Tetrahedron Letters,Vol.28,No.32,pp 3729-3732,1987
Printed in Great Britain

0040-4039/87 $3.00 + .00
Pergamon Journals Ltd.

NMR-SPI: A RELIABLE METHOD FOR DETERMINING THE MODE OF ESTER ATTACHMENT IN PYRROLIZIDINE ALKALOIDS

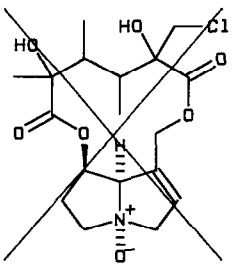
Martin W. Bredenkamp*[1] and Adriaan Wiechers[2]
Department of Chemistry, University of Pretoria,
PRETORIA 0002, Republic of South Africa

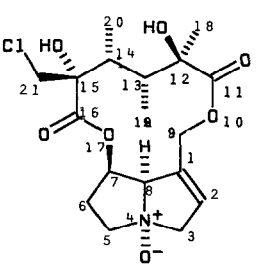
Abstract: The NMR-SPI technique proves to be more reliable than MS, and is generally applicable for determining the mode of ester attachment of the necic acids to the necine in pyrrolizidine alkaloids.

In the preceding communication the reliability of the mass spectrometric method for determining the mode of ester attachment in pyrrolizidine alkaloids was shown to be suspect. The existence of anomalies in the mass spectrometric method presented the need for a more reliable procedure. Basic ^{1}H NMR does not provide the desired information since there is no through-bond coupling between the protons of the necine moiety with those of the necic acid moieties. NMR techniques which depend on longrange through-bond connectivity between protons and ^{13}C nuclei deserved investigation. Selective population inversion (SPI) appeared to be well suited to indicate unambiguously the mode of ester attachment in macrocyclic pyrrolizidine alkaloids.[3]

SPI is used to determine the two- and three-bond connectivity pattern between protons and carbon nuclei, and could be used in determining the mode of ester attachment by demonstrating connectivity of strategic protons to the ester carbonyl carbon nuclei. This method was successfully used to prove the mode of ester attachment in merenskine N-oxide [1] (see Figure).[4]

Irradiation of the 9-*pro*-S-H signal effected the carbonyl carbon signal at δ_C178,17. Irradiation of the 21-H signal at δ_H3,706 effected the carbonyl carbon signal at δ_C173,44. Only the mode of ester attachment as illustrated in the Figure can satisfy these results. The reversed mode of ester attachment would have had both the above irradiation experiments effect the same carbonyl carbon signal. The mode of ester attachment in merenskine N-oxide [1] was confirmed





[1] Merenskine N-oxide

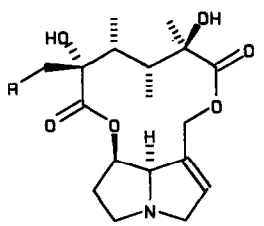
Figure

by irradiating the 18-H methyl signal, effecting the carbonyl carbon signal at δ_C178,17.

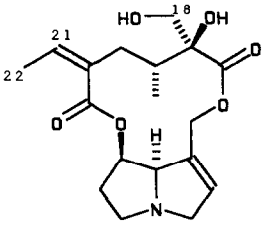
The generality of SPI for determining the mode of ester attachment in pyrrolizidine alkaloids was then investigated. The following alkaloids were available: The already mentioned merenskine N-oxide [1] represented the alkaloid N-oxides; the senecane alkaloids (12-membered macrocyclic) were represented by sceleratine [2],[5] retrorsine [3], jacobine [4] and merepoxine [5];[6] the crotalane alkaloids (11-membered macrocyclic) by monocrotaline [7]; the non-macrocyclic diester alkaloids by lasiocarpine [8]; and the monoester alkaloids by heliotrine [9] and echinatine [10]. The results are presented in the Table.

The following points should be borne in mind in planning SPI experiments for determining the mode of ester attachment in pyrrolizidine alkaloids:

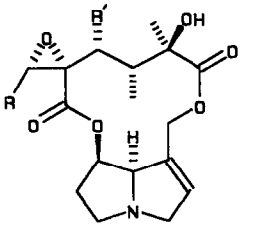
1. The stronger the magnetic field of the spectrometer, the smaller the chances of proton signal overlap. Irradiation of overlapping proton signals may ambiguously effect the carbonyl carbon signals. Our experiments were run on a Bruker WM500 (500 MHz) and the only case of proton signal overlap we experienced is the second entry of compound [10] in the table. The signals of 3'-H, 3-*pro*-S-H and 8-H coincide, nevertheless, the effected carbonyl carbon signal may be ascribed to irradiation of 3'-H only.
2. The correct assignment of the proton signals to be irradiated is essential. When uncertainty exists, respective irradiation of all the signals which may qualify for the proton to be irradiated, may help to identify the correct signal and simultaneously provide the desired information needed to deduce the mode of ester attachment. For monocrotaline [7], both the methyl singlets were irradiated of which the lower field signal effected a carbonyl signal.
3. All the unsuccessful SPI experiments are also listed in the table. Each experiment was attempted once only. The chances for success in a SPI experiment is proportional to the



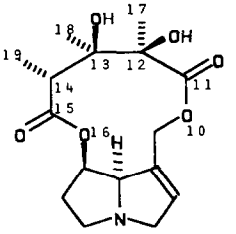
[2] Sceleratine, R=OH
[6] Merenskine, R=Cl



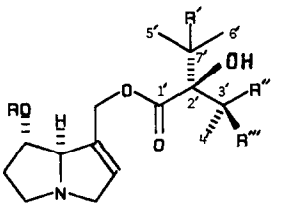
[3] Retrorsine



[4] Jacobine, R=Me, R'=H
[5] Merepoxine, R=H, R'=Me



[7] Monocrotaline



[8] Lasiocarpine, R=Angelyl, R'=OH, R''=OMe, R'''=H
[9] Heliotrine, R=R'=R''=H, R'''=OMe
[10] Echinatine, R=R'=R''=H, R'''=OH

Table: Three-bond ($^{13}C,^{1}H$) connectivities between selected protons and the carbonyl carbon nuclei

Alkaloid[a]	Irradiated proton	δ_H[b]	Carbonyl carbon nucleus effected	δ_C[c]
[1]	9-*pro*-S-H	5,404	11-C	178,17
	21*a*-H	3,706	16-C	173,44
	18-H	1,278	11-C	178,17
	7-H	5,636	-	-
[2]	9-*pro*-S-H	5,515	11-C	178,00
	21*a*-H	3,648	16-C	175,31
	18-H	1,237	11-C	178,00
[3]	21-H	5,665	16-C	167,33
	9-*pro*-S-H	5,444	11-C	175,53
	7-H	4,959	-	-
	18-*pro*-R-H	3,662	11-C	175,53
[4]	9-*pro*-S-H	5,527	11-C	177,47
	7-H	5,081	16-C	168,17
	21-H	2,894	-	-
	18-H	1,289	11-C	177,47
[5]	9-*pro*-S-H	5,557	11-C	178,03
	21*a*-H	2,730	-	-
	18-H	1,306	11-C	178,03
[7]	19-H	1,181	15-C	173,98
	17-H	1,401	11-C	173,49
	9-*pro*-S-H	4,854	11-C	173,49
[8]	3"-H	6,023	1"-C	167,55
	9-H	4,865	1'-C	173,70
	3'-H	3,749	1'-C	173,70
	5"-H	1,806	1"-C	167,55
[9]	9*a*-H	5,003	1'-C	174,87
	9*b*-H	4,613	1'-C	174,87
	3'-H	3,551	1'-C	174,87
	7'-H	2,103	1'-C	174,87
[10]	9*b*-H	4,769	1'-C	173,39
	3'-H	3,929	1'-C	173,39
	7'-H	2,149	-	-

[a] In $CDCl_3$ except [1] for which CD_3OD was used. [b] Signals were referenced to solvent peaks which were assigned chemical shifts δ_H7,240 for $CHCl_3$ and δ_H3,300 for CHD_2OD. [c] Signals were referenced to solvent peaks which were assigned chemical shifts δ_C77,00 for $CDCl_3$ and δ_C49,00 for CD_3OD.

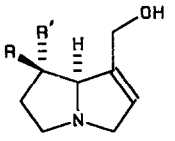
relative amplitude of the irradiated proton signal; i.e. greater success is obtained from signals of lower multiplicity and higher proton integral. Methyl signals are primary candidates. Irradiation of the characteristic 9-H transitions effected a carbonyl carbon signal every time in these experiments. Only one carbonyl carbon signal was definitely effected during three experiments in which 7-H transitions were irradiated.

4. Only two successful SPI experiments are necessary to prove the mode of ester attachment in pyrrolizidine alkaloid diesters: One by irradiating a necine proton and the other, a necic acid proton. It must, however, be stressed that if no carbonyl carbon signals are effected, the absence of such an atom within two or three bonds of the irradiated proton is

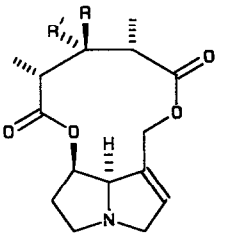
not implied.

In conclusion, the following remarks may be made regarding the generality of NMR-SPI for determining the mode of ester attachment in pyrrolizidine alkaloids:

1. NMR-SPI appears to be generally applicable to 9-monoester-, diester-, crotalane-, senecane- and the N-oxides of pyrrolizidine alkaloids where retronecine [11] or heliotridine [12] are the necine moieties.
2. NMR-SPI cannot be used to determine the mode of ester attachment in compounds such as fulvine [13] and crispatine [14] which have symmetrical necic acids.
3. NMR-SPI is certainly superior to the mass spectrometric method for determining the mode of ester attachment in pyrrolizidine alkaloids.



[11] Retronecine, R=OH, R'=H
[12] Heliotrine, R=H, R'=OH



[13] Fulvine, R=OH, R'=Me
[14] Crispatine, R=Me, R'=OH

Acknowledgments: We thank Mr Ivan Antonowitz of the CSIR for the execution of the NMR experiments, and Dr CCJ Culvenor of the CSIRO in Australia for providing alkaloids [4] and [7] - [10]. Furthermore, we thank the CSIR Foundation for Research Development and the University of Pretoria for financial aid.

NOTES AND REFERENCES:

1. Present address: NCRL, CSIR, PO Box 395, PRETORIA 0001, Republic of South Africa
2. Present address: Pennwalt Corporation, Technological Center, 900 First Avenue, KING OF PRUSSIA, PA 19406-0018, USA
3. KGR Pachler and PL Wessels, *J. Magn Reson.*, 1973, 12, 337; *ibid.*, 1977, 28, 53
4. MW Bredenkamp, A Wiechers and PH van Rooyen, *Tet. Lett.*, 1985, 26, 929
5. MW Bredenkamp, A Wiechers and PH van Rooyen, *Tet. Lett.*, 1985, 26, 5721
6. Merepoxine [5] is prepared from merenskine [6] by reaction with methanolic K_2CO_3, and was characterised by elemental analysis, IR, MS, ^1H and ^{13}C NMR

(Received in UK 15 June 1987)