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The mass spectral fragmentation of 4-isoxazolyl-1,4-dihydropyridines has been examined with the aid of linked metastable scanning. Three prominent pathways involve (i) O-N bond cleavage of the isoxazole followed by the loss of R^2CN , (ii) loss of carboalkoxy from the 3- and/or 5- position of the dihydropyridine and, (iii) loss of the 4-isoxazolyl-substituent.

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4-Aryl-dihydropyridines are of interest as antagonists of trans-membranal calcium influx [1]. Carbocyclic aryl groups, however, are generally not able to act as a reactive site for covalent affinity labeling. We are interested in a system which retains enough structural similarity to be an effective calcium entry antagonist, which also contains a labile functional group capable of acting as a covalent affinity label. Ideally, a covalent affinity label can be transformed into a reactive intermediate after binding the active site, thus providing structural information concerning the active site. A common mode of triggering this transformation is by means of photolysis, and photochemistry is also often correlated to mass spectral fragmentation patterns [2].

Recently we have reported the synthesis of a 4-isoxazolyl-1,4-dihydropyridine [3a] and subsequently have found that this class of compounds is active as calcium channel blockers [3b]. We wish to present here mass spectral evidence that the oxygen-nitrogen bond is labile, that this fragmentation is accompanied by metastable ions, and therefore, the isoxazolyl moiety has potential as a photo affinity label.

The mass spectrometry of isoxazoles [4-10] is usually characterized by cleavage of the oxygen-nitrogen bond

followed by fragmentation. Information is lacking in the literature, however, on the mass spectral fragmentation of compounds containing isoxazoles in the presence of other heterocyclic nuclei [11]. We have prepared a series of

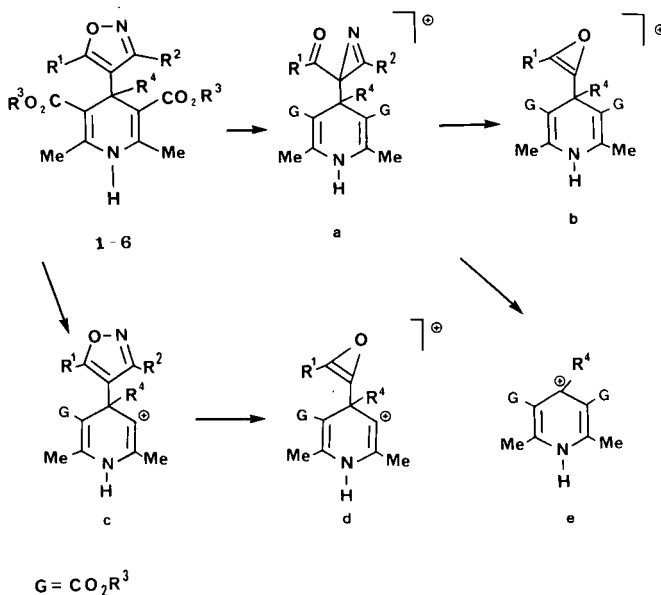


Table 1

4-Isoxazolyl-1,4-dihydropyridines

Entry	R ¹	R ²	R ³	R ⁴	mp [a]	Formula	mw	Anal. Found (Calcd.)	
								C	H
1	Me	Me	Et	H	151-153	C ₁₈ H ₂₄ N ₂ O ₅	348	ref [3]	
2	Me	Et	Et	H	152-154	C ₁₉ H ₂₆ N ₂ O ₅	362	63.27 (62.96)	6.98 (7.23)
3	Me	Ph	Et	H	200-202	C ₂₃ H ₂₆ N ₂ O ₅	410	67.15 (67.30)	6.44 (6.38)
4	Me	Me	Et	D	151-153	C ₁₈ H ₂₃ DN ₂ O ₅	349	[b]	
5	Et	Me	Et	H	142-144	C ₁₉ H ₂₆ N ₂ O ₅	362	62.76 (62.96)	7.16 (7.23)
6	Me	Me	Me	H	239-241 [c]	C ₁₆ H ₂₀ N ₂ O ₅	320	60.36 (59.99)	6.47 (6.29)

[a] Melting points are corrected, unless otherwise noted. [b] See experimental. [c] Uncorrected.

dihydropyridines (Table 1) and examined their fragmentation pathways and also report herein on linked metastable scanning [12] studies.

Results and Discussion.

The mass spectrum of **1**, as well as linked metastable scans, are shown in Figure 1. Compound **1** shows a respectable molecular ion in the EI spectrum (22% relative intensity) (Figure 1, spectrum i) and a base peak at m/z 234.

In order to elucidate the fragmentation pathway constant neutral loss, daughter ion and precursor ion linked metastable scans were examined. Linked scanning indicates that prominent ions arising from the M^+ (Figure 1, spectrum ii) occur at 333 (M-15); 319 (M-29); 275 (M-73) and 234 (M-114). Constant loss scans were examined for loss of $-CO_2Et$ (73) and CH_3-CN (41). The constant loss 73 linked scan (Figure 1, spectrum iii) gave rise to ions at 348, 307, 275 and 247. The constant loss 41 linked scan gave

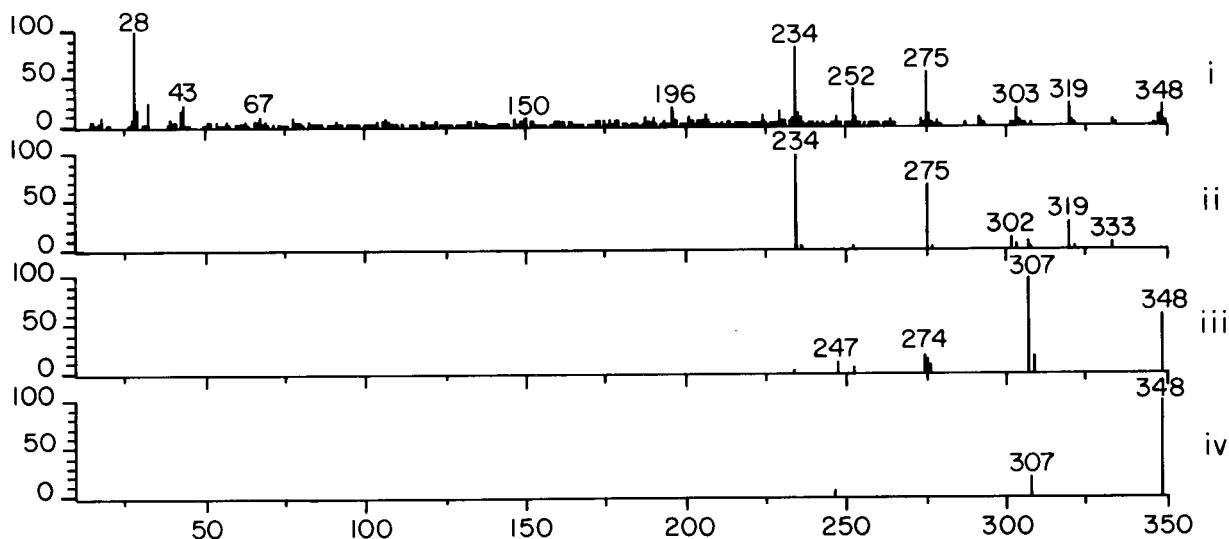


Figure 1. Mass Spectra, including linked metastable scanning study for **1**.

(i) Normal Scan. (ii) Daughter ion (B/E) scan of M^+ , m/z 348. (iii) Constant neutral loss (B/E $\sqrt{1-E}$) scan of m/z 73. (iv) Precursor (B²/E) scan of m/z 234.

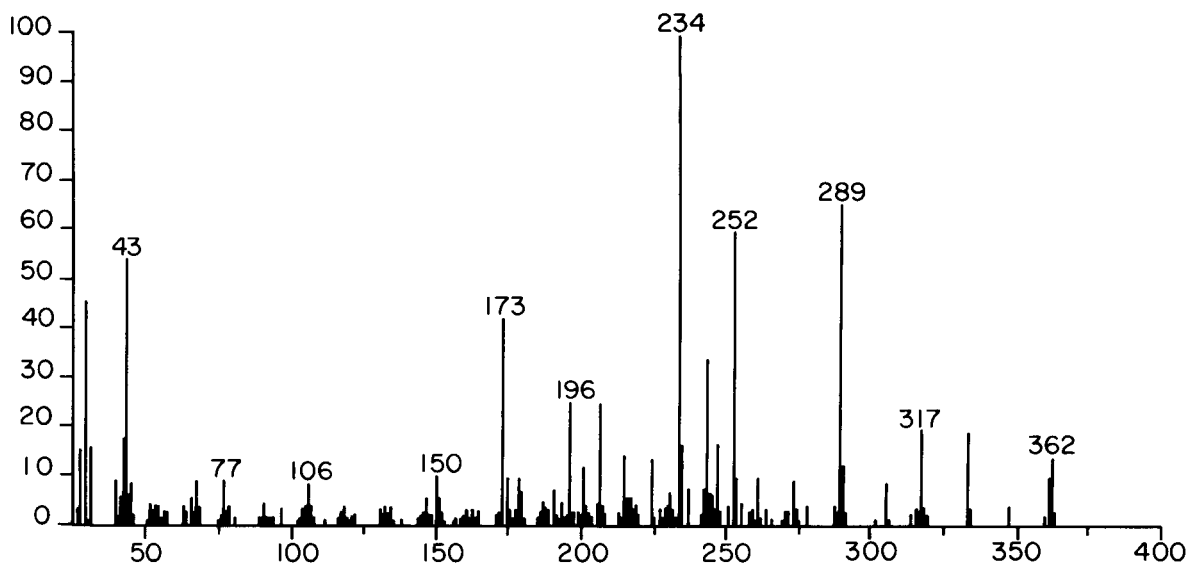


Figure 2. Mass spectrum for **2**

rise to ions of 348, 277, 274, 246, 228, 202 and 189. A precursor ion scan (Figure 1, spectrum iv) for ions which give rise to m/z 234 gave ions at 348 and 307. The results are consistent with cleavage of the oxygen nitrogen bond of the isoxazole **1** to give (a) which then undergoes loss of acetonitrile ($R^2 - CN$) to produce ion (b), m/z 307. This ion undergoes rapid loss of carboethoxy to produce the base ion, m/z 234. Direct loss of carboethoxy is evidenced by the ion (d) at m/z 275, in spectrum i.

In addition an ion is observed at m/z 252, this ion probably arises from loss of the isoxazolyl moiety to give ion (e). An ion of this type is commonly observed in the mass spectra of 1,4-dihydropyridines [15-17]. The mass spec-

trum of **2** shows similar features (Figure 2), a significant molecular ion and ions at m/z 234 (base ion) and 252.

The compound **3** differs only in the substitution at the R^2 position. The base ion of this compound is again observed at m/z 234 (Figure 3, i), thus the R^2 group is lost, most likely as benzonitrile. In addition the 252 ion is observed, as well as prominent M-41 (m/z 381) and M-73 (m/z 337). Linked scanning presents strong evidence that the compound **3** fragments by the analogous pathway. The base peak at m/z 234 arises from the molecular ion (Figure 3, spectrum ii), the ion of m/z 337 undergoes constant neutral loss of 103 to 234 (Figure 3, spectrum iii) and the complementary process, loss of 73 from m/z 307, is observ-

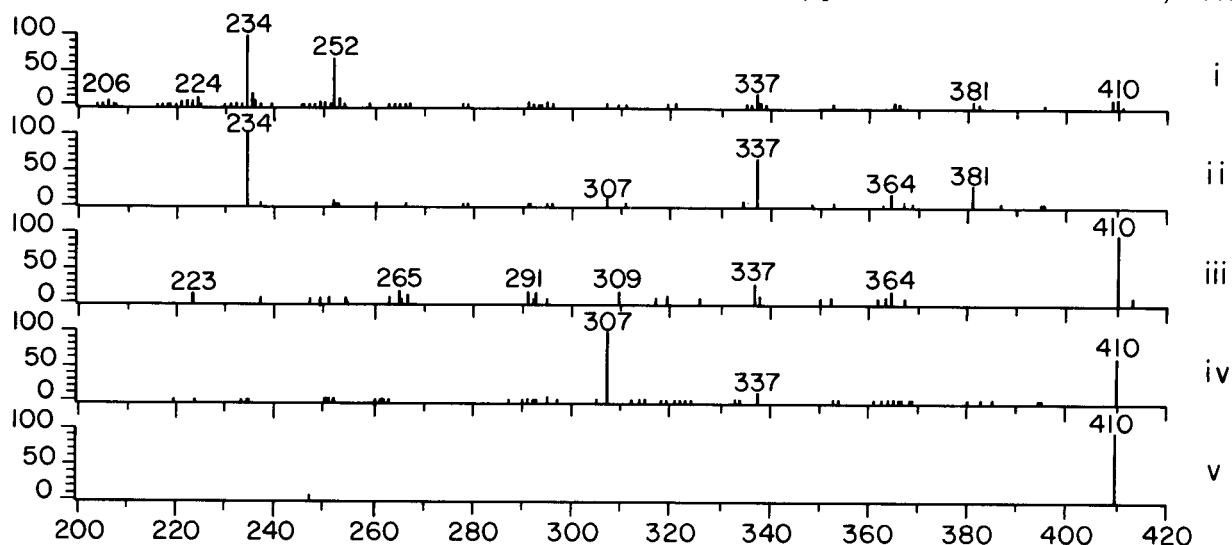


Figure 3. Mass spectra, including linked metastable scanning study for **3**.

(i) Normal scan. (ii) Daughter ion scan of M^+ , m/z 410. (iii) Constant neutral loss scan of m/z 103. (iv) Constant neutral loss scan of m/z 73. (v) Precursor scan of m/z 234.

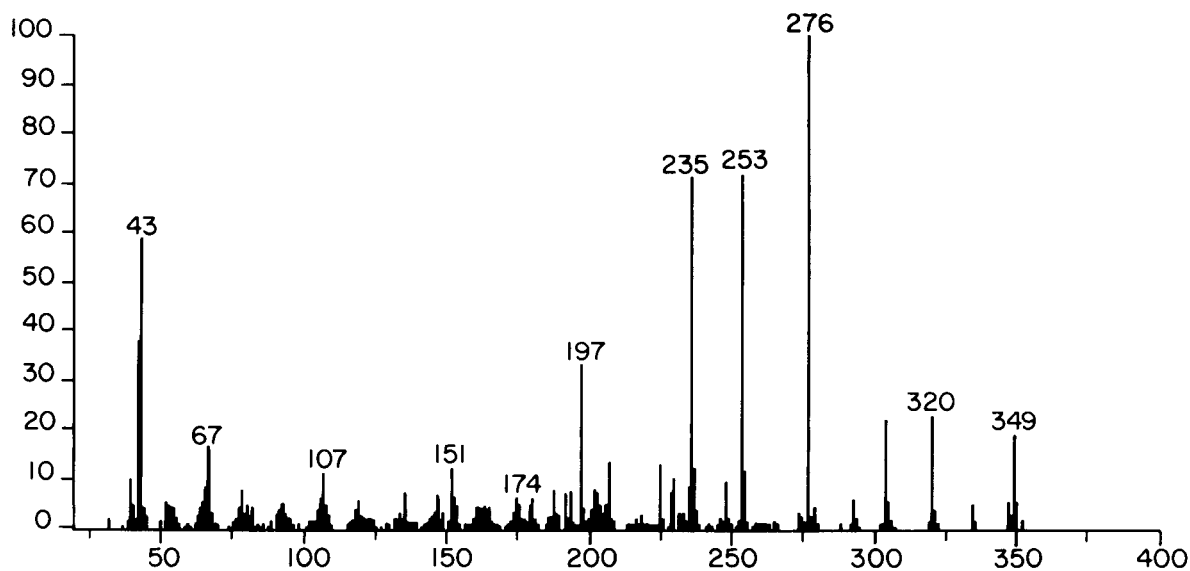


Figure 4. Mass spectrum for **4**.

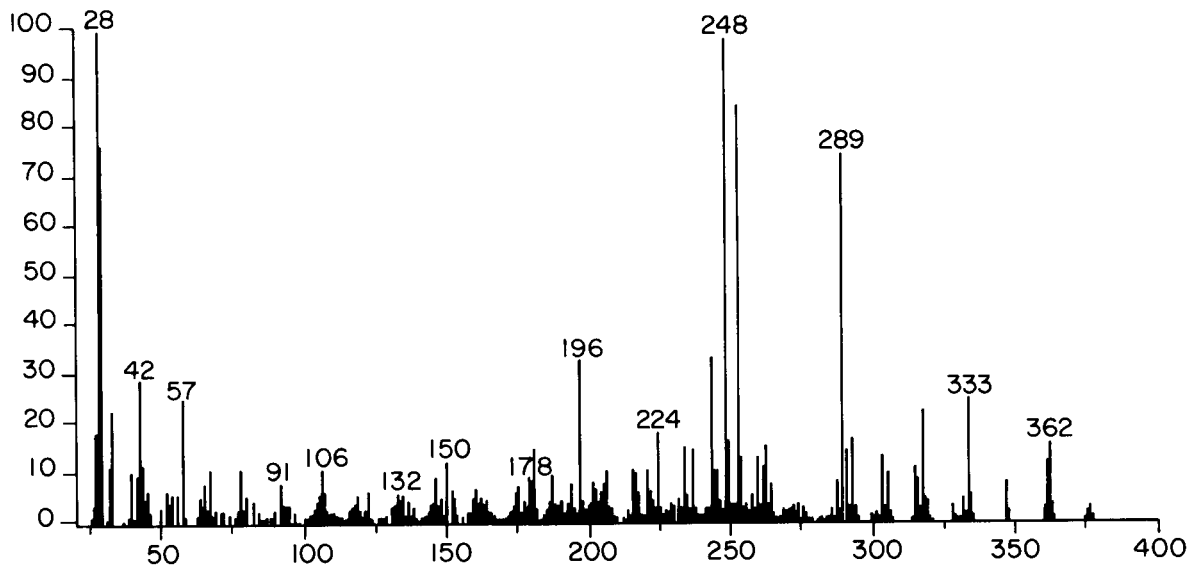


Figure 5. Mass spectrum for 5.

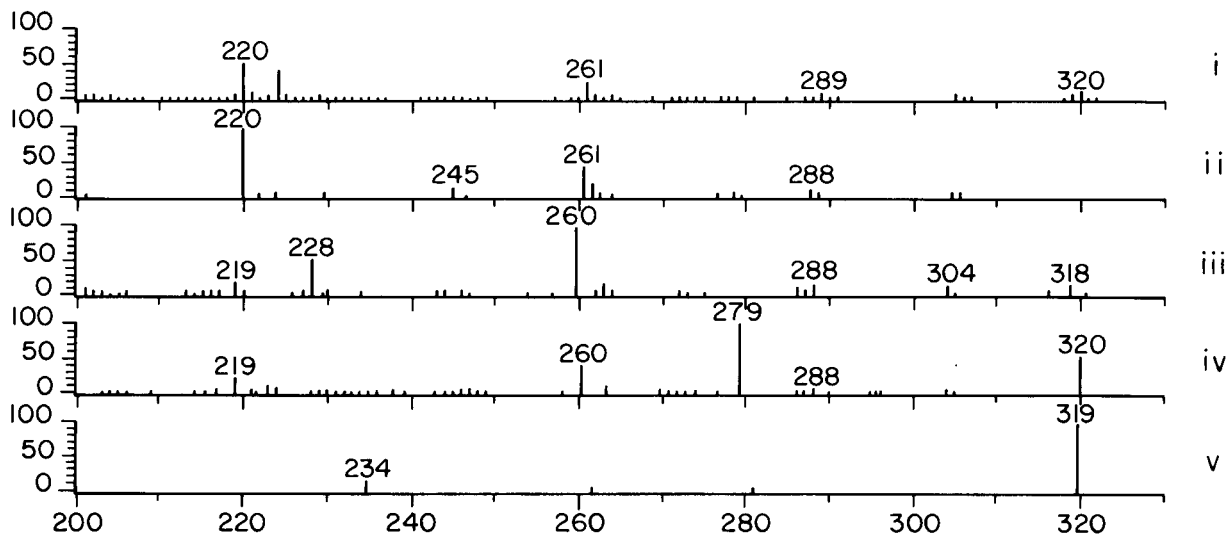


Figure 6. Mass spectra, including linked metastable scanning study for 6.

(i) Normal scan. (ii) Daughter ion scan of M^+ , m/z 320. (iii) Constant neutral loss scan of m/z 41. (iv) Constant neutral loss scan of m/z 59. (v) Precursor scan of m/z 220.

ed (Figure 3, spectrum iv). Thus the analogous fragmentation of **3** to (d) is indicated. Deuteration at the C-4 position of the dihydropyridine (Figure 4, mass spectrum of **4**) gives a base peak of m/z 276; however, the 235 and 253 ions are still quite prominent. For compound **5** (Figure 5) the base peak is observed at m/z 248. This provides further evidence that $M-R^2CN$ contributes to the base ion and that the R^1 group is retained in the ion. To elucidate the carboalkoxy fragmentation pathway the fragmentation of **6** was examined (Figure 6). A molecular ion is observed (m/z 320) and the base peak is observed at m/z 220. Prominent ions are observed at m/z 305, 289, 261 and 224. Thus loss of a single carboalkoxy to give the base ion is indicated.

Conclusion.

The mass spectral fragmentation of isoxazolyldihydropyridines **1-6** is characterized by cleavage of the isoxazoly O-N bond followed by an unusual loss of the R^2CN moiety. This fragmentation is accompanied by metastable ions; therefore, this transformation may be plausible as a photochemical reaction. Attempts to reduce this to practice as a synthetic tactic are now in progress.

EXPERIMENTAL

Mass spectra were obtained on a VG Micromass 70/70HS mass spectrometer with an 11/250 data system. Constant neutral linked magnetic

field - electric sector scanning was performed using the procedure described by Haddon [12,13].

The isoxazolyldihydropyridines **1-6** were prepared as previously described [3,14]. Purity was assessed by ¹H, ¹³C nmr, thin layer chromatography and combustion analysis. Combustion analysis for **1** has been reported, mp 151-153 (corrected) (lit [3] mp 155-157, uncorrected).

Compound **4** was prepared from 3,5-dimethylisoxazolecarboxylic acid in three steps. The acid was first reduced with lithium aluminum deuteride (THF, 0°) to give 3,5-dimethylisoxazole-4-d₂-carbinol (77% yield); gc-ms: m/z 129 (27% relative intensity), 111 (14), 82 (22), 73 (20), 43 (100). Oxidation of the d₂-alcohol to 3,5-dimethylisoxazole-4-d₂-carbaldehyde was performed according to Swern [18]. The product was obtained as an oil in 83% yield; ¹H nmr (deuteriochloroform): no detectable signal at 9.8 (-CDO), 2.6 (s, 3H), 2.4 (s, 3H); ms: m/z 126 (48% relative intensity), 111 (15), 84 (14), 83 (40), 82 (44), 70 (9), 55 (15), 43 (100). The d₁-aldehyde was subjected to the Hantzsch synthesis, as previously described [3], to give d₁-dihydropyridine (**4**).

Compound **6** had ¹H nmr (deuteriochloroform): 6.0 (br s, 1H), 4.9 (s, 1H), 3.6 (s, 6H), 2.4 (s, 9H), 2.2 (s, 3H); ¹³C nmr (deuteriochloroform): 167.6, 166.17, 159.62, 144.06, 119.69, 100.8, 50.86, 29.06, 18.98, 10.95, 9.93.

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