27. Detection of 9-Methoxy Substitution in Tetrahydroprotoberberine Alkaloids by Mass Spectrometry

by **Wilhelm J. Richter**[1])
Zentrale Funktion Forschung Ciba-Geigy AG. 4002 Basel, Switzerland
and **Elnar Brochmann-Hanssen**
School of Pharmacy University of California
San Francisco, Calif. 94143 U.S.A.

Herrn Prof. Dr. *H. Bretschneider*, Universität Innsbruck, in dankbarer Verehrung zum 70. Geburtstag gewidmet.

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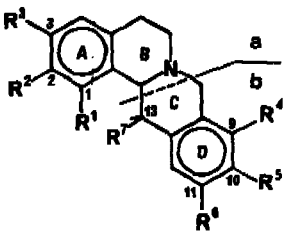
Summary. 9-Methoxytetrahydroprotoberberines are found to exhibit pronounced $(M-OCH_3)^+$ ions in their mass spectra as opposed to isomers or homologs lacking such substitution, yet carrying methoxyl substituents at other sites. Locating such substituents originally present, or produced from 9-hydroxy groups by treatment with diazomethane, allows to differentiate 9,10- from 10,11-oxygenation patterns in ring **D**, and thus to secure an important step in the structure elucidation of unknown alkaloids of this class.

As is quite generally true for nitrogen-containing natural products, mass spectrometry provides for the class of *tetrahydroprotoberberine alkaloids* a very efficient means of deducing more than incidental structural information from the frequently minute quantities of material available. Earlier studies [1–9] have ascertained that highly characteristic modes of fission of ring **C** permit not only the recognition of the basic skeleton and its substituents, but also a rough placement of the latter within certain areas of the structure, *e.g.* the ring moieties **A/B** or **C/D**, respectively:



a and b

In most cases, both parts of the molecule can be observed as abundant positive ions **a** and **b**, frequently associated with ions arising from gain or loss of hydrogen atoms during or after rupture of the ring (*cf.* Fig. 1 and 2). Ions of this type are obviously apt to furnish mutually corroborating evidence for the number and kind of substituents present at rings **A** and **C/D**. R^{2-6} substituents, usually –OH or $-OCH_3$ and occasionally $-OCH_2O-$, mark the two varieties of oxygenation patterns most frequently encountered within this class of alkaloids: 2,3,9,10 and 2,3,10,11. Less frequent is substitution at positions 1,5,12 and 13. In spite of newer data meanwhile

[1]) To whom inquiries should be directed.

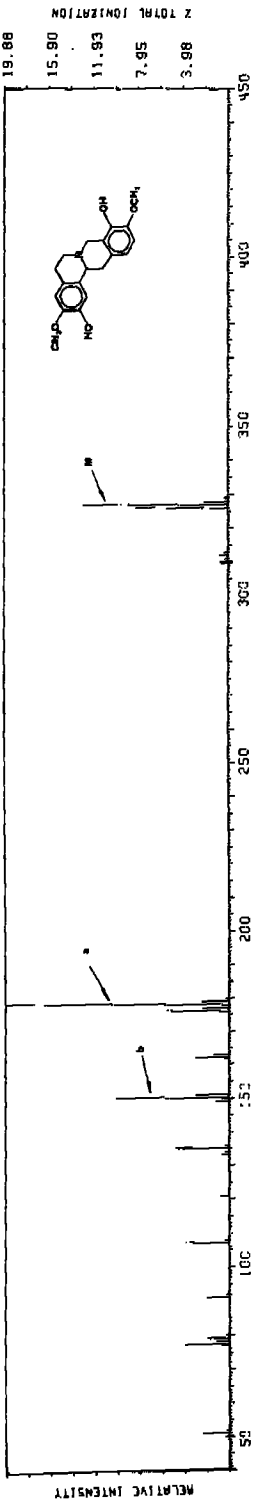


Fig. 1. *Mass Spectrum of (−)-Scoulerine (XVII)*

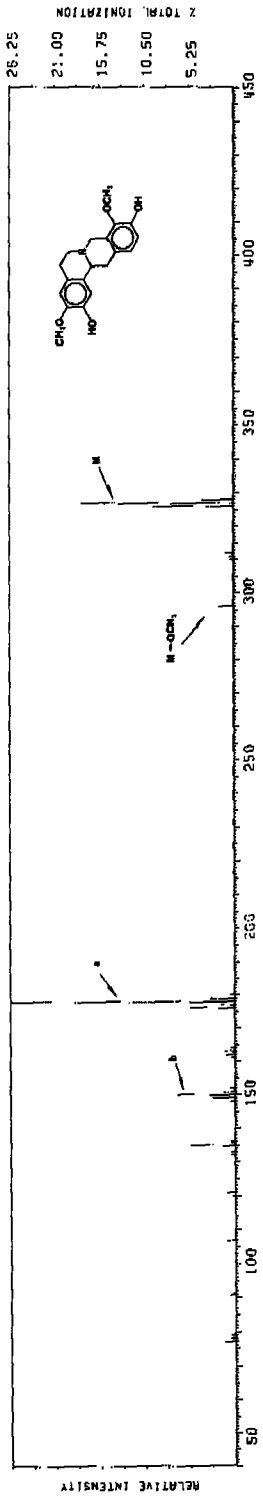


Fig. 2. *Mass Spectrum of (−)-Stepholidine (IX)*

accumulated, attempts at differentiating sites of substitution in full detail remain, however, largely elusive if solely based on mass spectrometry.

Loss of R^4 as a probe into substitution at C(9). – Nevertheless, a valuable exception to the inadequacy of the technique as to this important task pertains to the detectability of R^4 substituents, *i.e.* substituents placed at C(9). Thus, a significant increase of relative abundances of $(M-OCH_3)^+$ ions is observed for compounds in which R^4 is methoxyl, as compared to isomers or homologs carrying methoxyl groups at other positions. This behavior is illustrated in Fig. 1 and 2 by the mass spectra of the two alkaloids (–)-*scoulerine* and (–)-*stepholidine*. Of the two isomers, both belonging to the 2,3,9,10-oxygenated substitution type and differing only in ring **D** by a

Table 1. *Relative abundances of* $(M-OCH_3)^+$ *ions in 9-methoxytetrahydroprotoberberines*

Alkaloid	Substitution pattern	$(M-OCH_3)^+$ % of molecular ion	Ref.
(–)-tetrahydropalmatine (I)	$R^1 = R^6 = R^7 = H$ $R^2 = R^3 = R^4 = R^5 = OCH_3$	14.0	for (±)-compound *cf.* ref. [4]
(+)-corydaline (II)	$R^1 = R^6 = H$ $R^7 = CH_3$ $R^2 = R^3 = R^4 = R^5 = OCH_3$	12.5	[19]
(–)-corypalmine (III)	$R^1 = R^6 = R^7 = H$ $R^3 = OH$ $R^2 = R^4 = R^5 = OCH_3$	14.5	[13]
(–)-isocorypalmine (IV)	$R^1 = R^6 = R^7 = H$ $R^3 = OH$ $R^2 = R^4 = R^5 = OCH_3$	15.0	[17]
(±)-canadine (V)	$R^1 = R^6 = R^7 = H$ $R^2/R^3 = -OCH_2O-$ $R^4 = R^5 = OCH_3$	16.0	[4]
(–)-capaurine (VI)	$R^6 = R^7 = H$ $R^1 = OH$ $R^2 = R^3 = R^4 = R^5 = OCH_3$	19.0	[4] [8]
(±)-kikemanine (VII)	$R^1 = R^6 = R^7 = H$ $R^5 = OH$ $R^2 = R^3 = R^4 = OCH_3$	13.0	[7]
(–)-capaurimine (VIII)	$R^6 = R^7 = H$ $R^1 = R^5 = OH$ $R^2 = R^3 = R^4 = OCH_3$	12.0	[4] [8] [10] [11]
(–)-stepholidine (IX)	$R^1 = R^6 = R^7 = H$ $R^2 = R^5 = OH$ $R^3 = R^4 = OCH_3$	12.0	[18] [22]
(–)-discretamine (X)	$R^1 = R^6 = R^7 = H$ $R^3 = R^5 = OH$ $R^2 = R^4 = OCH_3$	13.0	[12] [23]

Table 2. *Relative abundances of* $(M - OCH_3)^+$ *ions in tetrahydroprotoberberines lacking 9-methoxy substitution* ($R_4 \neq OCH_3$)

Alkaloid	Substitution pattern	$(M-OCH_3)^+$ % of molecular ion	Ref.
(–)-xylopinine (XI) = norcoralydine	$R^1 = R^4 = R^7 = H$ $R^2 = R^3 = R^5 = R^6 = OCH_3$	2.0	[1] [4] [6]
O-methylcaseadine (XII)	$R^3 = R^4 = R^7 = H$ $R^1 = R^2 = R^5 = R^6 = OCH_3$	3.0	[4] [5]
(±)-stylopine (XIII)	$R^1 = R^6 = R^7 = H$ $R^2/R^3 = R^4/R^5 = -OCH_2O-$	3.0	[4]
(–)-discretine (XIV)	$R^1 = R^4 = R^7 = H$ $R^3 = OH$ $R^2 = R^5 = R^6 = OCH_3$	2.0	[12] [14]
O-ethyldiscretine (XV)	$R^1 = R^4 = R^7 = H$ $R^3 = OC_2H_5$ $R_2 = R^5 = R^6 = OCH_3$	3.0	[14]
(±)-nandinine (XVI)	$R^1 = R^6 = R^7 = H$ $R^2/R^3 = -OCH_2O-$ $R^4 = OH$ $R^5 = OCH_3$	1.0	[20]
(–)-scoulerine (XVII)	$R^1 = R^6 = R^7 = H$ $R^2 = R^4 = OH$ $R^3 = R^5 = OCH_3$	2.0	[15] for (±)-compound *cf.* ref. [4]
O,O-diethylscoulerine (XVIII)	$R^1 = R^6 = R^7 = H$ $R^2 = R^4 = OC_2H_5$ $R^3 = R^5 = OCH_3$	2.0 $(M - OC_2H_5)^+$ $= 15.0$[a]	
O,O-diethyl($-d_{10}$)scoulerine (XIX)	$R^1 = R^6 = R^7 = H$ $R^2 = R^4 = OC_2D_5$ $R^3 = R^5 = OCH_3$	3.0 $(M - OC_2D_5)^+$ $= 16.0$[a]	
(±)-coreximine (XX)	$R^1 = R^4 = R^7 = H$ $R^2 = R^6 = OH$ $R^3 = R^5 = OCH_3$	3.0	[16] [21]
O,O-diethylcoreximine (XXI)	$R^1 = R^4 = R^7 = H$ $R^2 = R^6 = OC_2H_5$ $R^3 = R^5 = OCH_3$	3.0 $(M - OC_2H_5)^+$ $= < 3.0\%$[a]	
O,O-diethyl($-d_{10}$)coreximine (XXII)	$R^1 = R^4 = R^7 = H$ $R^2 = R^6 = OC_2D_5$ $R^3 = R^5 = OCH_3$	3.0 $(M - OC_2D_5)^+$ $= < 3.0\%$[a]	
(–)-cascadine (XXIII)	$R^3 = R^4 = R^7 = H$ $R^1 = OH$ $R^2 = R^5 = R^6 = OCH_3$	3.0	[4] [5]

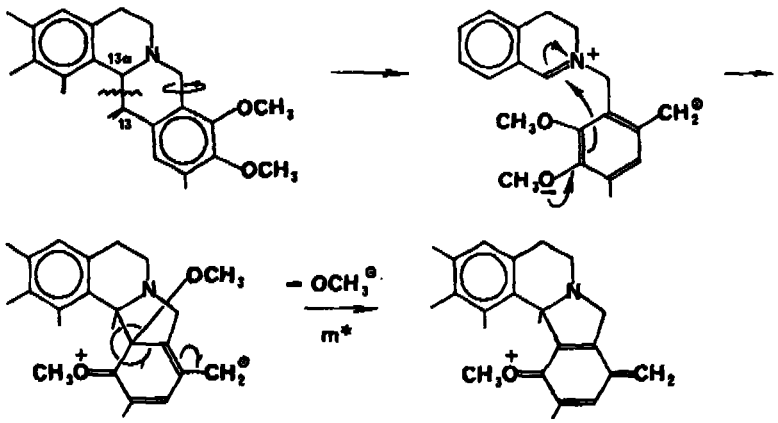
[a] *W. J. Richter* and *E. Brochmann-Hanssen*, unpublished results.

reverse placement of its substituents, only the 9-methoxy compound stepholidine exhibits a significant (M–OCH_3) peak at m/e 296. As can be seen from 23 tetrahydroprotoberberines in *Tables 1* and *2*, loss of methoxyl radicals is invariably enhanced by a factor of 5 to 10 (corresponding peaks with >10% of molecular ion intensity[2]) in all 9-methoxy compounds I to X over the quite small losses (<3% of molecular ion intensity[2]) in the compounds XI to XXIII which lack such substitution.

This correlation seems to apply equally well to the loss of R^4 substituents representing larger alkoxy moieties such as OC_2H_5, as can be concluded from the O,O-diethyl and O,O-diethyl-d_{10} derivatives of scoulerine (XVIII and XIX). In their spectra, (M–OEt) peaks are significant in contrast to the isomeric O,O-diethyl derivatives of coreximine (XXI and XXII) and the O-ethyl derivative of discretine (XV), carrying ethoxyl substituents at C(11) instead of C(9). The analogy does not, however, extend to the case R^4 = OH, as represented by nandinine (XVI) and scoulerine (XVII) itself. While the latter (Fig. 1) exhibits, in fact, a significant (M–17) peak, it was shown by high-resolution measurements to be due to loss of the elements CH_5 (CH_3/H_2 or H/CH_4) rather than loss of OH[3]). The fact that ejection of methoxy or alkoxy substituents takes, indeed, specifically place at C(9) is convincingly evidenced by the loss of C_2D_5O in the O,O-diethyl-d_{10} derivative of scoulerine (XIX).

It is interesting to note that the structure initially proposed for *capaurimine* ($R^1 = R^4 = OH$, $R^2 = R^3 = R^5 = OCH_3$, $R^6 = R^7 = H$) [10] is in clear contradiction to this finding, whereas its revised form VIII ($R^1 = R^5 = OH$, $R^2 = R^3 = R^4 = OCH_3$, $R^6 = R^7 = H$) [8] [11] is fully compatible with it. Reverse C(9)/C(10) substitution, suggested by NMR. evidence and confirmed by the synthesis of oxidative degradation products [11], would have been similarly indicated by its mass spectrum on the basis of this empirical correlation.

Generally, the observed ejection of an alkoxy radical from C(9) in marked preference to ejection of comparable substituents from other positions is very likely to



[2]) Due to considerable variation in the case of decomposition of M^+ as a function of substitution (*e.g.* **b** fragments gain predominance with increasing methoxy substitution of ring **D**) intensities of $(M—OCH_3)^+$ fragments relative to M^+ provide a more consistent basis of comparison than the commonly preferred intensities related to base peaks or total ionization.

[3]) *W. J. Richter & A. L. Burlingame*, unpublished results.

reflect aromatic substitution at this very site in the course of a more complex sequence of mechanistic events. Cleavage of the 13,13a-bond severing ring **C** is, for obvious energetic reasons, a most favorable introductory step probably responsible for most of the fragmentation processes observed within this class. One of the conceivable pathways enacting re-cyclization of the resulting 'open' molecular ion by ionic (in contrast to radical-type) attack at C(9) can be visualized proceeding as follows.

Eventual ejection of the 9-methoxy substituent, characterized in several cases by prominent metastable peaks *m** as result of rearrangement processes with low frequency factors, represents a consequence of re-aromatization of ring **D**.

Such substitution may be expected to be limited to this position by its accessibility for reactive centers formed within ring **B** especially through this type of initial cleavage. Radical- instead of ionic-type substitution at C(9) could be envisaged after intervention of hydrogen transfer between C(13) and C(6) in the initial cleavage product. Occurrence of such hydrogen abstraction from ring **B** has to be suspected as triggering event of other important fragmentation processes, *e.g.* the formation of $(M-1)^+$ ions[4]) as well as those **a** and **b** ions containing less or more hydrogen atoms than the ones derived from direct rupture of the ring. Irrespective of which of the resulting resonance forms with radical centers at C(6) or C(13a) initiates re-cyclization, C(9) will again be the expected best-accessible site of substitution.

The practical value of this correlation pertains to the structure analysis of very small amounts of samples of unknown alkaloids precluding the application of NMR. spectroscopy as the less sensitive, yet more informative technique with respect to the exact placement of oxygen substituents at aromatic rings. Its usefulness is further increased by the possibility of converting hydroxyl into alkoxy functions readily detectable from inspection of the mass spectrum when positioned at C(9).

In addition to distinction from the 10,11-oxygenated substitution pattern, differential positional analysis of the 9,10-oxygenated type becomes feasible for the frequent case of mixed hydroxy-methoxy substitution. As for any other empirically derived rule, careful attention must be paid to possible future exceptions in order to test its scope and limitations, and thus its validity. An example of application to an unsolved structural problem, *i.e.* the final placement of the four substituents in the dihydroxy-dimethoxytetrahydroprotoberberine (-)-*discretamine*, [12] will be presenteed in a forthcoming paper [23].

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28. The Structure of Discretamine, a Tetrahydroprotoberberine Alkaloid

by **Wilhelm J. Richter**[1])
Zentrale Funktion Forschung, Ciba-Geigy AG, 4002 Basel, Switzerland
and **Einar Brochmann-Hanssen**
School of Pharmacy, University of California, San Francisco, CA 94143, USA

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Summary. On the basis of mass spectrometric analysis, including comparison with closely related isomers, *discretamine* is proposed to have the structure of a 3,10-dihydroxy-2,9-dimethoxy-tetrahydroprotoberberine.

Discretamine, a tetrahydroprotoberberine alkaloid of only partially known structure, was isolated in 1959 by *Schmutz* [1] from *Xylopia discreta* (L. FIL.) SPRAGUE *et* HUTCHINS in too low a yield to permit the usual chemical determination of its exact substitution pattern by ethylation of phenolic hydroxyl groups and subsequent oxidative degradation [2] [3]. From the elemental analysis a $C_{19}H_{21}O_4N$ composition appeared likely, pointing towards the presence of two hydroxyl and two methoxyl groups at the cyclic moiety. The tetra-oxygenated pattern was shown to correspond to the 2,3,9,10 substitution type by conversion of the compound into (–)-*tetrahydropalmatine* ($R^1 = R^2 = R^3 = R^4 = CH_3$) through treatment with diazomethane [1].

[1]) To whom inquiries should be directed.