CHROM. 5325

Gas–liquid chromatography and mass spectrometry of various benzodiazepines

Several benzodiazepine derivatives have been shown to have powerful pharmacological and therapeutic effects[1]. Studies concerning the metabolism and tissue distribution of diazepam and three of its known metabolites in correlation with the anticonvulsant effects have been carried out in this laboratory[2–4] and required the availability of specific and precise micromethods for measuring the administered drug and its metabolites.

In previous papers a method for the gas chromatographic (GC) separation and the quantitative analysis of several benzodiazepines, including diazepam and some of its known metabolites, was described[5,6]. It was considered of interest to establish the identity of the GC peaks by using mass spectrometry (MS) coupled with GC. In addition, this report describes an improved GC procedure for benzodiazepine analysis which allows better separation, greater reproducibility and higher sensitivity.

Apparatus

GC analysis was carried out using a Carlo Erba Model GV instrument equipped with a flame ionization detector. The stationary phase used was OV-17, a more polar methyl phenyl silicone derivative which was found to be superior to the OV-1 phase used in the original method[5]. The column was a 2 m column of 3% OV-17 on Gas-Chrom Q (100–120 mesh) contained in glass tubing (2 mm I.D. × 4 mm O.D.). The carrier gas was N_2 at a flow rate of 37 ml/min.

The injection site temperature was 300°, the detector was 270° and the oven was 250°. The LKB Model 9000 instrument, fitted with a 2 m × 2 mm I.D. spiral glass column packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh) was employed for combined GC–MS experiments. Helium was used as the carrier gas and all the mass spectra were obtained at 12 eV and 70 eV. Other parameters were: injection site 300°, molecular separators 260°, ion source 290°, accelerating voltage 3 kV, ionizing current 20 μA. Only the 12 eV mass spectra or the 70 eV mass spectra were considered because of their greater simplicity in fragmentation patterns.

Chemicals

Synthesis of 6-chloro-4-phenylquinazoline-2-carboxaldehyde (QCA). 5 g of oxazepam were melted at 200° until steam development ceased (5–10 min). After cooling the compound was filtered and crystallized from benzene. Yellow prisms of QCA showing a m.p. of 177–178° and an 80% yield were obtained. The R_F was 0.42 on TLC using benzene–ethyl acetate (5:2) as development solvent; the I.R. spectrum had a $\nu_{max.}$ at 1728 cm^{-1}; the NMR spectrum showed adsorption at $\delta = 10.3$ (O=C–H). The mass spectrum is represented in Fig. 3.

QCA after crystallization from EtOH gives white crystals of hemiacetal. Both the former and the latter compound are identical to those synthesized by the method of STERNBACH *et al.*[7].

Synthesis of 7-chloro-1-deutero-3-hydro-3-deuteroxy-5-phenyl-2H-1,4-benzodiazepin-2-one (II) (Fig. 1). 0.01 mole of 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-

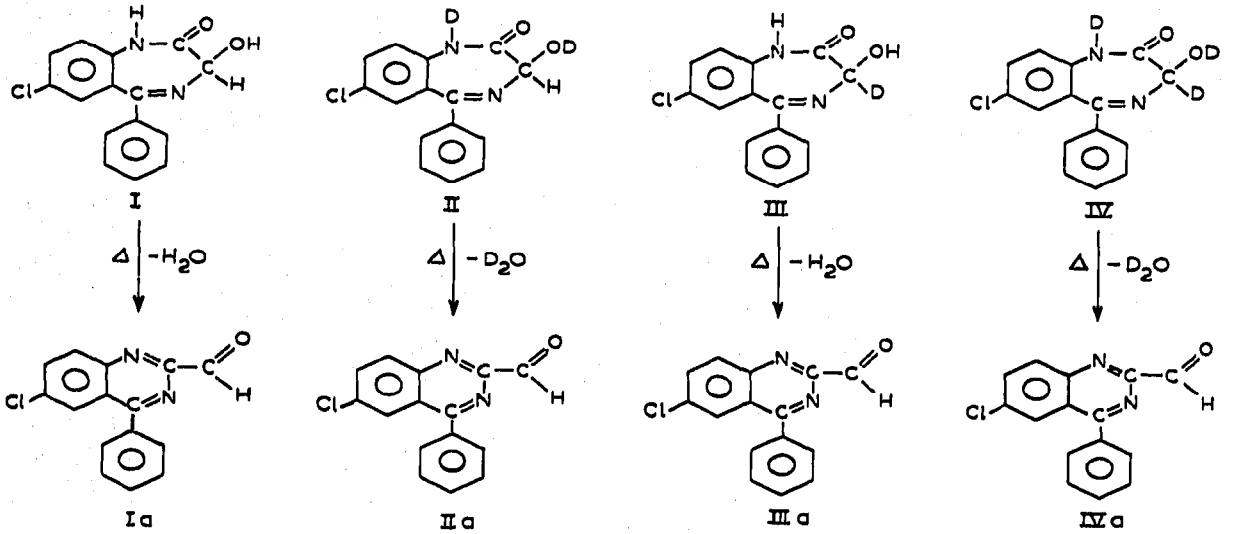


Fig. 1. Structural formulae of the compounds synthesised as described under *Chemicals* in the text.

1,4-benzodiazepin-2-one (I) was dissolved in dry dioxane; an amount of D_2O, slightly exceeding the stoïchiometric value, was added and the solution was shaken for 3–4 h.

The deuterated compound was precipitated by adding D_2O.

Synthesis of 7-chloro-1-hydro-3-deutero-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one (III) (Fig. 1). This was prepared according to a slightly modified method from HOFFMAN-LA ROCHE[8].

The mixture of 7-chloro-1-hydro-3,3-dideutero-5-phenyl-2H-1,4-benzodiazepin-2-one (Fig. 5) having 61% of dideuterated molecules (0.5 mmole), N-chlorosuccinimide (0.5 mmole) and benzoyl peroxide (2.5 mg) was refluxed, with stirring, for 1 h. Solids were removed by filtration and the filtrate was evaporated to a small volume. 0.1 mmole of sodium acetate in 1.9 ml of acetic acid was added and then the solution was heated in a water bath for 2 h; the suspension was evaporated to about half volume, and it was then cooled, and then an equal volume of water was added. The solids were removed by filtration, washed with water and then suspended, under agitation, in a small amount of acetic acid until a crystalline product was obtained.

The acetyl oxazepam obtained was washed with ether; the m.p. was 241–242°. To a suspension of acetyl oxazepam (0.5 mmole) in 2 ml of 95% ethanol, 0.11 ml of 9 *M* NaOH was added at room temperature. At first there was complete solution but this was followed by the formation of a precipitate. The suspension was diluted with 2 ml of water until complete solution and then acidified with acetic acid.

The crystalline oxazepam (III) (Fig. 1) precipitated, and after washing with water it was recrystallized from alcohol; m.p. 203–204°.

Synthesis of 7-chloro-1,3-dideutero-3-deuteroxy-5-phenyl-2H-1,4-benzodiazepin-2-one (IV). This compound was obtained by shaking the compound III with D_2O according to the procedure described above.

Results and discussion

The stationary phase used (OV-17) allows an efficient separation of the benzo-

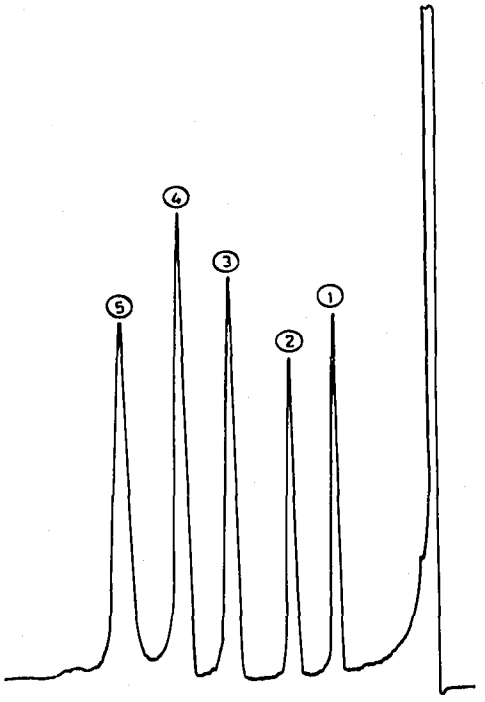


Fig. 2. Gas chromatographic separation of a mixture of five benzodiazepines. 1 = oxazepam; 2 = diazepam; 3 = N-demethyldiazepam; 4 = N-methyloxazepam; 5 = nitrazepam.

diazepines tested as indicated in Fig. 2. The sensitivity as well as the retention time are reported in Table I where a comparison is made between results obtained with OV-1 and OV-17. The identity of the GC peaks was established by MS. The mass spectra obtained after GC separation were analyzed for the following compounds: 7-chloro-1-methyl-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (diazepam); 7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (N-demethyldiazepam); 7-chloro-1-methyl-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one (N-methyloxazepam); 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one (oxazepam); 7-nitro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (nitrazepam). The fragmentation pattern of the examined benzodiazepines and the structural implications of this fragmentation will be published elsewhere. The mass

TABLE I

GAS CHROMATOGRAPHIC RETENTION TIME OF SEVERAL BENZODIAZEPINES USING OV-1 AND OV-17 PHASES

Drug	*Retention time*	
	OV-1	*OV-17*
Oxazepam	3 min (0.25)[a]	2 min 12 sec (0.25)
Diazepam	4 min 10 sec (0.20)	3 min 04 sec (0.20)
N-Demethyldiazepam	4 min 45 sec (0.40)	4 min 40 sec (0.20)
N-Methyloxazepan	6 min 12 sec (1.00)	5 min 44 sec (0.50)
Nitrazepam	8 min 30 sec (0.50)	7 min 12 sec (0.50)

[a] Figures in parentheses = sensitivity in μg (flame ionization detector).

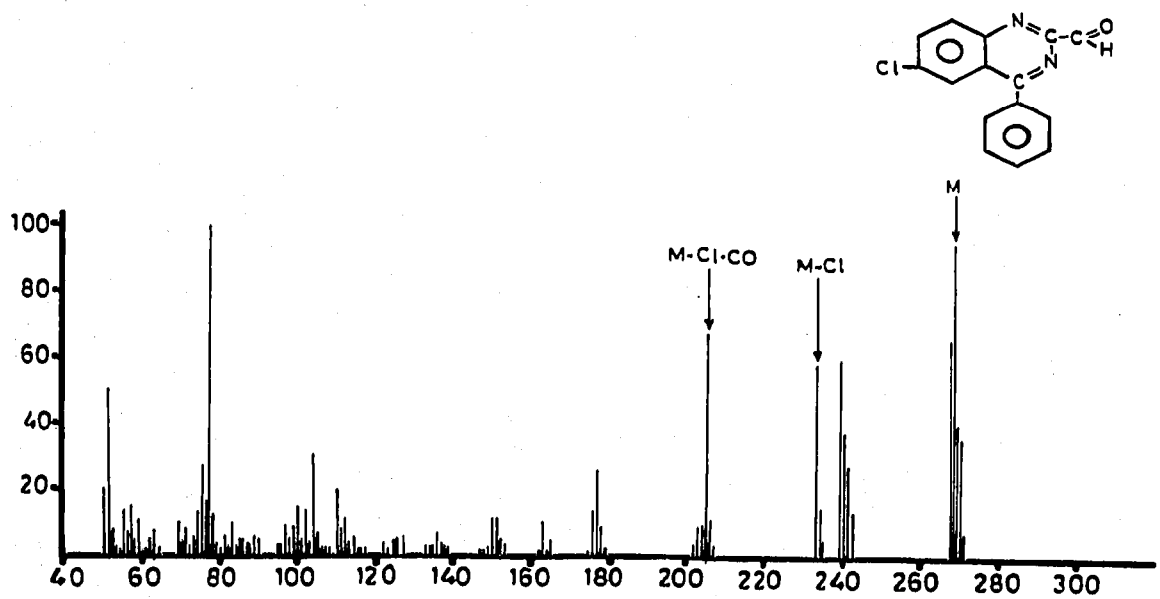


Fig. 3. Mass spectrum of 6-chloro-4-phenyl-2-carboxaldehyde; M = 268, 70 eV.

spectra of the GC peaks show that the structure of the analyzed benzodiazepines was not modified under the experimental conditions used with the exception of oxazepam. The latter compound, as a result of the heating, is rearranged to form 6-chloro-4-phenylquinazoline-2-carboxaldehyde with the loss of an H_2O molecule.

This compound, previously obtained by STERNBACH *et al.*[7] by treating oxazepam with HCl, was obtained in this laboratory as described under *Chemicals* with almost quantitative yields by heating oxazepam at 200°.

Its retention time in GC and its mass spectrum are identical to that obtained with the GC peak of oxazepam. The structure of 6-chloro-4-phenylquinazoline-2-

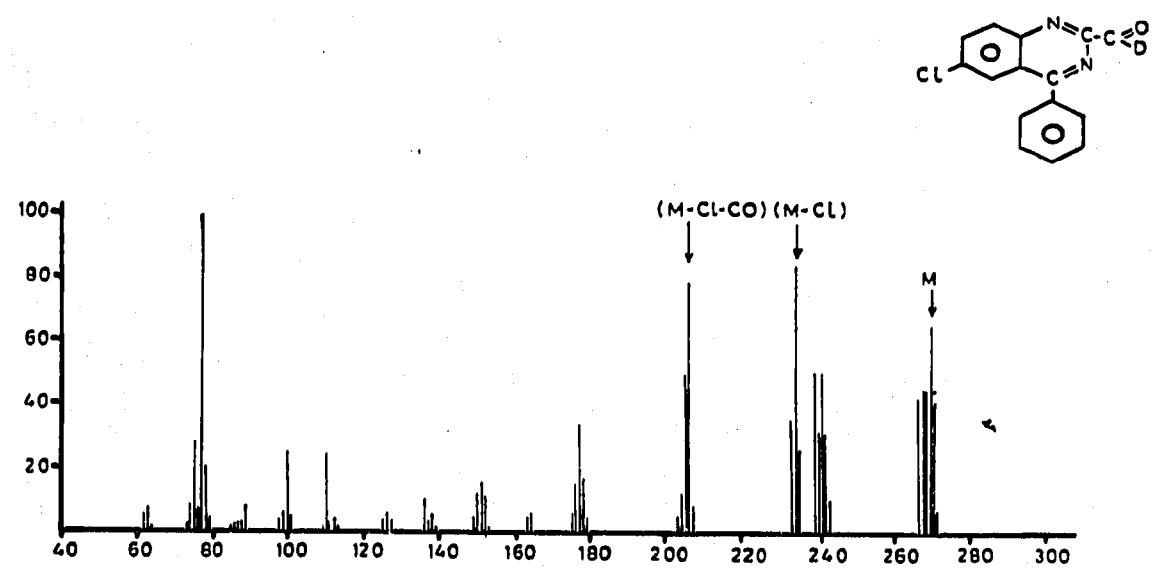


Fig. 4. Mass spectrum of monodeutero-substituted quinazoline carboxaldehyde; M = 269, 70 eV.

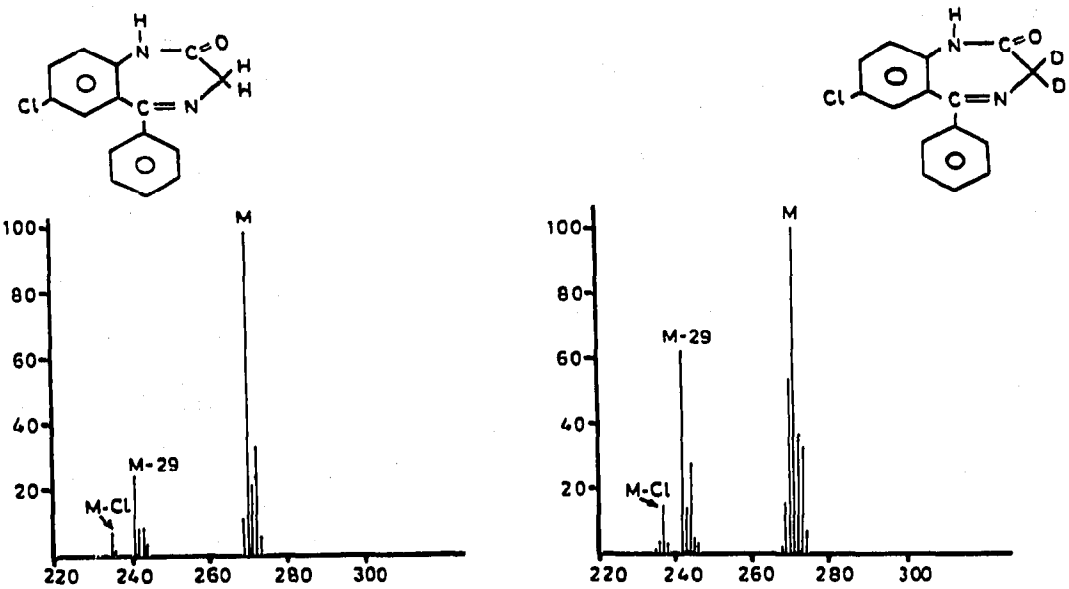


Fig. 5. Mass spectra of N-demethyldiazepam and 3-dideutero-substituted N-demethyldiazepam; M = 270, M = 272, 12 eV.

carboxaldehyde (QCA) has been further confirmed by means of NMR spectroscopy.

In order to investigate the dehydration mechanism of oxazepam under thermolytic conditions, benzodiazepines and deuterium-labelled benzodiazepines were synthesized. The molecules synthesized are shown in Fig. 1. The four compounds I, II, III, IV were transformed into the respective QCAs (Ia, IIa, IIIa, IVa) as described under *Chemicals*, and then analyzed in the mass spectrometer. SCHWARTZ[9] has previously reported the characteristic fragmentations of oxazepam: M, M—H_2O, M—HCO, M—$C_2H_2NO_2$. Some of these, namely M and M—HCO, also occur in the mass spectrum of the QCA derivative. Fig. 3 shows that the loss of HCO is a phenomenon similar to the one observed for oxazepam; the intensity of the molecular peak and the peaks deriving from the loss of chlorine and chlorine plus carbon monoxide are larger than those of the corresponding hydroxylated benzodiazepine (compound I). The QCAs obtained from compounds III and IV had the same mass spectra, whereas the one obtained from oxazepam II exhibited a mass spectrum identical to the unlabelled standard QCA.

TABLE II

DIFFERENCES IN THE FRAGMENTATION PATTERN OF IIa, IIIa, IVa QCAs WITH RESPECT TO THE STANDARD Ia

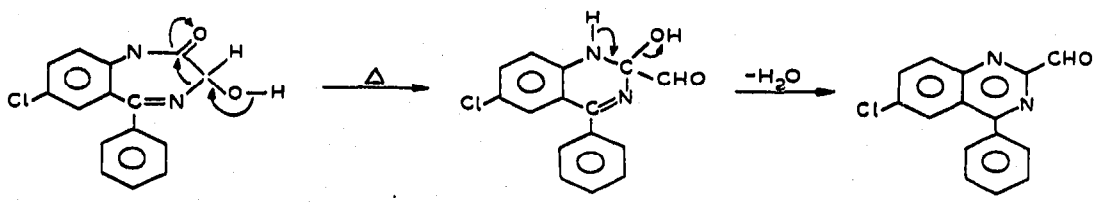
The *m/e* ratios of the compounds IIIa and IVa are shifted by one unit with respect to the standard (Ia) and IIa QCAs.

Formula	*Standard QCA m/e Ia*	*IIa m/e*	*IIIa m/e*	*IVa m/e*
M	268	268	269	269
M — Cl	233	233	234	234
M — Cl — CO	205	205	206	206

Table II reports on several simple fragmentations concerning unlabelled QCA and the aldehydes IIa, IIIa and IVa. The *m/e* ratios of compounds IIIa and IVa are shifted by one unit with respect to the standard and IIa compounds. The amount of deuterium found in the former two compounds (58%) is in agreement with the amount of deuterium (61%) in the starting material 7-chloro-3,3-dideutero-5-phenyl-2H-1,4-benzodiazepin-2-one.

For simplicity, the deuterated entity was calculated by utilizing the peaks corresponding to the molecular ion minus one atom of chlorine (M—Cl).

This finding suggests that one of the hydrogen atoms involved in the dehydration mechanism of the oxazepam molecule is bound to N_1 and the other is part of the hydroxylic group at C_3. As a result of the fact that N_4 is more nucleophilic than N_1 the possible dehydration mechanism may be represented as follows:



The results reported in Table II confirm that the 1-deutero-3-deuteroxy-substituted oxazepam II gives rise to an unlabelled aldehyde IIa.

Therefore, when the 3-deutero- or the 1,3-dideutero-3-deuteroxy-substituted benzodiazepines are used as starting material the same monodeuterated thermolytic transformation products (IIIa, IVa) are obtained.

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