Thermolysis of 1,4-Benzodiazepines during Gas Chromatography and Mass Spectroscopy

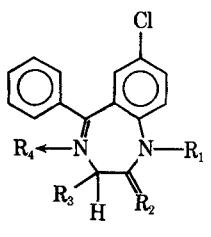
W. SADÉE* and E. VAN DER KLEIJN†

Abstract □ Diazepam (I), desmethyldiazepam (II), oxydiazepam (III), medazepam (IV), and oxazepam (V) were analyzed gas chromatographically using flame-ionization detection, electron-capture detection, and total ion-current detection (gas chromatograph connected to a mass spectrometer). 3-Hydroxy substitution of III and V induced a partial decomposition of III within the column, whereas V quantitatively rearranged to the quinazoline carboxaldehyde (X). This rearrangement occurred also under the conditions of electron impact. *N*-4-Oxides of benzodiazepines were thermally labile under the gas chromatography conditions. Thus, VII was converted partially to I by desoxygenation. The corresponding diazepam, 4,5-epoxide (XI), did not give the expected thermal conversion to the nitrone (VII), but rearranged to the 4-benzoylquinoxalinone (XII) as a major product.

Keyphrases □ 1,4-Benzodiazepines, thermolysis, rearrangement—during GC, mass spectroscopy analysis □ Thermolysis, 1,4-benzodiazepines—during GC, mass spectroscopy analysis □ Gas chromatography—thermolysis, rearrangement of 1,4-benzodiazepines, on column □ Mass spectroscopy—analysis

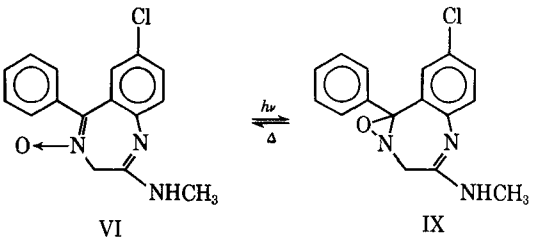
The group of 1,4-benzodiazepines is widely used as ataractics, hypnotics, central muscle relaxants, and anticonvulsants. Sensitive assays have been evaluated using gas chromatographic detection of these compounds in extracts of biological fluids. Primarily, the methods were based on hydrolysis to 2-aminobenzophenones (1–3). However, in recent publications it was shown that the intact benzodiazepines can also be separated by gas chromatography (GC) with reproducible results (4, 5). The detection in the low nanogram range was made possible using the ^{63}Ni-electron-capture detector (ECD).

Equimolar amounts of diazepam (I), desmethyldiazepam (II), oxydiazepam (III), and medazepam (IV) do not yield equal responses using flame-ionization detection (FID) and total ion-current detection (TICD). Preliminary investigations suggested that rearrangements of the diazepine ring can occur at the GC temperatures. Oxazepam (V) appeared in the GC spectrum between I and IV with the same retention time as desmethylmedazepam, although it possessed the highest polarity and should give the longest retention time. Chlordiazepoxide (VI), as well as the *N*-4-oxides of



	R_1	R_2	R_3	R_4
I	CH_3	O	H	-
II	H	O	H	-
III	CH_3	O	OH	-
IV	CH_3	H_2	H	-
V	H	O	OH	-
VII	CH_3	O	H	O
VIII	H	O	H	O

structures of benzodiazepines



VI IX

Scheme I

diazepam (VII) and of desmethyldiazepam (VIII), proved to be thermally labile. Compound VIII represents a major metabolite of VI. These nitrones were reported to be in photolytic and thermolytic equilibrium with the corresponding oxaziranes (*e.g.*, IX) (Scheme I) (6–8). The oxazirane–nitrone relation under the conditions of electron impact was discussed recently (9).

It is conceivable that there will be a correlation between the thermolysis during GC and some of the fragmentations observed during mass spectrometry (MS). The problem was approached by comparing mass spectra of directly inserted samples to mass spectra of samples subjected to GC analysis prior to MS. A GC–MS combination proved to be especially valuable for the present investigation.

MATERIALS AND METHODS

Mass spectra with high resolution (direct insertion) were taken on a MS-9, using the peak matching technique. The Finnigan system was used for the GC–MS analysis (a Varian GC model 1700 connected to a Finnigan quadrupole model 1015 with a Gohlke separator). The D/A model 150 data acquisition system (Digital Equipment Corp.) was used. The mass spectra were taken at 70 ev., 450 amp. ionization current, and the column bleeding spectra were automatically substracted. Mass ranges of 50–200, 200–300, and 300–400 were taken with a relative integration time of 1, 4, and 16. Mass spectra were normalized to the largest peak (=100). The resolution of the quadrupole instrument was about 500. The reconstructed gas chromatograms were based on the registration of the TICD in the MS. In some cases, a limited mass range was selected to get a clear gas chromatogram (*m/e* 250–310), which was normalized to the largest peak (=100).

GC Conditions—*TICD*—The following were used: glass columns, 0.9 m. × 0.32 cm. (3 ft. × 0.125 in.), 1.5% OV-1 on HP chrom G; temperature, 200–280°, linearly programmed; and carrier gas helium, 20 ml./min.

FID—The following were used: Varian hi-fi III series 1200; ss column, 1.8 m. × 0.32 cm. (6 ft. × 0.125 in.), 3% OV-17 on gas chrom W (AW); temperature, 245°; and carrier gas, nitrogen (70 ml./min.), hydrogen (40 ml./min.), and oxygen (700 ml./min.).

ECD—The following were used: Varian hi-fi III series 1200; ss column, 1.8 m. × 0.32 cm. (6 ft. × 0.125 in.), 3% OV-17 on gas chrom W (AW); temperature, 245°; and carrier gas, argon–methane (5%), 70 ml./min. The compounds were prepared by known methods or were provided.[1]

[1] By Hoffmann-La Roche Inc., N. J. (Compound X by Dr. L. H. Sternbach and Compounds XI and XIII by Dr. R. Y. Ning), and Wyeth Labs., Inc. (Compound XIV by Dr. S. C. Bell).

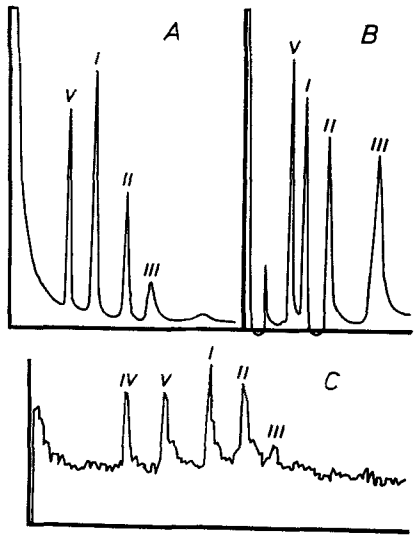


Figure 1—*Gas chromatograms of an equimolar mixture of I–V. Key: A, FID (8 × 10⁻⁹ M); B, ECD (16 × 10⁻¹¹ M); and C, TICD (8 × 10⁻⁹ M).*

RESULTS AND DISCUSSION

Figures 1 A, B, and C show the gas chromatograms of I–III, V, and, in one case (C), of IV, using FID, ECD, and TICD. Compound III gave only about 20% of the expected peak area with FID and TICD, whereas the response was about equal to the II area with ECD. This suggests a partial decomposition of III and an increased sensitivity to ECD.

The retention time of V was shorter than expected on the basis of its relative polar properties. It is conceivable that under the GC conditions, V will rearrange in a high yield to a product of lower polarity to give a well-resolved peak with a relatively short retention time. Indeed, the mass spectrum demonstrated that a thermolytic rearrangement occurred by loss of water.

Figure 2 shows the mass spectrum of oxazepam (V) and of the reaction product to which the structure of the known quinazoline carboxaldehyde (X) was assigned. An equivalent reaction was shown to occur in solution upon alkali treatment (10, 11). The authentic sample of X proved to give an identical retention time in the gas chromatogram and the same mass spectrum as the rearrangement product of V. The yield of X was practically 100%. The mechanism of the thermolysis can be described as shown in Scheme II.

The proposed mechanism is supported by the mass spectrum

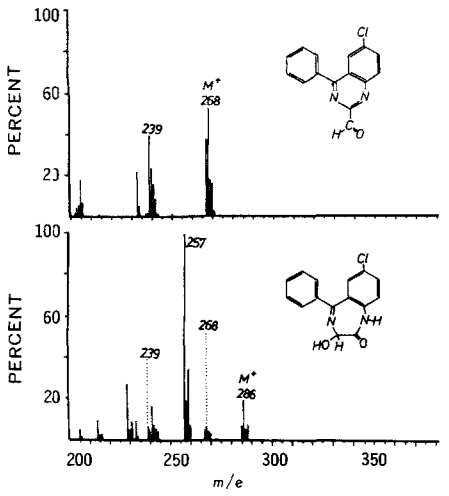


Figure 2—*Mass spectra of the quinazoline carboxaldehyde (X) (mol. wt. 268) and of oxazepam (V) (mol. wt. 286) on the MS-9 mass spectrometer (direct insertion).*

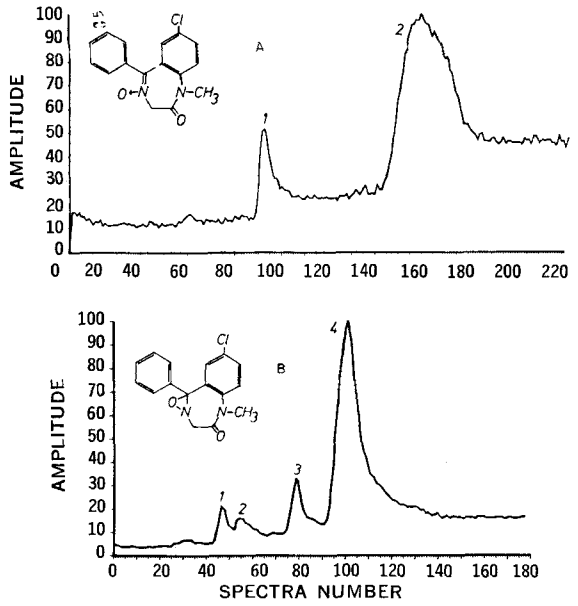
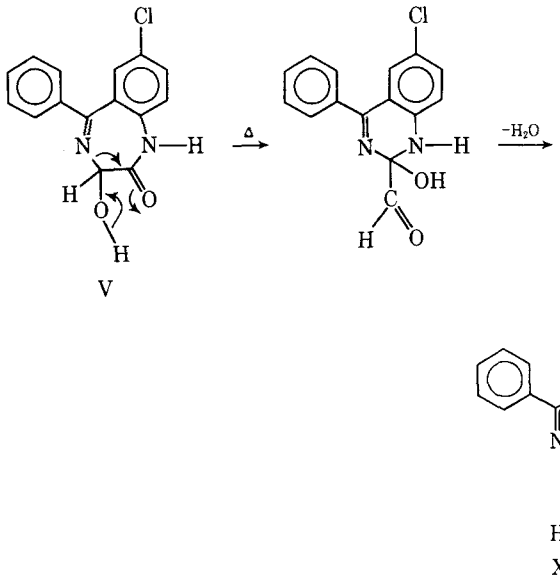


Figure 3—*Reconstructed gas chromatograms (TICD) of VII (A) and XI (B).*

of III, with the 0–18 label in position 3 (12). The peak at M^+-18 (loss of H_2O) is derived from the loss of the carbonyl oxygen rather than from the loss of the 3-OH-oxygen.

It can be postulated that the peaks at M^+-18 (*m/e* 268) as well as *m/e* 239 (M^+-H_2O, HCO) in the mass spectrum of V resulted from an initial thermolytic rearrangement to X and not from electron impact.

Another thermolytic and photolytic equilibrium was found with the nitrones (*e.g.*, VII) and oxaziranes (*e.g.*, XI) of benzodiazepines (6–8). Under the conditions of electron impact, the nitrones were reported to rearrange to oxaziranes and further to quinoxalinones such as XII (9). The same reaction occurred upon prolonged irradiation of oxaziranes (2, 4). Treatment of benzodiazepine *N*-4-oxides with $POCl_3$ also led to quinoxalinones (11). Therefore, VII and the corresponding oxazirane XI were analyzed with the GC–MS combination. Figure 3A shows the gas chromatogram of VII. The two peaks were readily identified as I and VII. Loss of oxygen was also observed during MS (9), and it appears that this reaction is at least partially thermolytic. No thermal rearrangement of the nitrone (VII) to the oxazirane (XI) could be detected.



Scheme II

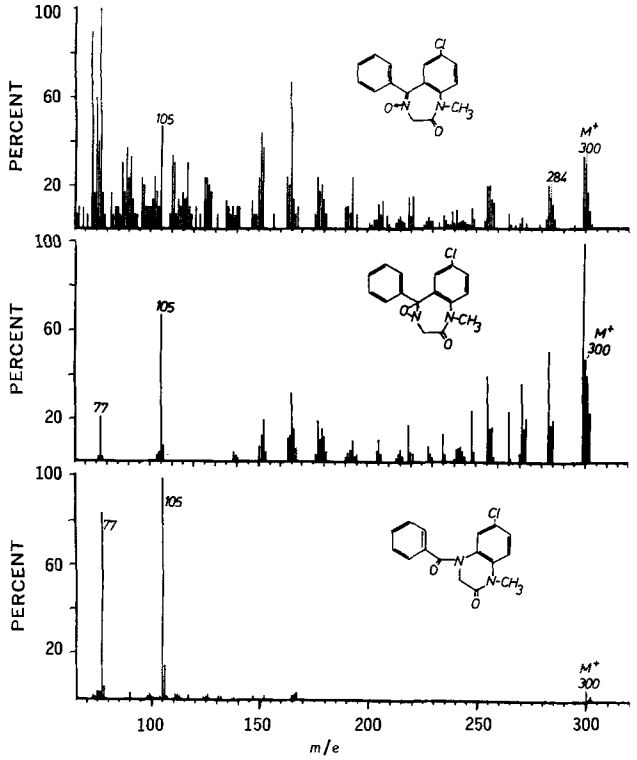
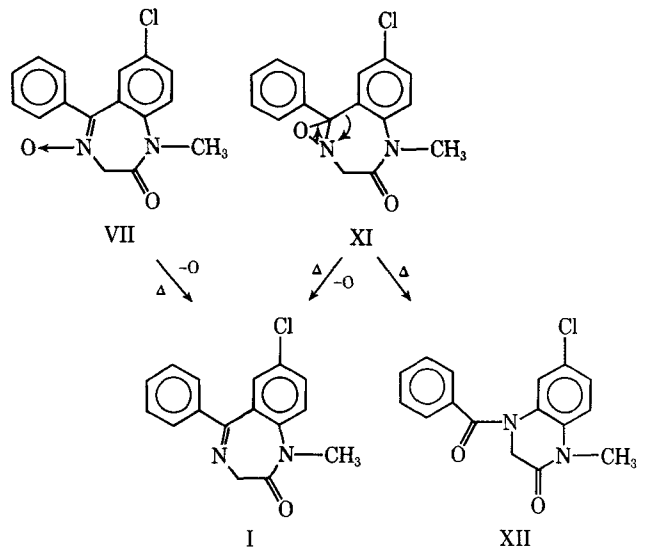


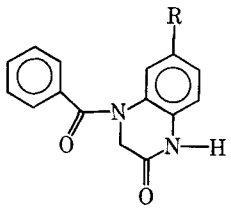
Figure 4—*Mass spectra of: A, VII (Finnigan system); B, XI (MS-9, direct insertion); and C, XII (Finnigan system).*

The gas chromatogram of the oxazirane (XI) proved to be more complex (TICD, Fig. 3B). At least four peaks were detected, of which 1 and 2 were not identified. Again, one peak was found to be I (3, ~10%), while the larger peak (4, ~85%) gave a mass spectrum with major fragments at *m/e* 51, 77, and 105 and a very small molecular ion peak at *m/e* 300. This suggested the formation of the 4-benzoylquinoxalinone (XII) in a Beckmann-type rearrangement (Scheme III).



Scheme III

The peak at *m/e* 105, which stands for the benzoyl ion, increased in relative abundance in the spectra of the nitrone (VII) to the oxazirane (XI) and to the 4-benzoylquinoxalinone (XII) (Fig. 4). This pattern supported the recently postulated rearrangement to quinoxalinones under electron impact, which was based on the presence of the peak at *m/e* 105 (9). This peak was present only in the spectrum of the nitrones and not in the spectrum of *N*-4-unsubstituted benzodiazepines. The mass spectra of the quinoxalinones (XIII and XIV) proved to possess the same fragmentations as XII. The thermolytic rearrangement of the benzodiazepin-2-one



XIII; R = Cl
XIV; R = H

4,5-epoxide (XI) was initiated by N—O bond fission. This is in contrast to the known formation of the nitrone (VI) from the corresponding oxazirane (IX) upon heating (7), which implied a C—O bond fission.

CONCLUSIONS

The benzodiazepines I, II, and IV and desmethylmedazepam were found to be thermally stable as judged by the mass spectra. 3-Hydroxy derivatives, *N*-4-oxides, and the corresponding 4,5-epoxides reacted by desoxygenation or by rearrangements to rather stable 1,3-quinazolines and quinoxalines at the GC temperatures. The formation of quinoxalines from *N*-4-oxides during MS is presumably only a result of electron impact, since this rearrangement did not occur at the GC conditions.

For the GC analysis of the nitrone (VII), the reaction of VII with acetic anhydride would result in a high yield to a 3-acetoxy benzodiazepine in a Polonovski-type rearrangement (13) which proved stable at the GC temperatures.

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* Present address: Pharmazeut. Institut, Freie Universitaet Berlin, 1-Berlin-33, Germany.

† Present address: Department of Pharmacology, Catholic University, Nijmegen, The Netherlands.