvent, the pH of the aqueous solution influences the photodecomposition; in irradiated solutions at pH <2 only one product was formed, but an increase in the pH of the solution resulted in an increase in the number of products.

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