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^{1}H NMR SPECTROSCOPY OF PYRROLIZIDINE ALKALOIDS

H. J. SEGALL* and J. L. DALLAS†

*Department of Veterinary Pharmacology and Toxicology; †Department of Biological Chemistry, University of California, Davis, CA 95616, U S A

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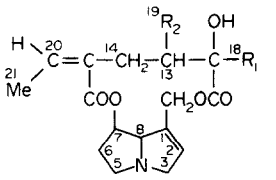
Abstract—The ^{1}H NMR spectra of three pyrrolizidine alkaloids of the macrocyclic diester type, retrorsine, seneciphylline and senecionine, plus their three *N*-oxides have been assigned. Previous ^{1}H NMR studies of these pyrrolizidine alkaloids have stressed the difficulties of spectral intrepretation. The results reported here will provide a useful resource for analysis of tertiary structure in these and related compounds.

INTRODUCTION

Retrorsine, seneciphylline and senecionine (Fig. 1) are members of a large group of phytotoxins known as pyrrolizidine alkaloids (PAs) that are native to *Senecio vulgaris* [1]. These three PAs are potent hepatotoxins [1, 2] and have exhibited genotoxicity [3, 4] as well as carcinogenicity [5].

In our attempt to isolate various *in vitro* hepatic metabolites of these complex macrocyclic PAs, we observed that the ^{1}H NMR data appeared to be somewhat lacking. Earlier reviews detailed the spectra of many of the nonesters, monoesters and diesters of PAs [6, 7]. Although an excellent ^{1}H NMR study of senecionine was recently published [8], many publications are somewhat incomplete in detailing the entire proton spectra of the macrocyclic PAs. This is quite often due to the difficulties in distinguishing the protons of overlapping or obscured signals. In addition, there are scattered reports of NMR data for the *N*-oxides which generally are the macrocyclic compounds found in the greatest abundance in the *Senecio* species [9].

Retrorsine, seneciphylline and senecionine are some of the most common PAs, as usually one and quite often two or more are found in many *Senecio* species [1]. They vary in the substitution (Me or CH_2OH) at the C-18 position (R_1) and their degree of unsaturation at the C-19 position (R_2). Consequently, it was felt that the ^{1}H NMR of macrocyclic PAs should be carefully examined under high resolution conditions.



Retrorsine	R_1 = CH_2OH,	R_2 = Me
Seneciphylline	R_1 = Me,	R_2 = CH_2
Senecionine	R_1 = Me,	R_2 = Me

Fig. 1 Macrocyclic pyrrolizidine alkaloid numbered according to the *Chemical Abstracts* method.

RESULTS AND DISCUSSION

The ^{1}H NMR spectra obtained in these studies exhibited the typical pattern of 12-membered macrocyclic PAs [10] with pertinent data reported in Tables 1 and 2. Complete assignments for all protons of senecionine were established by connectivity of nuclear spin coupling patterns and were found to agree with previously published spectra [8]. Additional assignments of the related compounds senecionine *N*-oxide, seneciphylline, seneciphylline *N*-oxide, retrorsine and retrorsine *N*-oxide, have been achieved by similar homonuclear decoupling experiments and comparison with the assignments of senecionine. Detailed simulations of complex spin multiplets were not performed Previous reports have expressed difficulty with assignments of H-3, H-5 and H-6 in the necine base due to insufficient spectral dispersion High resolution conditions at 360 MHz permitted the establishment of the connectivity for each band of resonances for these hydrogens. Comparison of spectra for senecionine, seneciphylline and retrorsine exhibited distinct variations in shielding of H-13, H-14, H-18 and H-19, which are characteristic of the substituent induced perturbations of the local magnetic environment. Since senecionine retains methyl groups at positions 18 and 19, we refer to Tables 1 and 2 for comparisons of spectral data relative to the senecionine model. Introduction of a hydroxyl substituent at C-18 (retrorsine) produced a nonequivalence of the two remaining H-18 protons and a decreased shielding effect of δ 2.4 relative to senecionine All other hydrogens of retrorsine exhibited identical shielding patterns and coupling constants when compared to senecionine, with the single exception that H-3 exhibited a simple first order pattern of vicinal coupling. The more drastic reduction of C-19 to a vinyl substituent (seneciphylline) produced perturbations to the shielding of H-14, H-18 and H-19. A characteristically small geminal coupling constant was observed for the two vinyl protons (2.0 Hz), as well as a decrease in shielding of δ4 relative to senecionine. A similar decreased shielding of δ 1–1.8 was observed for the

Table 1 ^{1}H NMR data for retrorsine, senecíphylline and senecionine

Proton(s) on carbon No	Retrorsine δ (ppm)	Retrorsine Proton	Senecíphylline δ (ppm)	Senecíphylline Proton	Senecionine δ (ppm)	Senecionine Proton
2	6 20	1H, *d*, *J* = 1 6 Hz	6 18	1H, *d*, *J* = 1 5 Hz	6 18	1H, *d*, *J* = 1 8 Hz
3a	3 94	1H, *d*, *J* = 15 9 Hz	3 93	1H, *d*, *J* = 15 7 Hz	3 93	1H, *m*
3b	3 39	1H, *ddd*, *J* = 15 9 Hz, *J* = 6 3 Hz, *J* = 1 8 Hz	3 38	1H, *d*, *J* = 2 0 Hz	3 38	1H, *m*
5a	3 26	1H, *t*, *J* = 8 7 Hz	3 26	1H, *t*, *J* = 8 0 Hz	3 25	1H, *t*, *J* = 1 9 Hz
5b	2 53	1H, *m*	2 53	1H, *m*	2 53	1H, *m*
6a	2 38	1H, *dd*, *J* = 14 0 Hz, *J* = 5 8 Hz	2 34	1H, *dd*, *J* = 13 7 Hz, *J* = 5 6 Hz	2 37	1H, *dd*, *J* = 14 1 Hz, *J* = 5 8 Hz
6b	2 15	1H, *m*	2 09	1 H, *m*	2 13	1H, *m*
7	5 00	1H, *t*, *J* = 3 3 Hz	4 95	1H, *t*, *J* = 3 0 Hz	5 00	1H, *m*
8	4 27	1H, *m*	4 24	1H, *m*	4 27	1H, *m*
9a	5 49	1H, *d*, *J* = 11 8 Hz	5 39	1H, *d*, *J* = 11 2 Hz	5 48	1H, *d*, *J* = 11 9 Hz
9b	4 09	1H, *d*, *J* = 11 8 Hz	4 01	1H, *d*, *J* = 11 2 Hz	4 03	1H, *d*, *J* = 11 9 Hz
13	1 64	1H, *m*	—	—	1 64	1H, *m*
14a	2 19	1H, *d*, *J* = 13 1 Hz	2 94	1H, *d*, *J* = 16 9 Hz, *t*, *J* = 2 0 Hz	2 16	1H, *dd*, *J* = 12 0 Hz
14b	1 73	1H, *m*	2 74	1H, *d*, *J* = 16 9 Hz, *m*	1 75	1H, *m*
18a	3 74	1H, *d*, *J* = 11 2 Hz	1 53	3H, *s*	1 31	3H, *s*
18b	3 62	1H, *d*, *J* = 11 2 Hz	—	—	—	
19a	0 85	3H, *d*, *J* = 6 4 Hz	5 23	1H, *d*, *J* = 2 0 Hz	0 90	3H *d*, *J* = 6 5 Hz
19b	—	—	5 04	1H, *d*, *J* = 2 0 Hz		—
20	5 71	1H, *q*, *J* = 7 3 Hz, *d*, *J* = 1 1 Hz	5 83	1H, *q*, *J* = 7 2 Hz, *d*, *J* = 1 0 Hz	5 71	1H, *m*
21	1 83	3H, *dd*, *J* = 7 1 Hz, *J* = 1 6 Hz	1 87	3H, *dd*, *J* = 6 8 Hz, *J* = 1 2 Hz	1 82	3H, *dd*, *J* = 7 2 Hz, *J* = 1 8 Hz

Table 2 ^{1}H NMR data for retrorsine *N*-oxide, senecíphylline-*N*-oxide and senecionine *N*-oxide

Proton(s) on carbon No	Retrorsine *N*-oxide δ (ppm)	Retrorsine *N*-oxide Proton	Senecíphylline *N*-oxide δ (ppm)	Senecíphylline *N*-oxide Proton	Senecionine *N*-oxide δ (ppm)	Senecionine *N*-oxide Proton
2	6 28	1H, *m*	6 28	1H, *m*	6 25	1H, *d*, *J* = 1 5 Hz
3a	4 67	1H, *d*, *J* = 17 1 Hz, *m*	4 55	1H, *m*	4 62	1H, *d*, *J* = 6 2 Hz, *m*
3b	4 51	1H, *d*, *J* = 17 1 Hz, *m*	4 55	1H, *m*	4 52	1H *d*, *J* = 6 2 Hz, *t*, *J* = 2 4 Hz
5a	4 04	1H, *m*	3 93	1H, *m*	3 99	1H, *m*
5b	3 68	1H, *m*	3 63	1H, *m*	3 67	1H, *d*, *J* = 12 0 Hz, *t*, *J* = 6 0 Hz
6a	2 97	1H, *m*	2 97	1H, *d*, *J* = 17 0 Hz	2 98	1H, *m*
6b	2 47	1H, *dd*, *J* = 14 4 Hz, *J* = 5 6 Hz	2 47	1H, *m*	2 46	1H, *m*
7	5 45	1H, *m*	5 44	1H, *m*	5 50	1H, *m*
8	4 94	1H, *m*	4 79	1H, *m*	4 89	1H, *m*
9a	5 51	1H, *d*, *J* = 12 2 Hz	5 42	1H, *m*	5 53	1H, *d*, *J* = 12 5 Hz
9b	4 21	1H, *d*, *J* = 12 2 Hz	4 12	1H, *m*	4 16	1H, *d*, *J* = 12 5 Hz
13	1 80	1H, *m*	—	—	1 60	1H, *m*
14a	2 18	1H, *d*, *J* = 13 9 Hz	2 90	1H, *d*, *J* = 16 1 Hz	2 14	1H, *d*, *J* = 13 7 Hz
14b	1 80	1H, *m*	2 72	1H, *m*	1 78	1H, *m*
18a	3 74	1H, *d*, *J* = 11 2 Hz	1 23	3H, *s*	1 31	3H, *s*
18b	3 68	1H, *d*, *J* = 11 2 Hz	—			
19a	0 84	3H, *d*, *J* = 6 7 Hz	5 23	1H, *d*, *J* = 1 5 Hz	0 90	3H, *d J* = 6 7 Hz
19b	—	—	5 01	1H, *d*, *J* = 1 5 Hz		
20	5 84	1H, *d*, *J* = 7 4 Hz, *q*, *J* = 0 5 Hz	5 94	1H, *q*, *J* = 6 9 Hz	5 84	1H, *d J* = 7 0 Hz, *q*, *J* = 1 2 Hz
21	1 86	3H, *dd*, *J* = 7 1 Hz, *J* = 1 4 Hz	1 89	3H, *d*, *J* = 7 0 Hz	1 85	3H, *dd*, *J* = 7 5 Hz, *J* = 1 6 Hz

H-14 protons of seneciphylline, as well as an increased H-14 geminal coupling constant

As reported previously [11, 12], the chemical shifts of the necine base hydrogens of the retronecine moiety are important parameters for the analysis of primary and tertiary structure in related compounds Furthermore, characteristic patterns in the deshielding of hydrogens in the retronecine moiety were recognizable in all of the *N*-oxides The H-3 resonances were the most strongly deshielded, shifting *ca* δ 1 5 relative to the reduced parent compound. In the parent PA the H-3 geminal pair yielded nonequivalent resonances, which exist in measurably different magnetic environments, whereas, in the *N*-oxides, the H-3 geminal pair of resonances were nearly unresolved at 360 MHz The H-5 geminal pair were deshielded by *ca* δ 0 8 relative to the parent compound with retention of the nonequivalent magnetic environments for each of the geminal protons The remaining hydrogens of the retronecine moiety H-6–H-8 all experienced deshielding effects of *ca* δ 0 5 when the *N*-oxide was formed. Other hydrogens, H-9 and components of the senecic acid moiety were virtually unaffected by oxidation of the nitrogen The significant perturbation to the H-3 resonances in the *N*-oxides indicated a significant restructuring of the magnetic environment of the unsaturated ring. It was noteworthy that H-5, H-8 and H-3 are all one bond removed from the nitrogen and yet H-3 was most strongly perturbed in the macrocyclic diester *N*-oxides. This is in marked contrast to the ^{13}C NMR of the macrocyclic diester *N*-oxides where C-8 exhibits the largest change in shielding upon oxidation of the nitrogen [13]

The ^1H NMR analysis of these macrocyclic PAs have been a great aid in the identification of toxic metabolites A recent *in vitro* microsomal study performed in our laboratory identified the compound 19-hydroxysenecionine, heretofore not previously reported in the literature [14]

EXPERIMENTAL

Pyrrolizidine alkaloids Retrorsine, seneciphylline and senecionine were extracted and isolated from *S vulgaris* using methods developed in our laboratory [15, 16] Standards of retrorsine *N*-oxide, seneciphylline *N*-oxide and senecionine *N*-oxide were prepared by published procedures [17–20]

360 *MHz* ^1H *NMR spectra* Spectra were recorded at 21–23° Optimization of the magnetic field homogeneity was achieved by computer adjustment of the room temp shim coils Spectral resolution is less than δ 0 002 All chemical shifts were referenced to TMS = δ 0 (residual $CHCl_3$ = δ 7 240 in $CHCl_3$-*d*) Resolution enhancement was achieved by double exponential apodization [21] All spectra were time-averaged for nominally 100 90° pulses Field-frequency lock was maintained by a time-shared ^2H heteronuclear lock

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REFERENCES

1 Bull, L B, Culvenor, C C J and Dick, A T (1968) *The Pyrrolizidine Alkaloids* North-Holland, Amsterdam
2 Culvenor, C C J, Edgar, J A, Jago, M V, Outteridge, A, Peterson, J E and Smith, L W (1976) *Chem Biol Interact* **12**, 299
3 Green, C E, Segall, H J and Byard, J L (1981) *Toxicol Appl Pharmacol* **60**, 176
4 Eastman, D F, Dimenna, G P and Segall, H J (1982) *Drug Metab Dispos* **10**, 236
5 Schoental, R, Head, M A and Peacock, P R (1954) *Br J Cancer* **8**, 458
6 Culvenor, C C J and Woods, W G (1965) *Aust J Chem* **18**, 1625
7 Mohanraj, S and Herz, W (1982) *J Nat Prod* **45**, 328
8 Wiedenfeld, H and Roder, E (1979) *Phytochemistry* **18**, 1083
9 Molyneux, R J, Johnson, A E, Roitman, J N and Benson, M E (1979) *J Agric Food Chem* **27**, 496
10 Culvenor, C C J (1966) *Tetrahedron Letters* 1091
11 Gelbaum, L T, Gordon, M M, Miles, M and Zalkow, L H (1982) *J Org Chem* **47**, 2501
12 Briggs, L H, Cambie, R C, Candy, B J, O'Donovan, C M, Russell, R H and Seelye, R N (1965) *J Chem Soc* 2492
13 Molyneux, R J, Roitman, J N, Benson, M and Lundin, R E (1982) *Phytochemistry* **21**, 439
14 Eastman, D F and Segall, H J (1982) *Drug Metab Dispos* **10**, 696
15 Segall, H J (1979) *J Liq Chromatogr* **2**, 429
16 Segall, H J. (1979) *J Liq Chromatogr* **2**, 1319
17 Kupchan, S M and Suffness, M I (1967) *J Pharm Sci* **56**, 541
18 Koekemoer, M J and Warren, F L (1951) *J Chem Soc* 66
19 Mattocks, A R (1969) *J Chem Soc* 1155
20 Craig, J C and Purushothaman, K K (1970) *J Org Chem* **35**, 1721
21 Ferrige, A G and Lindon, J C (1978) *J Magn Reson* **31**, 337