A General NMR Approach for the Structural Determination of Alkaloids: Application to 3-β-Hydroxylupanine

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The structure of 3-β-hydroxylupanine, a major alkaloid isolated from the seeds of *Lupinus mutabilis* S., was unequivocally established through dedicated ^{1}H and ^{13}C NMR experiments, thus revealing a novel general strategy for error-free structural assignment of lupine alkaloids.

Keywords: *Lupinus mutabilis; Leguminosae; structural analysis; quinolizidine alkaloids; NMR*

INTRODUCTION

A clear and unequivocal determination of the structure of lupine alkaloids is required to understand the phylogenetic relations between species as well as to establish reliable structure−function relationships in situations where these compounds exhibit biological activities. Although chromatographic methods can be used to assess the chemical purity of material obtained by extraction, the assignment of a given peak to a given chemical structure rests upon the availability of reliable standards.

At the present time, a combination of mass spectrometry and high-resolution NMR appears to be the optimal compromise to elucidate the chemical structure of unknown compounds, especially in situations where crystals cannot be obtained. The first method is basically used to derive the possible structures in terms of number and nature of substituents. NMR must then be used to provide the position of these substituents and the stereochemical features of substituted carbons.

In the field of lupine alkaloids, a large amount of ^{1}H as well as ^{13}C NMR data is available in the literature (Wink, 1993). Assignments are generally derived by analogy and can lead to potential discrepancies. A general error-free approach is therefore highly required.

Through the typical example presented here, we propose a general analytical strategy for the structural assignment of lupine alkaloids. Although the following approach is more demanding in terms of availability of material and in spectrometer specifications, it is designed to be virtually error-free and should be used to produce reliable data bases.

In the first step, the complete carbon backbone of the molecule can be reconstructed through natural abundance ^{13}C−^{13}C correlation. In the second step, all carbons having been assigned, a direct or reverse C−H correlation provides a staightforward and unequivocal assignment of all protons linked to carbons. A final check of the self-consistency of the C and the H assignments respective to the proposed chemical structure can be obtained from a H−H correlation experiment, preferably through the use of double-quantum transitions.

MATERIALS AND METHODS

General. TLC:silicagel (Kieselgel 60, F-254) of 0.25 mm layer thickness, elution with cyclohexane−diethylamine (7:3); the chromatograms were visualized by spraying with Dragendorff reagent. Analytical GC was performed at 207 °C on a CP Sil 5 CB column; FID detection (He, 1 mL/min, T_{inj} = 240 °C, T_{det} = 300 °C).

NMR measurements were performed in deuteriochloroform (Euriso-Top, Saclay, France) using a dual ^{13}C−^{1}H probe on a Bruker AMX500 spectrometer operating at 500 and 125.7 MHz for ^{1}H and ^{13}C, respectively. The concentration of the sample was 125 mg in 0.4 mL of $CDCl_3$. All 1D and 2D spectra were collected at 298 K. Processing and plotting of the data were performed on a Bruker X32 data station.

Extraction and Isolation. Dried seeds (3 kg) of *Lupinus mutabilis* grown in Ecuador ("chocho") were obtained from Latinreco Inc. 3-Fold extractions were performed with methanol−hydrochloric acid 1% v/v, the organic solvent being finally evaporated *in vacuo* to provide an acidic residue containing the alkaloid fraction. This aqueous phase was extracted with chloroform in order to discard the lipidic components and then basified (pH *ca.* 9.5) with dilute ammonia; the alkaloid fraction was recovered by liquid−liquid extraction with chloroform. This afforded the crude alkaloid extract (1.8% of the dry matter of seeds). The mixture of bases (54 g) was chromatographed on a silica gel column (SiO_2 60, ref 7734 Merck) using dichoromethane−methanol mixtures of increasing polarity as the eluent. At this stage, lupanine was co-eluted with **1**, but lupanine perchlorate crystallized in methanol, thus allowing isolation of *ca.* 30 g of this salt. The mother liquor was then treated on a silica gel column (SiO_2 60H, ref 7736 Merck). Elution was performed with benzene−diethylamine (98:2) and similar fractions (TLC monitoring) were gathered to afford **1** by crystallization from diethyl ether. Two additional crystallizations afforded 3 g of pure **1**. Mp 104 °C [Verdoorn (1991): mp 94−94.5 °C]. $[\alpha]_D$ 0° (*c* 2, EtOH) [Verdoorn (1991): idem]. IR η_{max} cm^{-1} (KBr): 3350 (OH) 2800, 2750 (Bohlmann bands), 1630 (lactam C=O). The retention features of alkaloid

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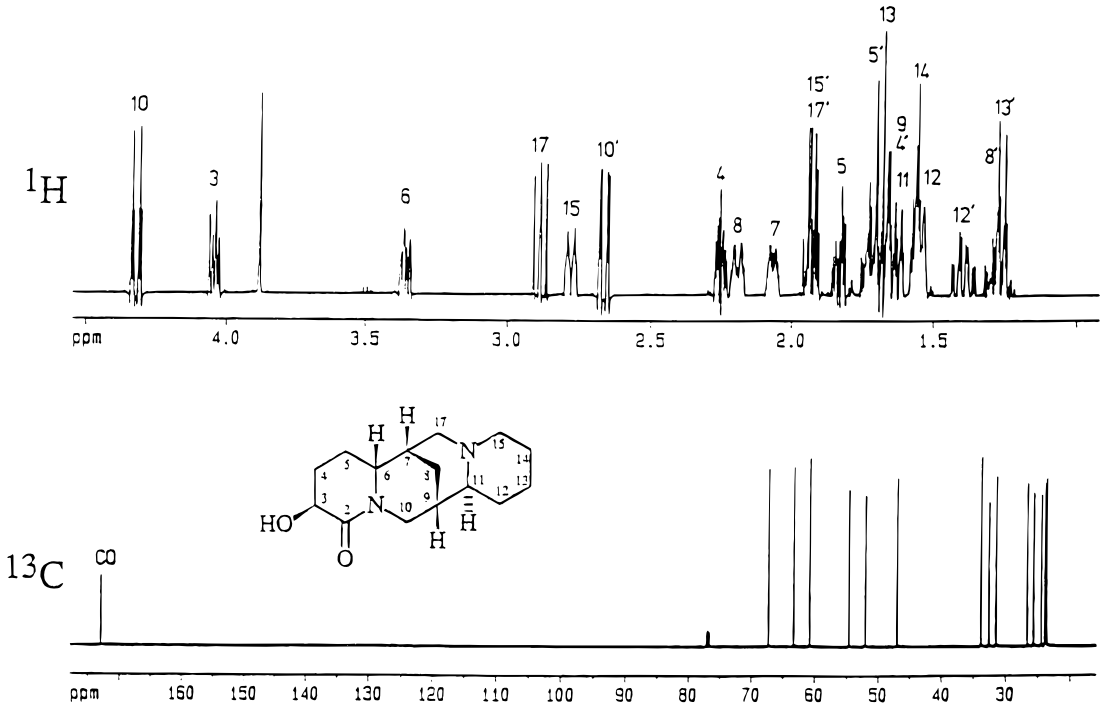

Figure 1. ^{1}H (a) and ^{13}C (proton-decoupled) (b) NMR spectra of **1** in $CDCl_3$ at 298 K. The assignments given for the proton spectrum are derived from experiments described in this work.

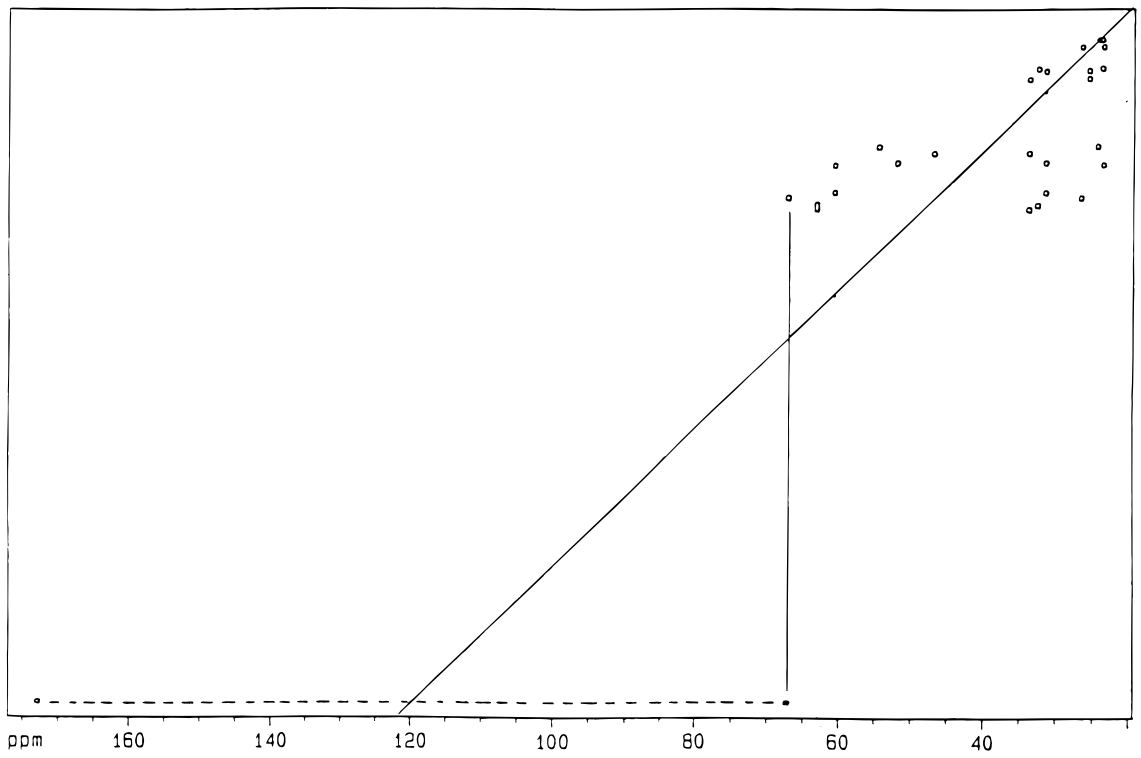

Figure 2. $^{13}C-^{13}C$ correlation map of **1** in $CDCl_3$ at 298 K. The solid line indicates the pseudo-diagonal. The dotted horizontal line shows the direct connectivity of the carbonyl carbon (172.9 ppm) with the neighboring carbon (C3) at 67.3 ppm.

1 (TLC R_f = 0.51; GC, 13 min) are close to those of lupanine (TLC R_f 0.61; GC, 11 min).

RESULTS AND DISCUSSION

L. mutabilis S. (Leguminosae) is an annual subligneous, one-meter high plant, composed of seven to nine glabrous above, glaucescent underneath, oblong folioles. Flowers are sweet-smelling, with a large white corolla, changing to pink, with a yellow-spotted standard. Seeds are white and shiny. The alkaloid content of the seeds of this species, cultivated in South America, has been largely studied (Von Baer et al., 1979; Hatzold et al., 1983). The present report deals with the isolation of a major quinolizidine alkaloid, 3-β-hydroxylupanine **1**, whose complete structural elucidation was performed using an adapted set of NMR spectroscopy experiments.

From the 50% methanolic extract of the seeds of *L.*

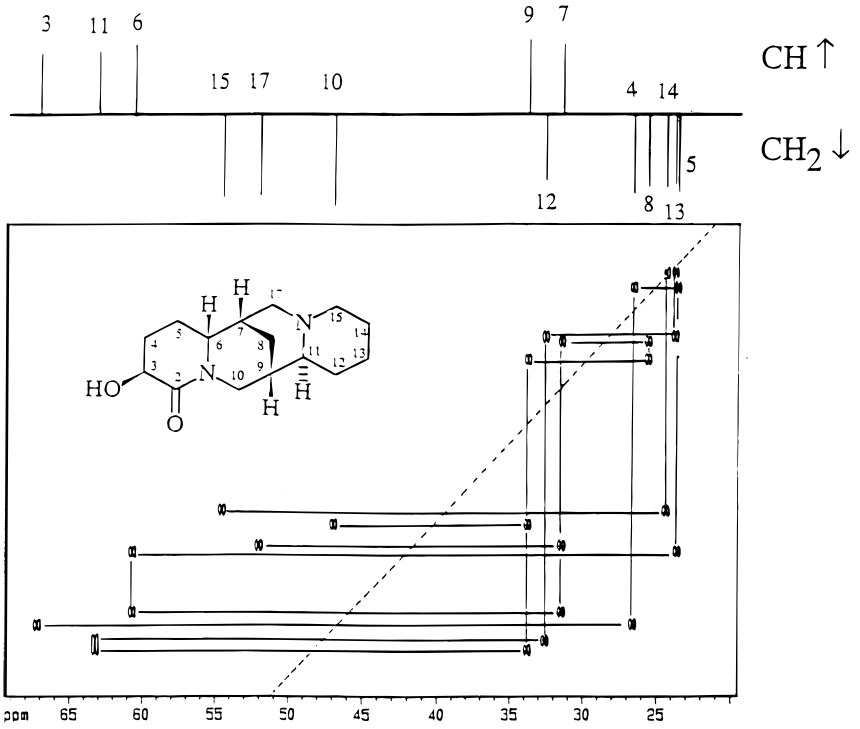

Figure 3. Partial $^{13}C-^{13}C$ correlation map (extension of Figure 2) with superimposed DEPT ^{13}C spectrum of **1** in $CDCl_3$. In the DEPT spectrum, methine carbons appear as positive signals and methylene carbons are negative.

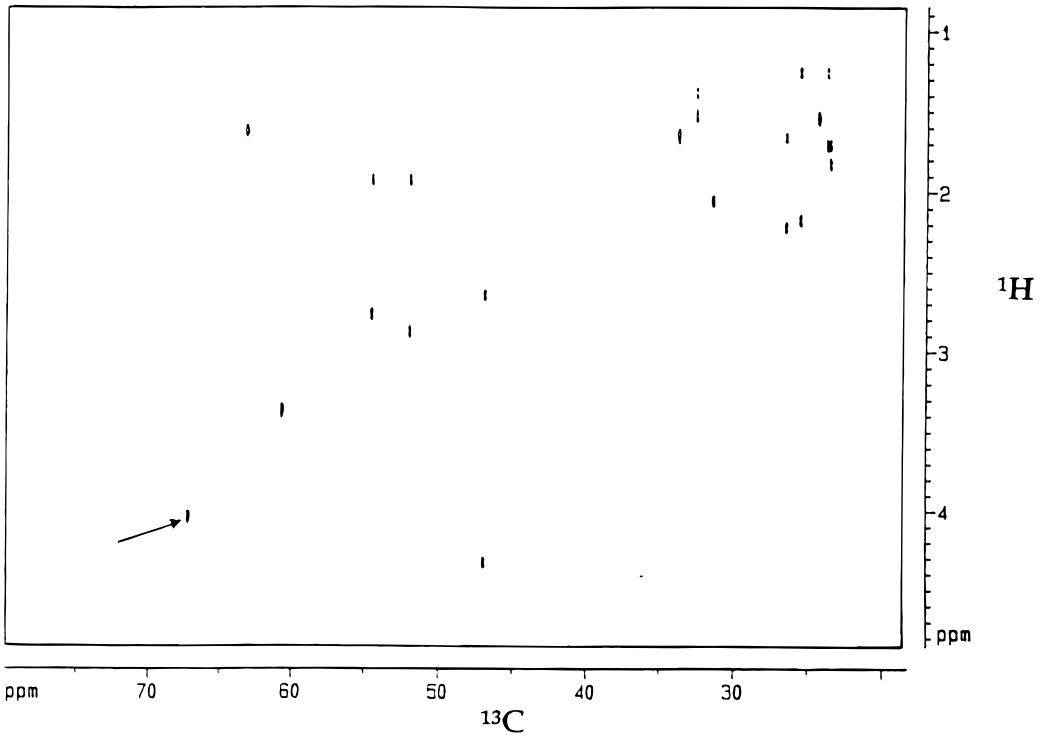

Figure 4. Contour plot of the direct $^{13}C-^{1}H$ correlation map for **1** in $CDCl_3$ at 298 K. The H−C correlation relating C3 to H3 is indicated by an arrow.

mutabilis S., 3-β-hydroxylupanine **1** was isolated along with other alkaloids, including lupanine. The HR-EI mass spectrum of **1** afforded the molecular formula $C_{15}H_{24}N_2O_2$ ($[M]^+$ *m/z* 264.1848, calcd 264.1838). In the EI mass spectrum, fragment ions at *m/z* 247 and 246, corresponding to $[M - OH]^+$ and $[M - H_2O]^+$, respectively, indicated the presence of one hydroxyl group, and this was confirmed by the 3350 cm^{-1} band in the IR spectrum.

The ^1H and ^{13}C spectra of compound **1** were recorded in $CDCl_3$ solution and are displayed on Figure 1. All

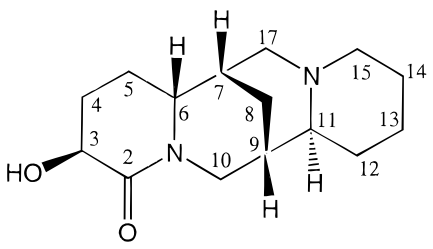

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Table 1. ^{1}H and ^{13}C Chemical Shifts of 1 in $CDCl_3$

position	carbon	proton
5	23.6	1.84; 1.70
13	23.9	1.69; 1.24
14	24.4	1.55
8	25.7	2.19; 1.28
4	26.6	2.25; 1.64
7	31.5	2.05
12	32.6	1.53; 1.40
9	33.8	1.65
10	47.0	4.32; 2.65
17	52.1	2.90; 1.95
15	54.6	1.95; 2.79
6	60.8	3.35
11	63.3	1.61
3	67.3	4.05
2	172.9	-

through the following section, the complete strategy for NMR assignment is described for **1** and is reported in Table 1.

Reconstitution of the Carbon Skeleton of 1. The most characteristic feature of the ^{13}C spectrum of **1** is the presence of a low-field signal (172.9 ppm) corresponding to a carbonyl group. This will be used as a starting point to reconstruct the carbon backbone of the molecule. The use of double-quantum (2Q) spectroscopy allows selection of these satellites since normal signals originating from $^{13}C-^{12}C$ pairs are unable to provide these 2Q transitions. The corresponding bidimensional experiment INADEQUATE (Mareci and Freeman, 1983) will hence select only $^{13}C-^{13}C$ satellites and allow a direct correlation between carbon signals.

The complete contour plot for the $^{13}C-^{13}C$ INADEQUATE experiment performed on **1** is displayed in Figure 2. The most important information derived from the complete contour plot concerns the fact that the carbonyl group is connected to one single other carbon atom, as is expected from the structure of **1**. The directly connected carbon C3 appears at 67.3 ppm. This point will be used in the next figure to derive the whole carbon backbone.

Figure 3 shows an extension (from Figure 2) with a complete assignment of the carbon skeleton. On the top of the contour plot of Figure 3, a DEPT experiment performed on the same sample is displayed. In the present case, only one spectrum that distinguishes between CH (positive signals) and CH_2 (negative signals) is displayed.

The direct connectivity of the carbonyl group to a carbon signal appearing at low field (67.3 ppm), assigned as a CH carbon from the DEPT experiment (Doddrell et al., 1982), suggests a substitution by one hydroxyl group at position 3. The assignment of all other carbons is straightforward from the contour plot of Figure 3. The derived assignments are reported on the DEPT spectrum, all methylene carbons next to a nitrogen atom (C15, C17, and C10) appearing at lower

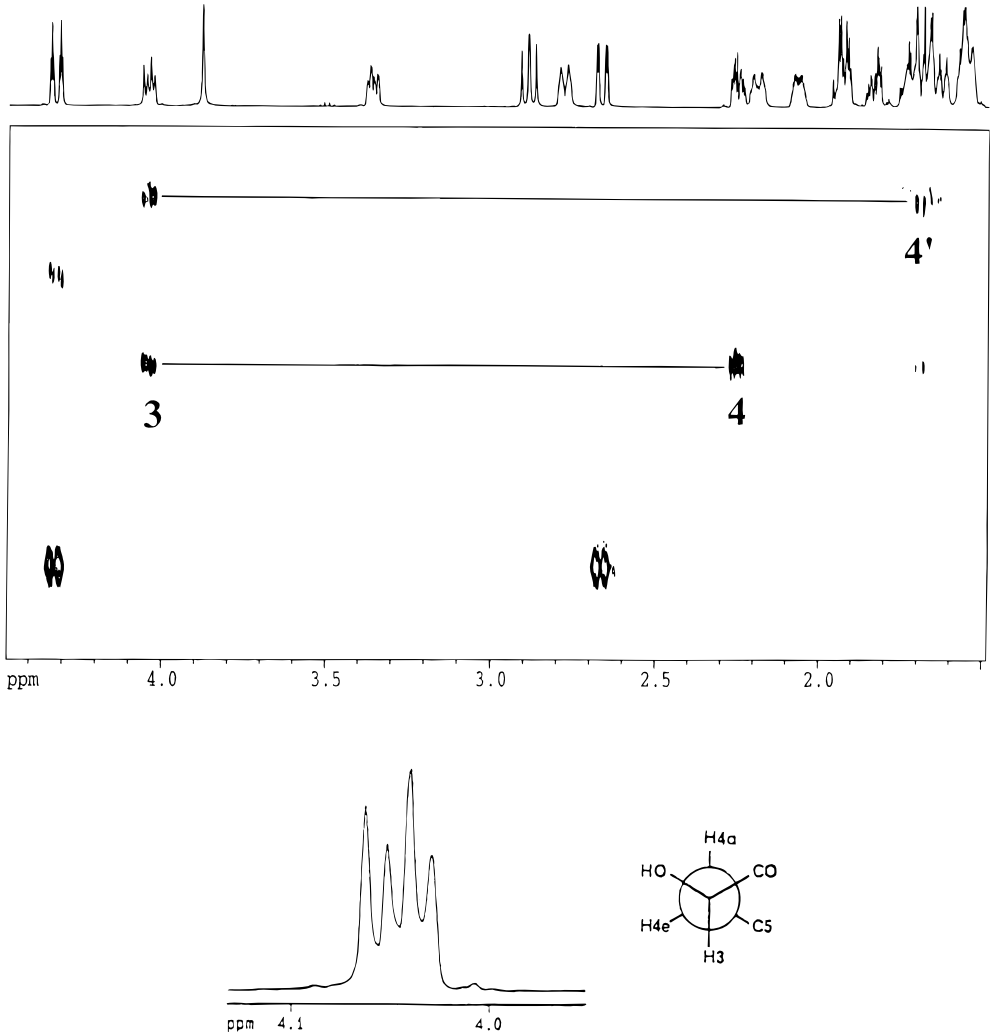

Figure 5. Partial contour plot of the $^{1}H-^{1}H$ double-quantum correlation experiment with extension of the proton signal from H3 and corresponding conformation.

field relative to C12, C4, C8, C14, and C5, as expected from the deshielding effects of nitrogen.

Assignment of Proton Signals from the Previously Assigned ^{13}C Spectrum. Once the ^{13}C backbone is reconstituted, the chemical shifts of protons directly bound to the assigned carbons can be determined using direct or inverse H−C bidimensional correlations.

Figure 4 shows the final contour plot of a direct C−H heteronuclear correlation (Rutas, 1984) with complete suppression of vicinal H−H couplings. In this experiment, any carbon atom can be related to the chemical shift(s) of the directly bound proton(s). For the sake of clarity, only information of importance to derive the position and orientation of the expected hydroxyl group will be detailed, all other data (for proton and ^{13}C) being presented in Table 1.

The most important point is that the carbon appearing at 67.3 ppm in ^{13}C (carbon C3 bearing the substituent) provides a correlation with a multiplet from the ^{1}H spectrum appearing at 4.05 ppm. Since this signal is clearly isolated on the spectrum, a detailed investigation of the multiplet structure can be performed to derive the respective orientations of the proton and the OH group on this carbon.

Assignment of the nearest neighbours of proton H3 is achieved using bidimensional ^{1}H−^{1}H double-quantum correlation. This technique provides basically the same information as COSY experiments but additionally suppresses all signals arising from uncoupled protons and all non-transferred magnetization.

Figure 5 shows an extension of this experiment along with the fine structure of multiplet of H3 and the corresponding configuration at C3. The quadruplet structure of H3 is consistent with the β orientation since the α configuration should lead to a triplet structure owing to the identity of expected couplings between H3 and both protons H4a and H4b. This implies that the hydroxyl substituent at C3 is oriented in the β configuration. Additionally, it is observed that the signal from the OH group appears at 3.85 ppm without giving rise to any coupling with H3. This spectral proximity is evidenced, however, by the second-order effects experienced by H3.

In conclusion, the novel NMR strategy disclosed in this report is proven highly efficient in securing without any ambiguity the 3-β-hydroxylated structure of compound **1**, to which a 4-OH structure had been erroneously assigned in previous work (Von Baer et al., 1979). In other respects, genuine 3-β-hydroxylupanine has been identified in Leguminosae of the genus *Pearsonia* (Verdoorn and Van Wÿk, 1990, 1991), whereas its 3-α-epimeric counterpart has been isolated from *Ammopiptanthus mongolicus* (Proksa et al., 1990).

Application of the strategy to additional alkaloids is expected to bring appreciable refinement of the NMR data collected thus far (Wink, 1993).

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