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# Fast Atom Bombardment Mass Spectrometry of Macrocyclic Diester Pyrrolizidine Alkaloid *N*-Oxides

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Fast atom bombardment mass spectra of macrocyclic diesters of retronecine *N*-oxide are characterized by an $(M + H)^+$ pseudomolecular ion base peak, an $(M + H - 16)^+$ ion, and a prominent ion series with $m/z$ 136, 120, 118, 106, 94, and 80.

Pyrrolizidine alkaloid *N*-oxides represent one of the largest and better known groups of naturally occurring alkaloid *N*-oxides (Phillipson and Handa, 1978). Electron impact (EI) mass spectra of pyrrolizidine alkaloid *N*-oxides often do not exhibit a molecular ion (Culvenor et al., 1975). Abdullaev et al. (1974) have noted that even when the molecular ion is absent, a characteristic triplet of ion peaks at M – 16, M – 17, and M – 18 is usually encountered and may be used to identify the molecular weight of the compound. Methane chemical ionization (CI) mass spectra show a useful pseudomolecular ion $(M + H)^+$ with simple C-9 mono- and C-7, C-9 diesters of retronecine *N*-oxide; however, macrocyclic diesters such as monocrotaline *N*-oxide do not show a significant $(M + H)^+$ ion in their CI mass spectra. The macrocyclic diesters, which are commonly encountered, are representative of the most difficult pyrrolizidine alkaloid *N*-oxides to analyze by conventional mass spectrometry. This paper describes the preliminary results obtained from the fast atom bombardment (FAB) mass spectral analysis of five macrocyclic diesters of retronecine *N*-oxide.

## EXPERIMENTAL SECTION

Parent pyrrolidizine alkaloids were obtained as previously described (Karchesy et al., 1984). Final purification was accomplished by preparative HPLC (Whatman C-8, methanol–0.1 M pH 6.0 phosphate buffer) and recrystallization from methanol. Identities were confirmed by melting points, GC–MS, and NMR. *N*-Oxides were prepared by oxidation of the parent pyrrolizidine alkaloid with hydrogen peroxide in methanol (Mattocks, 1969). The *N*-oxide products gave melting points in agreement with the literature (Bull et al., 1969; Culvenor et al., 1970). Samples for mass spectral analysis were prepared in glycerol and placed on a copper sample support. Spectra were obtained with a Varian CH-7 mass spectrometer modified to accept an Ion Tech, Ltd., saddle field ion source. Xe was used as the primary atom beam with the saddle field ion source operating at 7 keV. Spectra were calibrated with perfluorokerosene in the EI mode and recorded in the FAB mode with a System Industries 150 data system.

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**Table I. Key Ions of Macrocyclic Diester Pyrrolizidine Alkaloid *N*-Oxides Investigated, $m/z$ (Relative Intensity)**

| ion | *N*-oxide 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| $(M + H)^+$ | 342 (100) | 368 (100) | 352 (100) | 350 (100) |
| $(M + H - 16)^+$ | 326 (5) | 352 (15) | 336 (14) | 334 (18) |
| $m/z$ 136 | (24) | (27) | (22) | (33) |
| $m/z$ 120 | (30) | (56) | (28) | (55) |
| $m/z$ 118 | (30) | (50) | (33) | (55) |
| $m/z$ 106 | (14) | (24) | (13) | (26) |
| $m/z$ 94 | (17) | (28) | (16) | (29) |
| $m/z$ 80 | (12) | (24) | (14) | (26) |

## RESULTS AND DISCUSSION

FAB mass spectra of the *N*-oxides of the pyrrolizidine alkaloids monocrotaline (1), jacobine (2), senecionine (3), seneciphylline (4), and retrorsine (5) were readily obtained without special preparation or reagents to promote ionization. As shown in Table I and Figure 1, the spectra are characterized by an $(M + H)^+$ pseudomolecular ion as the base peak, an $(M + H - 16)^+$ ion, and a prominent ion series with $m/z$ 136, 120, 118, 106, 94, and 80. The prominent ion series is similar to, but not identical with

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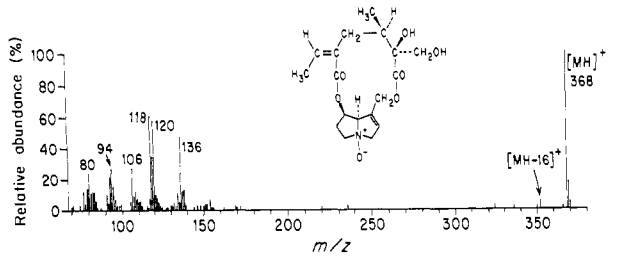


**Figure 1.** FAB mass spectrum of retrorsine *N*-oxide (**5**).

the characteristic ion series, $m/z$ 136, 120, 119, 93, and 80, seen in the EI mass spectra of all macrocyclic diesters of retronecine (Crout, 1969). Similar lower ion series are also observed in the EI mass spectra of nonmacrocyclic tertiary amine (Pedersen and Larsen, 1970), *N*-oxides (Culvenor et al., 1975; Abdullaev et al., 1974), and pyrrole (Mattocks, 1969) derivatives of retronecine esters. By analogy to the structures proposed for these ions, it is rationalized that the prominent ion series in the FAB spectra is also derived from the pyrrolizidine ring system.

In the EI mass spectra of retronecine *N*-oxide derivatives, a dual fragmentation pathway is observed that first involves loss of the *N*-oxide oxygen to give the tertiary base (M – 16) and the pyrrolic dehydro alkaloid (M–17, M–18), which act as precursors to the lower ion series (Culvenor et al., 1975; Abdullaev et al., 1974). These pathways may be operating under FAB conditions as well. In addition to the $(M + H - 16)^+$ ion noted above, a small ion at $(M + H - 18)^+$ with 2–3% relative abundance is also observed in the FAB mass spectra.

Presently macrocyclic diester pyrrolizidine alkaloid *N*-oxides are often routinely reduced in the isolation process to their tertiary bases (Phillipson and Handa, 1978; Huizing and Malingré, 1979). This is done at least in part to facilitate the analysis or structural elucidation of the parent *N*-oxide. Using FAB mass spectrometry, one can directly analyze the parent *N*-oxide.

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# Nitrite Inhibition of Acyl Transfer by Coenzyme A via the Formation of an *S*-Nitrosothiol Derivative

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At 25 °C and at pH 6 and below, coenzyme A reacts with nitrite in aqueous media to form an *S*-nitrosothiol (thionitrite) derivative. Spectroscopic observation shows that in a pH 2 buffer the reaction essentially reaches completion after 0.5 h. Raising the pH to 6 decreases, but does not completely suppress, both the rate and the extent of reaction. The derivative formed is stable with respect to hydrolysis over a wide range of pH (1–13). In the presence of phosphotransacetylase, this *S*-nitrosothiol derivative, in contrast to coenzyme A itself, is incapable of accepting activated acetyl groups. These results suggest a possible coenzyme A related mechanism that may contribute, in part, to the inhibition of the outgrowth of bacteria such as *Clostridium botulimum* in processed food products.

The mechanism by which nitrite inhibits bacterial growth is still unknown. Recent work with aerobic bacteria has shown that oxidative phosphorylation may be inhibited by the presence of nitrite (Rowe et al., 1979; Yarbrough et al., 1980). On the other hand, nitrite was found to inhibit the phosphorylation reactions associated with the phosphoroclastic system in the extracts of *Clostridium sporogens* (Woods et al., 1981). A recent electron spin resonance study with *Clostridium botulinum* (Reddy et al., 1983) suggests that the interaction between nitrite and proteins containing iron–sulfur clusters may be the source of the effects on the energy metabolism processes mentioned above. After treatment with nitrite, bacterial cultures of *Clostridium perfringens* were found to exhibit substantially decreased activity of glycolytic enzymes, e.g., glyceraldehyde-3-phosphate dehydrogenase and aldolase (O'Leary and Solberg, 1976). However, whether nitrite interacts directly or indirectly with these enzymes remains unresolved.

Under appropriate conditions, nitrite reacts with simple thiol compounds to produce *S*-nitrosothiol (thionitrite) derivatives (Byler et al., 1983; Kresze and Uhlich, 1959; Oae et al., 1978). Accompanying the nitrite-induced decrease of glycolytic enzymatic activities, there is also a significant decrease of free –SH concentration in soluble

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