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Short Communication

Structure elucidation of 6-chloro-2-methyl-4(1H)-quinazolinone, a photodecomposition product of midazolam*

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Introduction

The photodecomposition of midazolam was recently studied in aqueous solutions both under irradiation from a high-pressure mercury lamp and exposed to normal daylight [1]. The main decomposition product in daylight exposed solution was 6-chloro-2-methyl-4-(2-fluorophenyl)-quinazoline. In this solution TLC experiments also showed another major decomposition product, which was isolated by flash chromatography. On the basis of conventional ^{1}H- and ^{13}C-NMR, and mass spectra, the structure of the compound was assumed to be 6-chloro-2-methyl-4(1H)-quinazolinone (Fig. 1). The aim of the present study was to verify the structure of this compound by IR analysis and by using two-dimensional NMR techniques: homonuclear correlation spectrum (COSY), heteronuclear correlation spectrum (HMQC) and heteronuclear multiple bond coherence spectrum (HMBC).

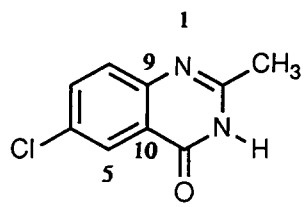

Figure 1
Structure of 6-chloro-2-methyl-4(1H)-quinazolinone.

Experimental

Materials

Midazolam was kindly supplied by Hoffman-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. 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 [2]. 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 IR spectrum was recorded with a Unicam SP3-200 infrared spectrometer (KBr disc). A Jeol JMS-SX 102 mass spectrometer with direct inlet

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(electron energy 70 eV) was used to obtain the mass spectra.

NMR spectra were produced on a Bruker AMX-400 spectrometer. The NMR sample was prepared by dissolving about 2 mg of the compound in 0.8 ml of solvent (CD_3OD or $CDCl_3$, TMS internal standard). The measuring temperature was 29.5°C and measuring conditions were as follows. ^{1}H-NMR in $CDCl_3$: spectral width 5051 Hz, number of scans 32 and pulse width 6.2 μs (45°), ^{1}H-NMR in CD_3OD: spectral width 4854 Hz, number of scans 128 and pulse width 6.2 μs (45°). ^{13}C{1H}NMR in $CDCl_3$ and in CD_3OD: spectral width 23810 Hz, number of scans 16384 and pulse width 2.6 μs (33°). DEPT-135-NMR in $CDCl_3$: spectral width 23810 Hz, number of scans 8192 and pulse widths 7.0 μs (13C, 90°) and 12.4 μs (1H, 90°). DQF-filtered COSY in CD_3OD: F_2 and F_1 spectral width 2673.8 Hz, number of scans 32, number of points (F_2) 2048 and (F_1) 256 (zero filled to 1024) and pulse width 12.4 μs (1H, 90°), sine bell window function was applied in both dimensions. HMQC in CD_3OD with TPPI phase incrementation. F_2: spectral width 3546 Hz, number of scans 384, number of points 2048 (zero filled to 4096), pulse width 7.2 μs (1H, 90°). F_1: spectral width 14590 Hz, number of points 128 (zero filled to 1024), pulse width 12.0 μs (13C, 90°). 50% shifted sine square bell window function was applied in both dimensions. HMBC in CD_3OD. F_2: spectral width 3937 Hz, number of scans 640, number of points 2048 (zero filled to 4096), pulse width 7.2 μs (1H, 90°). F_1: spectral width 20628 Hz, number of points 256 (zero filled to 1024), pulse width 12.0 μs (13C, 90°). A 50% shifted sine square bell window function was applied in both dimensions.

TLC experiments were performed on precoated 0.25-mm silica gel $60F_{254}$ aluminium sheets and the spots were detected under UV light (254 nm). Three different solvent systems were used in unsaturated chambers: toluene–2-propanol (7:3, v/v) (A), chloroform–acetone (9:1, v/v) (B) and ethyl acetate–methanol–diethylamine (9:0.5:0.5, v/v/v) (C). Length of the run was 8 cm.

Photodecomposition studies

Midazolam in buffer pH 3.9 (Britton–Robinson buffer according to Brezina and Zuman [3]) was exposed to normal daylight as reported before [1]. A 3-mM solution of midazolam in buffers pH 3.3 and 1.9 was also prepared, and 10-ml portions of these solutions were exposed to normal daylight on a southern windowsill in 10-ml clear glass vials for 1 year. A 1.8-mM solution of 6-chloro-2-methyl-4(2-fluorophenyl)-quinazoline in buffer pH 3.3 was similarly exposed to daylight during the same time. From time to time during the experiment the vials were shaken.

Isolation of 6-chloro-2-methyl-4(1H)-quinazolinone

Isolation of the compound from solution pH 3.9 has been reported [1] and the same method was used for solution pH 3.3.

Characterization of 6-chloro-2-methyl-4(1H)-quinazolinone

HRMS: $C_9H_7ON_2Cl$ calculated 194.0246, found 194.0246. MS *m/z* (% rel.int.): 196 (33, M + 2), 195 (12), 194 (100, M), 179 (7), 153 (19), 124 (8), 86 (38), 84 (52), 75 (7), 58 (12), 51 (14), 49 (45), 45 (10). IRν_{max}: 3180, 3080, 3040, 2930, 2895, 1680 (C = O), 1630, 1610, 1470, 1300, 830, 815 cm^{-1}. TLC R_f 0.57 (A), 0.16 (B), 0.30 (C). ^{1}H-NMR data in Table 1 and ^{13}C-NMR data in Table 2.

Results and Discussion

The main decomposition product in midazolam solutions exposed to daylight was 6-chloro-2-methyl-4-(2-fluorophenyl)-quinazoline, which mostly precipitated during the experiment. Among other photoproducts, TLC experiments of each exposed solution (pH 1.9–3.9) revealed one decomposition product in considerable amount, which was separated by flash chromatography. The proposed structure of this product was 6-chloro-2-methyl-4(1H)-quinazolinone (Fig. 1) based on a mass spectrum, and conventional ^{1}H- and ^{13}C-NMR spectra of the compound. In the aromatic region of the ^{1}H-NMR spectrum there were only signals of three protons, and the COSY spectrum was used to assign these signals to protons in the same benzene ring. The signals of the protons showed no further splitting indicating the absence of fluorine in the same ring as the protons. In addition to the aromatic protons, the spectrum showed a three-proton singlet at 2.56 ppm, which corresponded to the methyl group attached in position 2 of the molecule. The chemical shift and the multiplicity of this signal are indicative

Table 1
^{1}H-NMR data of 6-chloro-2-methyl-4(1H)-quinazolinone (s = singlet, d = doublet, dd = doublet of doublets)

Number of H	Solvent $CDCl_3$ δ^1H (ppm)	$J_{H,H}$ (Hz)	Solvent CD_3OD δ^1H (ppm)	$J_{H,H}$ (Hz)
3	11.24 (s)		—	
5	8.23 (d)	$^4J_{5,7} = 2.4$	8.11 (dd)	$^4J_{5,7} = 2.5$ $^5J_{5,8} = 0.4$
7	7.70 (dd)	$^3J_{7,8} = 8.7$ $^4J_{7,5} = 2.4$	7.76 (dd)	$^3J_{7,8} = 8.8$ $^4J_{7,5} = 2.5$
8	7.62 (d)	$^3J_{8,7} = 8.7$	7.59 (d)	$^3J_{8,7} = 8.7$
11	2.56 (s)		2.44 (s)	

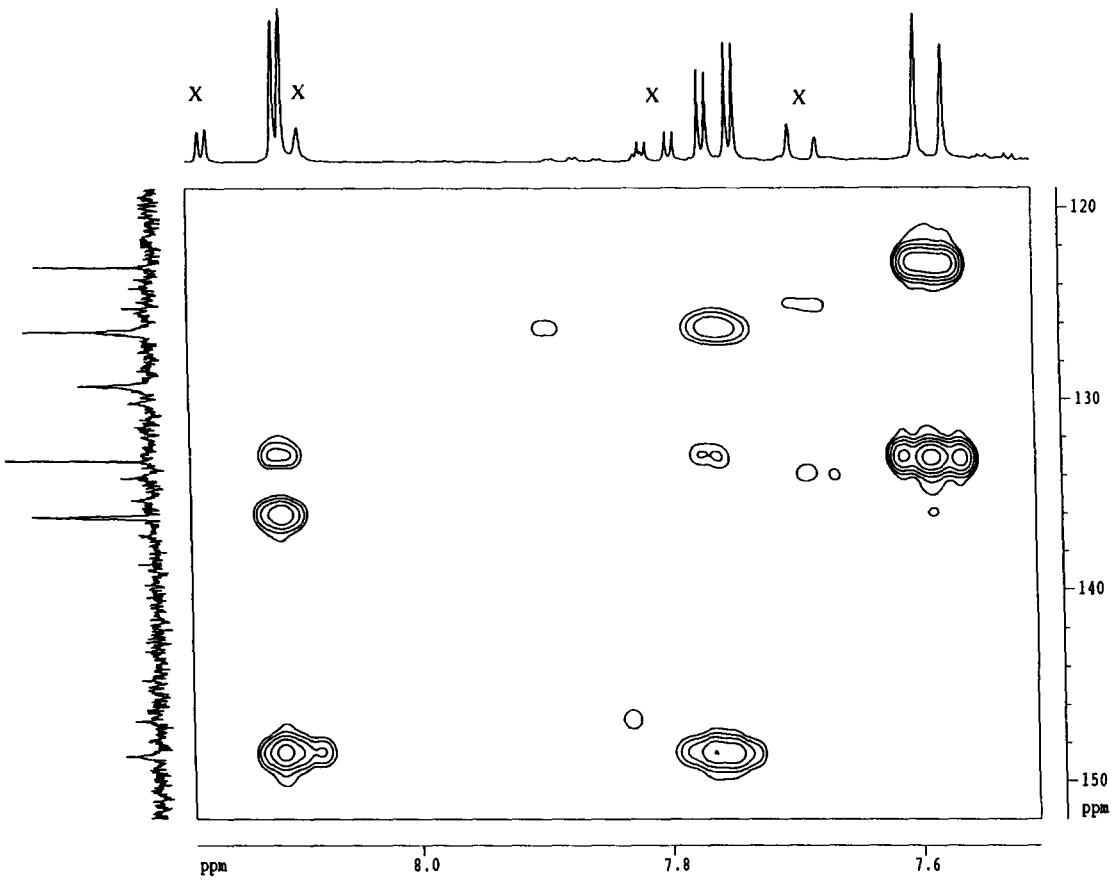

Figure 2
Aromatic region of the two-dimensional HMBC-NMR spectrum of 6-chloro-2-methyl-4(1H)-quinazolinone (X = impurity).

Table 2
^{13}C-NMR data of 6-chloro-2-methyl-4(1H)-quinazolinone

Number of C	Solvent $CDCl_3$ δ^{13}C (ppm)	Solvent CD_3OD δ^{13}C (ppm)
2	153.2	156.9
4	162.8	163.2
5	125.7	126.4
6	132.4	133.2
7	135.4	136.1
8	128.8	129.2
9	147.9	148.7
10	121.4	123.0
11	22.2	21.5

of a methyl group attached to a quaternary carbon atom in a heterocyclic ring. In the ^{1}H-NMR spectrum taken in $CDCl_3$ there was further a broad signal at 11.24 ppm, which may be due to an OH- or more likely to a NH-proton. The absence of this signal in CD_3OD supported the interpretation. Since the IR spectrum showed no absorption bands in the region of OH-groups, and there were absorptions between 3180 and 3080 cm^{-1} and in the amide carbonyl region (1680 cm^{-1}), the structure element of a secondary amide was accepted.

The assignment of the three methine carbons was based on a heteronuclear correlation spectrum (HMQC) associating the signals of directly bonded ^{13}C and ^{1}H. The quaternary carbons showed $^{3}J_{CH}$ (and possibly $^{2}J_{CH}$) couplings as cross-peaks in a heteronuclear multiple bond coherence spectrum (HMBC), which was used in the assignment of these carbons (Fig. 2). C-2 (156.9 ppm) had no correlations with the aromatic protons, only a two-bond coupling with methyl protons and the carbonyl carbon C-4 (163.2 ppm) had coupling to H-5 (absent in Fig. 2). As expected, chlorine-substituted C-6 had cross peaks in the spectrum with H-5, H-7 and H-8. Quaternary carbon in position 9 exhibited two three-bond couplings (H-5 and H-7) and one two-bond coupling with H-8, while C-10 showed only one $^{3}J_{CH}$ (H-8). The chemical shifts of carbons showed excellent correlation with those reported for a related compound, 2-methyl-3-propyl-4-quinazolone [4].

The structure of 6-chloro-2-methyl-4(1H)-quinazolinone suggested it to be a further decomposition product of 6-chloro-2-methyl-4-(2-fluorophenyl)-quinazoline, which is the main product in the photodecomposition of midazolam in normal daylight. To determine whether this was so, quinazoline solutions were exposed to daylight. TLC experiments showed the appearance of the quinazolinone and a trace of another decomposition product. When quinazoline solutions were exposed to a high-pressure mercury lamp, no quinazolinone was revealed by TLC.

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