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Original article

Napelline-type C_{20}-diterpenoid alkaloid iminiums from an aqueous extract of "fu zi": Solvent-/base-/acid-dependent transformation and equilibration between alcohol iminium and aza acetal forms

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ABSTRACT

Three new napelline-type C_{20}-diterpenoid alkaloids, named aconicarmichinium A and B trifluoroacetates (**1** and **2**) and aconicarmichinium C chloride (**3**), were isolated from an aqueous extract of "fu zi", the lateral roots of *Aconitum carmichaelii*. Their structures were elucidated by extensive spectroscopic data analysis. Compounds **1**–**3** represent the first examples of napelline-type C_{20}-diterpenoid alkaloid alcohol iminiums, of which the structures were fully characterized. In addition, transformation and equilibration between the alcohol iminiums (**1**–**3**) and the aza acetals **1a**–**3a** were investigated by measurements of the NMR spectra in protic and aprotic deuterium solvents including alkali pyridine-d_5, along with evaporation under reduced pressure and gradual additions of TFA, AcOH, and HCl. The results demonstrated that the transformation and equilibration were solvent-, base-, and acid-dependent. Especially, in aqueous biological fluid, these C_{20}-diterpenoid alkaloids would more likely exist as the alcohol iminiums accompanied by anion counterparts in biosystems to increase their solubility, bioavailability, transportations, and functions. The absolute configurations of **1**–**3** were confirmed by X-ray crystallographic analysis of **2a**.

1. Introduction

Diterpenoid alkaloids are a large group of natural products, which are classified as C_{20}-, C_{19}-, and C_{18}-categories and mainly isolated from plants of several genera belonging to the Ranunculaceae, Rosaceae, Asteraceae, Garryaceae, Escalloniaceae, and Polygonaceae families [1–4]. Because of diversities of complex structures and biological activities, these molecules continuously attract great interests of scientists in phytochemistry, synthesis, medicinal chemistry, and pharmacology for more than a century [5]. Taking advantage of reversible replacement reactions, acidification and saponification with a variety of acids and bases are classic procedures in extraction and separation of the diterpenoid alkaloids and application of acids and/or bases is almost unavoidable [6–11]. Although a majority of diterpenoid alkaloids were finally obtained and structurally characterized as free base forms [1–11], their hydrophobic and alkali properties suggest that, under *in vivo* hydrophilic conditions, the basic alkaloids have to interact with various acidic molecules to increase solubility, bioavailability, transportations, and functions, as well as to maintain a relatively stable physiological pH of aqueous biological systems. In addition, the properties suggest that there are dynamic equilibrations between different forms/species of the alkaloids in the biological systems, and the equilibrations, along with the forms/species with structural alterations, may play important biological roles. The suggestion have been demonstrated by studies on several bioactive benzo[*c*]phenanthridine alkaloids, including natural products sanguinarine and chelerythrine [11–19], as well as synthetic analogues [20]. However, detailed interactions of the diterpenoid alkaloids with solvents, bases, and acids, as well as the structural characterization of the associated forms/species in solution systems, are not investigated yet.

"Fu zi", the lateral roots of *Aconitum carmichaelii* Debx. (Ranunculaceae), is an indispensable ingredient of formulations in traditional Chinese medicine for the treatment of cardianeuria, neuralgia, and rheumatalgia in China, Japan, and Korea [21–23]. Previous studies have shown that toxic aconitine C_{19}-diterpenoid

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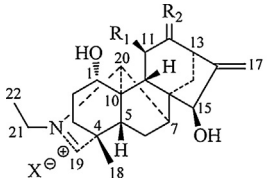

1 R_1 = H; R_2 = αOH, βH; X = TFA
2 R_1 = H; R_2 = O; X = TFA
3 R_1 = OH; R_2 = αOH, βH; X = Cl

Fig. 1. Structures of **1**–**3**.

alkaloids are the main active constituents of "fu zi", while more than a hundred of compounds were reported from various extracts of *A. carmichaelii* [9,24–32]. However, the previous investigations of the plant materials including raw and prepared "fu zi" were mainly extracted with organic solvents, such as benzene, $CHCl_3$, methanol, and ethanol [22–31]. This is inconsistent with a practical application of decocting the formulations. Therefore, an aqueous extract of the raw lateral roots of *A. carmichaelii* was investigated as part of a program to systematically study the chemical diversity of traditional Chinese medicines and their biological effects [33–50]. In previous papers, we reported four new hetisan-type C_{20}- and twenty-one new aconitane-type C_{19}-diterpenoid alkaloids, two new 2-(quinonylcarboxamino)benzoates, and seven new aromatic acid derivatives [51–53] from the aqueous extract. A continuation of the investigation has resulted in the isolation and structural characterization of three new napelline-type C_{20}-diterpenoid alkaloids (**1**–**3**) (Fig. 1), which were obtained as the alcohol iminiums. Since the chemical transformation from the aza acetal-containing napelline-type C_{20}-diterpenoid alkaloids to the alcohol iminium salts was reported [1,5,54,55], the alcohol iminiums in **1**–**3** could readily be formed from the corresponding aza acetals, and the anion counterparts were obviously induced by using hydrochloride (HCl) and trifluoroacetic acid (TFA) in fractionation and HPLC separation steps of this study. However, the only alcohol iminium salt "songoramine hydrochloride" was previously obtained and characterized only by IR spectroscopic data [55] with the absence of experimental details. This, together with the above mentioned multiple properties of alkaloids and equilibrations between the iminium and alkanolamine forms of sanguinarine and chelerythrine in the biological systems, especially biological interaction variations of the different forms with proteins [11–19], inspired us to fully characterize the structures of **1**–**3** and to preliminarily investigate influences of protic and aprotic solvents including the alkali pyridine-d_5, as well as common acids TFA, AcOH, and HCl, on the transformation and equilibration between the alcohol iminium and aza acetal forms of these napelline-type C_{20}-diterpenoid alkaloids. Reported herein are the details.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a P-2000 polarimeter (JASCO, Tokyo, Japan). UV spectra were recorded on a V-650 spectrometer (JASCO). CD spectra were measured on a JASCO J-815 CD spectrometer (JASCO). IR spectra were recorded on a Nicolet 5700 FT-IR Microscope spectrometer (FT-IR Microscope Transmission). 1D NMR and 2D NMR spectra were obtained at 600 or 500 MHz for ^1H; 150 or 125 MHz for ^{13}C; and 470 MHz for ^{19}F, respectively, on an Inova 500 MHz, a SYS 600 MHz (Varian Associates Inc., Palo Alto, CA, USA), a Bruker 600 MHz spectrometer (Bruker Corp., Karlsruhe, Germany), or a WNMR-I 500 MHz (Wuhan Zhongke Niujin Magnetic Resonance Technology Co., Ltd., Wuhan, China), with TMS or solvent peaks as references. ESIMS and HR-ESIMS data were obtained on Agilent 1100 Series LC-MSD-Trap-SL and Agilent 6520 Accurate-Mass Q-TOFL CMS spectrometers (Agilent Technologies, Ltd., Santa Clara, CA, USA), respectively. Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), reversed phase C-18 silica gel (W. R. Grace & Co., Maryland, USA), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and MCI gel (CHP20P, 75–150 mm) (Mitsubishi Chemical Corporation, Tokyo, Japan). HPLC separation was performed on a system consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual absorbance detector (Waters Corporation, Milford, MA, USA) or a Smartline RI detector (KNAUER, Berlin, Germany), using an Ultimate XB-Phenyl semi-preparative column (250 mm × 10 mm i.d.) packed with phenyl-silica gel (5 μm) (Welch, shanghai, China) or a YMC-Pack Ph column (250 mm × 10 mm i.d.) packed with phenyl-silica gel (5 μm) (YMC Co., Ltd, Kyoto, Japan). The pH values were measured using a FiveEasyPlus™ FE28-Standard pH meter equipped with an Inlab science pH electrode (Mettler-Toledo, Zurich, Switzerland) which was calibrated from pH 0 to pH 12 with standard solutions. When needed, the pure TFA (99.9%, J&K Chemica, Germany), AcOH (99.8%, J&K Chemica, Germany), or standard solutions of TFA, AcOH, or HCl (Beijing Chemical Works, Beijing, China) in MeOH-d_4 (Cambridge Isotope Laboratories, Inc., MA, America) or D_2O (Beijing Easobio Technology Co., Ltd, Beijing, China) was added to the sample in the NMR tube using a 10 μL syringe or finnpipette. The maximum volume of the standard solutions added to the samples is 8 μL. TLC was conducted on precoated silica gel GF_{254} plates. Spots were visualized under UV light (254 or 365 nm) or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating or with a Dragendorff's reagent. All other chemicals were purchased from Sigma–Aldrich or J&K Chemica.

2.2. Plant material

See Refs [51,52].

2.3. Extraction and isolation

For extraction and preliminary fractionation of the extract, see ref 51. Fraction C2-1 (200 g) was dissolved in H_2O (500 mL), basified with concentrated ammonium hydroxide (25 mL) to pH 10, then extracted with EtOAc (500 mL × 4). The EtOAc phase was concentrated under reduced pressure to give C2-1-A. The aqueous layer was acidified with 6 mol/L HCl (66 mL) to pH 4, and partitioned with n-butanol (500 mL × 3). Evaporation of the *n*-butanol phase under reduced pressure yielded C2-1-B (12 g).

Fraction C2-1-A (60.0 g) was chromatographed over basified silica gel (pH 8–9), eluting with a gradient of petroleum ether-Me_2CO-diethylamine (15:1:1–4:1:1) mixture to afford C2-1-A-1–C2-1-A-6. Fraction C2-1-A-6 (8.3 g) was further fractionated by reverse phase (RP) flash charomatography (20–90% MeOH in H_2O, containing 0.1% TFA) to give C2-1-A-6-1–C2-1-A-6-3. Separation of C2-1-A-6-3 (1.40 g) by CC over Sephadex LH-20 (50% MeOH in H_2O) yielded C2-1-A-6-3-1–C2-1-A-6-3-2, of which C2-1-A-6-3-2 (112 mg) was purified by HPLC (YMC-Pack Ph column, 20% MeCN in H_2O containing 0.1% TFA, 1.5 mL/min) to yielded **2** (33.4 mg, t_R = 18 min).

Fraction C2-2 (200 g) was separated by CC over Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) yielded C2-2-1–C2-2-8. Fraction C2-2-4 (9.5 g) was chromatographed over silica gel (150 g) eluting with a gradient of petroleum ether-Me_2CO-diethylamine (5:2:1–2:2:1) to give C2-2-4-1–C-2-2-4-7, of which C2-2-4-4 (420 mg) was further separated by CC over silica gel, eluting with a gradient of $CHCl_3$ (saturated with ammonia water)-MeOH (30:1–5:1), to yield C2-2-4-4-1–C-2-2-4-4-6. Isolation of C2-2-4-4-5 (200 mg) by preparative TLC [$CHCl_3$ (saturated with ammonia water)-MeOH, 5:1] afforded C2-2-4-4-5-1–C-2-2-4-4-5-3. HPLC purification of