

**MASS SPECTRAL FRAGMENTATION OF ORELLANINE AND ITS
TETRAMETHYL ETHER WITH REGARD TO THEIR FACILE
THERMAL AND PHOTOCHEMICAL DEOXYGENATION**

**Wiesław Z. Antkowiak*, Róża Antkowiak, Elżbieta Wyrzykiewicz, and
Grzegorz Czerwiński**

Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

This paper is dedicated to Dr. Arnold Brosst on the occasion of his 70th birthday.

Abstract - The Elms fragmentation of orellanine, its tetramethyl ether, and orelline were investigated. Fragmentation pathways, the elucidation of which was assisted by accurate mass measurements and metastable transitions, are proposed. A mechanism of the facile *N*-oxide oxygen elimination as $\cdot\text{OH}$ or $\cdot\text{OCH}_3$ found to be a primary process is discussed.

The mushroom alkaloid orellanine (**1**) of 3,3',4,4'-tetrahydroxy-2,2'-bipyridine-*N,N'*-dioxide structure¹ and several model compounds whose molecules contain an oximinopropenoxy [*N*-O,3'-OR] system were found to eliminate oxygen easily at elevated temperature or upon uv irradiation. It has been suggested that this process is preceded by a sigmatropic rearrangement with [1,5]-oxygen shift from *N*-oxide to the hydroxyl group at C-3 of the second ring and a formation of an unstable peroxide intermediate² In the recent years mass spectral fragmentation data of **1** were occasionally quoted^{3,4} and at an earlier stage of our study on this alkaloid constituted the fundamental evidence in the structure assignment.⁵ In 1989 Richard and Ulrich reported⁶ some data, not fully consistent with our observations, concerning the EI mass spectra of **1** in comparison with those of some related bipyridine-*N*-oxides: 2,2'-bipyridine-*N*-oxide, 2,2'-bipyridine-*N,N'*-dioxide and 4,4'-dihydroxy-2,2'-bipyridine-*N,N'*-dioxide.

According to our recent results, under electron impact conditions the 3,3'-dihydroxy- and 3,3'-dimethoxy-2,2'-bipyridine-*N,N'*-dioxides, contrary to the corresponding 4,4'-isomers, revealed the preference of $\cdot\text{OH}$ or $\cdot\text{OCH}_3$ and $\cdot\text{OH}$ radicals elimination, respectively (accompanied by the thermally induced oxygen loss) indicating the special reactivity of the oximinopropenoxy system of the 3,3'-derivatives also in this case. The *N*-oxide (**1**) and its tetramethyl ether (**2**)⁷ containing both the 3,3'- and 4,4'-substituents in each molecule create a possibility of comparing the reactivity of the systems under identical Elms energetic conditions.

The principal mode of fragmentations⁸ of the natural orellanine (**1**) determined in particular on the basis of the exact mass measurements and the MIKE spectra of its molecular ion (m/z 252, Figure 1a) and several fragment ions are presented in Scheme 1. In our opinion the sequence of the fragmentation of the molecular ion of **1** ($a \rightarrow b \rightarrow c \rightarrow d$) reveals a great similarity to those determined for 3,3'-dihydroxy-2,2'-bipyridine-*N,N'*-dioxide and indicates a preference for the initial fragmentation due to the $[N \rightarrow O, 3'-OH]$ system without a significant contribution of the remaining hydroxyl groups. The MIKE spectrum of the molecular ion (m/z 256) of tetradeuterated orellanine, 3,3',4,4'-tetradeuterioxy-2,2'-bipyridine-*N,N'*-dioxide (**1-d₄**),⁹ also showed beyond any doubt the initial elimination of the deuterioxy radical as the only process (apart from the thermal oxygen ejection), which gave the fragment ion of m/z 238 (Figure 1b).

It has to be pointed out that, except for the appearance of the ion $[M - \cdot OH]^+$ at m/z 235, there are significant differences between the MIKE spectrum of the molecular ion of **1** obtained by us and that published by Richard and Ulrich.⁶ According to our findings, in the normally applied EIms conditions, the ions $[M - \cdot OH]^+$ at m/z 235 and $[M - O]^+$ at m/z 236 are the only intermediates of the further fragmentation of **1** consisting mainly (but not exclusively) in the successive elimination of the second $\cdot OH$ radical and CO molecules (additionally, $\cdot H$ in the case of ion q' , Figures 1c, 1d).

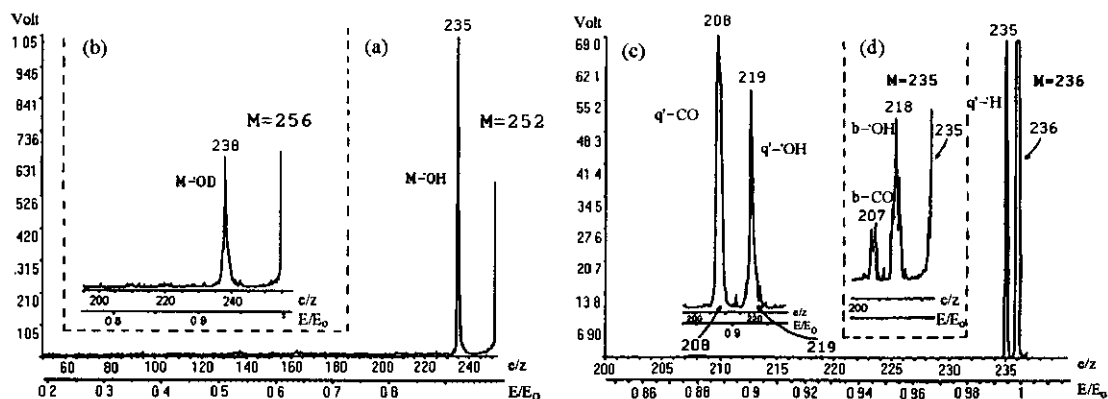
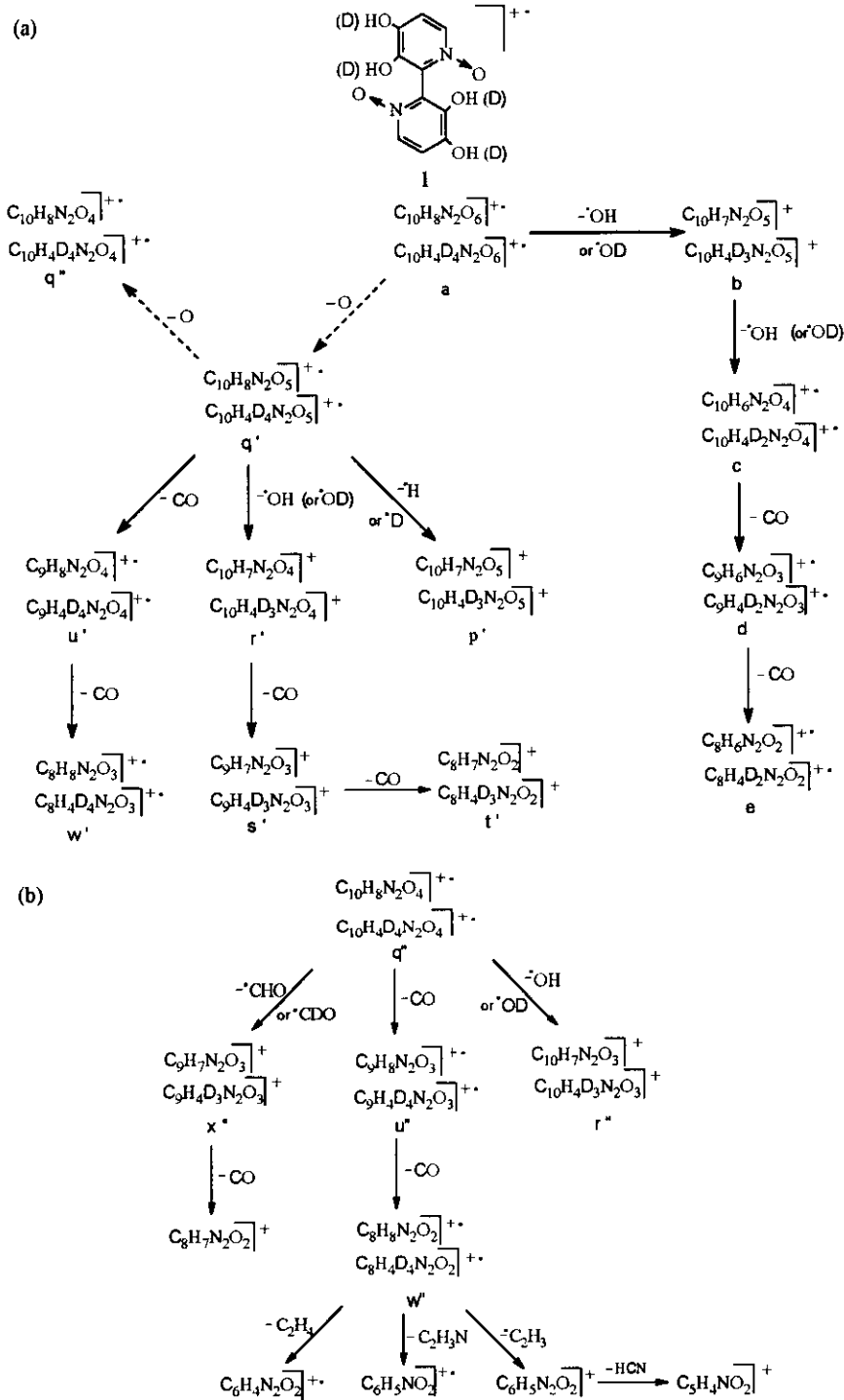


Figure 1. MIKE spectra of ions at: (a) m/z 252, M^+ of **1**, (b) m/z 256, M^+ of **1-d₄**, (c) m/z 236, $[M-O]^+$ from **1** (main fragment), (d) m/z 235, $[M-\cdot OH]^+$ from **1** (main fragment).

The structures of the fragment ions **b**, **c** and **e** (Scheme 1) may be presented as shown in Figure 2. The further fragmentation pattern of ion q'' (resulting from **1** and **1-d₄**) is superimposable on q' that found for the free base, orelline¹⁰ (or its tetradeuterio analogue,⁹ respectively) and is shown in Scheme 1(b). Thus, it is quite clear that the deoxygenation of orellanine takes place not only in the pyrolytic process prior to ionization (as not observed



Scheme 1. Mass spectral fragmentation pattern of (a) 1 and (b) the free base, orelline, q⁺.

by MIKES; indicated by dotted lines, Scheme 1, ions q' and q''), but also in the EI induced process. The latter takes place by the elimination of $\cdot\text{OH}$ radicals, bearing oxygen from the *N*-oxide function (since it preceded the

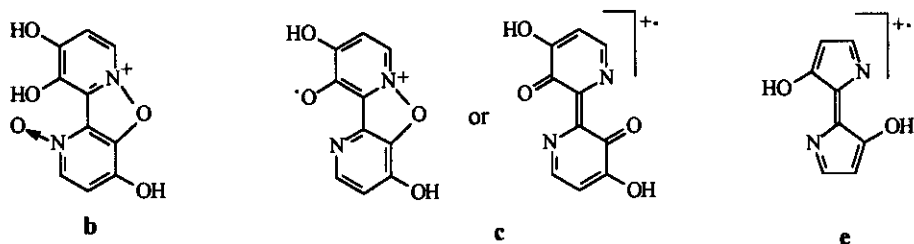


Figure 2. Structure of the fragment ions **b**, **c**, and **e** from **1**.

phenolic CO elimination) and hydrogen transferred most probably from the hydroxyl group located at the position 3' of the bipyridine skeleton as follows from the fragmentation of **1-d₄** (Figure 1b)

It is interesting to note that a real EI mass spectrum of orellanine can only be recorded at the lowest possible temperature ($\sim 150^\circ\text{C}$). At a higher temperature ($\sim 200^\circ\text{C}$) the thermal *N*-oxide oxygen expulsion proceeded

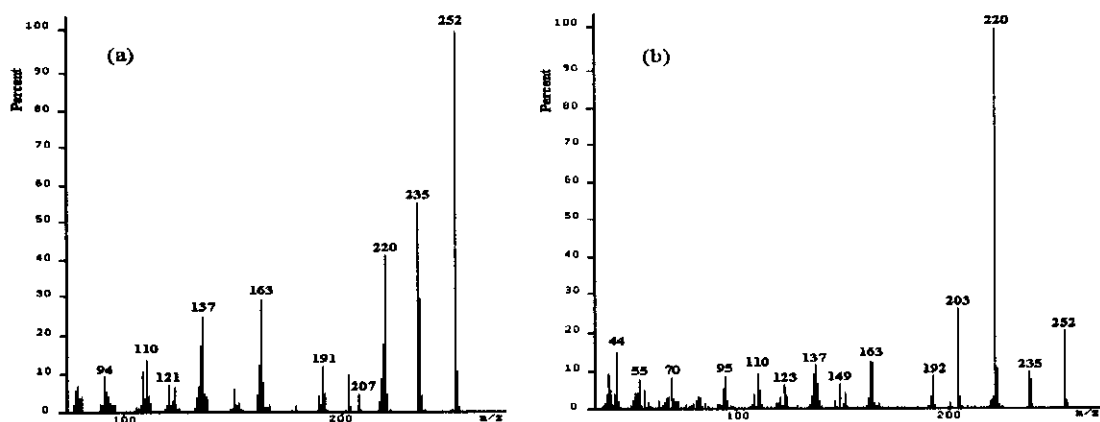
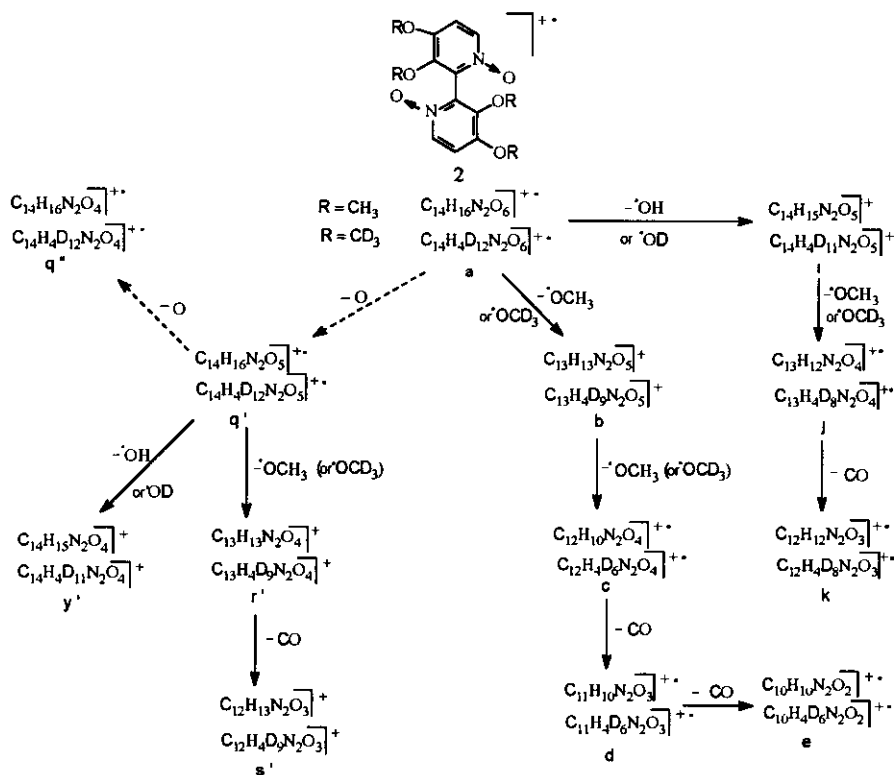


Figure 3. Elms of orellanine (**1**) recorded at (a) 155°C , (b) 210°C .

so fast that the obtained spectrum actually revealed a superimposed pattern of the fragmentation of the free base orelline, its mono-*N*-oxide, orellanine, and the *N,N'*-dioxide **1** (Figure 3).

A similar preference for the fragmentation with the main contribution of only $[\text{N}\rightarrow\text{O}, 3'\text{-OR}]$ system in the initial steps was observed in the case of **2** (Scheme 2), whose mass spectrum closely resembled that of 3,3'-dimethoxy-2,2'-bipyridine-*N,N'*-dioxide. As it followed from the MIKE experiments (Figure 4c) the molecular ion (m/z 308) decomposed by the elimination of the hydroxyl radical (ion **i**, m/z 291) and, above all, the methoxy group (ion **b**, m/z 277, 100% in EI mass spectrum). This pattern was confirmed by the

fragmentation of the deuterated analogue, 3,3',4,4'- tetra(trideuteromethoxy)-2,2'-bipyridine-*N,N'*-dioxide¹¹



Scheme 2 Mass spectral fragmentation pattern of compound (2).

(2-d₁₂, Figure 4a), the molecular ion of which (m/z 320, Figure 4b) yielded only the ions: [M - OD]⁺ (m/z 302) and [M - OCD₃]⁺ (m/z 286).

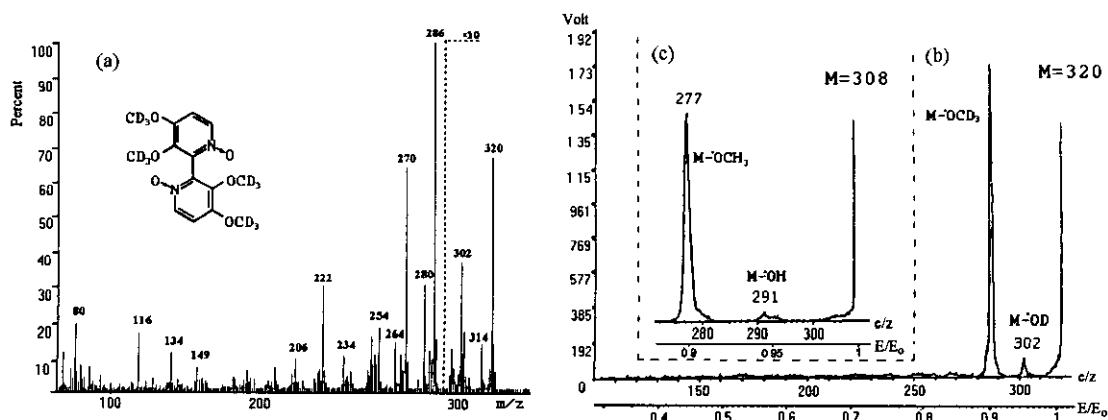
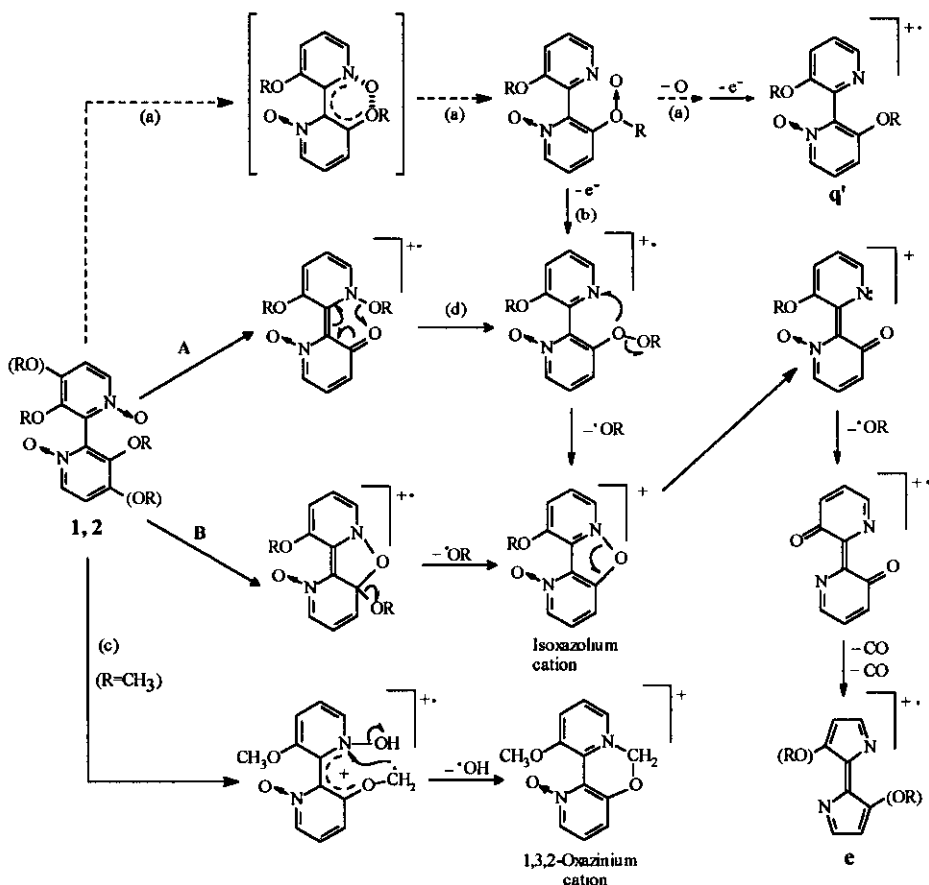


Figure 4. Mass fragmentation of 2-d₁₂: (a) EIms, (b) MIKES of M⁺ at m/z 320 [and that of M⁺ from 2 at m/z 308 inserted as(c)].

Finally, it has to be pointed out that the electron impact induced elimination of the first oxygen as $\cdot\text{OR}$ (hydroxyl or hydroxyl and methoxyl, respectively) from **1** and **2** (or their deuterated analogues) is the only fragmentation of the molecular ion and is not accompanied even by a trace of any other process (except for the thermal deoxygenation). These are the only fragment ions obtained in this way which underwent further decomposition, the $\cdot\text{OH}$ ($\cdot\text{OD}$) or $\cdot\text{OH}$ and $\cdot\text{OCH}_3$ ($\cdot\text{OD}$ and $\cdot\text{OCD}_3$) elimination from the second oximinopropenoxy [$\text{N}\rightarrow\text{O},3'$ -OR] system included. Therefore, the elimination of the first oxygen has to be much faster than the



Scheme 3. Alternative (A and B) interpretations of the initial steps of the fragmentation of compound (1) and (2) under the EI/MS conditions. (For the sake of simplicity, the substitution inside the "bay-region" was indicated only)

fragmentation of (and characteristic for) the substitution in 4,4'-positions (no $\cdot\text{D}$, $\cdot\text{OH}$ or $\cdot\text{CDO}$ elimination from the molecular ion of **2-d₁₂**). The different rates of fragmentation of the 3,3'- and 4,4'-substituted part of the bipyridine-*N,N'*-dioxide molecule can be only explained by the specific interaction inside the "cavity" of the planar

conformation delimited by the oximinopropenoxy [N→O,3'-OR] system (Scheme 3). Our interpretation of the deoxygenation mechanism of these compounds under the EIms conditions consists in the separate treatment of the thermal and electron impact induced decomposition of the *N*-oxide function. However, in both cases the process is supposed to be controlled by the oximinopropenoxy system of the "bay-region". Due to the electronic and steric factors, this system creates (in contrast to the [N→O,4'-OR] system) an opportunity to lower the energy requirements, the result of which is an evidently faster elimination of oxygen (as ·O or ·OR, respectively). The interpretation of the evidently much faster elimination of ·OR in comparison with other fragmentations could include the preliminary *N*-oxide oxygen rearrangement with the formation of a peroxide intermediate. Both the sigmatropic rearrangement and the peroxide decomposition should be a low energetic process. To a certain degree this mechanism resembles that suggested for the thermally and photochemically induced deoxygenation of orellanine-like compounds.² Alternatively, in the mass spectrometry conditions the initial ·OR elimination could be preceded by the formation of an isoxazolium cation which in the next step would rearrange to a ketone by the N-O bond cleavage. The ·OH elimination in the case of orellanine tetramethyl ether could also be a result of a preliminary H transfer from OCH₃ to N-O oxygen due to the proximity effect;¹² the possible subsequent cyclization would yield a stable 1,3,2-oxazinium cation. The rationale of these possible transformations is shown in Scheme 3

ACKNOWLEDGEMENT

The authors are grateful to the Research Committee whose financial support in the form of the KBN Grant No 2 0721 91 01 made this work possible. Thanks are duly proffered also to the MS laboratory Staff of the Organic Chemistry Institute, P.A.Sc., Warsaw, especially to Dr E Baranowska and Dr. W.Danikiewicz for their kindness and the highly professional MS spectra recording. The authors would also like to point out with appreciation the profound contribution of Dr W.P.Gessner to the MS studies of orellanine at an earlier stage of our interest in this topic; the results of those studies were only wholly published in his Ph.D Thesis.

REFERENCE

1. W.Z Antkowiak and W.P.Gessner, *Tetrahedron Lett.*, 1979, 1931.
2. R.Antkowiak and W.Z.Antkowiak, in "The Alkaloids" (A.Brossi ed), Vol. 40, Chap 2, p.189, Academic Press, San Diego, California, 1991, and references cited therein.
3. E.V.Dehmlöv and H.-J.Schulz, *Tetrahedron Lett.*, 1985, 26, 4903.

4. E.V. Dehmlov and A. Slegers, *Liebigs Ann. Chem.*, 1992, 953.
5. W.P. Gessner, Ph.D. Thesis, A. Mickiewicz University, Poznań, Poland, 1980
6. J.-M. Richard and J. Ulrich, *Biomed. Environ. Mass. Spectrom.*, 1989, **18**, 1
7. M. Tiecco, M. Tingoli, L. Testaferri, D. Chianelli, and E. Wenkert, *Tetrahedron*, 1986, **42**, 1475.
8. Low- and high-resolution mass spectra were recorded on a AMD 604 two-sector mass spectrometer (ionizing voltage 70 eV, accelerating voltage 8 kV, resolution 20 000). Samples were introduced by a direct insertion probe at the source temperature of 150°C. The elemental compositions of the ions were determined by a peak matching method relative to perfluorokerosene and using the same instrument. All measured masses agreed with the calculated to within 2 ppm. Mass analysed ion kinetic energy (MIKE) spectra were obtained on the same AMD 604 mass spectrometer. Low resolution mass spectra and metastable peaks in the first field free region were also obtained on a Jeol JMS-D-100 mass spectrometer linked to a Texas Instruments 980B Computer (ionizing voltage 75 eV, ionizing current 300 μ A, accelerating voltage 3 kV, resolution 8000).
9. Synthesized by an exhaustive treatment of the protonated compound with D₂O (ref.5); refers to 1-d₄ and orelline-d₄ preparation, respectively.
10. Orelline was obtained by the Pt catalyzed hydrogenolysis of **1** (ref 5). Recently the total synthesis of orelline was reported by F. Trecourt, M. Mallet, O. Mongin, B. Gervais, and G. Queguiner, *Tetrahedron*, 1993, **49**, 8373.
11. The tetra-trideuteromethyl analogue of **2** (2-d₁₂), was obtained by the general procedure of the synthesis of this compound except for using the corresponding deuterium labelled reagents. However, an attempt to prepare the regioselectively deuterated tetramethyl ether of **2** (2-3,3'-d₆) by this method failed; due to a strong activation of the C-3 position by the ortho NO₂ the sodium methoxide treatment of the 2-bromo-3-trideuteromethoxy-4-nitropyridine-*N*-oxide intermediate caused a fast nucleophilic exchange (in 70%) of the OCD₃ on OCH₃ before the substitution at C-4 took place.
12. F. Uchamaru, S. Okada, A. Kosasayama, and T. Konno, *J. Heterocycl. Chem.*, 1971, **8**, 99

Received, 18th March, 1994