International Journal of Pharmaceutics, 3 (1979) 205–220

PHOTOCHEMICAL DECOMPOSITION OF 1,4-BENZODIAZEPINES. CHLORDIAZEPOXIDE *

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(Received September 7th, 1978)
(Revised version received February 5th, 1979)
(Accepted February 28th, 1979)

SUMMARY

Irradiation of 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-oxide(I, chlordiazepoxide; the active compound of Librium Roche) with UV light provides a mixture of 1-benzoyl-7-chloro-1,2-dihydro-3-methylaminoquinoxaline(III) and 9-chloro-5-methylamino-2-phenyl-4H-benzo[g]-1,3,6-oxadiazocine(IV), while 7-chloro-2-methylamino-4,5-epoxy-4,5-dihydro-5-phenyl-3H-1,4-benzodiazepine(II, oxaziridine) is isolated as an intermediate. The quinoxaline and benzoxadiazocine derivatives (the photoisomers III and IV) decompose photochemically: the former to 2-methylamino-6-chloroquinoxaline(V), 2-methylamino-5-benzoyl-6-chloroquinoxaline(VI) and 2-methylamino-3-phenyl-6-chloroquinoxaline(VII), and the latter to 9-hydroxy-7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine(VIII).

INTRODUCTION

Chlordiazepoxide belongs to the group of 1,4-benzodiazepines, which are used frequently as hypnotic or as tranquillizer (Sternbach et al., 1968). It is remarkable that the photochemical activity of this group of compounds has received little attention, especially as some 1,4-benzodiazepines, including chlordiazepoxide, proved to be phototoxic (Magnus, 1976). The phototoxicity of chlordiazepoxide was established by clinical observations (Fitzpatrick et al., 1974), while the injurious influence of light was also demonstrated with albino mice (Ison et al., 1969; Ljunggren et al., 1978), bacteria (Daniels, 1965) and yeast (Ison et al., 1969). When, after administration of chlordiazepoxide, albino mice are exposed to UV light, erythema, dermatitis or oedema and venous

* Dedicated to Prof. Dr. E. Havinga on the occasion of his 70th brithday.

distention develops (Ison et al., 1969). Because glass, which only transmits light above 300 nm, is used as filter, long-wave UV light is responsible for the occurrence of phototoxicity.

Phototoxicity occurs if an unwanted biological effect appears after administration of chlordiazepoxide in combination with light, thus after a photochemical reaction. The molecule in an excited electronic energy level, obtained after absorption of light, initiates this reaction (Calvert et al., 1966; Cox et al., 1971). A number of processes, indicated in Fig. 1 by a, b and c, can be initiated by the molecule in this excited state. These processes, which may be highly important for photobiology, can be distinguished in: (a) the dissociation of the molecule into radicals or an isomerization reaction (decomposition products); (b) a reaction with another molecule and (c) a process, by which the molecule (donor) transfers the energy to another molecule (acceptor) through which the acceptor reaches an excited state. With this excited acceptor molecule, the same type of processes as mentioned for the donor molecule can commence. The decomposition products may lead to the demonstrated unwanted biological effect, because they have unwanted physiological properties, eventually after reaction with body compounds (Beijersbergen van Henegouwen et al., 1978; Kockevar et al., 1977; Musajo et al., 1972) or because they can easily transfer energy to body compounds (Rahn et al., 1974). However, it is also possible that the drug itself is the molecule that is able to transfer energy to or react with body compounds, resulting in unwanted effects.

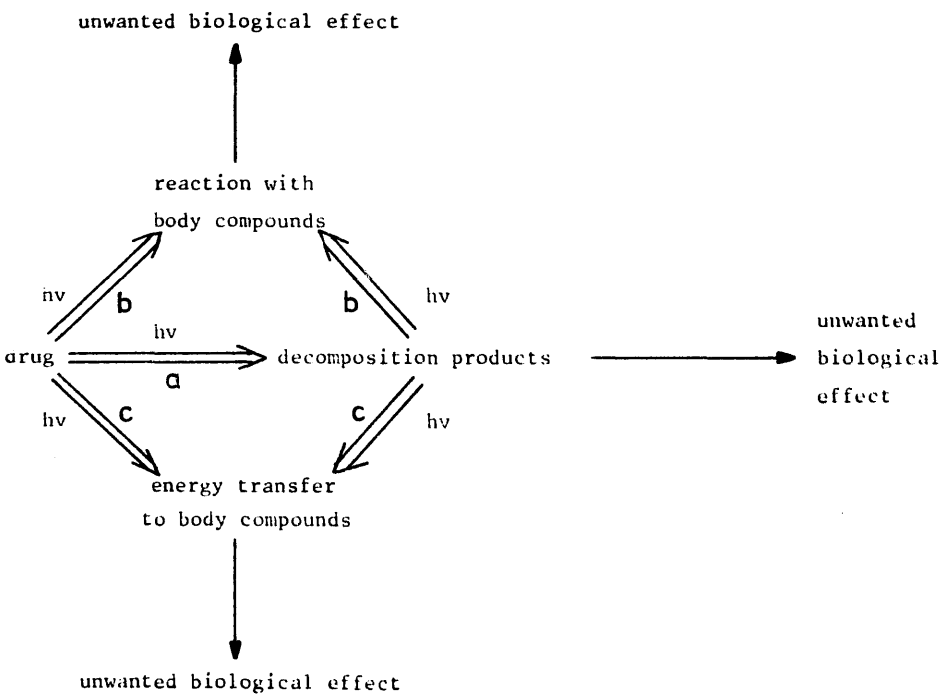

Fig. 1. Possible processes (a, b and c) in biological systems initiated by drug molecules after absorption of light energy.

The foregoing prompted us to investigate the photochemical decomposition of the phototoxic chlordiazepoxide. Some attention has already been paid to the photochemical decomposition of this compound. Under the influence of light chlordiazepoxide decomposes into two photoisomers, a quinoxaline and a benzoxadiazocine derivative (Field et al., 1968), while an oxaziridine can be isolated as an intermediate (Sternbach et al., 1962). However, it is significant to identify the structures of decomposition products which are present beside these two photoisomers in an irradiation mixture of chlordiazepoxide, because the appearance of phototoxicity may be the result of energy transfer (Fig. 1: process c) in which case compounds of low concentration may be involved. The possibility also exists that certain decomposition products present in low concentration may have a biological activity, which importantly exceeds that of the drug itself. Finally, it may not be excluded that a compound, under certain circumstances present as a main product, is a by-product under other circumstances; the reverse may also occur.

MATERIALS AND METHODS

Chlordiazepoxide was obtained from Hoffmann-LaRoche and used as such in our study. The quality of the solvents was 'chemically pure' and they were further purified by distillation. For column chromatography 'Kieselgel 60 GF_{254} for thin layer chromatography (Merck)' was used, the preparative thin layer chromatography was performed with 'DC-Fertigplatten Kieselgel 60 F_{254} (Merck)' and thin layer chromatography with 'DC-Plastikrolle Kieselgel 60 F_{254} (Merck)'.

The NMR spectra were recorded with a Jeol-JNM-PS-100 and a Jeol-PFT-100 spectrometer (Funds for the Fourier transform attachment were made available by the Netherlands Organization for the Advancement of Pure Research – Z.W.O.). Spectral parameters of the aromatic protons H_A, H_B, H_C and H_D of compounds III, V, VI and VII were obtained by computer simulation using a Jeol-NMR simulation program (EC-computer system). The resulting chemical shifts were reported with an accuracy of 0.001 ppm. Coupling constants were estimated to be accurate to 0.1 Hz. $Eu(Fod)_3$-d_{27} was used as a shift reagent, while $CDCl_3$, C_6D_6, DMSO-d_6 and D_2O were used as solvents. Chemical shifts were represented in ppm and tetramethylsilane was used as a standard.

The mass spectra were recorded with an AEI MS-902. The m/e of the molecular ions were determined with the peak matching method. The difference between the experimental and calculated values was not more than 4–5 ppm in all cases.

The UV spectra were recorded with a Perkin Elmer EPS-3T spectrometer (isopropanol and methanol as solvents).

A Rayonet Photochemical Reactor (RPR-208) was used for the irradiation experiments in which a cylindrical quartz vessel (diameter 6.5 cm), filled with a solution of chlordiazepoxide in methanol, was placed centrally and surrounded either by 8 lamps of 254 nm (RUL-2537 A), or by 8 lamps of 300 nm (RUL-3000 A) or by 5 lamps of 350 nm (RUL-3500 A). During the irradiation the solution was stirred with a magnetic stirrer.

For the conversion of the chlordiazepoxide (I) into the oxaziridine (II), a solution (10^{-3} M) was irradiated with light of 350 nm (irradiation time 20 min). The reaction mix-

ture contained practically pure oxaziridine, which could be obtained after removal of the solvent under reduced pressure. Qualitative analysis of the reaction mixture was performed with reversed phase thin layer chromatography, with ethanol as mobile phase. The percentage of the undecomposed chlordiazepoxide was determined with HPLC (Spectra Physics SP 3500 B liquid chromatograph with Spectra Physics SP 8200 UV detector (254 nm)). The column (SS) dimensions were: o.d. = 6.2 mm, i.d. = 2.8 mm, and length 100 mm. Packing was of Lichrosorb RP2-d_{50} (=6 μm) (Merck). As mobile phase methanol : water (70 : 30) was used. The analysis was performed at room temperature. The flow rate was 0.4 ml/min and the chart speed was 10 mm/min.

The 1,2-dihydroquinoxaline (III) and the benzoxadiazocine derivative (IV) were obtained in a high yield by irradiating a solution of 1.67×10^{-3} M chlordiazepoxide (I) with light of 300 nm for 3.5 h. Besides the photoisomers III and IV, the irradiation mixture also contained decomposition products of these photoisomers. After irradiation the solvent was removed under reduced pressure and the residue dissolved in 5–10 ml ethyl acetate : hexane (90 : 10). For the separation of the compounds column chromatography was applied. Length and diameter of the column were 23 and 3 cm, respectively. Because of the small pellet size of the silica gel, pressure was needed (7 cm Hg, nitrogen). The chromatographic separation was started with 500 ml ethyl acetate : hexane (90 : 10) as eluent. Then the eluent was changed to ethyl acetate. After 200 ml the chromatographic separation was continued with 600 ml ethyl acetate : ethanol (96%) (90 : 10). During the chromatographic separation effluent fractions of 15 ml were collected and analyzed by thin layer chromatography with ethyl acetate as mobile phase in a saturated tank (the R_f values obtained are mentioned below for the pure, separated compounds only). Based on the chromatographic results, 5 effluent fractions were obtained of which fractions A and B were mixtures.

Fraction A contained the oxaziridine (II) and three quinoxaline derivatives (V, VI and VII). Fraction B contained the benzoxadiazocine derivative (IV) and an isomer (IX). Fraction C contained 1,2-dihydroquinoxaline (III; $R_f = 0.31$). Fraction D contained a 1,4-benzodiazepine derivative (VIII; $R_f = 0.25$) and fraction E contained undecomposed chlordiazepoxide (I; $R_f = 0.16$). Of the main components, compound III was obtained pure after evaporation of the solvent of fraction C, but compound IV was mixed with its isomer IX. To obtain pure compound IV, the solvent from fraction B was evaporated and the residue separated by column chromatography with ethyl acetate : chloroform (2 : 1) as eluent. Length and diameter of the column were 23 and 3 cm, respectively (pressure was applied, as above). Effluent fractions of 10 ml were collected and analyzed by thin layer chromatography with ethyl acetate : chloroform (2 : 1) as mobile phase. In this way the benzoxadiazocine derivative (IV, for this solvent system $R_f = 0.19$, for the ethyl-acetate system $R_f = 0.40$) was obtained pure.

The different decomposition compounds formed from the photoisomers III and IV; namely the compounds V–IX can be obtained in larger amounts and in a less complicated mixture by separate irradiation of the photoisomers III and IV.

By irradiation of 10^{-3} M 1,2-dihydroquinoxaline (III) in methanol with light of 254 nm during 7.5 h the quinoxaline derivatives (V, VI and VII) were obtained. The solvent was removed under reduced pressure and the residue dissolved in 5–10 ml of ethyl acetate : chloroform (1 : 1). The compounds were isolated with column chromatography.

Length and diameter of the column were 24 and 3 cm, respectively (pressure was applied as above). Ethyl acetate : chloroform (1 : 1, 900 ml) was used as eluent. Effluent fractions of 10 ml were collected and analyzed by thin layer chromatography with ethyl acetate : chloroform (2 : 1) as mobile phase. In this way the quinoxaline derivatives (V, $R_f = 0.33$; VI, $R_f = 0.41$; VII, $R_f = 0.52$) were obtained in a pure state. The column chromatography was followed with a Mineralight UVSL 25, fitted with lamp J_{204} (360 nm), with which the fluorescent bands of the quinoxaline derivatives V, VI and VII were visualized.

By irradiation of 10^{-3} M benzoxadiazocine derivative (IV) in methanol with light of 254 nm during 7.5 h the compounds VIII and IX were obtained. For the separation of the compounds (IV, VIII and IX) the same procedure was used as described for the resolution of fraction B. The effluent fractions were analyzed by thin layer chromatography with ethyl acetate : chloroform (2 : 1) as mobile phase (compound IV, $R_f = 0.19$; compound VIII, $R_f = 0.10$; compound IX; $R_f = 0.24$).

After hydrolysis of compound VIII in alkaline medium (1 N NaOH), the mixture was separated by means of preparative thin layer chromatography on silica gel with benzene : ethyl acetate (8 : 2) as mobile phase, thus resulting in pure benzophenone derivative X.

RESULTS AND DISCUSSION

The irradiations of chlordiazepoxide were performed with light of 350, 300 and 254 nm. The results prove that the decomposition scheme of chlordiazepoxide is not affected by the wavelength of the light. In an irradiation mixture only the concentration of the formed products is affected by the wavelength used. This fact has been used to obtain sufficient amounts of the different decomposition products.

For instance, to obtain an optimum amount of oxaziridine (II; Fig. 2), chlordiazepoxide was irradiated with light of 350 nm, which is the preferable wavelength in this case. From the UV spectra (Fig. 3) of chlordiazepoxide and oxaziridine it is clear that the ratio of the absorptions of these compounds, respectively, is the largest at 350 nm. Consequently, the product, which itself is photolabile, is, in comparison to chlordiazepoxide, exposed to light as little as possible.

The oxaziridine (II) is quite easily reconverted into chlordiazepoxide with dilute acid (Sternbach et al., 1962). This conversion also takes place during thin layer chromatography on silica gel. For this reason reversed phase thin layer chromatography is used, by which method the presence of the oxaziridine along chlordiazepoxide can easily be proved. By means of HPLC it was found that after 20 min irradiation only 3% chlordiazepoxide remained. The UV spectrum (isopropanol) of the irradiated solution was then in agreement with that of the oxaziridine (Sternbach et al., 1962).

In the mass spectrum the difference between the chlordiazepoxide and oxaziridine isomers finds expression in the intensity of the fragment m/e 105 ($C_7H_5O^+$), which is higher for the latter compound. Beside the fragment m/e 282 ($M^{+\cdot}-OH^{\cdot}$), which is present for both compounds, the spectrum of the oxaziridine shows the fragment m e 194 ($M^{+\cdot}-C_7H_5O^{\cdot}$), while the spectrum of chlordiazepoxide shows the fragment m/e 205 (m/e $282-C_6H_5^{\cdot}$).

The NMR spectrum of the oxaziridine in $CDCl_3$ (Fig. 4) shows a doublet at 2.70 ppm

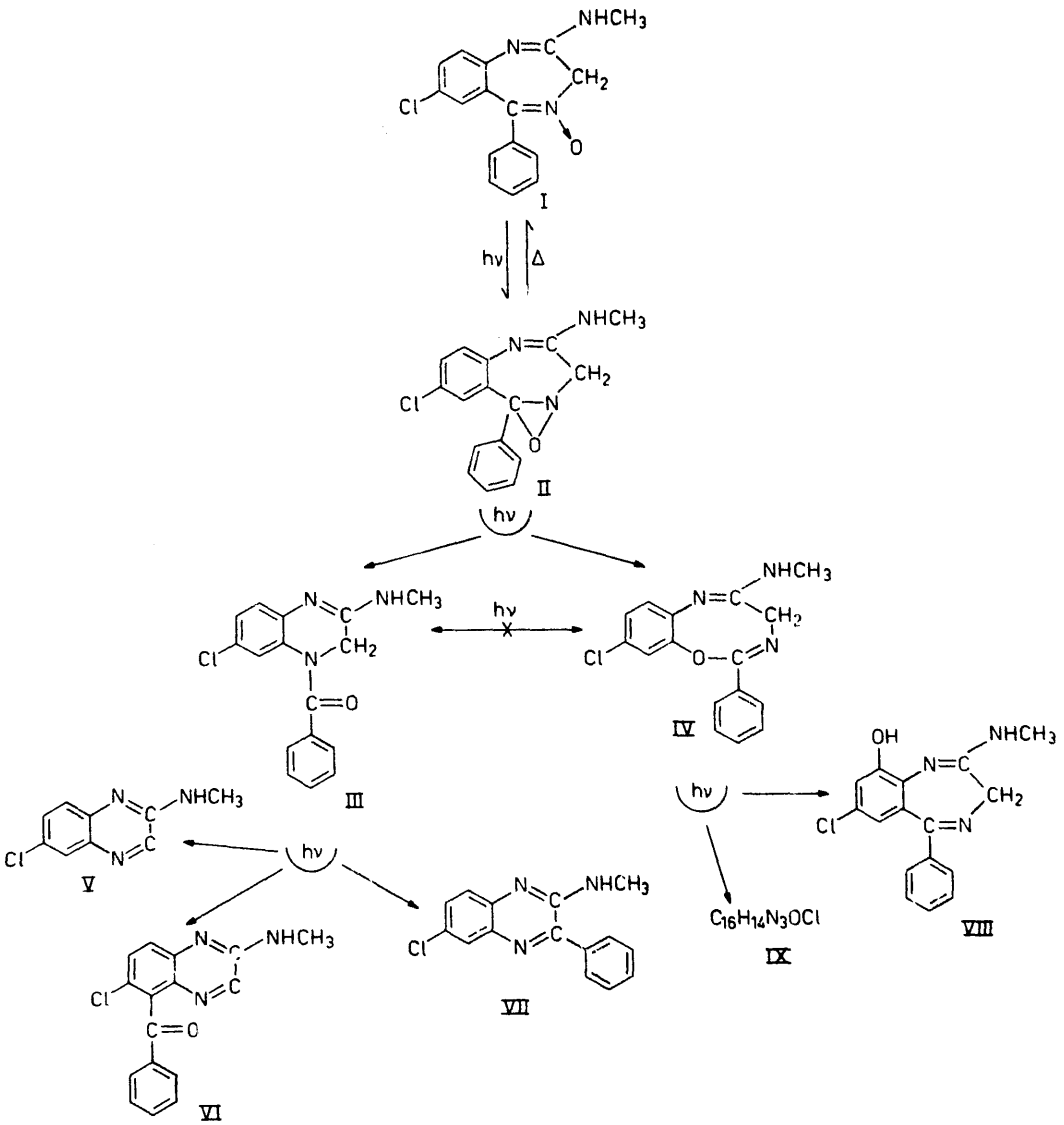

Fig. 2. Photochemical decomposition products of chlordiazepoxide (I).

(3 protons, J = 4.9 Hz), a broad signal at 5.40 ppm (N–H proton), a multiplet at 7.00–7.42 ppm (8 protons) and a pair of doublets (AB-spectrum) arising from the methylene protons. The NMR spectrum of chlordiazepoxide in $CDCl_3$ (Fig. 5) shows a doublet at 2.86 ppm (3 protons, J = 4.9 Hz), a multiplet at 6.95–7.70 ppm (8 protons) and two broad signals at 4.20 ppm and 4.60 ppm arising from the methylene protons. For the identification of the oxaziridine it is also possible to make use of NMR temperature dependency of chlordiazepoxide (Nuhn et al., 1967; Bley et al., 1968). By lowering the temperature of a chlordiazepoxide solution to −15°C, the broad signals split into a pair of doublets at 4.10 ppm and 4.70 ppm. (AB-spectrum) while by raising the temperature

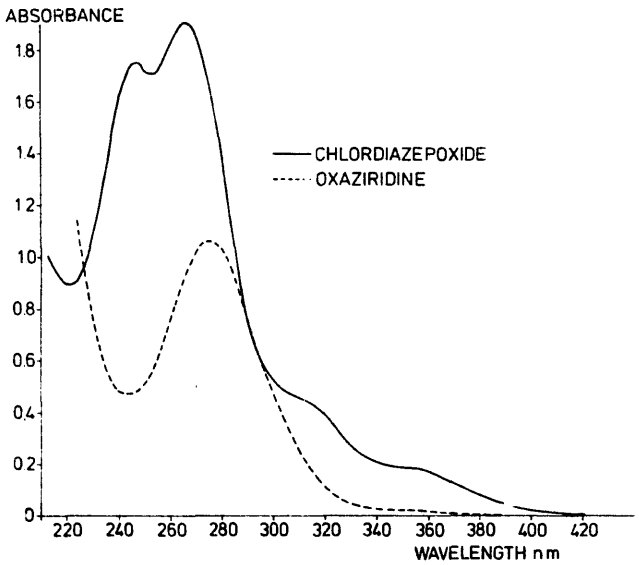

Fig. 3. UV spectra of chlordiazepoxide (I) and the oxaziridine (II) in methanol.

(60°C) the broad signals sharpen into a single peak at 4.35 ppm. By raising the temperature of a solution of the oxaziridine in $CDCl_3$ to 60°C no alteration in the NMR spectrum is observed. When the temperature is raised to 130°C (solvent DMSO-d_6) and cooled to room temperature, the spectrum of chlordiazepoxide is obtained. This thermal

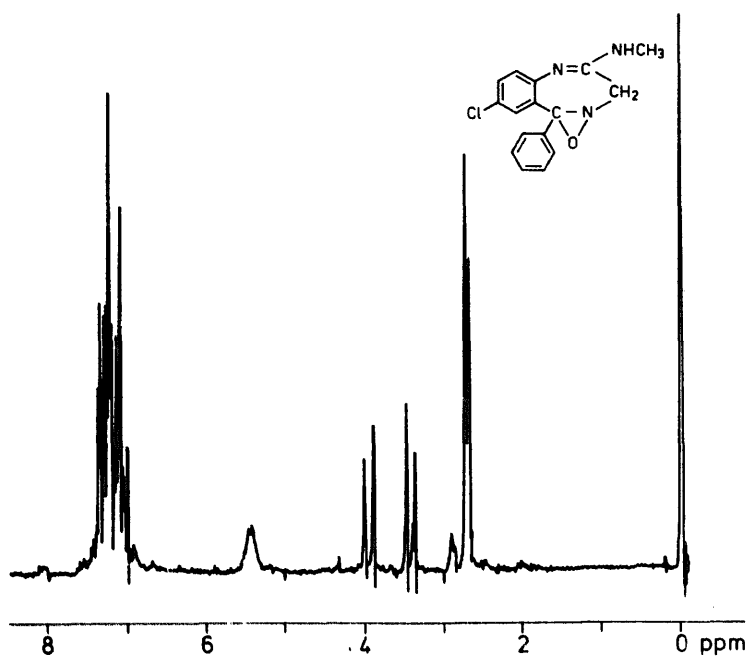

Fig. 4. NMR spectrum of the oxaziridine (II) in $CDCl_3$.

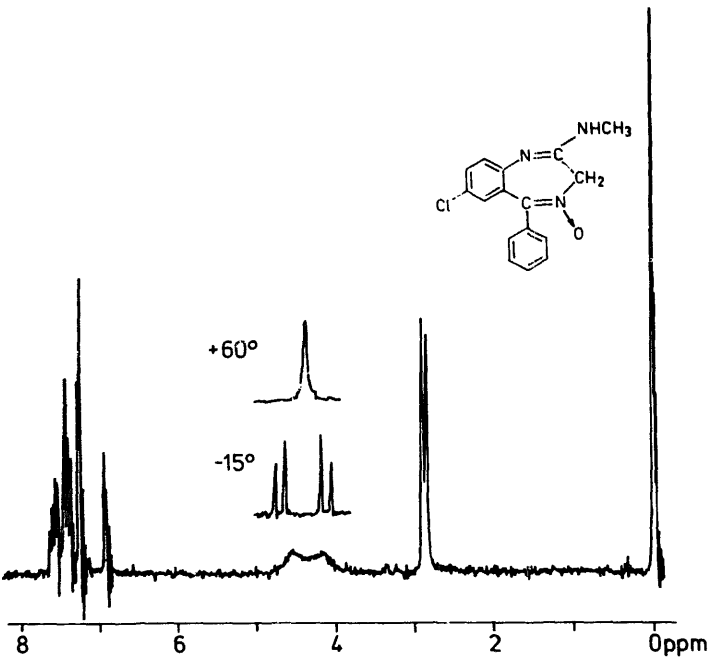

Fig. 5. NMR spectrum of chlordiazepoxide (I) in $CDCl_3$.

conversion of the oxaziridine into the chlordiazepoxide is in agreement with the literature (Sternbach et al., 1962).

The two photoisomers, the 1,2-dihydroquinoxaline (III) and the benzoxadiazocine derivative (IV), are formed by irradiation of the oxaziridine (II; Fig. 2). Beside these main products compounds V to IX are present in minor amounts. This situation is identical to that obtained by irradiation of chlordiazepoxide. To obtain the photoisomers III and IV in optimum amounts, the irradiation is performed with light of 300 nm. At this wavelength the oxaziridine is easily converted, while the light absorption of the photoisomers is much less than with irradiation at 254 nm (Field et al., 1968).

The UV and mass spectra of both compounds (III and IV) and the NMR spectrum of compound IV are in complete agreement with the structure of 1-benzoyl-7-chloro-1,2-dihydro-3-methylaminoquinoxaline and 9-chloro-5-methylamino-2-phenyl-4H-benzo[g]-1,3,6-oxadiazocine respectively (Sternbach et al., 1968). The NMR spectrum of compound III in $CDCl_3$ shows a singlet at 3.00 ppm (3 protons, $N-CH_3$), a singlet at 4.42 ppm (2 protons, $-CH_2-$) and a multiplet at 6.37–7.40 ppm (8 protons). These data are in agreement with the 1,2-dihydroquinoxaline structure mentioned above. Further a coupling pattern (an approximation of a first-order spectrum) is observable in the aromatic multiplet, which is a result of the substitution pattern of the 3 protons (H_A, H_B and H_C) attached to the condensed benzene ring (Fig. 6). The correct spectral parameters of the aromatic protons H_A, H_B and H_C, reproduced in Table 1, are obtained by computer simulation. This spectrum is reproduced in Fig. 6.

An internal conversion under influence of light, resulting in a change of compound III into IV or the reverse, could not be detected.

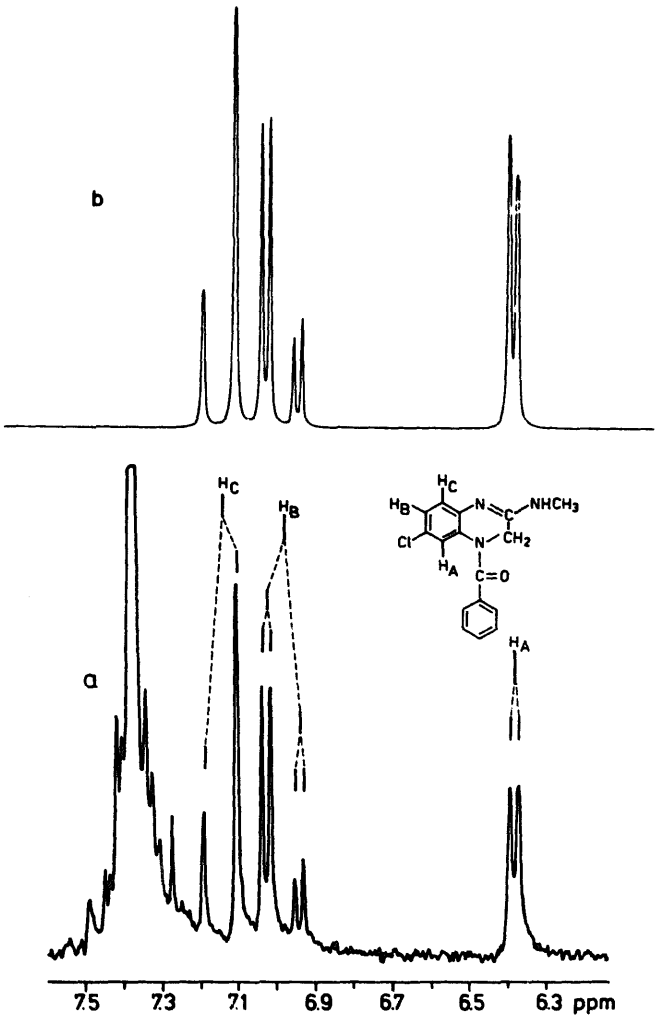

Fig. 6. NMR spectrum (aromatic multiplet) of 1,2-dihydroquinoxaline (III). a: experimental; b: simulated.

TABLE 1

CHEMICAL SHIFTS (ppm) AND COUPLING CONSTANTS (Hz) OBTAINED BY COMPUTER SIMULATION

Compound	H_A	H_B	H_C	H_D	J_{AB}	J_{AC}	J_{BC}	Solvent
III	6.380	6.985	7.125	–	2.2	<0.3	8.3	$CDCl_3$
V	7.834	7.495	7.623	8.165	2.3	0.5	9.0	$CDCl_3$
VI	–	7.569	7.714	8.024	–	–	9.0	$CDCl_3$
VI	–	7.473	7.661	7.794	–	–	9.0	$CDCl_3/C_6D_6$
VII	7.831	7.239	7.634	–	2.3	<0.2	9.1	C_6D_6

On irradiation of the quinoxaline derivative (III) compounds V, VI and VII are formed (Fig. 2). The empirical formulae, determined by mass spectrometry, are $C_9H_8N_3Cl$ (compound V), $C_{16}H_{12}N_3OCl$ (compound VI) and $C_{15}H_{12}N_3Cl$ (compound VII). By determining the number of double bounds and rings from these empirical formulae and by taking into account the structure of the starting material, the presumption existed that the heterocyclic ring changed into an aromatic ring, thus the compounds could be derivatives of quinoxaline ($C_8H_6N_2$). That the compounds V, VI and VII can be structurally related also becomes obvious from the NMR spectra (solvent $CDCl_3$), which show a large mutual agreement (Table 2). The presence of the methylamino group in the respective compounds appears from a broad signal between 5.12 and 5.45 ppm (N–H proton) and a doublet between 3.05 and 3.12 ppm (3 protons, J = 5 Hz), which, in agreement with the NMR spectra of chlordiazepoxide (doublet at 2.86 ppm) and the oxaziridine (doublet at 2.70 ppm), is attributed to a methyl group attached to a nitrogen atom. Concerning the aromatic multiplet, compounds V and VI are distinguished from compound VII by a singlet (1 proton) at low field. This proton is not observed with compound VII.

For a definite clarification of the structures, use was made of NMR data from the aromatic multiplet and the observed coupling pattern of the protons H_A, H_B and H_C of the quinoxaline derivative III (see Fig. 6). For compound V these signals in the aromatic multiplet can be attributed to 4 protons, of which 3 show the pattern of ortho- and meta-coupled protons, in agreement with the structure of 2-methylamino-6-chloroquinoxaline. The chemical shifts of these protons in relation to each other are in agreement with those of quinoxaline and of 6-chloroquinoxaline (Brignell et al., 1967). The fourth proton in the aromatic multiplet is visible at lower field as a singlet. This proton is attached to the C-3 position (H_D). That its signal is shifted upfield with respect to the signal of the H_D proton of quinoxaline (8.71 ppm – 8.17 ppm) is explained by the presence of an electron donating group; see 2-methyl-quinoxaline (Matsuura et al., 1963). The spectral parameters, obtained by computer simulation, are reproduced in Table 1.

The NMR spectrum of compound VI again shows a singlet at low field, which, in analogy to compound V, is assumed to be caused by the presence of a proton at the C-3 position (H_D). The solubility in organic solvents, as well as the volatility, which is sufficient to obtain a mass spectrum, are in contradiction with the presence of a quarternary nitrogen atom at the C-4 position in the molecule. For this reason, it was assumed that removal of the benzoyl group had taken place. That the benzoyl group is attached to the C-5 position is obvious from NMR data. In the aromatic multiplet two protons

TABLE 2

NMR DATA OF THE QUINOXALINE DERIVATIVES IN $CDCl_3$

	Aromatic protons	N–H	N–CH_3
2-Methylamino-6-chloroquinoxaline (V)	7.43–7.84 (m) and 8.17 (s)	5.12	3.10 (d), J=5
2-Methylamino-5-benzoyl-6-chloroquinoxaline (VI)	7.41–7.87 (m) and 8.02 (s)	5.22	3.05 (d), J=5
2-Methylamino-3-phenyl-6-chloroquinoxaline (VII)	7.51–7.76 (m)	5.45	3.12 (d), J=5

can be observed, of which the signals are split into a doublet. This indicates that these protons are orientated ortho in relation to each other on the aromatic ring (C-7 and C-8 positions), which is supported by a coupling constant of 9 Hz (also found between the two ortho hydrogen atoms H_B and H_C in 2-methylamino-6-chloroquinoxaline). The fact that the signals of these protons are not split further means that the meta position (C-5) lacks a hydrogen atom. That the signal splitting (J = 9 Hz) is only caused by the coupling of the protons H_B and H_C can be seen when C_6D_6 is added to the solvent $CDCl_3$, in which case an exclusive shifting of the split signals is observed, while the coupling constant of 9 Hz is maintained. Because the NMR spectrum is not a first-order spectrum (just like the spectra of the compounds V and VII), the correct spectral parameters are obtained by computer simulation (see Table 1).

In the NMR spectrum of compound VII the singlet at low field is missing. Since this singlet can be seen for 2-methylaminoquinoxaline and 2-methylamino-5-benzoyl-6-chloroquinoxaline, but also for quinoxaline, 6-chloroquinoxaline and quinoxaline-5-acetate (Brignell et al., 1967), it is assumed that a proton at the C-3 position is absent. In the NMR spectrum of compound VII only aromatic protons are observed, beside the protons of the methylamino group. Considering that the empirical formula of compound VII ($C_{15}H_{12}N_3Cl$) differs from the related compound V ($C_9H_8N_3Cl$) with a fragment C_6H_4, the supposition is justified that compound VII is 2-methylamino-3-phenyl-6-chloroquinoxaline. However, in the aromatic multiplet the coupling pattern of the C-5, C-7 and C-8 protons (H_A, H_B and H_C) is not observed (solvent $CDCl_3$). Also, with addition of $Eu(FOD)_3$-d_{27} as shift reagent, the coupling pattern is not visible. However, with C_6D_6 as solvent, a coupling pattern can be seen that may be a result of the substitution pattern of the 3 protons, if one of the two doublets of proton H_B (caused by coupling with proton H_A) is located under the signal of the solvent. That this supposition is correct is confirmed by studying the change in chemical shift of proton H_B in the NMR spectrum of 2-methylamino-6-chloroquinoxaline, by which the solvent ($CDCl_3$) is enriched dropwise with C_6D_6. In C_6D_6 the spectra of compound VII and 2-methylamino-6-chloroquinoxaline, regarding the signal of proton H_B, becomes identical. The obtained data confirm that compound VII is 2-methylamino-3-phenyl-6-chloroquinoxaline. The spectral parameters, obtained by computer simulation, are reproduced in Table 1.

On irradiation of the benzoxadiazocine derivative (IV) two isomers (VIII and IX) are also formed (Fig. 2). The NMR spectrum of compound VIII in $CDCl_3$ (Fig. 7) shows a temperature dependence just as for chlordiazepoxide (Fig. 5). Beside a doublet at 2.76 ppm (3 protons, J = 5 Hz), a broad signal at 6.04 ppm arising from a N–H proton and an aromatic multiplet between 6.73 and 7.64 ppm (7 protons), the spectrum shows at 27°C a broad signal at 4.11 ppm. However, by lowering the temperature to –45°C, two doublets appear at 3.57 ppm and 4.86 ppm. At 45°C the broad signal sharpens to a singlet at 4.10 ppm. An analogous temperature dependence of the NMR spectrum, as observed with chlordiazepoxide, points to the presence of a methylene group in the molecule. In the aromatic multiplet two doublets (AB spectrum) are visible, which show a coupling of 2.3 Hz ($CDCl_3$) or 2.4 Hz (DMSO-d_6), thus indicating a meta coupling. The C-6 and C-8 protons of chlordiazepoxide also have a coupling of 2.4 Hz (DMSO-d_6).

The temperature dependence of the signal of the methylene protons of compound VIII, in analogy to the 1,4-benzodiazepines (Linscheid et al., 1967; Sadée, 1969; Sadée et

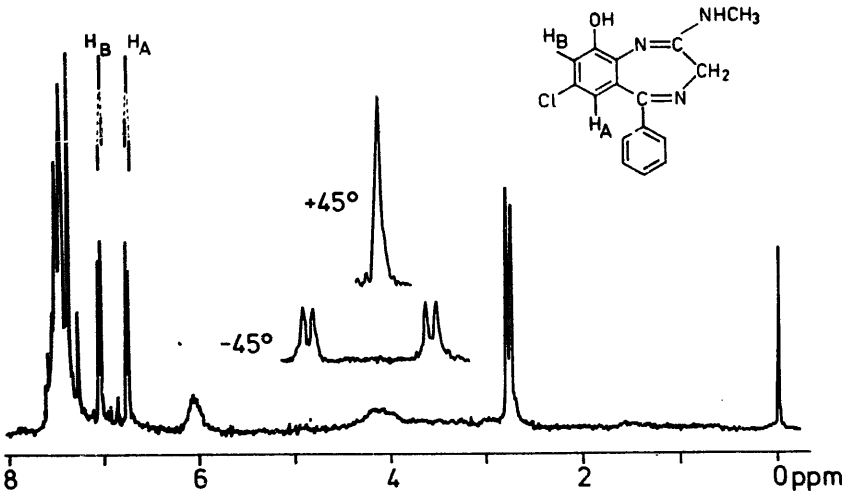

Fig. 7. NMR spectrum of 9-hydroxy-7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine (VIII) in $CDCl_3$.

al., 1973; Sarrazin et al., 1975), and the presence of two protons, which have a coupling of 2.4 Hz (meta), are in agreement with a 1,4-benzodiazepine structure of compound VIII, of which the C-9 position must be substituted. Considering the empirical formula ($C_{16}H_{14}N_3OCl$) showing the presence of an oxygen atom, the substituent could be a hydrogen group. That this is true, becomes evident after hydrolysis of compound VIII by which a benzophenone derivative (X) is formed (Fig. 8). The NMR spectrum shows two meta-coupled protons (J = 2.4 Hz) and is identical with the NMR data of 2-amino-3-hydroxy-5-chlorobenzophenone (Garcia et al., 1972). Also the UV spectrum and mass spectrum ($M^{+\cdot}$: $C_{13}H_{10}NO_2Cl = 247$) are completely in agreement with 2-amino-3-hydroxy-5-chlorobenzophenone (Yasumura et al., 1971) for which in the mass spectrum the specific fragmentation pattern (m/e 77 – $C_6H_5^+$; m/e 105 – $C_7H_5O^+$; m/e 142 – $C_6H_5NOCl^+$; m/e 170 – $C_7H_5NO_2Cl^+$) is found for a benzophenone derivative. Likewise, this benzophenone formation is in agreement with the hydrolysis of 1,4-benzodiazepines (Mayer et al., 1972, 1974; Maulding et al., 1975; Han et al., 1976, 1977), by which the corresponding benzophenone derivative is formed. This means that compound VIII is 9-hydroxy-7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine. When supposing that in compound VIII the chemical shift of the protons H_A and H_B with regard to each other is the same as for chlordiazepoxide, it also means that the chemical shift of the protons H_A and H_B of compound VIII with regard to TMS are opposite to those of compound X. This is in analogy to chlordiazepoxide and 2-amino-5-chlorobenzophenone. The designation of the protons H_A and H_B is simple in the NMR spectra of the last mentioned compounds, because the ortho-coupling between protons H_B and H_C is present.

The structure of compound IX ($C_{16}H_{14}N_3OCl = 299$) is not yet elucidated. By means of NMR spectrometry it is already established that the methylamino group and also a methylene group is present in the molecule. However, more data, including for instance [^{13}C]NMR, are necessary for a definite structure elucidation.

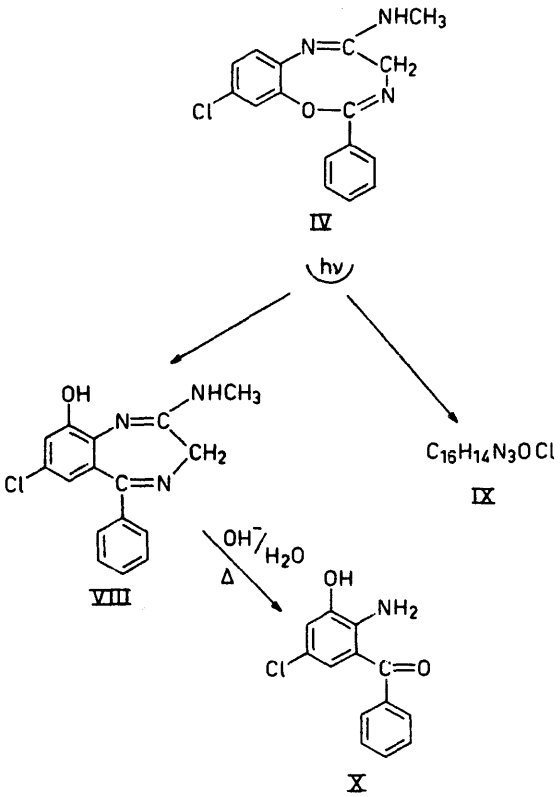

Fig. 8. Photochemical decomposition products of the benzoxadiazocine derivative (IV) and a benzophenone derivative (X), obtained by hydrolysis of compound VIII.

In stability research a distinction can usually be made between the thermochemical and photochemical decomposition concerning the character of the products formed. This is certainly valid for several 1,4-benzodiazepines. In contrast with the thermochemical decomposition (Mayer et al., 1972, 1974; Maulding et al., 1975; Han et al., 1976, 1977), where, dependent on the pH, two products are formed, namely a benzophenone and a quinolone derivative, the photochemical decomposition progresses entirely different. Even when the photochemical decomposition of chlordiazepoxide is compared with structurally related compounds such as nitrazepam (Roth et al., 1969, 1973) and diazepam (Cornelissen et al., 1978) no resemblance appears to exist.

As the photochemical activity of the 1,4-benzodiazepines differs so much mutually it can be expected that the unwanted physiological processes will differ as well, certainly when they are caused by decomposition products. The purpose of this study was to investigate the photochemical decomposition of chlordiazepoxide, because decomposition products, which can be formed through interaction with light after administration of the drug to the body, may be cause of phototoxicity (Beijersbergen van Henegouwen, 1977).

To what extent and in what way the decomposition products of chlordiazepoxide (Fig. 2) are responsible for the demonstrated phototoxicity, have to be proved by further experiments. Not only the unwanted biological activity caused by decomposition

products, but also processes by which transfer of energy (process c) play a part, have to be considered. At this moment only some general remarks can be given on the activity of the decomposition products formed from chlordiazepoxide. The oxaziridine (II) resembles an epoxide, which type of compound is always suspect for its possible irreversible reaction with DNA, RNA and protein (Oesch, 1976). Two decomposition products, the 1,2-dihydroquinoxaline (III) and the benzoxadiazocine derivative (IV), have antiinflammatory, anticonvulsant and antibacterial properties (Field et al., 1971). To what extent the quinoxaline derivatives V, VI and VII have their own biological activities has to be investigated. However, it can be expected that these compounds are active, because the biological activity of great numbers of other quinoxaline derivatives are known. Of quinoxaline itself it is also known that on irradiation with light of longer wavelength (>300 nm), it can act as a photosensitizer (Takeshita et al., 1972). Whether the quinoxaline derivatives, formed during photochemical decomposition of chlordiazepoxide, have the same properties has still to be investigated. If this is the case, they could cause unwanted activity by transfer of the absorbed energy.

Not only chlordiazepoxide itself, but also metabolites may be responsible for the appearance of phototoxicity. Demoxepam and N-desmethylchlordiazepoxide are structurally related to chlordiazepoxide and have an N-oxide function as well (Hackman et al., 1974). Demoxepam also decomposes photochemically to form an oxaziridine (Ning et al., 1975). The possibility of oxaziridine formation by desmethylchlordiazepoxide, is being studied.

ACKNOWLEDGEMENTS

We thank Drs. W. Luijten for the recording of mass spectra and Mr. C. Erkelens for the recording and the dicussion of NMR spectra. Miss A. Schriever and Mr. R. Dekkers are thanked for their contribution to the experimental work. The gift of chlordiazepoxide from Hoffman-LaRoche, Basle, is gratefully acknowledged.

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