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J. Nat. Prod., **1992**, 55 (12), 1764-1767• DOI: 10.1021/np50090a008 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 12, pp. 1764-1767, December 1992

PYRROLIZIDINE AND SECOPYRROLIZIDINE ALKALOIDS FROM *SENECIO RACEMOSUS*

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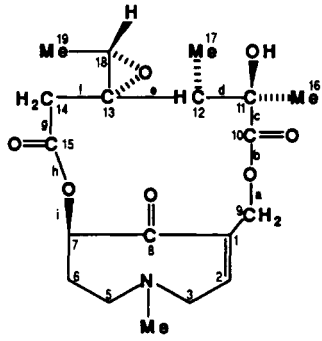
ABSTRACT.—Studies of the alkaloidal constituents of *Senecio racemosus* have resulted in the isolation of sarracine and 9-angelylplatynecine, along with a new secopyrrolizidine base, senecioracenine [**1**]. The structure **1** has been assigned on the basis of spectral studies including 2D nmr.

Senecio racemosus DC (Compositae) is widely distributed in the temperate regions and the hills of the tropics. The medicinal importance of the pyrrolizidine alkaloids of the *Senecio* species (1–5) has prompted us to study the alkaloidal constituents of this species; a literature survey showed that only one pyrrolizidine alkaloid has so far been reported from this plant (6). As a result of our investigation on the fresh and undried plant material of Turkish origin, we have isolated, besides sarracine and 9-angelylplatynecine, a new secopyrrolizidine base named senecioracenine, to which the structure **1** has been assigned on the basis of extensive ms and nmr studies including homonuclear 2D ^{1}H-nmr (COSY-45°, *J*-resolved), HMQC, ^{13}C-nmr, and DEPT experiments.

Senecioracenine [**1**] gave a positive reaction with Dragendorff's reagent. The molecular ion peak at *m/z* 381.1775 (hrms) corresponded to a molecular formula $C_{19}H_{27}NO_7$ (calcd 381.1787). The ir spectrum showed the presence of diester functions (1740 cm^{-1}, 1720 cm^{-1}), a transannular carbonyl function (1600 cm^{-1}) (7,8), and a hydroxyl group (3500 cm^{-1}).

The ms fragmentation pattern of **1** was characteristic of secopyrrolizidine alkaloids very similar to otosenine [**2**] (9,10). The diagnostic fragments at *m/z* 364.1560 ($C_{19}H_{26}NO_6$) and 366.1755 ($C_{18}H_{24}NO_7$) resulted from the losses of tertiary hydroxyl and N-Me groups, respectively (11). The macrocyclic diester function was revealed by the characteristic loss of CO_2 (9,11,12) from the molecular ion peak giving a fragment at *m/z* 337.1877 ($C_{18}H_{27}NO_5$). The peak at *m/z* 168.0993 ($C_9H_{14}NO_4$) was likely formed by cleavage of diester bonds to give the free secopyrrolizidine system. Further peaks at *m/z* 151.1002



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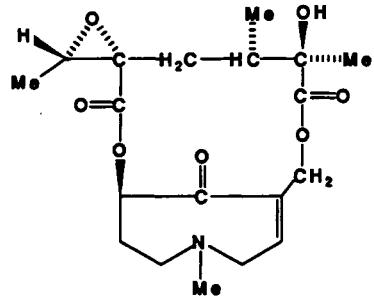

2

($C_9H_{13}NO$), 150.0730 ($C_9H_{12}NO$), 149.1320 ($C_9H_{11}NO$), 123.1143 (C_7H_9NO), 122.1003 (C_7H_8NO), 110.0602 (C_6H_8NO), 96.0925 (C_5H_6NO), and 94.0648 (C_5H_4NO) were common to the secopyrrolizidine base otosenine [**2**] (7,9,11,12), reflecting a similarity in the basic necine system of the two alkaloids.

Senecioracenine [**1**] thus differs from otosenine [**2**] (9,10) in having a different macrocyclic ring. This was also revealed by the ^{1}H-nmr spectrum of **1**, which showed a singlet at δ 2.3 (3H) due to an *N*-Me group while another 3H singlet was observed at δ 1.33. A pair of double doublets (1H each) at δ 5.44 and 4.23 (J = 11.4 Hz) were due to non-equivalent methylene protons and showed cross peaks with each other in the COSY-45° spectrum. These could be assigned to the C-9 of necines bearing a macrocyclic diester (7,9,10,12,14,15). Other proton resonances at δ 3.60 and 3.75 (1H each, distorted doublets), δ 3.00 and 3.24 (1H each, multiplets) were due to methylenes vicinal to nitrogen. Further confirmation was provided by the COSY-45° spectrum. The signals at δ 3.60 and 3.75 showed cross peaks with the olefinic proton at δ 6.16 and hence could be assigned to protons of C-3. The protons at δ 3.00 and 3.24 showed connectivities to the signals at δ 2.20 (1H, m) and 2.70 (1H, m), whose chemical shifts were characteristic of protons at the C-6 of secopyrrolizidine alkaloids (7,10,12), and allowed us to identify the C-5 protons.

The most downfield distorted triplet, integrating for one proton, at δ 6.16 confirmed 1,2-unsaturation in the molecule (7,11,12). Another methine resonance at δ 4.88 (broad singlet) was assigned at C-7 carrying an ester function (7,10,12,14,15). The signals due to the macrocyclic ring were very similar to otosenine (10,11) or petasitenine (14), particularly the chemical shift of the methine proton of the epoxide ring at δ 3.05 (q, J = 5.8 Hz) and the doublet of the Me group attached to the epoxide carbon (δ 1.22, J = 5.8 Hz). The only notable difference was the signal of the methylene group forming an AB quartet with geminal coupling of 12 Hz rather than a complex multiplet observed for otosenine (10,11).

Further structural evidence was provided by the ms fragmentation of the macrocyclic ring of **1**. The peaks resulting from the cleavages at the ad and ae bonds at *m/z* 294.1705 ($C_{16}H_{23}NO_4$) and 266.1379 ($C_{14}H_{19}NO_4$), respectively, were common in composition to otosenine [**2**], confirming this part of the structure (9). The similarity was further confirmed by the existence of nOe interactions between the Me protons at C-11 and C-12 indicating their cis disposition. Moreover, the chemical shifts of C-11 and C-12 as well as the chemical shifts and coupling constants of H-12 showed very close agreement to

TABLE 1. HMQC Spectrum of Compound **1**.[a]

Position	^{13}C nmr	^{1}H nmr
1	134.57	—
2	136.22	6.16
3	61.45	3.60, 3.75
5	57.54	3.00, 3.24
6	35.69	2.20, 2.70
7	80.81	4.88
8	191.40	—
9	63.67	4.23, 5.44
10	177.79	—
11	78.41	—
12	39.88	1.90
13	65.38	—
14	39.77	2.16, 1.10
15	169.95	—
16	24.33	1.33
17	13.87	1.07
18	57.48	3.05 [2.95[b], 2.97[c], 2.98[d]]
19	12.71	1.22 [1.24[b,d], 1.27[c]]
20	42.79	2.30

[a]In $CDCl_3$; chemical shifts are in ppm with reference to TMS as internal standard.
[b]Values from Asada and Furuya (12).
[c]Values from Liu and Röeder (13).
[d]Values from Cava *et al.* (11).

TABLE 2. Comparison of Ms Fragmentation Patterns of Otosenine [**2**] and **1**.

Type of cleavage	Compound	
	2 *m/z*	**1** hrms *m/z* [calcd]
ac	337 ($C_{18}H_{27}NO_5$)	337.1877 [337.1889] ($C_{18}H_{27}NO_5$)
ad	294 ($C_{16}H_{23}NO_4$)	294.1705 [294.17051] ($C_{16}H_{23}NO_4$)
ae	266 ($C_{14}H_{20}NO_4$)	266.1379 [266.13921] ($C_{14}H_{20}NO_4$)
af	250 ($C_{13}H_{16}NO_4$)	182.1179 [182.11808] ($C_{10}H_{16}NO_2$)
id	238 ($C_{12}H_{16}NO_4$) − H	238.1058 [238.0791] ($C_{12}H_{16}NO_4$) − H
bc	353 ($C_{18}H_{27}NO_6$)	353.1830 [353.18381] ($C_{18}H_{27}NO_6$)
ai	150 ($C_9H_{12}NO$)	150.0730 [150.09187] ($C_9H_{12}NO$)
ah	168 ($C_9H_{14}NO_2$)	168.0993 [168.10243] ($C_9H_{14}NO_2$)

those of otosenine [**2**], allowing us to assign the same relative configuration at C-11 and C-12 in compound **1** as in **2** (13). On the other hand, cleavage at the af bond gave a peak at *m/z* 182.0737 ($C_{10}H_{16}NO_2$). Similar cleavage in otosenine gives the corresponding peak at *m/z* 250.1085 ($C_{13}H_{16}NO_4$) (9) allowing us to assign the epoxide ring to C-13, rather than C-14 as in otosenine, which was also confirmed by the multiplicity of the adjacent methylene protons.

The remaining problem was to ascertain the orientation of the epoxide ring in **1**. By comparing ^{1}H- and ^{13}C-nmr chemical shifts of the methine proton of the epoxide ring and the Me group attached to it with the reported data (11–13), an α orientation has been assigned to it, and the stereostructure of **1** is, therefore, as presented.

Evidence confirming the assignments of structure **1** was provided by the ^{13}C-nmr spectrum, HMQC, and homodecoupling experiments. Selective irradiation either at δ 2.16 or 1.10 changed the doublet of the other into a singlet, without affecting any other proton resonance. Similarly, irradiation at δ 3.05 (H-18) caused simplification of the doublet (J = 5.8 Hz) at δ 1.22 (H-19) into a singlet, while a singlet was observed instead of a quartet (J = 6.4 Hz) at δ 1.9 (H-12) when the resonance at δ 1.07 (H-17) was irradiated.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded on a JASCO A-302 spectrophotometer. The hrms were recorded on a Finnigan MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The nmr spectra were recorded on a Bruker AM-300 spectrometer with TMS as the internal reference. Tlc experiments were performed on Si gel (GF-254, 0.2 mm) cards (Riedel-De Haen), and flash chromatography was carried out on Si gel (230-400 mesh, E. Merck). The 2D COSY-45° experiment was acquired at 300 MHz with a sweep width of 4000 Hz (2K data points in ω_2) and 2000 Hz (256 t_1 values zero-filled to 1K) in ω_1. The heteronuclear 2D ^{1}H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12,820 Hz (2K data points in ω_2) and 1024 Hz (256 t_1 values zero-filled to 1K) in ω_1. In both the 2D experiments a 2-sec relaxation delay was used and 16 transients were performed for each t_1 value. In HMQC experiments interpulse delays optimized for a $^1J_{CH}$ of 0.0037 Hz [D2 = (1/2J) × H, J = 135 Hz].

EXTRACTION AND ISOLATION OF ALKALOIDS.—*S. racemosus* was procured from the suburbs of Ankara and identified by Prof. Dr. Bilge Sener, Department of Pharmacognosy, Faculty of Pharmacy. A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey.

The crude MeOH extract (1.5 kg) from the fresh and undried plant material (45 kg) was partitioned between H_2O (2 liters) and hexane (10 liters). The aqueous layer, which gave a positive Dragendorff's test, was basified with 20% aqueous NH_3 solution (pH = 8.0), and the liberated crude alkaloids were extracted with $CHCl_3$. Evaporation of the $CHCl_3$ extract gave a gummy residue (2 g), which was fractioned by cc over Si gel (180 g, 70–230 mesh, Merck) using C_6H_{14}-$CHCl_3$ (1:1) to give six fraction, followed by

$CHCl_3$-MeOH (9:1) to yield ten fractions, $CHCl_3$-MeOH (8.5:1.5) to give twelve fractions, and finally $CHCl_3$-MeOH (7:3) to afford fourteen fractions. From tlc of each fraction, compound **1** was found to be in the fractions using $CHCl_3$-MeOH (7:3). These fractions were further purified by flash chromatography over Si gel (230–400 mesh, Merck) using $CHCl_3$-MeOH (8:2) as eluent, thus obtaining compound **1** (20 mg, gummy solid). The column fraction eluted in $CHCl_3$-MeOH (8.5:1.5) contained one major and two minor alkaloids. To purify the major compound this fraction was subjected to mplc (Si gel, 230–400 mesh, Merck), and eluted in $CHCl_3$-MeOH (9:1) to yield an almost pure compound with lingering traces of impurities. It was finally purified on tlc cards using $CHCl_3$-MeOH (9.1:0.9) to yield compound **2** (25 mg, colorless crystals from EtOH). The eluents obtained in $CHCl_3$-MeOH (9:1) were combined and chromatographed over Si gel (230–400 mesh, Merck) using a Buchner column and $CHCl_3$-MeOH (9.3:0.7) as eluent. Final purification was achieved on tlc cards using $CHCl_3$-MeOH (9.5:0.5) to afford 9-angelylplatynecine (15 mg, colorless oil).

Senecioracenine [**1**].—Oil, $[\alpha]_D$ +50° (c = 0.2 in $CHCl_3$); ir ($CHCl_3$) ν max 3500, 1740, 1720, 1600 cm^{-1}; hreims *m/z* 381.1775 ($C_{19}H_{27}NO_7$ requires 381.1787) (0.5); eims *m/z* (% rel int) 366.1755 ($C_{18}H_{24}NO_7$) (1), 364.1560 ($C_{19}H_{26}NO_6$) (5), 337.1877 ($C_{18}H_{27}NO_5$) (2.2), 182.0737 ($C_{10}H_{16}NO_2$) (15), 168.0993 ($C_9H_{14}NO_2$) (78), 151.1002 ($C_9H_{13}NO$) (100), 150.0730 ($C_9H_{12}NO$) (36), 149.1320 ($C_9H_{11}NO$) (8), 123.1143 (C_7H_9NO) (94), 122.1003 (C_7H_8NO) (70), 110.0602 (C_6H_8NO) (80), 96.0925 (C_5H_6NO) (90), 94.0648 (C_5H_4NO) (54); ^1H nmr ($CDCl_3$, 300 MHz, δ ppm) 6.16 (1H, distorted triplet, H-2), 3.60, 3.75 (2H, distorted doublet, 1 H each, H_2-3), 3.00, 3.24 (2H, m, 1H each, H_2-5), 2.20, 2.70 (2H, m, 1H each, H_2-6), 4.88 (1H, distorted br s, H-7), 5.44 (1H, d, J = 11.4 Hz, H-9), 4.23 (1H, d, J = 11.4 Hz, H-9), 3.05 (1H, q, J = 5.8 Hz, H-18), 2.16 (1H, d, J = 12 Hz, H-14), 1.10 (1H, d, J = 12 Hz, H-14), 1.33 (3H, s, H-16), 1.22 (3H, d, J = 5.8 Hz, H-19), 1.07 (3H, d, J = 6 Hz, H-17), 1.90 (1H, q, J = 6.4 Hz, H-12); ^{13}C nmr see Table 1. The assignments were made by comparison with published ^{13}C- and ^1H-nmr data of related bases (10, 12, 14, 15) and confirmed in each case by ^1H-^{13}C correlated spectroscopy (HMQC).

Sarracine [**2**].—Crystals from EtOH: mp 45–47°; $[\alpha]_D$ −120.5° (EtOH); hreims *m/z* 337.1898 ($C_{18}H_{27}NO_5$ calcd 337.1889) (5); eims *m/z* (% rel int) 237 (20), 222 (15), 139 (30), 138 (90), 122 (36), 96 (30), 95 (40), 83 (39), 82 (100). The physical and spectral data coincided with reported data for sarracine (16–18).

9-Angelylplatynecine.—Oil: $[\alpha]_D$ −75° (EtOH); hreims *m/z* 239.1530 ($C_{13}H_{21}NO_3$ calcd 239.1521) (2); eims *m/z* (% rel int) 224 (2.5), 221 (15), 156 (25), 139 (20), 113 (30), 99 (36), 96 (45), 95 (60), 83 (55), 82 (100). The physical and spectral data of this compound identified it as 9-angelylplatynecine (16, 19).

ACKNOWLEDGMENTS

The authors thank the Ghee Corporation of Pakistan and Abbott Laboratories, Karachi, for financial support.

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Received 10 March 1992