NPC Natural Product Communications

2013
Vol. 8
No. 12
1711 - 1712

Supinidine Viridiflorates from the Roots of *Chromolaena pulchella*

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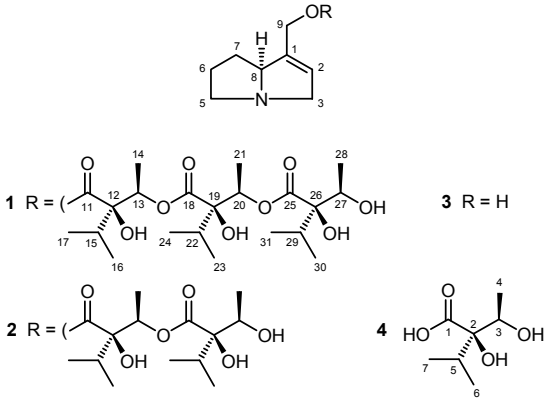
Received: September 13th, 2013; Accepted: September 18th, 2013

The alkaloid extract from the roots of *Chromolaena pulchella* provided two new pyrrolizidine alkaloids, elucidated as (–)-supinidine triviridiflorate (**1**) and (–)-supinidine diviridiflorate (**2**) based on their physical and spectroscopic properties. Their absolute configuration was determined by chemical correlation with (–)-supinidine (**3**) and (+)-viridifloric acid (**4**).

Keywords: *Chromolaena pulchella*, Asteraceae, Pyrrolizidine alkaloids, Absolute configuration, NMR spectroscopy.

Pyrrolizidine alkaloids (PAs), although widely distributed, are characteristic of certain genera of the Boraginaceae, Leguminosae, and Asteraceae/Compositae families [1]. Many PAs are known to produce hepatic toxicity and there are several records of livestock poisoning [1a,b]. Chemical studies of some *Chromolaena* species (Asteraceae) showed the presence of this class of natural molecules. Thus *N*-oxides of 7-angeloylretronecine, intermidine, licopsamine, echinatine, 3´-acetylrinderine, and supinine have been identified in *C. odorata* [1c]. The present work describes the isolation of the new PAs supinidine triviridiflorate (**1**) and supinidine diviridiflorate (**2**) (Figure 1) from the crude alkaloid extract of the roots of *C. pulchella*, for which we recently reported the isolation of labdanes and *ent*-clerodanes from the aerial parts [1d].



Compound **1**, a pale yellow oil, showed a $[M + 1]^+$ ion at *m/z* 572.3427 in its HRESI/APCI mass spectrum revealing the molecular formula $C_{29}H_{49}NO_{10}$ + H (calcd *m/z* 572.3435). The ^{1}H NMR chemical shift values of the alkaloid moiety were in agreement with those for a supinidine type pyrrolizidine ring system [2]. The complete ^{1}H and ^{13}C data, shown in Table 1, gave characteristic signals for a 1,2-unsaturated pyrrolizidine alkaloid with a necic acid esterified at C-9. The ^{1}H NMR spectrum of **1** showed signals assignable to a vinylic proton at δ_H 5.71 (H-2) and an AB system (J = 13.6 Hz) at δ_H 4.72 and 4.60 due to protons of the C-9 hydroxymethylene group, whereas the signal for the hydrogen atom attached to bridgehead C-8 was observed at δ_H 4.15. The signals for the methylene groups at C-3 and C-5, bearing the nitrogen atom, were observed at δ_H 3.91 and 3.37, and at 3.11 and 2.53, respectively. Moreover, signals for a trimeric α-isopropyl-α,β-dihydroxybutyric acid residue were observed as quartets at δ_H 5.35 (J = 6.2 Hz, H-13), 4.98 (J = 6.6 Hz, H-20), and 4.09 (J = 6.6 Hz, H-27) showing strong correlation in the COSY spectrum with the doublets at δ_H 1.39 (Me-14), 1.22 (Me-21), and 1.21 (Me-28), respectively. The ^{13}C and APT NMR spectra showed signals for three carbonyl carbon atoms at δ_C 174.0 (C-18), 173.6 (C-25), and 173.3 (C-11), for two vinylic carbons at 136.9 (C-1) and 126.1 (C-2), for three quaternary carbons bearing oxygen atoms at 82.6 (C-26), 81.7 (C-12), and 80.3 (C-19), for seven methine carbons, three of them bearing oxygen atoms at 76.4 (C-13), 71.5 (C-20) and 69.4 (C-27), one bearing the nitrogen atom at 71.5 (C-8), and three owing to the isopropyl groups at 33.8 (C-15), 32.7 (C-29) and 30.9 (C-22), for five methylene carbons, one of them bearing an oxygen atom at 61.9 (C-9), two bearing the nitrogen atom at 61.6 (C-3) and 56.6 (C-5), and two at 30.2 (C-7) and 25.7 (C-6), and for six methyl groups owing to three isopropyl groups at 17.3, 17.0, 16.9, 16.8, 16.6 and 15.3. Esterification at the C-9 position was confirmed by the HMBC correlation between H_2-9 and the carbonyl group C-11, while the individual assignments for the three acid residues were supported from HMBC correlations of H-13 with C-11 and C-12, of H-20 with C-18 and C-19, and of H-27 with C-25 and C-26.

Compound **2** exhibited in its HRESI/APCI mass spectrum a $[M + 1]^+$ ion at *m/z* 428.2646 in agreement with the molecular formula $C_{22}H_{37}NO_7$ + H (calcd *m/z* 428.2648). The complete ^{1}H and ^{13}C data shown in Table 1 demonstrated high structural similarities

Table 1: ^{13}C and ^{1}H NMR data for **1** and **2** (100 and 400 MHz, $CDCl_3$).[a]

	1		**2**	
Position	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)
1	136.9, C		136.9, C	
2	126.1, CH	5.71, br s	125.7, CH	5.73, br s
3	61.6, CH_2	3.91, br d (15.8)	61.2, CH_2	3.95, br d (15.8)
3'		3.37, dd (15.8, 4.4)		3.40, br d (15.8)
5	56.6, CH_2	3.11, dt (9.9, 5.5)	56.6, CH_2	3.18, dt (10.0, 5.1)
5'		2.53, dt (9.9, 7.0)		2.54, dt (10.0, 7.0
6	25.7, CH_2	1.80, m	25.7, CH_2	1.81, m
7	30.2, CH_2	1.98, m	29.9, CH_2	2.04, m
7'		1.50, m		1.54, m
8	71.5, CH	4.15, br m	71.5, CH	4.25, br m
9	61.9, CH_2	4.72, br d (13.6)	62.2, CH_2	4.80, br d (13.5)
9'		4.60, br d (13.6)		4.74, br d (13.5)
11	173.3, C		174.0, C	
12	81.7, C		81.2, C	
13	76.4, CH	5.35, q (6.2)	73.9, CH	5.26, q (6.2)
14	13.1, CH_3	1.39, d (6.2)	13.9, CH_3	1.32, d (6.2)
15	33.8, CH	1.98, sept (7.0)	32.6, CH	2.09, sept (7.0)
16	17.0, CH_3	1.05, d (7.0)	16.4, CH_3	0.94, d (7.0)
17	17.3, CH_3	0.96, d (7.0)	17.1, CH_3	0.96, d (7.0)
18	174.0, C		174.3, C	
19	80.3, C		82.4, C	
20	71.5, CH	4.98, q (6.6)	69.2, CH	3.95, q (6.6)
21	14.6, CH_3	1.22, d (6.6)	17.5, CH_3	1.17, d (6.6)
22	30.9, CH	2.09, sept (7.0)	32.3, CH	2.08, sept (7.0)
23	15.3, CH_3	0.86, d (7.0)	16.4, CH_3	0.92, d (7.0)
24	16.9, CH_3	0.74, d (7.0)	16.9, CH_3	0.87, d (7.0)
25	173.6, C			
26	82.6, C			
27	69.4, CH	4.09, q, (6.6)		
28	17.2, CH_3	1.21, d (6.6)		
29	32.7, CH	2.09, sept (7.0)		
30	16.8, CH_3	0.94, d (7.0)		
31	16.6, CH_3	1.00, d (7.0)		

[a]Assigned by gHMQC and gHMBC.

with the aforementioned PA **1**, implying that compounds **1** and **2** belong to the same class of alkaloids. Compound **2** differed from **1** in the absence of the third *α*-isopropyl-*α*,*β*-dihydroxybutyric acid residue, since in the ^{1}H NMR spectrum only two methine signals for protons bearing oxygen atoms were observed at δ_H 5.26 (q, *J* = 6.2 Hz, H-13), and 3.95 (q, *J* = 6.6 Hz, H-20), whereas in the ^{13}C NMR spectrum only two carbonyl groups signals were observed at δ_C 174.0 (C-11), and 174.3 (C-18). As in the case of compound **1**, 2D NMR spectroscopy was employed to completely assign the ^{13}C and ^{1}H spectra. Alkaline hydrolysis of a mixture of compounds **1** and **2** gave the necine base (–)-supinidine (**3**) [2,3a] and the necic acid residue (+)-viridifloric acid (**4**) [3b].

Experimental

General: Optical rotation, Perkin-Elmer 341 polarimeter; IR, Perkin-Elmer 16F PC IR-FT spectrophotometer using thin films of compounds deposited on a CsI crystal; Low-resolution MS, either Agilent 1100 LC/MSD or Varian Saturn 2000 spectrometers; HRMS, Agilent LCTOF instrument; NMR, JEOL Eclipse 400 spectrometer; CC, Merck silica gel 40; TLC, silica gel 60 precoated glass plates.

Plant material: Specimens of *C. pulchella* (H.B.K.) R.M. King & H. Rob. (Asteraceae) were collected near km 61 of Morelia-Zacapu federal road 15, in the municipality of Constitución, State of Michoacán, México, during October 2005. A specimen (No. 192522) is deposited at the Herbarium of Instituto de Ecología A. C., Centro Regional del Bajío, Pátzcuaro, Michoacán, Mexico, where Prof. Jerzy Rzedowski kindly identified the plant material.

Extraction and isolation: Air-dried and powdered roots of *C. pulchella* (870 g) were extracted with MeOH (3.5 L) under reflux for 6 h. Filtration and evaporation of the extract afforded a yellow viscous oil (38.6 g) which gave a positive Dragendorff test. To this product aq. HCl 2% (300 mL) and zinc powder (40 g) were gradually added under stirring for 12 h. The acidic aqueous solution was treated with aq. KOH 5% to obtain a phase with pH 10 and then extracted with $CHCl_3$ (3 × 200 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated to provide 2.6 g of residue. A portion of this (876 mg) was subjected to chromatography on silica gel 60 (20 g) using $CHCl_3$–MeOH–diethylamine (98:2:1, 96:4:1, 90:10:1, and 80:20:1). Fractions of 100 mL of each polarity were collected, monitored by TLC, and analyzed by ^{1}H NMR spectroscopy. The resulting material from each fraction was labelled as A (138 mg), B (354 mg), C (112 mg) and D (116 mg). From fractions A, C, and D fatty materials were isolated. Separation of fraction B (178 mg) by means of preparative TLC using $CHCl_3$–MeOH (9:1) as the mobile phase gave pure **1** (45 mg, R_f 0.5). Another portion of fraction B (138 mg) was purified by TLC ($CHCl_3$–MeOH–H_2O, 200:50:7) affording **2** (20 mg, R_f 0.2).

Supinidine triviridiflorate (1)

Pale yellow oil; $[\alpha]_D^{20}$: –1.5 (*c* 3.5, $CHCl_3$)
IR (film): 3518, 2972, 2937, 2878, 1725, 1454, 1386 cm^{-1}.
^{1}H and ^{13}C NMR: Table 1.
MS (EI, 70 eV): *m/z* (%) 572 $[M + 1]^+$ (40), 428 (2), 410 (4), 284 (3), 266 (19), 140 (19), 122 (100), 110 (10), 107 (6), 94 (25), 70 (38); HRESI/APCIMS: *m/z* $[M + 1]^+$ calcd for $C_{29}H_{49}NO_{10}$ + H: 572.3435; found: 572.3427.

Supinidine diviridiflorate (2)

Pale yellow oil; $[\alpha]_D^{20}$: –8.2 (*c* 2.0, $CHCl_3$).
IR (film): 3024, 2970, 2945, 1727, 1456, 1389 cm^{-1}.
^{1}H and ^{13}C NMR: Table 1.
MS (EI, 70 eV): *m/z* (%) = 428 $[M + 1]^+$ (2), 396 (2), 382 (2), 284 (28), 224 (6), 140 (19), 122 (100); HRESI/APCIMS: *m/z* $[M + 1]^+$ calcd for $C_{22}H_{37}NO_7$ + H: 428.2648; found: 428.2646.

Hydrolysis of compounds 1 and 2: A mixture of **1** and **2** (40 mg) in MeOH (3 mL) was treated with NaOH (24 mg) in H_2O (0.25 mL) and heated to reflux for 15 min in a micro-wave system working at 100 W. The mixture was treated with H_2O (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL) to give (–)-supinidine (**3**) (3.5 mg) as a yellow oil, which showed $[\alpha]_D$ –9.1 (*c* 0.16, EtOH) lit. $[\alpha]_D$ –10.4 (*c* 2.64, EtOH) [3a]. The aq. phase was acidified with 2% HCl and extracted with CH_2Cl_2 (2 × 10 mL) to give (+)-viridifloric acid (**4**) (24 mg) [3b,4].

Acknowledgments - Partial financial support from Conacyt, Mexico (grant No. U2-80555), from ICYTDF (grant No. 337/2010), and PROMEP-UMSNH (grant No. 332) is acknowledged.

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