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PHOTOCHEMICAL DECOMPOSITION OF 1,4-BENZODIAZEPINES. DIAZEPAM *

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SUMMARY

On irradiation of 7-chloro-1,3-dihydro-1-methyl-5-phenyl-(2H)-1,4-benzodiazepin-2-one (diazepam) with UV light of 254 nm benzophenones, 4-phenylquinazolinones, 4-phenylquinazolines and glycine are formed.

INTRODUCTION

The photochemical activity of 1,4-benzodiazepines has received little attention up to the present. This is strange in view of the fact that some 1,4-benzodiazepines, like chlordiazepoxide (Ison and Davis, 1969; Magnus, 1976) and nitrazepam (Magnus, 1976), are phototoxic. Phototoxicity occurs when after administration of the drug, irradiation with light causes an unwanted biological effect. As causes of phototoxicity a number of types of photochemical activity can be mentioned, such as the creation of the very reactive singlet oxygen, when the energy absorbed by the drug is transferred to oxygen. It is also possible that the drug decomposes photochemically under the skin, in which case compounds are formed with undesirable biological effects. Some attention has been paid to the photochemical decomposition of chlordiazepoxide (Sternbach et al., 1962; Field and Sternbach, 1968) and nitrazepam (Roth and Adomeit, 1969, 1973). Diazepam also appears to be photochemically unstable and although it is therapeutically frequently used and its structure is related to chlordiazepoxide and nitrazepam, it is remarkable that the photochemical decomposition of diazepam has never been studied.

MATERIALS AND METHODS

Diazepam is obtained from Hoffman La Roche and used as such in our study. The quality of the solvents is 'chemically pure' and they are further purified by distillation. For column chromatography 'Kieselgel 60 GF_{254} for thin layer chromatography (Merck)' is used, while the preparative thin layer chromatography is performed with 'DC-Fertig-

* Active component of Valium Roche.

platten Kieselgel 60 F_{254} (Merck)'. The NMR spectra are recorded with a Jeol-JNM-PS-100; solvent $CDCl_3$, while $EU(FOD)_3$-d_{27} is used as a shift reagent. The mass spectra are recorded with a AEI MS-902. The m/e of the molecular ions are 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 are recorded with a Perkin Elmer EPS-3T spectrometer (methanol as solvent). A Rayonet Photochemical Reactor (RPR-208) is used for the irradiation experiment, in which a cylindrical quartz vessel (diameter 6.5 cm), filled with a solution of diazepam in methanol (0.85×10^{-3} M), is placed centrically and surrounded by eight lamps of 254 nm (Rul-2537 A); during the irradiation time of 17 hr the solution is stirred with a magnetic stirrer.

After irradiation the solvent is removed under reduced pressure and the residue dissolved in 5–10 ml benzene : ethanol (96%) (100 : 20). For the first separation of the compounds column chromatography is applied. Length and diameter of the column are 14 and 3 cm, respectively. The chromatographic separation is started with 240 ml benzene : ethanol (96%) (100 : 20) as eluent. Then the eluent is changed to benzene : ethanol (96%) (80 : 20). After 200 ml the chromatographic separation is continued with 200 ml benzene : ethanol (96%) (50 : 50) and at last with 350 ml ethanol (96%). Five effluent fractions are obtained of which fractions A, B and E are mixtures.

Fraction A contains benzophenone derivatives and a part of the undecomposed diazepam. By means of preparative thin layer chromatography on silica with benzene as mobile phase this mixture is further separated, resulting in the benzophenone derivatives I, II and III in a pure form.

Fraction B contains quinazolinone derivatives as well as the remainder of the undecomposed diazepam. After evaporation of the eluent the residue is separated by column chromatography with benzene : ethanol (96%) (100 : 5) as eluent. Length and diameter of the column are 14 and 3 cm, respectively. In this way the quinazolinone derivatives V and VI are obtained in a pure state. Fractions C and D (the quinazoline derivaties VII and VIII, respectively) are eluted in a pure state.

The last fraction E contains a third quinazoline derivative with its analogue without chlorine of which the structures are not yet elucidated.

After the irradiation, when the solvent is removed and the residue is dissolved in chloroform : water (2 : 1) mixture, the presence of the amino acid glycine (compound IV) is demonstrated in the water layer by means of thin layer chromatography on silica with butanol : water : acetic acid (12 : 5 : 3) as mobile phase and with ninhydrin as coloring agent.

RESULTS AND DISCUSSION

From a solution of diazepam, irradiated with UV light, the compounds given in Fig. 1 are isolated and identified. They belong to the following types of compounds: benzophenones, 4-phenylquinazolinones, 4-phenylquinazolines and glycine. The percentage of the different types of compounds formed depends on the solvent used, the concentration of the solution, the irradiation time, the intensity and the wavelength of the light. Although phototoxicity is usually caused by sunlight, which contains at sea level mainly wavelengths above 290 nm, the use of light of 254 nm is justified. Concerning diazepam,

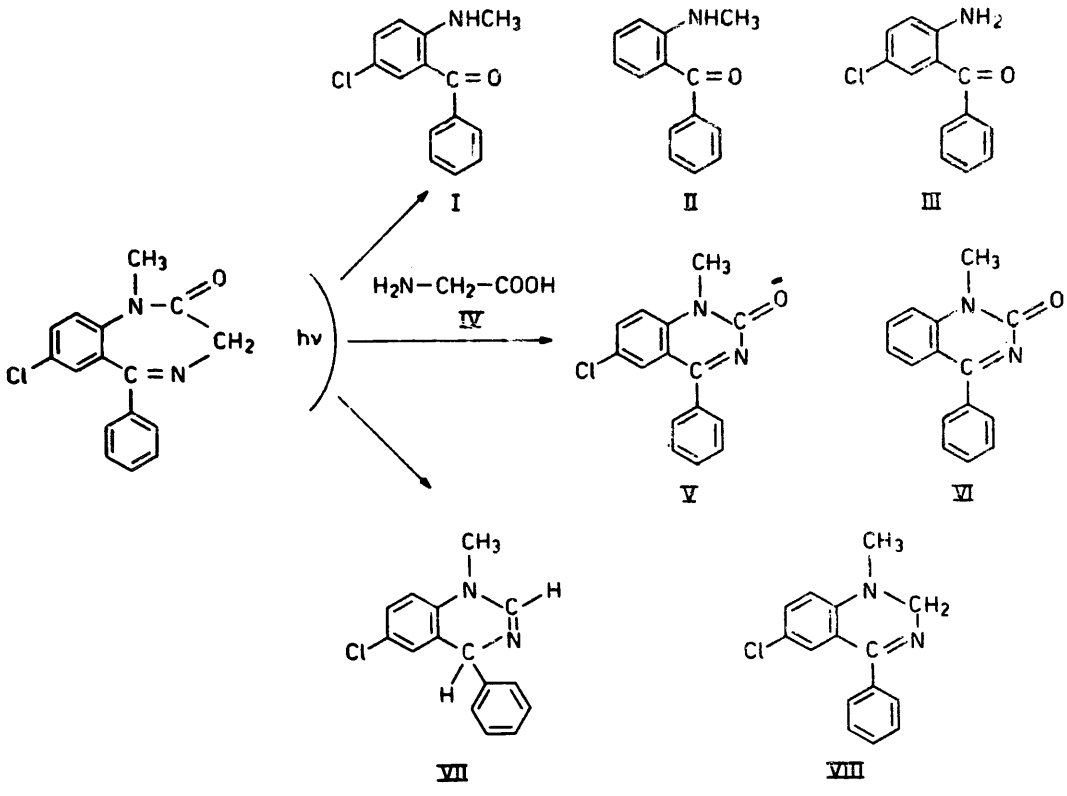


Fig. 1. Photochemical decomposition products of diazepam.

we have established that the wavelength does not determine the character of the products, but only the concentration of the products formed. It was found that an irradiated solution obtained in the way described above, contained about 8% benzophenones, 15% 4-phenylquinazolinones and 70% 4-phenylquinazolines, calculated in relation to the amount of decomposed diazepam.

The identification of the benzophenones is performed as follows: the compounds I, II and III show an identical mass fragmentation pattern (Fig. 2), whereby the difference in m/e of the fragments is caused by the different substituents on the 2 and 5 positions in the benzophenone molecule. The fragments' m/e 105, 77, 51 and the metastable fragment at 56.5, corresponding with the transition 105 → 77, indicate the presence of a benzoyl group in the molecule. This information together with the molecular formula of the parent ion, obtained from the m/e as determined by peak matching, gives strong evidence for a benzophenone structure, whereby the substituents are found in one of the two benzene nuclei only. The metastable fragments at 116.7, 83.9 and 103.1, corresponding with the transition 168 → 140 (compound I), 134 → 106 (compound II) and 154 → 126 (compound III) support the existence of a benzophenone structure. For further structure elucidation use is also made of the data obtained from UV, IR and NMR spectroscopy and thin layer chromatography. As a result the compounds I and III are found to be identical with 2-methylamino-5-chlorobenzophenone and 2-amino-5-chlorobenzophenone, respectively. The final proof was obtained by comparison with authentic samples of these

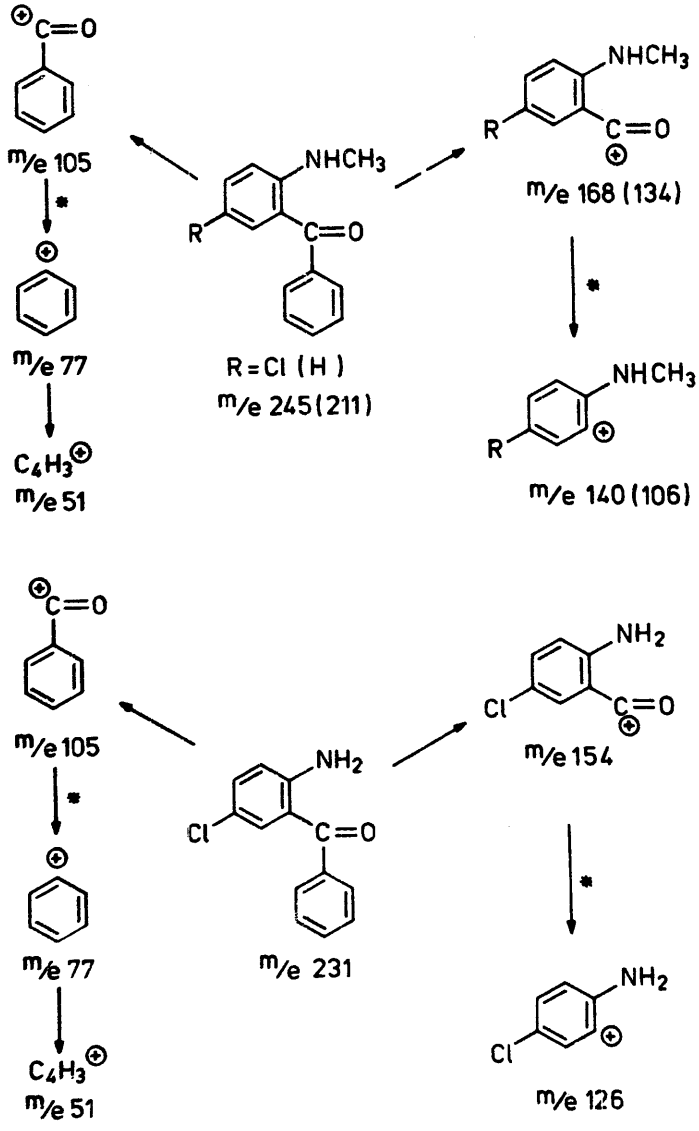


Fig. 2. The mass spectra of 2-methylamino-5-chlorobenzophenone (R=Cl), 2-methylaminobenzophenone (R=H) and 2-amino-5-chlorobenzophenone.

compounds. These are obtained for 2-methylamino-5-chlorobenzophenone by acid hydrolysis of diazepam (Mayer et al., 1972; Seitzinger, 1975b), while 2-amino-5-chlorobenzophenone is an intermediate in a possible synthetic route of diazepam (Angel, 1912). Moreover the UV spectrum of compound I and the mass spectrum of compound III correspond with the spectral data of the respective compounds given in literature (Seitzinger, 1975a; Rendic et al., 1975). From the m/e of the parent ion of compound II ($C_{14}H_{13}NO$ = 211) and the conformity of the fragmentation patterns of the compounds I, II and III, whereby the differences in m/e of the fragments are only caused by the presence of the chloro atom or the methyl group, it follows that compound II is 2-methylaminobenzophenone. This conclusion is supported by the conformity of the UV absorption data of

TABLE 1

UV ABSORPTION DATA OF BENZOPHENONE DERIVATIVES DISSOLVED IN METHANOL

Compound	λ_{max}(nm)
2-methylamino-5-chlorobenzophenone (I)	237, 410
2-methylamino-benzophenone (II)	237, 400
2-amino-5-chlorobenzophenone (III)	237, 385

compound II with those of compounds I and III (Table 1). The formation of the benzophenone derivative 2-methylamino-5-chlorobenzophenone, which is the same product as obtained after thermochemical decomposition of diazepam (Mayer et al., 1972), means that glycine, as the complementary part of the diazepam molecule, must also be present in the irradiation mixture. This is identified by means of thin layer chromatography, with glycine as reference substance.

The second type of compounds formed, is characterized by a 4-phenylquinazolinone structure. The mass spectrum of compound V is identical with the mass spectrum of 6-chloro-1-methyl-4-phenyl-2(1H)-quinazolinone (Rendic et al., 1975). The NMR spectrum is in agreement with this quinazolinone structure: singlet at 3.8 ppm (3 protons) and a multiplet (8 protons) at 7.3–8.0 ppm. The evidence that compound VI also possesses a 4-phenylquinazolinone structure is found by comparing the mass spectra (Fig. 3)

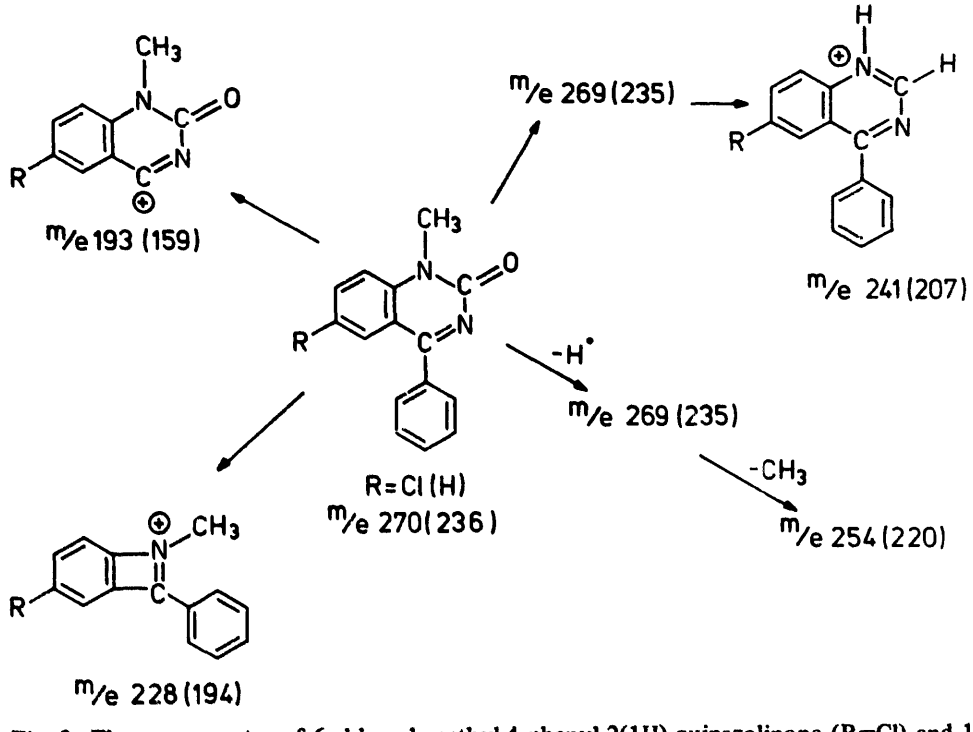
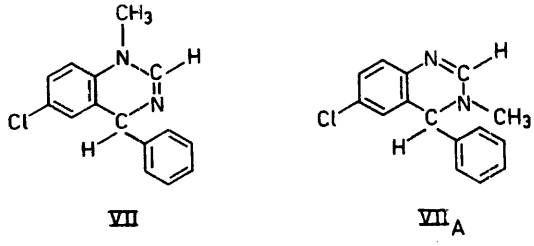


Fig. 3. The mass spectra of 6-chloro-1-methyl-4-phenyl-2(1H)-quinazolinone (R=Cl) and 1-methyl-4-phenyl-2(1H)-quinazolinone (R=H).

of compounds V and VI. They produce identical fragmentation patterns, while the difference in m/e between the corresponding fragments is attributed to the chlorine atom. The molecular formula ($C_{15}H_{12}N_2O$) of this quinazolinone is in agreement with the m/e (= 236) of the parent ion.

The problem with the structure elucidation of the third type of compounds is that some of these compounds are isomers with the same parent ion: $C_{15}H_{13}N_2Cl = 256$. The mass spectra of these compounds are completely the same with regard to the fragmentation patterns. The only difference is observed in the intensity of the fragments m/e 255 (loss of $H^{\cdot}$) and m/e 179 (loss of $C_6H_5^{\cdot}$). For one of the three isolated isomers the structure elucidation is not yet completed. However, from the same fraction the analogue without a chlorine atom is also obtained, in accordance with the decomposition pattern of the other types of compounds, e.g. benzophenones (compounds I and III) and 4-phenylquinazolinones (compounds V and VI). From the ratio of carbon to hydrogen and the absence of oxygen in the molecular formula of compounds VII and VIII ($C_{15}H_{13}N_2Cl = 256$), it can be concluded that these compounds do not have the quinazolinone structure, but are most probably 4-phenylquinazoline derivatives. When the NMR spectra of compound VII and 6-chloro-2-methyl-4-phenyl-3,4-dihydroquinazoline are compared, it appears that a signal at 5.6 ppm is present in both spectra, which in the case of the 3,4-dihydroquinazoline is attributed to the proton at the C-4 position (Oelschläger and Hoffmann, 1967; Knollmüller, 1970). Further, the NMR spectrum of compound VII shows a signal between 3 and 4 ppm (3 protons), which, in agreement with the NMR spectra of diazepam and the quinazolinones, is attributed to a methyl group attached to a nitrogen atom. Lastly there is a multiplet (9 protons) at 6.64–7.40 ppm. Based on the NMR data and the molecular formula two structures VII and VII_A (Fig. 4) are still possible for this compound. However, after hydrolysis in 1 N hydrochloric acid 2-methylamino-5-chlorobenzophenone is formed, thus the methyl group is at the N-1 position. Formula VII: 6-chloro-1-methyl-4-phenyl-1,4-dihydro-quinazoline, represents the correct structure.

From the spectral data of compound VIII the idea was formed that this compound could be the 6-chloro-1-methyl-4-phenyl-1,2-dihydroquinazoline. From the literature (Hromatka et al., 1969) it is known that on reduction of 7-chloro-5-phenyl-1,3-dihydro-2,1,4-benzothiadiazepine-2,2,4-trioxide ($VIII_A$) with triethylphosphite, 7-chloro-5-phenyl-1,3-dihydro-2,1,4-benzothiadiazepine-2,2-dioxide ($VIII_C$) is formed together with 6-chloro-4-phenyl-1,2-dihydroquinazoline ($VIII_B$; Fig. 5). Therefore, starting from compound $VIII_A$, its *N*-methyl derivative is prepared with methyl iodide and this is subsequently reduced with triethylphosphite. From the reaction mixture the 6-chloro-1-



VII VII_A

Fig. 4. Two possible structures for compound VII based on NMR data and molecular formula.

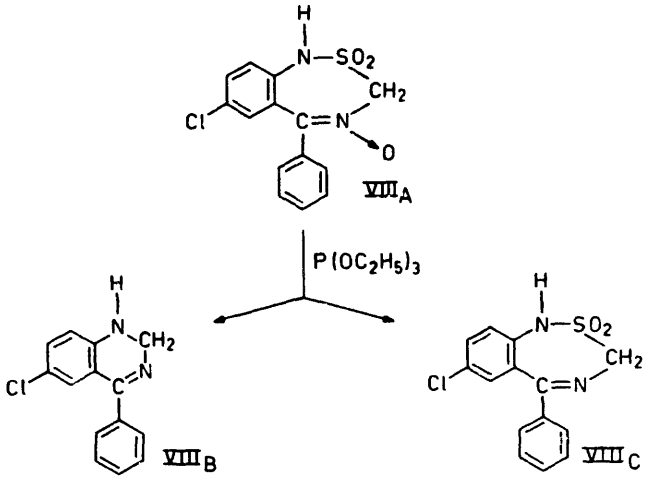


Fig. 5. Reduction of 7-chloro-5-phenyl-1,3-dihydro-2,1,4-benzothiadiazepine-2,2,4-trioxide ($VIII_A$) to 7-chloro-5-phenyl-1,3-dihydro-2,1,4-benzothiadiazepine-2,2-dioxide ($VIII_C$) and 6-chloro-4-phenyl-1,2-dihydroquinazoline ($VIII_B$).

methyl-4-phenyl-1,2-dihydroquinazoline is obtained in a pure form. The position of the methyl group is proved after hydrolysis with 1 N hydrochloric acid, giving the expected 2-methylamino-5-chloro-benzophenone. The NMR spectrum shows a singlet of 5 protons at 3.34 ppm and a multiplet of 8 aromatic protons between 6.7 and 7.4 ppm. After addition of a shift reagent ($EU(FOD)_3$) the singlet of 5 protons is split in a singlet of 2 protons ($-CH_2-$) and a singlet of 3 protons ($-NCH_3$). By means of thin layer chromatography, UV, NMR and mass spectrometry it is proved that compound VIII is identical with 6-chloro-1-methyl-4-phenyl-1,2-dihydroquinazoline. The research concerning the clarification of the structure of the third quinazoline isomer with its analogue without chlorine, will be continued.

When the photochemical decomposition of diazepam is compared with structurally related compounds (Sternbach et al., 1962; Field and Sternback, 1968; Roth and Adomeit 1969, 1973) it is evident that no resemblance exists. This is in contrast to the thermochemical decomposition (Mayer et al., 1972, 1974; Wesley et al., 1976) where, depending on the water concentration, two products are formed: a benzophenone and a quinolone derivative (Fig. 6). Phototoxicity can be caused by decomposition products even when formed in relatively small concentrations. In the case of diazepam little is known about the physiological effects of the different decomposition products. Only compound V, 6-chloro-1-methyl-4-phenyl-2(1 H)-quinazolinone, is known as a uricosuric, analgesic and antiphlogistic agent (Yamamoto et al., 1974; Ishizumi et al., 1974; Ishizumi et al., 1975). The 4-phenylquinazolines generally have analgesic, depressive and anticonvulsant properties (Bell and Wei, 1972), but to what extent the 4-phenylquinazoline derivatives obtained are active has to be examined. Benzophenone itself is often used as a photosensitizing agent in photochemical experiments, because an intersystem crossing of 100% can take place into the triplet state. In a biological system this relatively long-living triplet may cause undesired effects, such as reaction with or energy transfer to another molecule. It is known that benzophenone, after photosensitization at 313 nm,

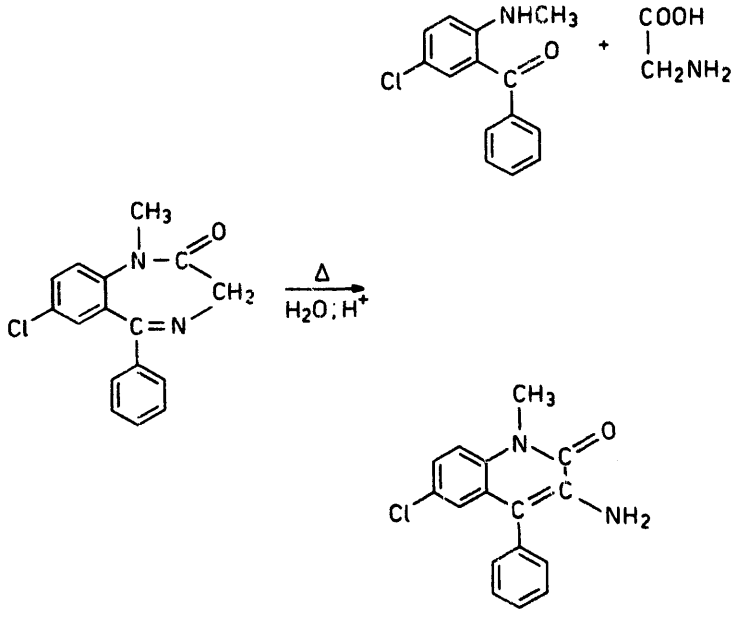


Fig. 6. Thermochemical decomposition products of diazepam.

can cause chain cleavage and thymine dimer formation in DNA (Rahn et al., 1974). It will be studied if the benzophenone derivatives, formed during photochemical decomposition of diazepam, have the same properties as benzophenone itself.

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