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ACCURATE ASSIGNMENTS IN PMR AND ^{13}C NMR SPECTRA OF ANHYDROLYCOCTONINE USING 2D SPECTROSCOPY

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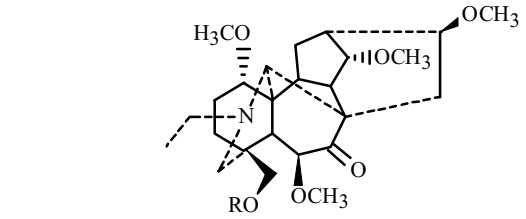
Resonances in PMR and ^{13}C NMR spectra of anhydrolycoctonine were fully assigned based on a series of 1D and 2D NMR experiments. The conformation of ring A was concluded to be a distorted boat with H-1β from a comprehensive analysis of chemical shifts, SSCC, and the NOE for two possible conformations.

Key words: norditerpene alkaloids, anhydrolycoctonine, 1D and 2D NMR.

We earlier reported the isolation from the weakly basic total alkaloids from roots of *Aconitum septentrionale* K. of the new alkaloid anhydrolycaconitine (**1**) [1], which has a broad spectrum of pharmacological activity (antiarrhythmic, anti-inflammatory, psychostimulant, antidepressant, spasmolytic, ganglion-blocking, etc.) [2]. The isolation from roots of this plant of another alkaloid of a similar type, acoseptine (**2**) was also described [3]. However, assignments of certain resonances were uncertain for **1** and **2** because of their complicated 1D NMR spectra. This prompted us to perform a detailed NMR analysis (2D COSY, HSQC, HMBC, and NOESY [4]) of the product of alkaline hydrolysis of **1** with a simpler structure, anhydrolycoctonine (**3**), in order to finalize the assignments of all resonances.



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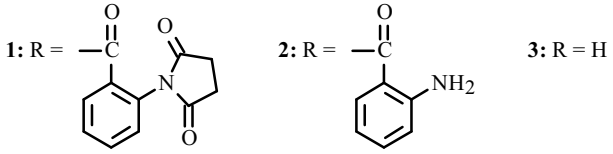

Resonances in PMR and ^{13}C NMR spectra of **3** were assigned in detail starting with a fragment having characteristic NMR parameters, namely, the C-7 ketone, the resonance of which in the ^{13}C NMR spectrum was observed at weak field at 201.7 ppm. Cross peaks between C-7 and protons of four CH groups (H-5, H-6, H-9, H-17) and one CH_2 group were observed in the 2D HMBC spectrum tuned to through-space ^{1}H—^{13}C SSCC (Fig. 1).

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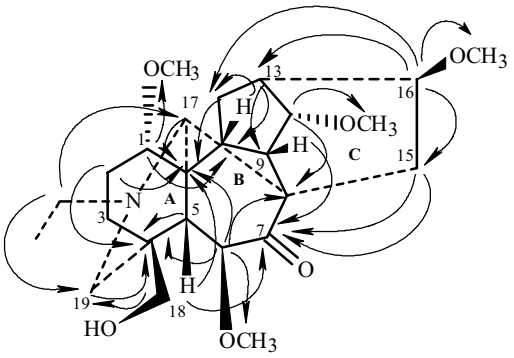

Fig. 1. Principal HMBC correlations from protons to C atoms for **3**.

These CH_2 protons are unambiguously located on C-15. Therefore, starting with them and based on the 2D COSY spectrum, protons of the five-membered ring and H-16 were found. Furthermore, a five-spin system consisting of one CH and two CH_2 groups (from DEPT and 2D HSQC) could be identified in the 2D COSY spectrum. These belonged to the CH-1, CH_2-2, and CH_2-3 protons, respectively.

The CH_2-18, CH_2-19, and *N*-ethyl protons were also identified in the 2D COSY spectrum. Carbon atoms directly bonded to protons in **3** were unambiguously determined from the 2D HSQC spectrum.

All resonances in the PMR and ^{13}C NMR spectra were completely assigned, including resonances for C atoms that were not bonded to H atoms, using the 2D HMBC spectrum. The CH-14 and CH_2-15 protons were coupled to quaternary C-8 at δ 59.2 ppm and the CH_2-15 protons were coupled to C-13 and C-16 (Fig. 1).

Furthermore, there was a strong cross peak between one of the CH_2-15 protons and the C atom of the CH group that we assigned as C-17 because the 2D HMBC experiment was tuned to detect coupling between atoms separated by a maximum of three bonds. The resonance of H-17 was determined using the 2D HSQC spectrum.

The 2D HMBC spectrum showed cross peaks between H-17 and C-1, C-15, $CH_3\underline{CH_2}N$; and between H-17 and the C atom of a CH group (C-5 or C-6) (Fig. 1). This resonance was assigned to C-5 because H-17 was separated from C-5 by three bonds whereas C-6 was four bonds away. Thus, proton H-5 was identified from the 2D HSQC spectrum.

The resonance of H-6 was established based on the 2D COSY spectrum, in which a cross peak between H-5 and H-6 was noted. The resonance for C-6 was assigned using the 2D HSQC spectrum.

Cross peaks between protons H-5, H-6, H-17, CH_2-2, and CH_2-12 and quaternary C-11 at 51.3 ppm were observed in the 2D HMBC spectrum; between protons CH_2-2, CH_2-3, H-5, H-6, CH_2-18, and CH_2-19 and the resonance of quaternary C-4 at 40.3 ppm; and between H-6 and C-8 (Fig. 1). The group of protons H-1, H-6, H-14, and H-16, and methoxy C atoms could be differentiated using cross peaks in the 2D HMBC spectrum. The proton resonances corresponding to them were determined using the 2D HSQC spectrum. Furthermore, correlations between the CH_2 protons of the *N*-ethyl and C-17 and C-19 were observed in the 2D HMBC spectrum (Fig. 1). The presence of the last cross peak enabled CH_2-18 and CH_2-19 to be differentiated. The 2D HMBC spectrum also showed coupling between protons of CH_2-19 and C-3, C-5, $CH_3\underline{CH_2}N$, C-17, and C-18; between protons of CH_2-18 and C-3, C-5, and C-19.

Thus, several homo- ($^1H—^1H$) and heteronuclear ($^1H—^{13}C$) correlations were used to assign unambiguously resonances of all C and H atoms (Table 1) and to establish the chemical structure of **3**.

In addition, we estimated chemical shifts (CS) of ^1H and ^{13}C in the most flexible fragment of **3** for two possible conformations, ring A (chair and distorted boat) with two orientations of H-1, in order to check the capabilities and limitations of quantum-chemical methods for calculating NMR parameters for establishing structures of new and complicated alkaloids (GIAO B3LYP/6-31G(d)//HF/6-31G) [5, 6].

In general, the calculated ^{13}C CS agree well with the experimental values for all four versions (R^2 = 0.99-1.00). This was completely normal because the CS of these nuclei are more sensitive to changes in the molecular framework and much less to the conformation [5b-d, 6]. Furthermore, the good agreement of the calculated and experimental CS for ^{13}C is additional proof that the established chemical structure and the resonance assignments are correct for **3**. However, a conclusion about the conformation of ring A and the orientation of the substituent on C-1 is difficult to make based on the ^{13}C data.

TABLE 1. Experimental Chemical Shifts (CS)[a] in PMR and ^{13}C NMR Spectra of **3**

C atom	δ_C, ppm	δ_H, ppm	C atom	δ_C, ppm	δ_H, ppm
1	79.40	3.34	14	83.34	3.49
2	20.45	1.95; 1.46	15	26.05	2.29
3	28.97	1.86; 1.30	16	83.07	2.07
4	40.35		17	66.03	3.53
5	45.64	1.88	18	69.60	3.45; 3.8
6	83.71		19	56.10	3.21; 2.79
7	201.67	3.88	CH_2(Et)	48.30	1.91; 2.55
8	59.17		CH_3(Et)	9.79	0.87
9	42.55	3.38	OCH_3-1	55.56	3.25
10	49.11	2.10	OCH_3-14	59.52	3.53
11	51.26		OCH_3-16	56.65	3.26
12	31.50	2.07; 1.44	OCH_3-18	57.00	3.36
13	39.57	2.31	OH		1.77

[a]CS are measured relative to the solvent resonance at 303 K.

In principle the CS of ^1H are more sensitive to conformational changes [5b-d, 6]. However, in this instance the lack of strong anisotropic groups, the stereoselective effects of which could be used to correlate with the structure, does not enable the conformation of ring A to be found by analyzing the ^1H CS.

An attempt to solve this structural problem could be made by analyzing vicinal SSCC of protons on C-1, C-2, and C-3, the values of which, according to Karplus [7], should also depend on the dihedral angles between these protons.

In fact, the SSCC for these protons were observed to be stereospecific. However, they were in general consistent with both the distorted boat conformation of ring A with H-1β and the chair with H-1α. Thus, additional information about the conformation of this ring or the orientation of proton H-1 was needed in order to unambiguously define the molecular structure.

In this instance, the orientation of H-1 relative to certain protons of the C ring could be easily determined from the NOE. The presence of a NOE between H-1 and H-10 is consistent with the β-orientation for H-1. Then, taking into account the results for the SSCC, it can be concluded that the distorted boat conformation with H-1β exists for **3**.

The presence of a large number of nonequivalent protons and the good spread of the PMR makes the use of the NOE exceedingly useful for determining the three-dimensional structure of this compound. In particular, the NOE between H-2a—H-5 and H-3a—H-19 determines unambiguously the distorted boat conformation for ring A. The presence of effects between H-6 and the CH_2-18 protons is possible only for the α-orientation of H-6. The NOE between H-9—H-10, H-9—H-14, and H-10—H-14 also defines unambiguously the orientation of these protons in ring C; between H-17—H-16, the *exo* orientation of the methoxy fragment.

Thus, the chemical and conformational structures of **3** in solution were established based on the detailed NMR analysis. The resulting structure agrees with the XSA [3].

EXPERIMENTAL

PMR and ^{13}C NMR spectra of **3** in $CDCl_3$ were recorded on an Avance-600 (Bruker) NMR spectrometer [600 MHz (^1H) and 150.926 MHz (^{13}C)] at 30°C. CS in the PMR and ^{13}C NMR spectra were calculated using B3LYP/6-31G(d)//RHF/6-31G and the Gaussian 98 program set [8]. Exact ^1H—^1H SSCC for **3** were determined by simulating the spectrum and comparing it with the experimental one. The Daisy program (Bruker TopSpin) was used for the calculation.

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