IMPROVED LABELLING METHODS FOR C9-^{2}H-RETRONECINE

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Abstract - The necine base retronecine (**1a**), common to numerous toxic pyrrolizidine alkaloids, has previously been labelled with ^{3}H at C-9 for use in metabolic studies. The current method for labelling retronecine is inefficient, and we report here an improved method of which utilizes the Dess-Martin periodinane reagent. Characterization of the resulting aldehyde (**2**) is described. The overall yield of C9-^{2}H-retronecine (**1b**) is 71% (from **1a**).

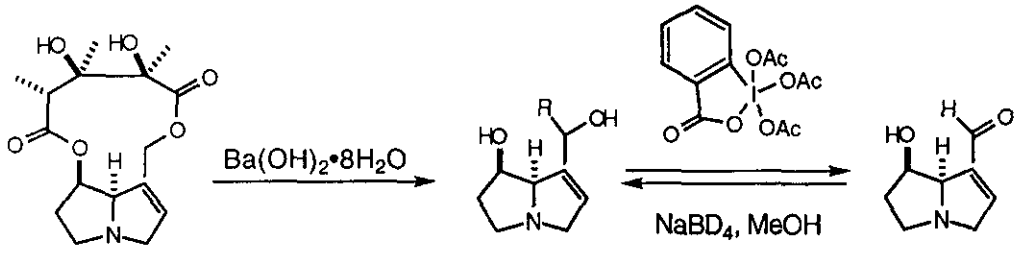
INTRODUCTION

Toxic plants containing pyrrolizidine alkaloids are common throughout the world and are responsible for the death of livestock as well as humans.[1,2] All pyrrolizidine alkaloids contain an amine portion, known as a necine base, that is usually attached through one or more ester linkages to a necic acid portion. There are some 6,000 plants that contain a total of at least 180 known pyrrolizidine alkaloids.[1,4] The most toxic of these are cyclic diesters, also known as macrolactone pyrrolizidine alkaloids, usually containing the unsaturated necine base retronecine (**1a**).[1,3,4]

Irreversible liver cirrhosis occurs in cattle when they ingest pyrrolizidine alkaloid containing plants at as little as 5% of their body weight.[1a,5] In contrast, the ingestion of these same plants by sheep at 200-300% of their body weight produces no such effect.[2b,5c,5e] The resistance of sheep to these toxins has been attributed to a consortium of bacteria contained within sheep rumen, that detoxify pyrrolizidine alkaloids before they come into contact with the liver.[6,7] We required retronecine for our research in order to determine how sheep ruminal bacteria detoxify pyrrolizidine alkaloids.

Studies involving pyrrolizidine alkaloids are often hindered by the difficulty in obtaining large quantities of the pure compounds.[3,6a,8,9] For this reason, semisynthetic derivatives of pyrrolizidine alkaloids, both labelled and unlabelled, have been used in several metabolic studies to simulate the toxic effects of pyrrolizidine alkaloids and to elucidate the origin of their toxicity.[5,8] Since the necine base has been implicated in the toxicity of pyrrolizidine alkaloids, several studies have employed the more accessible necine base retronecine.[5,8a]

The current method for labelling retronecine is inefficient (10-30% overall yield).[11,12a] The only published method involves a selective manganese dioxide oxidation[8a,10-13] followed by reduction of the resultant aldehyde with a labelled reducing reagent, such as $LiAl^3H_4$[11] or NaB^3H_4.[8a,10,12] The manganese dioxide oxidation affords a variety of undesired side products and the isolation is further complicated because the desired aldehyde (**2**) can not be stored or purified.[13] We report an improved method for isolating retronecine (**1**) and for deuterating retronecine at C-9 by utilizing a more selective method of oxidation.



3 **1a** R=H **b** R=D **2**

RESULTS AND DISCUSSION

Monocrotaline is efficiently hydrolyzed with $Ba(OH)_2$ as previously reported, but involves a tedious isolation procedure.[14] The crude product mixture obtained after hydrolysis and treatment with CO_2 is most efficiently purified by column chromatography on silica gel using a ternary solvent system ($CHCl_3$:MeOH:Et_3N). With this new protocol, we have obtained pure retronecine in up to 95% yield on a 5 gram scale.

Having obtained large quantities of retronecine we next developed an efficient method for preparing C9-^{2}H-retronecine. We reasoned that a chemoselective oxidation of retronecine would be more efficient and might be achieved using the Dess-Martin periodinane reagent. This reagent could potentially differentiate between the two alcohol functions because of their difference in steric environments. In addition, the enhanced reactivity of the Dess-Martin periodinane with benzylic alcohols[15] suggested that the rate of reaction with the allylic alcohol moiety may be faster than with the alcohol function at C7.

In practice, oxidation of retronecine with Dess-Martin periodinane proceeded cleanly and quickly (less than 1 h). Our experiments indicate that oxidizing retronecine with periodinane yields two products, the major product being the desired aldehyde (**2**). The aldehyde (**2**) has been reported previously, although the respective nmr signals have not been unambiguously assigned (shown in Figure 1). Table 1 contains the complete chemical shift assignments for the aldehyde (**2**), as well as those for compounds (**1a**) and (**1b**), that correspond well with recently published nmr data.[16] The overlap between protons 7-H and 3-H_a and protons 3-H_b and 5-H_a in compound (**2**) prevented the coupling constants from being determined. Further characterization of **2** was complicated by instability under various conditions, including but not limited to, heat, silica gel, and storage at greater than 0°C. For compounds (**1a**) and (**1b**), many of the signals were insufficiently resolved to allow extraction of coupling constants, although the large coupling between 3-H_a and 3-H_b was clearly visible (see Experimental section).

The reduction of aldehyde (**2**) closely followed earlier methods.[10,12] We have, however, found that the reduction can be readily performed in a MeOH-Et_2O solvent mixture, avoiding isolation and retarding decomposition of the rather unstable aldehyde (**2**). Treatment of **2** with NaB^2H_4 affords C9-^{2}H-retronecine in 71% overall yield (from **1a** to **1b**). Figure 2 shows the proton nmr spectra for compounds (**1a**) and (**1b**) in the region affected by the deuterium substitution. The signal centered

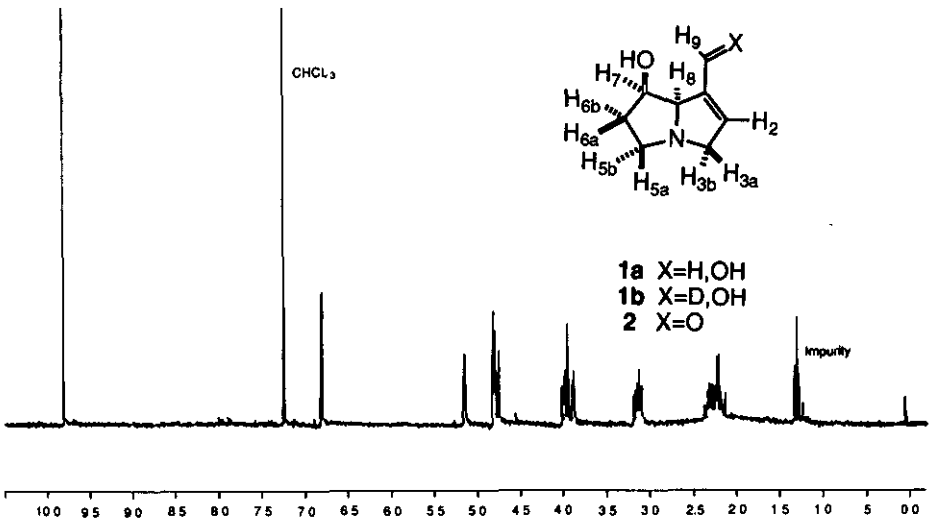


Figure 1. ^{1}H nmr of compound (**2**).

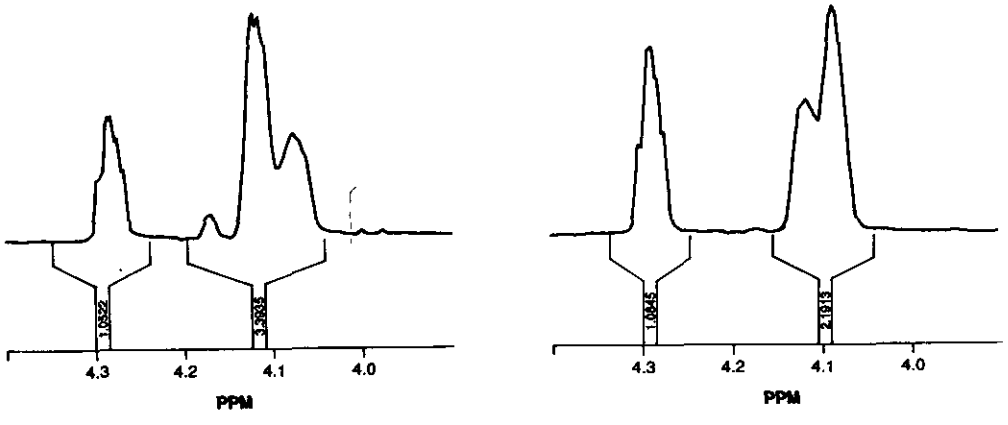
Table 1. ^{1}H Nmr Chemical Shifts in ppm (δ).

	1a*	**1b***	**2****
2-H	5.63, s	5.64, s	6.81, q
3-H_a	3.72, dt	3.73, dd	4.78, br m
3-H_b	3.29, ddd	3.29, dt	3.94, br m
5-H_a	3.13, br m	3.14, br m	3.94, br m
5-H_b	2.60, br m	2.61, br m	3.14, br m
6-$H_{a,b}$	1.84, br m	1.85, br m	2.25, br m
7-H	4.28, br m	4.29, br m	4.78, br m
8-H	4.12, br m	4.10, br m	5.15, d
9-$H_{a,b}$	4.12, br m	4.10, br m	9.80, s

*Taken in D_2O. **Taken in $CDCl_3$.

at 4.3 ppm is due to 7-H, while the signal centered at 4.1 ppm is from the three protons 8-H and 9-H. The integration in the 3.9-4.2 ppm region clearly indicates the presence of three protons in **1a**, but only two protons in **1b**. The deuterium nmr of compound (**1b**) consists of two singlets with equal integration. This occurs because the reducing agent (NaB^2H_4) is not stereoselective and gives rise to both the R and S configurations at C-9 in approximately equal amounts.

We anticipate using the deuterium labelled retronecine to investigate the detoxification of pyrrolizidine alkaloids by sheep ruminal bacteria. Detection of the deuterium label in the degradation products will allow us to determine the degradation pathway(s). The greater efficiency involved in labelling retronecine will greatly facilitate our studies of the biological degradation of retronecine, and will also facilitate other workers in this area.



1a **1b**

Figure 2. ^1H Nmr detail of compounds (**1a**) and (**1b**).

EXPERIMENTAL

Melting points were measured on a Büchi melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 5DBX FT-IR spectrophotometer. Optical rotations were measured in 1-dm cells (1 ml capacity) on a Perkin Elmer Model 243 polarimeter at ambient temperature. Nuclear magnetic resonance spectra were recorded on a Bruker AM-300 spectrometer in D_2O or $CDCl_3$ for ^1H and ^{13}C nmr, and in $CHCl_3$ for ^2H nmr. Chemical shifts are given in ppm relative to the solvent peak, either HOD in D_2O or $CHCl_3$ in $CDCl_3$ for the ^1H and ^{13}C nmr, or natural abundance $CDCl_3$ in $CHCl_3$ for the ^2H nmr. Mass spectra and exact mass determinations were obtained on a Varian MAT 311 spectrometer.

Retronecine (1a)

A mixture of a saturated aqueous solution of $Ba(OH)_2$ prepared from $Ba(OH)_2 \bullet 8H_2O$ (4.0 g, 13 mmol) and water (20 ml), and crystalline monocrotaline (1.9667 g, 6.04 mmol) was stirred for 2 h at 40-50°C, and then refluxed for 2 h. After cooling to room temperature, solid CO_2 was added, and the resultant mixture was then kept at room temperature for 16 h. The solution was filtered through filter paper, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$:MeOH:Et_3N (13:5:2)), which yielded 0.8523 g (91%) of a light tan solid. Recrystalization from hot acetone produced pure retronecine as white crystals with mp 108°C. $[\alpha]^{20}_D$ +52.7° (c=0.67, EtOH). Ir ν(film): 3400, 2940, 2850, 1635 cm^{-1}. ^1H Nmr (D_2O) δ 1.84 (2H, br m), 2.60 (1H, br m), 3.13 (1H, br m), 3.29 (1H, ddd, J=2.0, 4.9, 15.5 Hz), 3.72 (1H, dt, J=3.5, 15.4 Hz), 4.12 (3H, br m), 4.28 (1H, br m), 5.63 (1H, s). ^{13}C Nmr (D_2O) δ 35, 53, 58, 61, 71, 76, 125, 137. High resolution mass spectra calculated for $C_8H_{13}NO_2$ 155.0946, found 155.09460.

[9-^{2}H]-Retronecine (1b)

To a stirred dichloromethane solution (5 ml) of Dess-Martin periodinane (305.8 mg, 0.721 mmol) and CF_3CO_2H (60 μl, 0.616 mmol) was added a dichloromethane solution (25 ml) of retronecine (100.6 mg, 0.648 mmol). The resultant mixture was stirred for 1 h, after which dry ether (100 ml) was added. The resultant precipitate was filtered and washed successively with ether. The volume of the filtrate was reduced below 15°C under reduced pressure to approximately 3 ml to afford a solution of the crude, unstable, aldehyde (**2**) which was used without purification. Ir ν(film): 3400, 1680 cm^{-1}. ^{1}H Nmr ($CDCl_3$) δ 2.25 (3H, br m), 3.14 (1H, br m), 3.94 (2H, br m), 4.78 (2H, br m), 5.15 (1H, d, J=1.7 Hz), 6.81 (1H, q, J=2.0, 4.1 Hz), 9.80 (1H, s). High resolution mass spectra calculated for $C_8H_{11}NO_2$ 153.0790, found 153.07900.

Methanol (10 ml) was added to the crude aldehyde solution (≈3 ml) and the mixture was cooled to 0°C, followed by the addition of NaB^2H_4 (175 mg, 4.18 mmol). The reaction was monitored by tlc (silica gel, $CHCl_3$:MeOH:Et_3N (13:5:2)), until all of the starting material was consumed (≈30 min). The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (silica gel, $CHCl_3$:MeOH:Et_3N (13:5:2)), to yield a light tan solid (71.9 mg, 71% from **1a**). Ir ν(film): 3400, 2940, 1640 cm^{-1}. ^{1}H Nmr (D_2O) δ 1.85 (2H, br m), 2.61 (1H, br m), 3.14 (1H, br m), 3.29 (1H, dt, J=5.0, 15.5 Hz), 3.73 (1H, dd, J=1.6, 15.5 Hz), 4.10 (2H, br m), 4.29 (1H, br m), 5.64 (1H, s). ^{13}C Nmr (D_2O) δ 35, 53, 58 (t), 61, 71, 76, 125, 137. ^{2}H Nmr ($CHCl_3$) δ 4.18 (1D, s), 4.38 (1D, s). High resolution mass spectra calculated for $C_8H_{12}DNO_2$ 156.1009, found 156.10090.

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REFERENCES

1. (a) L. B. Bull, C. C. R. Culvenor and A. T. Dick, "The Pyrrolizidine Alkaloids", North-Holland Publishing Company, Amsterdam, 1968. (b) A. R. Mattocks, "Chemistry and Toxicology of Pyrrolizidine Alkaloids", Academic Press, Inc., London, 1986.

2. (a) S. Snyder, *Ore. Agri. Rec.*, 1972, **255**, 3. (b) J. M. Kingsbury, "Poisonous Plants of the United States and Canada", Prentice-Hall, Inc., Englewood Cliffs, NJ, 1964.

3. A. R. Mattocks, *Chem.-Biol. Interactions*, 1981, **35**, 301.

4. World Health Organization, "Pyrrolizidine Alkaloids", No. 80, Environmental Health Criteria, Geneva, 1988.

5. (a) A. M. Craig, E. G. Pearson, C. Meyer, and J. A. Schmitz, *Am. J. Vet. Res.*, 1991, **52**, 1969. (b) E. J. H. Ford, H. E. Ritchie, and E. Thorpe, *J. Comp. Pathol.*, 1968, **78**, 207. (c) P. R. Cheeke, *Can. J. Anim. Sci.*, 1984, **64**(Suppl.), 201. (d) E. Thorpe and E. J. H. Ford, *J. Comp. Path.*, 1968, **78**, 195. (e) J. W. Dollahite, *Southwest Vet.*, 1972, **25**, 222.

6. (a) A. M. Craig, L. L. Blythe, E. D. Lassen, and M. L. Slizeski, *Isr. J. Vet. Med.*, 1986, **42**, 376. (b) G. W. Lannigan, *J. Gen. Microbiol.*, 1976, **94**, 1.

7. (a) D. E. Wachenheim, L. L. Blythe, and A. M. Craig, *Appl. Environ. Microbiol.*, 1992, **58**, 2559. (b) D. E. Wachenheim, L. L. Blythe, and A. M. Craig, *Vet. Hum. Toxicol.*, 1992, **34**, 513. (c) A. M. Craig, C. J. Latham, L. L. Blythe, W. B. Schmotzer, and O. A. O'Connor, *Appl. Environ. Microbiol.*, 1992, **58**, 2730.

8. (a) A. R. Mattocks, *Xenobiotica*, 1988, **7**, 665. (b) D. F. Eastman, G. P. Dimenna, and H. J. Segall, *Drug Metab. Dispos.*, 1982, **10**, 236.

9. H. J. Segall, C. H. Brown, and D. F. Paige, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 671.

10. J. R. Piper, P. Kari, and Y. F. Shealy, *J. Labelled Compd. Radiopharm.*, 1981, **18**, 1579.

11. I. C. Hsu and J. R. Allen, *J. Labelled Compd.*, 1975, **11**, 71.

12. (a) A. R. Mattocks, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 479. (b) R. C. Shumaker, M.-T. S. Hsia, J. L. Seymour, and J. R. Allen, *J. Labelled Compd. Radiopharm.*, 1978, **15**, 227.

13. A. R. Mattocks, *J. Chem. Res. (S)*, 1977, 40.

14. W. M. Hoskins and D. H. G. Crout, *J. Chem. Soc., Perkin Trans. 1*, 1977, 538.

15. (a) D. M. Dess and J. C. Martin, *J. Org. Chem.*, 1983, **48**, 4155. (b) D. M. Dess and J. C. Martin, *J. Am. Chem. Soc.*, 1991, **113**, 7277.

16. L. Röder, K. Liu, and T. Bourauel, *Fresenius J. Anal. Chem.*, 1992, **342**, 719.

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