The *m/z* 91 Rearrangement Ion in the Mass Spectra of Some 1,4-Benzodiazepines†

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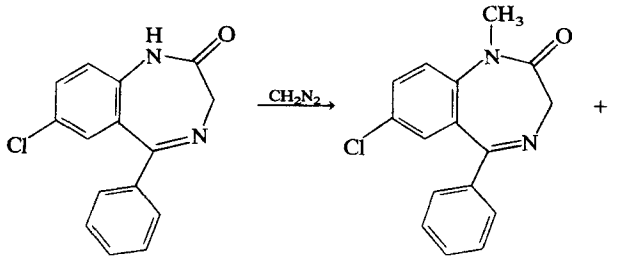
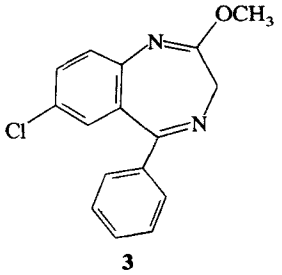
With the aid of isotope labeling and by substituent shifts, the relatively strong *m/z* 91 ion in the mass spectra of 7-chloro-2-methoxy-5-phenyl-3*H*-1,4-benzodiazepine and related compounds was shown to contain the 5-phenyl ring and the 3-CH_2 group. Mechanisms involving the opening of the 7-membered ring and the migration of the phenyl ring from C-5 to C-3 are postulated for the formation of this ion. This rearrangement ion was also observed in the mass spectra of some 1-alkyl-7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-ones.

INTRODUCTION

In our search for suitable derivatives for the gas chromatographic analyses of 1,4-benzodiazepines, we observed that the reaction of **1** with diazomethane gave two products, **2** and **3** (Scheme 1). The electron



1 **2**



3

Scheme 1

impact mass spectra of **3** showed a strong fragment ion at *m/z* 91. A strong *m/z* 91 ion has also been observed in the mass spectra of analogs of **2** which contain an ethyl or *t*-butyl group on N-1. This report describes the characterization of this ion and suggests possible mechanisms for its formation.

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RESULTS

The major ions in the mass spectra of **3** and similar compounds are summarized in Table 1. A strong fragment ion at *m/z* 91 is observed in the mass spectra of **3** and its dechloro analog, **4**. High resolution measurements of **3** showed this to be $[C_7H_7]^+$. Substitution of two deuterium atoms on the 2′,6′ positions of **3** (**3a**) or on C-3 of compound **4** (**4a**) shifts the ion to *m/z* 93. These results indicate that the *m/z* 91 ion consists of the 5-phenyl ring, the two hydrogens on C-3 and one other carbon atom.

The two obvious candidates for the unidentified carbon are C-3 and C-5. The mass spectra of a 2-methoxy-1,4-benzodiazepine containing 33% ^{14}C enrichment at C-5 (**5a**) and the unlabeled compound (**5**) are shown in Fig. 1. If C-5 contributes to the rearrangement ion, the ratio [*m/z* 127]/[*m/z* 125] would be larger in the ^{14}C enriched compound **5a** than in **5**. Since the observed ratios for **5** (0.37) and **5a** (0.38) are essentially equivalent, C-5 is not present in the rearrangement ion. Strong support for C-3 as the missing carbon was obtained from the mass spectrum of **6** (methyl group on C-3) which shows a strong *m/z* 105 ion and a very weak *m/z* 91 ion. No metastable ions providing information about the precursor(s) of *m/z* 91 were observed in the mass spectrum of **3**.

As seen in Table 1, the abundance of *m/z* 91 was observed to depend on the nature of the 7- substituent (**3**, **4**, **7**, **8**, **9** and **10**). The intensity of *m/z* 91 was greatest when the 7-substituent was NO_2 (**10**) and least when it was NH_2 (**7**). Hammett plots of the log of the intensity ($\%\Sigma_{40}$) values of [*m/z* 91]/[M]$^{+\cdot}$ versus σ_p and σ_{p^+} gave correlation coefficients of 0.90 and 0.92 and slopes of +1.0 and +0.7, respectively. The correlation coefficient for σ_m was lower (0.76). Interestingly, a better correlation (0.96) was observed from the plot of the log of the intensity ($\%\Sigma_{40}$) of *m/z* 91 versus σ_{p^+} (slope = 0.33).

An *m/z* 91 ion was also observed in the mass spectra of some 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-ones (Table 2). The abundance

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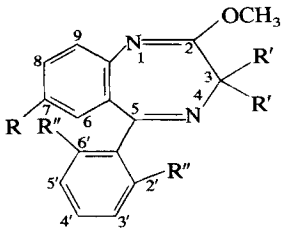
Table 1. Mass spectral data of some substituted 2-methoxy-5-phenyl-3*H*-1,4-benzodiazepines



Compound	R	R′	R″	m/z (%Σ_{40}) $[M]^{+\cdot}$[a]	$[M-H]^{+}$	Rearrangement ion	Other ions[b]
3	Cl	H	H	284(8.6)	283(14.8)	91(6.6)	249(2.1)
3[c]	Cl	H	H	284(7.4)	283(18.1)	91(6.4)	249(2.0)
3a	Cl	H	D	286(6.2)[d]	285(4.5)[a]	93(5.7)	284(12.0), 92(1.2), 91(0.5)
4	H	H	H	250(10.6)	249(20.0)	91(6.3)	77(2.3)
4a	H	D	H	252(9.8)	251(19.8)	93(5.4)	250(2.7), 92(0.8), 91(0.7)
6	Cl	H, CH_3	H	298(7.0)	297(14.2)	105(5.4)	283(2.0), 91(0.2), 77(2.2)
7	NH_2	H	H	265(17.4)	264(21.4)	91(3.9)	249(2.7), 237(2.7)
8	OCH_3	H	H	280(10.1)	279(23.9)	91(5.3)	264(2.4), 252(2.7)
9	CH_3	H	H	264(9.9)	263(19.5)	91(6.6)	249(2.9)
10	NO_2	H	H	295(6.6)	294(11.2)	91(8.1)	278(2.0), 249(2.6), 248(7.6)

[a] Corrected for ^{13}C contribution.
[b] Ions with $\%\Sigma_{40} > 2$ or ions related to the rearrangement ion.
[c] MS data obtained on the same day as **3a**, but one month before the other samples in the table.
[d] Corrected for ^{37}Cl contribution.

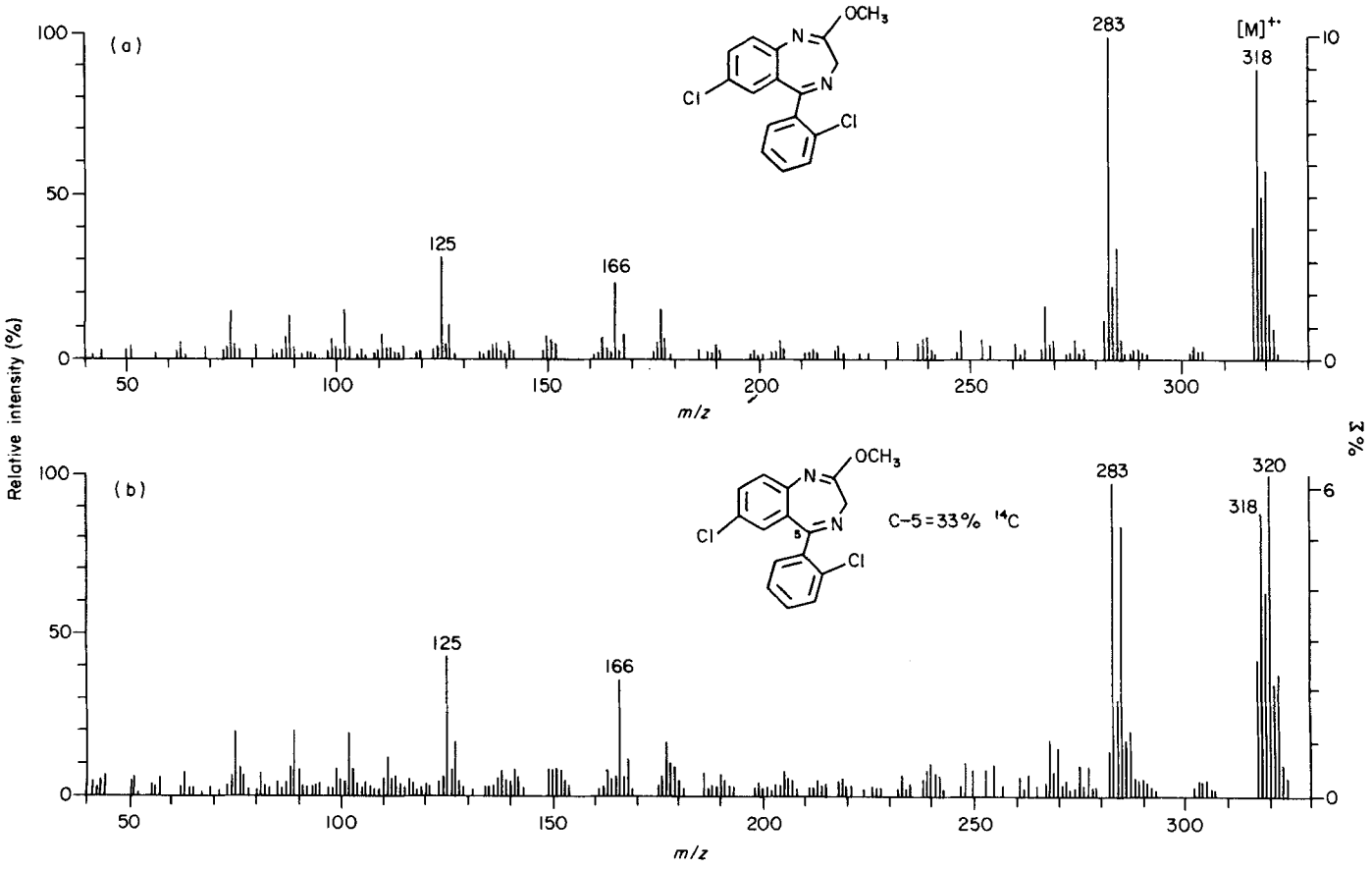
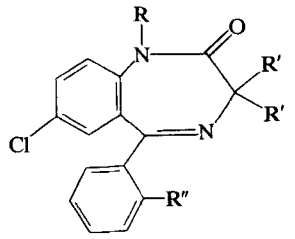


Figure 1. Mass spectra of (a) **5** and (b) **5a**. Compound **5a** contains 33% ^{14}C enrichment at C-5. The spectra are an average of 5 scans. Peaks with less than 1.5% relative abundance are not plotted.

Table 2. Mass spectral data of some substituted 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-ones



Compound	R	R'	R''	$[M]^{+\cdot}$ [a]	$[M-H]^+$	m/z (%Σ_{40}) Rearrangement ion	Other ions[b]
1	G	H	H	270(5.9)	269(6.4)	91(0.7)	242(7.9), 241(7.1), 77(2.2)
2	CH_3	H	H	284(6.7)	283(8.7)	91(1.0)	256(10.3), 255(4.2)
11	CH_2CH_3	H	H	298(5.7)	297(9.3)	91(2.7)	270(10.3), 269(5.2), 241(2.1)
12	$C(CH_3)_3$	H	H	326(0.6)	—	91(10.1)	270(10.2), 269(24.4), 242(3.3), 241(2.8), 57(3.9)
13	CH_2CH_3	H	F	316(6.5)	315(8.8)	109(1.5)	297(3.6), 288(8.7), 287(4.7), 259(2.1), 91(0.7)
14	CH_2CH_3	H, CH_3	H	312(2.7)	311(8.7)	105(1.9)	283(2.6), 271(9.3), 270(8.3), 91(0.3)

[a] Corrected for ^{13}C contribution. [b] Ions with %$\Sigma_{40}>2$ or ions related to the rearrangement ion.

of this ion was dependent on the substituent on N-1. When the N-1 group was hydrogen (**1**) or methyl (**2**), the *m/z* 91 ion was insignificant (%$\Sigma_{40}\leq 1$). However, the intensity of *m/z* 91 increased to 2.7 when the N-1 group was ethyl (**11**) and to 10.1 when the N-1 group was *t*-butyl (**12**). Again, the *m/z* value of this ion shifts in the mass spectra of compounds having a substituent on the 5-phenyl ring (**13**) or on C-3 (**14**).

The low electron energy mass spectral results for **3** and **12** are summarized in Table 3. As the electron energy is lowered, the abundance of a rearrangement ion is usually observed to increase relative to simple cleavage ions.[2] $[M-C_4H_8]^{+\cdot}$, the McLafferty rearrangement ion in the mass spectrum of **12**, does follow this trend. However, the intensities of the *m/z* 91 ions in the mass spectra of **3** and **12** fall off as fast as, or faster than, the other major fragment ions.

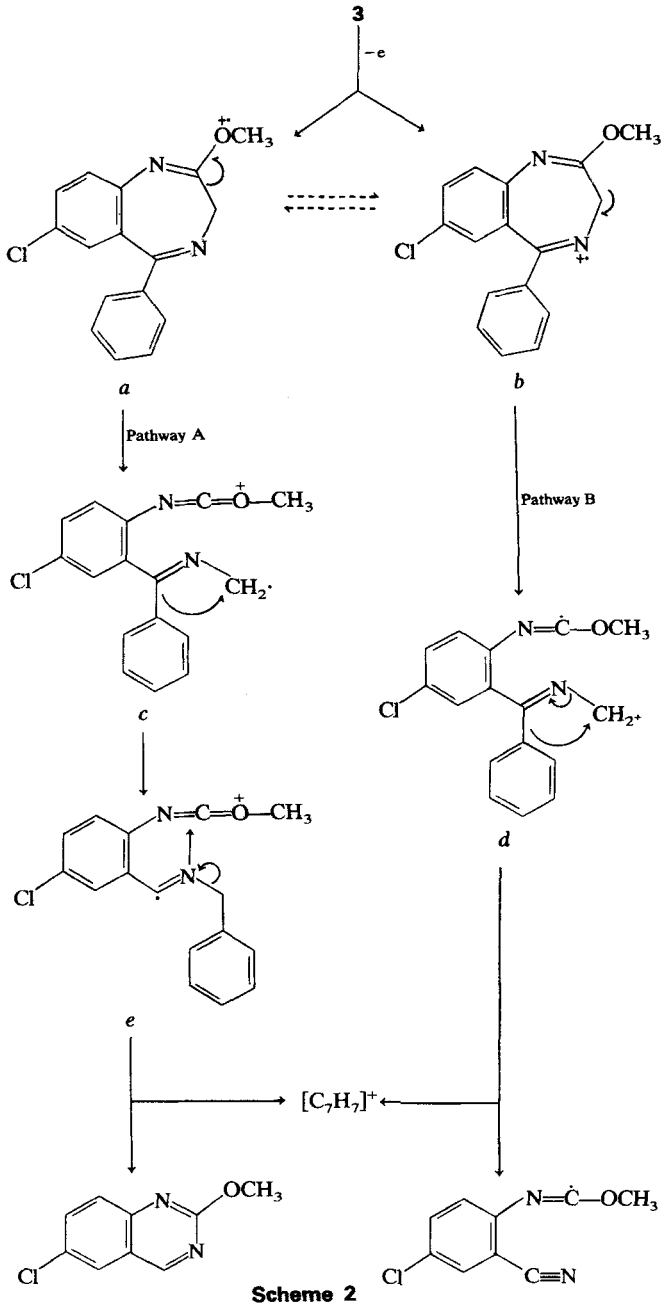
DISCUSSION

For pictorial convenience we have postulated in Scheme 2 two possible mechanisms for the formation

Table 3. Ionization voltage studies of 3 and 12

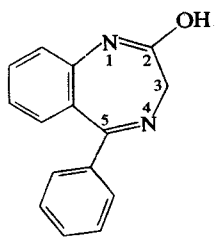
Compound	m/z	Ion[b]	eV[a]	70.0	29.5	24.5	22.0	19.5	17.0	14.5
				%Σ_{40}[c]						
3	284[d]	$[M]^{+\cdot}$		9.9	15.4	20.5	24.7	31.3	39.6	54.4
	283	$[M-H]^+$		18.3	24.4	26.5	26.3	24.8	17.2	5.4
	249	$[M-Cl]^+$		3.0	3.3	3.8	4.1	4.0	3.7	1.5
	231			0.2	0.6	0.8	1.1	1.3	2.4	1.3
	91	$[C_7H_7]^+$		5.7	6.3	3.1	1.6	0.4	0	0
12	326	$[M]^{+\cdot}$		0.7	1.7	1.9	2.4	3.6	5.2	9.6
	270[d]	$[M-C_4H_8]^{+\cdot}$		6.2	9.7	13.3	16.1	20.9	29.6	40.0
	269	$[M-C_4H_9]^+$		21.1	26.9	29.7	31.8	30.6	23.9	12.1
	242			2.6	3.3	3.6	3.5	3.0	2.6	1.1
	241			3.2	2.8	2.5	2.0	1.0	0.4	0
	91	$[C_7H_7]^+$		9.2	11.4	7.5	5.3	2.4	0.5	0
	57			3.2	5.0	3.8	2.8	1.4	0.4	0

[a] The ionization voltages are nominal values obtained from the meter.
[b] Ions were identified by high resolution mass spectra (error≤ 5 mmu).
[c] Fragment ions with %$\Sigma_{40}>2$ are reported.
[d] Corrected for ^{13}C contribution.

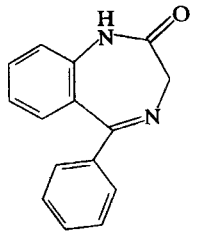


Scheme 2

Table 4. CNDO/2 calculations on the ground states of the neutral molecules and the positively charged (equilibrium) molecular ions of two 5-phenyl-1,4-benzodiapines



15a



15b

Compound	Charge density N-1	C-2	2-O	OH/NH	C-3	3-H	N-4	C-5	Aromatic carbons	Aromatic hydrogens
15a[a]	−0.27	+0.28	−0.24	+0.16	+0.05	0	−0.17	+0.14	−0.03→+0.10	−0.01→0
[15a]$^{+\cdot}$	−0.01	+0.38	−0.15	+0.25	+0.07	+0.10	+0.01	+0.25	−0.01→+0.14	+0.01→0.08
[15a]$^{+\cdot}$**−15a**	+0.26	+0.10	+0.09	+0.09	+0.02	+0.10	+0.18	+0.11	−0.02→+0.06	+0.01→0.08
15b[a]	−0.21	+0.35	−0.34	+0.12	+0.02	0	−0.16	+0.12	−0.04→+0.01	−0.01→0
[15b]$^{+\cdot}$	−0.10	+0.42	+0.04	+0.22	+0.06	+0.10	−0.01	+0.25	0→+10.13	+0.01→0.07
[15b]$^{+\cdot}$**−15b**	+0.11	+0.07	+0.38	+0.10	+0.04	+0.10	+0.15	+0.13	−0.01→+0.07	+0.02→0.07

[a] Using Koopman's theorem,[10] the ionization potentials of the highest occupied molecular orbital in **15a** and **15b** were calculated to be 9.77 and 9.70 eV, respectively.

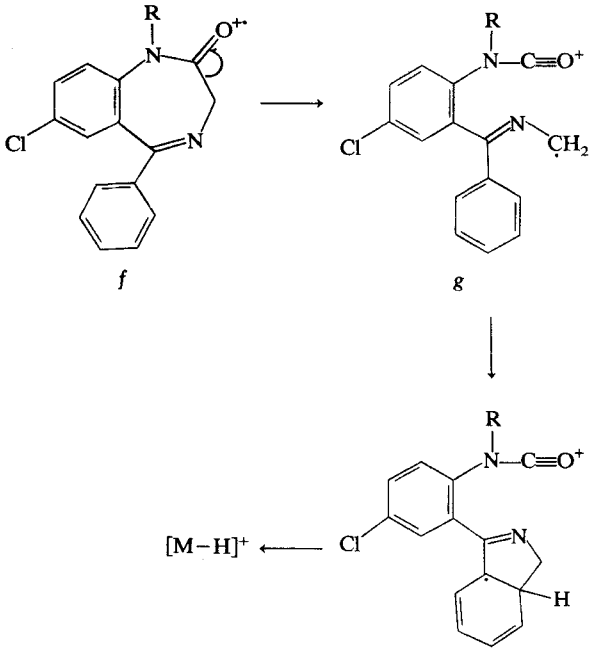
of *m/z* 91 from **3**. Since CNDO/2 calculations (Table 4) on the neutral and positively charged molecules of **15a**, a model compound for **3**, indicated that most of the charge is equally localized on the (N-1)=(C-2)—O group and on the (N-4)=(C-5) group, we have represented [**3**]$^{+\cdot}$ by *a* and *b*. A possible mechanism for the fragmentation of *a* to *m/z* 91 is shown in pathway A. This mechanism includes migration of the phenyl group to a radical and is complicated by the remoteness of the charge from the benzyl group (ion *e*). Pathway B includes the migration of the phenyl to a carbonium ion and it appears to be simpler and more favorable than pathway A. The migration of the phenyl and the cleavage of the (N-4)—(C-3) bond could be one step as shown, or two steps.

In both pathways the molecular ions are postulated as undergoing cleavage of the (C-2)—(C-3) bond, isomerization about the (N-4)=(C-5) bond and possibly phenyl migration before formation of the *m/z* 91 ion. If one of these processes had a high activation energy and was the rate determining step in the formation of *m/z* 91, it could explain why *m/z* 91 does not behave like a 'normal' rearrangement ion. For example, the behavior of *m/z* 91 with decreasing electron energy would be a reflection of the rate determining step and not the last step where *m/z* 91 is being formed. Second, if the rate of fragmentation is dependent upon the rate of formation of an intermediate molecular ion species and if that intermediate does not have a sufficient lifetime to be metastable, then a metastable peak is not expected.[3]

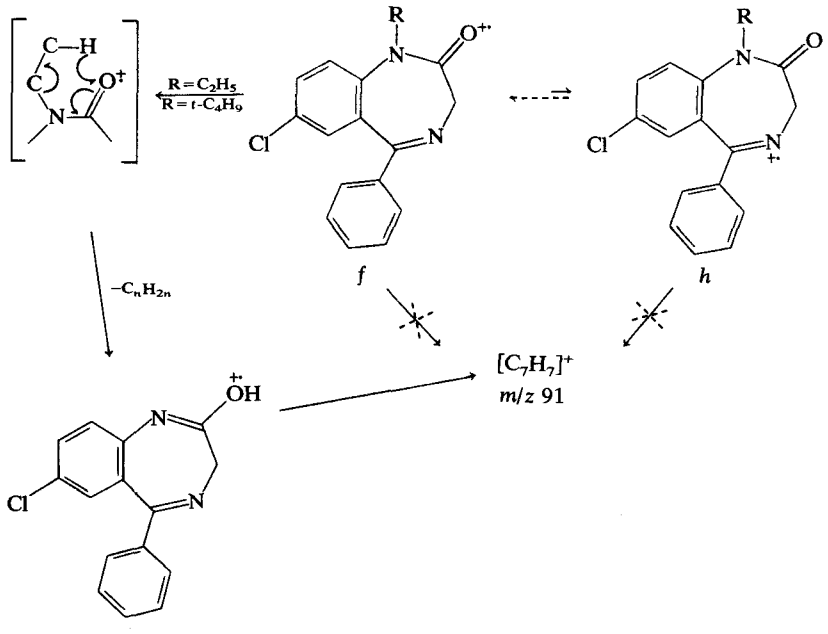
The good correlation observed in the Hammett plots of the log of the intensity values of [*m/z* 91]/[M]$^{+\cdot}$ versus σ_p and σ_{p^+} could be interpreted as favoring pathway A where the 7-substituent is *para* to the cation. We are reluctant to come to this conclusion without the corresponding Hammett parameters from the 8-substituents. An insufficient number of compounds prevented us from making this study.

A major fragment ion in the mass spectra of all compounds listed in Table 1 is [M−H]$^+$. Analysis of the mass spectrum of **3a**, the 2′,6′-dideutero analog of **3**, indicates that most (≥65%) of the hydrogen is lost from the 2′,6′ positions of the 5-phenyl ring. We reported previously[4] that the preferential loss of the 2′,6′ hydrogens to form [M−H]$^+$ in the mass spectra of 5-phenyl-1,4-benzodiazepin-2-ones could be explained by the mechanism shown in Scheme 3. Ion g is similar to ion *c* in pathway A of Scheme 2. Therefore, *c* may be fragmenting to [M−H]$^+$ by a mechanism similar to that shown in Scheme 3.

In Scheme 4 we have attempted to rationalize the variation in the abundance of *m/z* 91 in the mass



Scheme 3



Scheme 4

spectra of the 5-phenyl-1,4-benzodiazepin-2-ones containing different N-1 groups. CNDO/2 calculations on **15b** and $[\mathbf{15b}]^{+\cdot}$ (Table 4) suggest that *f* is a better representation of the molecular ion than *h*. The low abundance of *m/z* 91 in the spectra of compounds where R=H (**1**) or CH_3 (**2**) suggests that *f* and *h* do not undergo significant fragmentation via mechanisms similar to pathways A and B (Scheme 2). When R= ethyl (**11**) or *t*-butyl (**12**), the mass spectra show a relatively strong *m/z* 91 and a strong McLafferty rearrangement ion at *m/z* 270. Therefore, it seems reasonable that compounds containing a β-hydrogen on the N-1 group first undergo a McLafferty rearrangement to form *i*. Then ion *i*, which is very similar to *a* (Scheme 2), fragments to *m/z* 91 via pathway A or B.

The elucidation of the *m/z* 91 ion described in this paper should be helpful in the characterization of metabolites of 5-aryl-1,4-benzodiazepin-2-one drugs. Reaction of the metabolites with diazomethane and the mass spectral analyses of the *O*-methyl products should differentiate between hydroxylation on the 5-phenyl ring, on the fused benzene ring and at C-3.

EXPERIMENTAL

Mass spectrometry

All low resolution mass spectral data, with the exception of the low voltage spectra, were obtained on an Hitachi RMU-6L mass spectrometer interfaced with a Watson-Biemann separator to a Perkin-Elmer Model 990 gas chromatograph. The ionization voltage was 70 eV. The temperature of the source and interface were 200–220 °C and 260–290 °C, respectively. The column (1.8 m × 4 mm i.d.) was packed with 3% SE-30 on 80/100 Chromosorb Q (Applied Science Laboratories). The initial GC oven temperature was 180 or 200 °C and the temperature program rate was 4 °C min^{-1}. Helium was used as the carrier gas (30 ml min^{-1}). The data were collected on a Varian 620i computer. The intensities of 5 scans were averaged for each spectrum.

High sensitivity mass spectra of **3** (for observation of metastable peaks) were obtained on the Hitachi RMU-6L mass spectrometer at 70 eV using the direct insertion probe.

Low voltage spectra of **3** and **12** were obtained on a Varian MAT CH5 mass spectrometer using the direct insertion probe. The intensities of 5–8 scans were averaged.

High resolution MS data were obtained with a CEC 21-110 mass spectrometer using the direct insertion probe. The spectra were recorded on Ionomet photoplates at 70 eV.

Variations in ion intensities were observed in the mass spectra obtained of the same sample on different days. To minimize these variations, samples of any important series, i.e. deuterated vs nondeuterated compounds and low voltage studies, were run on the same day.

Reference compounds

Compounds **3a** and **4–10** were prepared by reacting the corresponding 1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (0.5 mg in 0.1 ml of Mallinckrodt Nanograde methanol) with 0.5 ml of ethereal diazomethane overnight in a stoppered vial at room temperature. The samples were blown dry with a stream of nitrogen and the residue was reconstituted in 0.2 ml of methanol. Gas chromatographic analyses indicated that the *N*-methyl and *O*-methyl products were formed in about equal amounts. When diazoethane was used, alkylation of the oxygen was

favored by 2:1. The *O*-alkyl products had shorter GC retention times and better peak shapes than the *N*-alkyl products.

Diazomethane[4] and diazoethane[5] were prepared from *N,N*-nitrosomethylurea (supplied by Hoffmann-La Roche Inc.) and *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co.), respectively, using filtration procedures.

Compounds **1**, **2**, **3**, **11**, **12** and **13** and the precursors for **3a**, **4–10**, and **14** were obtained from the Chemical Research Department, Hoffmann-La Roche Inc. All compounds gave satisfactory elemental analyses. The precursor to **5a**, which contained 33% ^{14}C enrichment at C-5, was prepared by an unambiguous synthesis. The deuterium content of the precursors to **3a** (0% d_0, 11% d_1, 89% d_2) and **4a** (1% d_0, 5% d_1, 94% d_2) were determined. No significant loss of deuterium was observed in the reaction of the deuterated precursors with diazomethane. The computer program used to calculate the isotopic content of **3a**, **4a**, **5a** and their precursors, and the ratio of M−H to M−D in **3a**, has been described previously.[6]

CNDO/2 calculations

The molecular orbital program was written by A. Chung-Phillips, Miami University, Oxford, Ohio, and was obtained from the Quantum Chemistry Program Exchange.[7] Most of the atom coordinates were taken from the X-ray crystallography data reported by Sternbach *et al.*[8] Standard bond lengths and angles from Pople and Gordon[9] were used for N-1, C-2, 2-O and O—H in **15a**.

Acknowledgments

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