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^{13}C, ^{15}N CPMAS NMR and GIAO DFT calculations of stereoisomeric oxindole alkaloids from Cat's Claw (*Uncaria tomentosa*)

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ABSTRACT

Oxindole alkaloids, isolated from the bark of *Uncaria tomentosa* [Willd. ex Schult.] Rubiaceae, are considered to be responsible for the biological activity of this herb. Five pentacyclic and two tetracyclic alkaloids were studied by solid-state NMR and theoretical GIAO DFT methods. The ^{13}C and ^{15}N CPMAS NMR spectra were recorded for mitraphylline, isomitraphylline, pteropodine (uncarine C), isopteropodine (uncarine E), speciophylline (uncarine D), rhynchophylline and isorhynchophylline. Theoretical GIAO DFT calculations of shielding constants provide arguments for identification of asymmetric centers and proper assignment of NMR spectra. These alkaloids are 7*R*/7*S* and 20*R*/20*S* stereoisomeric pairs. Based on the ^{13}C CP MAS chemical shifts the 7*S* alkaloids (δ C3 70–71 ppm) can be easily and conveniently distinguished from 7*R* (δC3 74.5–74.9 ppm), also 20*R* (δC20 41.3–41.7 ppm) from the 20*S* (δC20 36.3–38.3 ppm). The *epiallo*-type isomer (3*R*, 20*S*) of speciophylline is characterized by a larger ^{15}N MAS chemical shift of N4 (64.6 ppm) than the *allo*-type (3*S*, 20*S*) of isopteropodine (δN4 53.3 ppm). ^{15}N MAS chemical shifts of N1–H in pentacyclic alkaloids are within 131.9–140.4 ppm.

1. Introduction

Uncaria tomentosa (Cat's Claw, Unâ de Gato, Vilcacora) is a large, woody vine with hook-like thorns that resemble the claws of a cat. It is indigenous to the Amazon rainforest and other tropical areas of South and Central America. The vine has been used medicinally by native tribes for at least 2000 years in treating inflammation, arthritis, bone pain, asthma, deep wounds, and cancer [1]. Since the early 1990s Cat's Claw has been used in Peru and Europe as an adjunctive treatment for diseases that target the immune system. Numerous investigations have been carried out to isolate and determine its bioactive components. Over 50 compounds have been identified including oxindole alkaloids [2], ursane type pentacyclic triterpenes, ursolic and quinovic acid derivatives, sterols [3–5] and procyanidins [6]. The pentacyclic oxindole alkaloids: mitraphylline, isomitraphylline, pteropodine, isopteropodine, speciophylline and uncarine F have been especially associated with immunomodulatory and cytotoxic activities [7,8]. In Europe, the measurement of isopteropodine (uncarine E), the most immunostimulating alkaloid, is used for the purpose of standardization of Cat's Claw products [9]. The content of the alkaloids in the commercial extracts should be standardized to 1.3–1.75% by weight, out of which 97% should constitute pentacyclic alkaloids. The presence of alkaloids is most frequently tested by chromatographic methods [10–12].

The absolute stereochemistry has been determined by single crystal X-ray crystallography for pteropodine, isopteropodine [13], mitraphylline, speciophylline and rhynchophylline [14]. In 1993, eleven natural heteroyohimbine-type pentacyclic oxindole alkaloids, isolated from *Uncaria* species, were analyzed by means of ^{1}H, ^{13}C NMR, including 2D NMR correlation experiments [15]. These stereoisomeric alkaloids have also been studied by ^{15}N NMR spectroscopy [16].

The present study deals with the characterization of the most abundant alkaloids of *U. tomentosa* by solid-state NMR technique. Their structures are illustrated in Fig. 1 and stereochemical data are listed in Table 1. To our knowledge, this is the first report of ^{13}C and ^{15}N CPMAS NMR chemical shifts for *Uncaria* oxindole alkaloids. The study provides theoretical calculations of shielding constants using the DFT/GIAO approach.

2. Experimental

Pentacyclic oxindole alkaloids are usually isolated following a procedure published previously [17]. Cat's Claw alkaloids were

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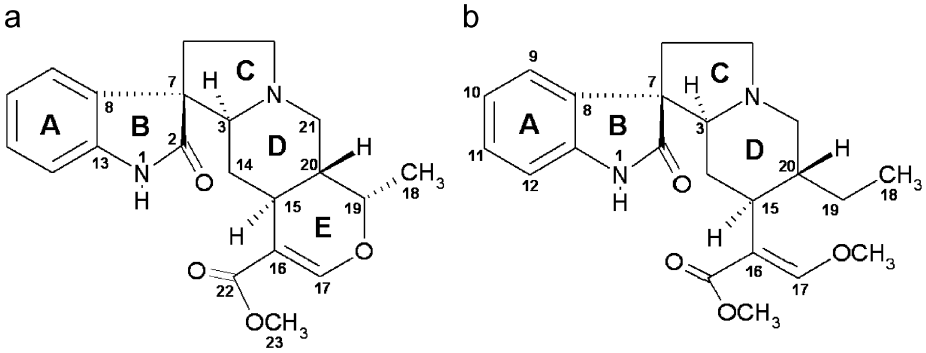

Fig. 1. The structure of pentacyclic (a) and tetracyclic (b) alkaloids.

Table 1
Characterization of stereochemistry of pentacyclic **1–5** and tetracyclic **6**, **7** alkaloids

	Alkaloid	Type						D/E ring
1	Mitraphylline	*Normal*	3*S*	7*R*	15*S*	19*S*	20*R*	*trans*
2	Isomitraphylline	*Normal*	3*S*	**7*S***	15*S*	19*S*	20*R*	*trans*
3	Pteropodine (Uncarine C)	*Allo*	3*S*	7*R*	15*S*	19*S*	**20*S***	*cis*
4	Isopteropodine (Uncarine E)	*Allo*	3*S*	**7*S***	15*S*	19*S*	**20*S***	*cis*
5	Speciophylline (Uncarine D)	*Epiallo*	**3*R***	7*S*	15*S*	19*S*	**20*S***	*cis*
6	Rhynchophylline	*Normal*	3*S*	7*R*	15*S*	–	20*R*	–
7	Isorhynchophylline	*Normal*	3*S*	7*S*	15*S*	–	20*R*	–

purified from a commercial extract of *U. tomentosa* provided by Advanced Nutra Inc., Redding, CA, lot # AN C9800F2. Typically, the extract was suspended in water and treated with an excess of sodium carbonate. The alkaline solution was then quickly extracted with chloroform. The chloroform layer was then washed with 3% sulfuric acid. In this operation, the alkaloids, but not neutral components dissolved in the aqueous phase. After neutralization of the acid the alkaloids were again extracted into chloroform and recovered from it by evaporation *in vacuo*. The mixture of alkaloids was purified into individual components using centrifugal partition chromatography (CPC), model FCPC1 from Kromaton Technologies Inc., France. Details of the purification will be published elsewhere.

The ^{1}H and ^{13}C chemical-shift spectra were recorded for a $CDCl_3$ solution on a Bruker DSX-500 spectrometer. Standard pulse programs from Bruker library were used for COSY, TOCSY, HMQC and HMBC experiments. The 2D ^{1}H–^{13}C correlations were performed using the phase-sensitive gradient-selected (PFG) inverse technique; the HMBC experiment was optimized for $J = 5$ Hz. Chemical shifts are reported in ppm relative to internal TMS.

Cross polarization (CP) magic angle spinning (MAS) solid-state ^{13}C and ^{15}N NMR spectra were recorded at 100.6 and 40.6 MHz, respectively on a Bruker DSX-400 WB MHz spectrometer; powder samples packed in a 4 mm ZrO_2 were spun at 10 kHz (^{13}C) and 3.5 kHz (^{15}N). For the acquisition of ^{13}C spectra a contact time (t_{CP}) of 2 ms, a repetition time (t_R) of 8 s and spectral width of 25 kHz were used, and 400–600 scans were accumulated. ^{13}C chemical shifts were calibrated indirectly through the glycine CO signal recorded at 176.0 ppm, relative to TMS. Dipolar dephasing (DD) pulse sequence (with 50 μs delay time (t_D) inserted before acquisition) was used to observe selectively the non-protonated carbons. Two-dimensional phase adjusted spinning sideband (2D PASS) experiments (introduced by Anzutkin et al. [18,19]) were performed at a spinning rate of 2 kHz, 1000 scans for each spectrum were accumulated. The ^{13}C δ_{ii} parameters were calculated using the WINMAS program.

Accumulation of the natural abundance ^{15}N MAS NMR spectra used ^{1}H decoupling, a 10 s recycle delay and a contact time (t_{CP}) of 5 ms; typically, 3200 scans were accumulated in overnight experiments. Chemical shifts for ^{15}N were calibrated indirectly on glycine resonance $\delta^{15}N$ 10.0 ppm, and referenced to nitromethane $\delta^{15}N = 380.2$ ppm (liquid NH_3 $\delta^{15}N = 0$).

The geometry of alkaloids was adopted from crystallographic data of: **1** (CCSD MUTZEG), **3** (CCSD refcodes QIKGOG and KIKXIL), **4** (CCSD refcode QIKGUM), and **6** (CCSD refcodes MUTYUV, NIFDUB). Since the positions of hydrogen atoms in X-ray crystal structures are inaccurate, energy minimization was performed on the hydrogen atoms of the crystal structures using the semi-empirical PM3 method (from HyperChem 7.0 [20]). A full structure relaxation was subsequently performed when the geometry was optimized at density functional theory (DFT) level using procedures implemented in the GAUSSIAN-03 package [21]. The final low-energy structures were used for calculations of NMR shielding constants. Shielding tensors were computed using the GIAO-DFT (gauge-independent atomic orbital) with the 6-311G** basis set approach. Gaussian-03 calculations provide absolute shielding values, which were assigned such that $\sigma_{33} \geqslant \sigma_{22} \geqslant \sigma_{11}$ ($\delta_{11} \geqslant \delta_{22} \geqslant \delta_{33}$), the latter component being along the direction of greatest shielding. The shielding constants are frequently converted to NMR chemical shifts by calculating reference compounds. The systematic offset of these data by several ppm makes such recalculation somewhat arbitrary, but the values of theoretical chemical shifts are easy to compare with experimental ones. The calculations of carbon shielding constants were performed for a set of compounds, including CH_3NH_2, CH_3OH, CH_3CN or urea [22,23]. The results depend on the method and basis set applied, as illustrated for tetramethylsilane: $\sigma_{TMS} = 183.2$ ppm, B3LYP/6-311++(3df,3dp)] [24], $\sigma_{TMS} = 202$ ppm, GIAO CPHF 6-31G(d,p) [25]. The absolute shielding values calculated for peptides were converted to chemical shifts relative to the absolute shielding of liquid TMS of 184.1 ppm [26].

Theoretical isotropic ^{13}C chemical shifts δ_{iso} and the principal elements of chemical shift tensor (CST) δ_{ii} are given relative to