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USPALLATINE, A PYRROLIZIDINE ALKALOID FROM *SENECIO USPALLATENSIS*

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Key Word Index—*Senecio uspallatensis*; Compositae; pyrrolizidine alkaloids; uspallatine.

Abstract—A new pyrrolizidine alkaloid uspallatine was isolated from roots of *Senecio uspallatensis* together with the previously known retrorsine. The structure of the new alkaloid was established by spectroscopical data and chemical transformation.

INTRODUCTION

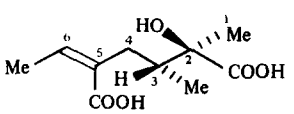
As part of our chemical systematic study of the genus *Senecio* (Senecioneae: Compositae) which grows in the western region of Argentina, we have investigated the roots and aerial parts of *S. uspallatensis* with regard to its alkaloid content. *Senecio uspallatensis*, commonly known as 'chachacoma', is a common plant in the mediun Andes cordillera and is particularly frequent in Paramillos de Uspallata. Its infusion is drunk by the villagers instead of mate [1]. From this species a new macrocyclic pyrrolizidine diester alkaloid, which was named uspallatine, was isolated together with the previously known retrorsine. Uspallatine is a diester of a new amino alcohol, 6α,7β-dihydroxy-1-hydroxymethyl-1,2-dehydro-8α-pyrrolizidine (**1**), hereafter called uspallatinecine.

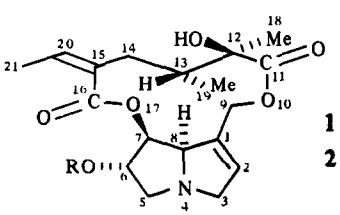
Up to now three pyrrolizidine triols have been reported. Eight esters of crotanecine (unsaturated triol pyrrolizidine necine) have been characterized in *Crotalaria* sp. [2, 3]. Croalbinecine (saturated pyrrolizidinetriol necine) was isolated as the base constituent of croalbidine from *C. albida* [4]. Rosmarinecine, a stereoisomer of croalbinecine, has been isolated from *S. angulatus* as a constituent of rosmarinecine and angularine [4]. Thus, this is the first report of the occurrence of unsaturated necine triols in *Senecio*.

RESULTS AND DISCUSSION

The empirical formula of uspallatine (**1**), is $C_{18}H_{25}NO_6$ (from the high resolution mass spectrum). The peaks at *m/z* 307, 154, 152, 137, 136, 135, 95 and 94 indicated that it is an alkaloid of the pyrrolizidine type with an unsaturated triol base, esterified by a C-10 dicarboxylic acid forming a macro ring. Several of these peaks are prominent also in the spectra of anacrotine and madurensine [2].

Important information regarding its structure was obtained from NMR analysis. The ^{1}H NMR data are given in Table 1 and that for ^{13}C NMR in Table 2. The ^{1}H NMR showed signals appropriate to senecic acid or its diastereoisomers (*cis*-grouping CH_3–CH=C [3], CH_3–C–OH, CH_3–CH) and also exhibited the character-





1 R = H
2 R = CO – Me
22 23

Table 1. ^{1}H NMR spectrum of uspallatine (60 MHz, deuterochloroform)

Carbon containing proton	Shift of proton 1	Shift of proton 2
2	6.13, 1H, *br s*	6.21, 1H, *br s*
5d	3.23, 1H, *dd*, $J \simeq 10$, 2 Hz	—
5u	2.66, 1H, *dd*, $J \simeq 10$, 4 Hz	3.00, 1H, *dd*, $J \simeq 10$, 4 Hz
6	4.56, 1H, *br d*, $J \simeq 4$ Hz	5.46, 1H, *br d*, $J \simeq 4$ Hz
7	4.83, 1H, *dd*, $J \simeq 4$, 2 Hz	5.06, 1H, *br s*
8	3.91, 1H, *br s*	—
9d	5.47, 1H, *d*, $J \simeq 11$ Hz	5.47, 1H, *d*, $J \simeq 11$ Hz
9u	4.07, 1H, *br d*, $J \simeq 11$ Hz	—
18	1.33, 3H, *s*	1.33, 3H, *s*
19	0.91, 3H, *d*, $J \simeq 6$ Hz	0.91, 3H, *d*, $J \simeq 6$ Hz
20	5.73, 1H, *q*, $J \simeq 7$ Hz	5.83, 1H, *q*, $J \simeq 7$ Hz
21	1.83, 3H, *d*, $J \simeq 7$ Hz	1.85, 3H, *d*, $J \simeq 7$ Hz
23	—	2.18, 3H, *s*

Table 2. ^{13}C NMR spectrum of uspallatine (20 MHz, deuterochloroform)

Carbon No.	Shift 1	Shift 2
1	131	130.7
2	135.9	136.4
3	62.4	62.5
5	60.3	57.9
6	78.6	78.1
7	76.7	75.6
8	74.6	75.2
9	60.0	60.2
11	177.8	177.8
12	76.6	76.4
13	38.2	38.2
14	37.9	37.9
15	132.4	132.2
16	166.9	169.5
18	24.8	24.8
19	10.8	10.9
20	134.7	135.2
21	14.9	14.9
22	—	166.7
23	—	20.8

istic AB pattern due to the H-9 protons of macrocyclic diesters [3]. The most downfield broad singlet at δ6.13 was ascribed to an olefinic proton in the pyrrolizidine nucleus. Irradiation of this signal sharpened the H-9d broad doublet. The small allylic coupling constant suggested a double bond between C-1 and C-2. The ^{1}H NMR showed two additional signals downfield, a broad double-doublet attributable to a proton geminal to an acyl group and a broadened doublet at δ4.56 due to a carbinol methyne proton. The presence of a secondary hydroxyl group was confirmed by acetylation to give a monoacetate, **2**. The ^{1}H NMR spectrum of **2** showed a typical acylation shift on the signal at δ4.56 ($\Delta\delta$0.90). Spin decoupling showed that the broadened doublet at δ5.46 was coupled with the neighbouring methylene protons at C-5, thus supporting the presence of the hydroxyl group being at C-6, which was in good agreement with the downfield shift upon acetylation of the vicinal H-7 ($\Delta\delta$0.23) [5]. This conclusion was further supported by the fragmentation data in the high resolution mass spectrum of uspallatine [2] and by the ^{13}C NMR spectrum [6]. From the above data, the structure of **1** was similar to that of anacrotine isolated previously from *Crotalaria anagyroides* [2].

The ^{13}C NMR chemical shifts of **1** were found to closely resemble those of anacrotine [6]; however, C-5–C-7 were shifted upfield (C-5: δ60.3 → δ58.48; C-6: δ78.6 → δ74.77 and C-7: δ76.7 → δ75.49), and C-3 and C-16 were shifted downfield (C-3: δ62.4 → δ63.57 and C-16: δ166.4 → δ169.58), suggesting a stereoisomeric change. The ^{1}H NMR spectrum was also similar to that of anacrotine [2] except for the chemical shift of the H-7 signals and for the form of the multiplet due to H-5u [3]. The H-7 was shifted upfield (H-7: δ5.24 → 4.83), and the H-5u signal appeared as a double-doublet (J = 10 and 4 Hz) at δ2.66; the geminal J value (10 Hz) was more consistent with an *exo* buckled anacrotine type but not with an *endo* buckled madurensine type structure [2, 3].

The relative configuration at C-6 was deduced from vicinal $J_{5,6}$ values ($J_{5\beta,6}$ = 4 Hz, $J_{5\alpha,6} \approx$ 2 Hz). These only were consistent with an α-orientation of the hydroxyl group at C-6. The downshift of H-7 on acetylation ($\Delta\delta$0.23) due to the acyl effect [5] provides further support to the 'syn' relationship between OH-6 and H-7.

Alkaline hydrolysis gave senecic acid (**3**), identified by its mp, $[\alpha]_D^{20}$, ^{1}H NMR and mass spectrum, and uspallatinecine (**4**), identified by ^{1}H NMR and mass spectrometry.

Lithium aluminium hydride reduction of the tosylate of **1**, obtained as described in refs [7, 8], gave the known amino alcohol retronecine, thus establishing the relative stereochemistry of H-7 and H-8.

EXPERIMENTAL

S. uspallatensis Hook et Arn was collected in April 1984 in Villavicencio (lendoza) by José A. Ambrosetti and Luis A. Del Vitto (Merl No. 32.489, Ladiza, Mendoza). Milled plant material (roots, 3800 g) was extracted with MeOH. The extract was

processed in the usual way for alkaloid extraction [9], obtaining 4.6 g (0.12%) of crude alkaloid. The crude alkaloid extract was chromatographed on a column (0.35 × 20 cm) of silica gel 60 HF_{254} (75 g) Fractions (25 ml) were collected. The solvent system, $CHCl_3$–MeOH–NH_3 (85:14:1), was used According to the results of TLC, fractions (13–18) (0.082 g) were combined, the same as fractions 20–27 (0.350 g)

From the first fraction group retrorsine was identified by comparison with an authentic sample. Fractions 20–27 were purified by repeated chromatography on silica gel 60 HF_{254} and Sephadex LH-20 to give 0.3 g uspallatine.

Uspallatine, **1** Mp 205–207° (MeOH–$CHCl_3$), $[\alpha]_D^{20}$ +4.11° (MeOH; *c* 0.0764). Calc for $C_{18}H_{25}NO_6$: M_r 351.1682 Found: M_r (MS) 351.1624

Hydrolysis of **1** Compound **1** (0.1 g) was refluxed with $Ba(OH)_2$ in H_2O (0.5 g, 5 ml) for 3 hr. After cooling, the soln was satd with CO_2 and the $BaCO_3$ removed by filtration. The soln was acidified to Congo Red with HCl and extracted with Et_2O. The combined Et_2O extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo* to yield *ca* 0.045 g crystalline acid

Senecic acid, **3**. Mp 145–146° (EtOAc–petrol), $[\alpha]_D^{20}$ +12.7° (EtOH, *c* 0.0834) Calc. for $C_{10}H_{16}O_5$. M_r 216.2349. Found. M_r (MS) 216.2332. ^{1}H NMR (60 MHz, DMSO): δ0.73 (3H, *d*, *J* = 6 Hz, Me-3), 1.16 (3H, *s*, H-1), 1.83 (3H, *d*, *J* = 7 Hz, Me-6), 5.80 (1H, *q*, *J* = 7 Hz, H-6). Other significant peaks in the MS were, 70 eV, *m/z* (rel. int.): δ198 $[M-18]^+$ (30.0), 180 (12.1), 154 (66.6), 153 (54.6), 123 (100), 111 (24 2), 109 (33.1), 83 (64.2), 81 (65.1).

After extraction of the acid component, the hydrolysis soln was made basic with NaOH, extracted with Et_2O and filtered. The Et_2O extract was evapd to dryness to yield 0.024 g of base.

Uspallatinecine. Calc for $C_8H_{13}NO_3$· M_r 171.0913. Found: M_r (MS) 171.0903 ^{1}H NMR (60 MHz, DMSO): δ2.96 (1H, *dd*, *J* ≈ 10, 4 Hz, H-5u), 3.33 (1H, *dd*, *J* ≈ 10, 2 Hz, H-5d), 6.16 (1H, *br s*, H-2), 4.08 (5H, *br s*, H-6, H-7, H-8, H-9u and H-9d)

Uspallatine acetate, **2** Colourless oil (MeOH–$CHCl_3$)

$$[\alpha]_D^{20} = \frac{+2.8 \quad +2.87 \quad +3.32 \quad +5.97 \quad +6.12}{584 \quad 578 \quad 546 \quad 436 \quad 365} \text{ (MeOH; } c \text{ 0.0407)}$$

Calc for $C_{20}H_{27}NO_7$. M_r 395.4498. Found: M_r (MS) 395.4479. Other significant peaks in the MS were, 70 eV. *m/z* (rel. int.): δ349 $[M-44]^+$ (5.0), 334 (9.6), 306 (16.4), 278 (30.1), 197 (13.7), 196 (28.5), 195 (16.4), 194 (65.4), 178 (30.10), 177 (30.2), 153 (45.2), 137 (15.0), 136 (28.7), 135 (24.6), 134 (20.5), 133 (34.2), 120 (47.9), 119 (23.3), 118 (100), 117 (30.1), 115 (26.0), 94 (46.6), 93 (42.5), 81 (19.2), 80 (50.7).

Retronecine from **1**. Compound **1** (0.1 g) in C_5H_5N (2 ml) was treated with a soln of TsCl (0.100 g) in C_5H_5N (2 ml) [7] to give the Ts derivative of **1** which was immediately treated with a soln of $LiAlH_4$ in Et_2O (2 ml, 1.6 M) and worked-up as in ref. [8]. The residue was chromatographed over basic Al_2O_3 and deactivated with EtOAc to give a product (0.03 g). The product was crystallized from MeOH. The product was identical with retronecine.

Retronecine. Mp 114.3–115.1° (MeOH), $[\alpha]_D^{20}$ +50.3° (EtOH; *c* 0.0517) Calc for $C_8H_{13}NO_2$ M_r 155.1935. Found: M_r (MS) 155.1923. Other significant peaks in the MS were, 70 eV, *m/z* (rel. int.): δ111 (77.6), 95 (14.1), 94 (24.7), 93 (10.6), 81 (18.8), 80 (100), 73 (29.4).

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