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TROPANE ALKALOIDS FROM *SCHIZANTHUS LITORALIS*

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Key Word Index—*Schizanthus litoralis*; Solanaceae; leaves; tropane alkaloids.

Abstract—Six new tropane alkaloids have been isolated from the leaves of the endemic Chilian plant *Schizanthus litoralis* (Solanaceae). The new structures include a new tropanol dimer of itaconic acid together with 6β-senecioyloxytropan-3α-methylmesaconate, 6β-cinnamoyloxytropan-3α-methylmesaconate, 6β-senecioyloxytropan-3α-ol and *cis–trans N*-(4-hydroxyphenethyl) ferulamides. Other known alkaloids were also isolated. Their structures were elucidated by spectroscopic methods. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

Schizanthus litoralis belongs to the tribe Salpiglossidae native to Chile. The plant grows up to 1 m high and possesses doubly pinnatifid leaves and large butterfly-like blue blossom [1, 2]. Previous chemical work on *Schizanthus* has shown that this genus accumulates a number of tropane-derived alkaloids, including hydroxytropane esters, hygrolines, dimeric tropane diesters of mesaconic and itaconic acids, cyclobutane tricarboxylic triesters and pyrrolidine alkaloids [3–6].

In this communication, we wish to report the isolation and characterization of new tropane alkaloids from *S. litoralis*, including the dimeric tropane diester of itaconic acid (**1**). The new alkaloids, 6β-senecioyloxytropan-3α-methylmesaconate (**2**), 6β-cinnamoyloxytropan-3α-methylmesaconate (**3**), 6β-senecioyloxytropan-3α-ol(**4**) and *cis-trans N*-(4-hydroxyphenethyl) ferulamides (**5A**, **5B**), together with 3α-senecioyloxytropan-6β-ol, (−)-hygroline and (+)-pseudohygroline, already reported from *S. pinnatus* [7] and *S. hookeri* [8], were also isolated from this species.

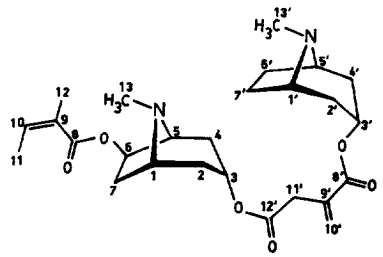
RESULTS AND DISCUSSION

The ^{1}H NMR data of compounds **1**–**4** are presented in Table 1. The data of diagnostic value are (a) the chemical shifts and multiplicities of the olefin proton signals of the corresponding acid or diacid, (b) the position and multiplicity of the skeletal proton resonances at the point of attachment of the ester moiety to the tropane nucleus and (c) the number of protons corresponding to each signal in the spectrum [3].

Thus, in **1** the signals for H-10′ at δ 5.78, 6.33 (1H each) and for H-11′ 3.36 (2H) indicated the presence of itaconic acid, whereas the multiplet centred at δ 6.04 (H-10) showed the presence of one angelic acid residue. On the other hand, two triplets (H-3 and H-3′ respectively) at δ 5.04 (J = 5.0 Hz) and 5.07 (J = 5.3 Hz) (1H each) showed that both tropane units are present in **1** ($C_{26}H_{38}N_2O_6$) and are linked to a C-3 α ester group, whereas the attachment of the third ester residue at C-6 was evident from its characteristic multiplet (*dd*) at δ 5.45 (1H, J = 7.5, 3.2 Hz). These assignments were corroborated by additional resonances for two *N*-Me groups at δ 2.28 and 2.48 and the Me-11, 12 of angelic acid (Table 1) at δ 1.98 (3H, *d*, J = 7.2 Hz) and 1.86 (3H, *d*, J = 16 Hz), respectively.

The ester linkages to the tropane residues in **1** were determined by correlation of the skeletal and acyl protons to the corresponding ester carbonyl groups in 2D long-range ^{13}C–^{1}H chemical shift correlation experiments (Table 2).

The EI-mass spectrum of **1** showed the well established fragmentation pattern of 3,6-diacyloxytropane esters [4] and the relative intensities of the ions at m/z 238, 222, 138 and 122 agree with the proposed structure: HR-mass spectrometry gave m/z 474.2769



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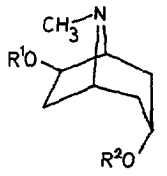
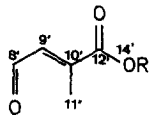
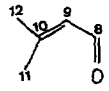
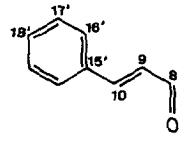


	R^1	R^2
2	Senecioyl	Methylmesaconate
3	Cinnamoyl	Methylmesaconate
4	Senecioyl	H



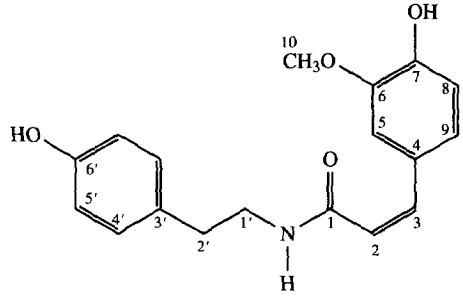
R = CH_3



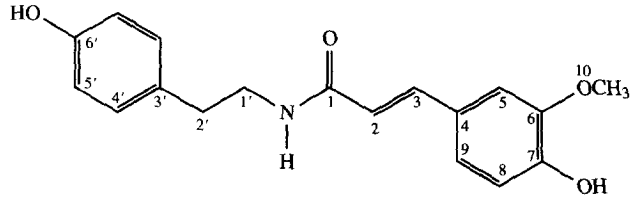


$[M]^+$ (calc. for $C_{26}H_{38}N_2O_6$, 474.2431). The assignment of the ^{13}C NMR spectrum of **1** was based upon C–H correlation experiments and comparison with values for related alkaloids reported in the literature [4, 5, 9] (Table 3).

The bases **2** and **3** were 3,6-diacyloxytropanes, as inferred from the characteristic ^1H NMR signals assigned to H-3 and H-6, [3, 10] at δ 5.13 (*t*, $J = 5.0$ Hz), 5.10 (*t*, $J = 5.0$ Hz), and 5.45 (*dd*, $J = 7.5$, 3.3 Hz), 5.55 (*dd*, $J = 7.5$, 3.4 Hz), respectively, and the typical mass spectral fragments at *m/z* 96, 95, 94, 82 and 81. The nature of the esters was also evident from their characteristic signals in ^1H NMR spectra; for **2**, δ 6.75–6.80 (1H, *qq*, $J = 1.6$ Hz, H-9′) and 2.30–2.31; (3H, *d*, $J = 1.6$ Hz, H-11′) for mesaconic esters; 2.17–2.18 (3H, *d*, $J = 1.2$ Hz, H-11-12) and 5.68–5.61 (1H, *m*, W



5A



5B

Table 1. ^{1}H NMR data for compounds **1**–**4**

H	**1**	**2**†	**3**†	**4**
H-1	3.30, *s*, (*br*) (W 1/2 = 20)	3.38, *s*, (*br*) (W 1/2 = 20)	3.45, *s*, (*br*) (W 1/2 = 15)	3.30, *s*, (*br*) (W 1/2 = 8)
H-2	1.70; 2.20	2.25; 1.67	2.30; 1.67	2.14; 1.64
H-3	5.04, *t* (5.0)*	5.13, *t* (5.0)	5.10, *t* (5.0)	4.03, *t* (5.0)
H-4	1.90; 2.20	2.25; 1.90	2.30; 1.94	1.92; 2.1
H-5	3.15, *s* (*br*) (W 1/2 = 15)	3.23, *s* (*br*) (W 1/2 = 10)	3.30, *s* (*br*) (W 1/2 = 8)	3.5, *m* (W 1/2 = 15)
H-6	5.45, *dd* (7.5; 3.2)	5.45, *dd* (7.5; 3.3)	5.55, *dd* (7.5; 3.4)	3.48, *dd* (7.5; 3.1)
H-7	2.20; 2.50	2.17; 2.53	2.25; 2.58	2.21; 2.70
H-9		2.68, *m* (W 1/2 = 3.0)	6.45	5.61, *m* (W 1/2 = 2.5)
H-10	6.04, *q* (7.2)		7.66	
H-11	1.98, *d* (7.2)	2.17, *d* (1.2)		2.18, *d* (1.2)
H-12	1.86, *d* (*J* = 16)	1.90, *d* (1.2)		1.88, *d* (1.2)
H-1′	3.15, *s*, (*br*) (W 1/2 = 20)			
H-9	3.36, (*s*)	6.75, (*d*, 1.6)	6.80, (*q*, 1.6)	
H-3′	5.07, *t* (5.3)			
H-5′	3.15, *s* (*br*) (W 1/2 = 20)			
H-9′		6.75, *qq* (1.6)	6.80, *qq* (1.6)	
H-10′	5.78, *s* 6.33, *s*			
H-11′	3.36, *s*	2.30, *d* (1.6)	2.31, *d* (1.6)	
H-13	2.48, *s*	2.53, *s*	2.55, *s*	2.43, *s*
H-13′	2.28, *s*			
H-14′		3.82, *s*	3.82, *s*	
H-16′			7.50, *d* (7.0)	
H-17′			7.36	
H-18′			7.36	

*J values in parentheses.
†Recorded at 500 MHz.

1/2 = 3) for senecioyl esters. Additionally, **3** showed signals at δ 7.36–7.50 (5H, Ar) and δ 6.45 (1H, *d*, J = 16 Hz, H-9) for cinnamoyl esters.

The mass spectra of **2** and **3** showed the well established fragmentation pattern of 3,6-diacyloxytropane derivatives and the relative intensities of the ions at *m/z* 266, 238, 222, 138 122, 96, 95 [4, 9] showed that the senecioyl residue was at C-6 in **2**, while the fragments at *m/z* 269, 140, 131 were consistent with a cinnamic acid ester attached to C-6 in **3**. (−)-6β-Senecioyloxytropane-3α-ol (**4**) was also isolated and its structure readily assigned on the basis of its spectroscopic properties.

Table 2. ^{13}C–^{1}H long-range connectivities in compound **1**

H	δ	C
H-3	5.04	C-2, C4, C1, C-5, C-12′
H-6	5.45	C-8′, C-10′
H-10′	5.78	C-11′, C-8′
H-12	6.33	C-9′, C-8′
H-3′	5.07	C-8′, C-9′

Compounds **1** to **4** add to the already long list of tropane alkaloids having the slight structural variations already described [11]. However, **3** is a cinnamate not previously reported from the Solanaceae, with a transposed substitution pattern with a respect to that described for the genus *Erythroxylum* [12].

Compounds **5A** and **5B** were isolated as a mixture of geometrical isomers. Their ^{1}H NMR spectra in CD_3OD showed all the signals doubled with the same coupling constants and intensities in the ratio 3:4 (Table 4). Thus, there are characteristic resonances arising from α,β-unsaturated aromatic systems corresponding to a

Table 3. ^{13}C NMR data of compounds **1–4**

C	1	2†	3†	4
1	60.0	59.4	59.0	60.1
2	33.3	33.4	33.1*	36.7*
3	67.8	67.1	65.0	64.3
4	34.8*	31.7	31.8*	37.0*
5	66.1	65.7	63.1	66.0
6	79.0	77.8	77.8	79.9
7	34.6*	35.6	35.8	35.8
8	167.7	166.6	166.0	166.0
9	127.7	116.0	118.0	116.4
10	137.6	157.5	145.0	157.4
11	15.8	20.2	—	20.3
12	20.6	27.5	—	27.1
13	40.4	39.2	39.8	40.6
1′, 5′	60.1	—	—	—
2′, 4′	36.9	—	—	—
3′	69.0	—	—	—
6′, 7′	25.8	—	—	—
8′	166.1	164.9	—	—
9′	133.4	126.6	126.5	—
10′	129.0	144.3	144.2	—
11′	38.6	14.4	14.8	—
12′	167.7	167.5	167.8	—
13′	40.2	—	—	—
14′	—	52.6	53.5	—
15′	—	—	134.3	—
16′	—	—	127.2	—
17′	—	—	128.4	—
18′	—	—	130.5	—

*Assignments may be interchangeable.
†Recorded at 125 MHz.

cis–trans mixture of cinnamic acid derivatives (δ_H 5.80, 6.60 and 6.38, 7.43; $J = 15.7$ Hz) [13, 14], the high-field region of the proton spectrum showed two AB systems characteristic of two methylenes (δ 2.67, 3.37 and 2.75, 3.43) and the methyl groups linked to heteroatoms at δ 3.37 ($\delta c = 42.5$) and δ 3.43 ($\delta c = 43.0$). Additionally, there were signals arising from two AMX systems at δ_H 6.73, 6.92, 7.35 and δ_H 6.78, 7.03, 7.10, typical of 1,3,4-trisubstituted aromatic systems.

The ^{13}C NMR spectra of **5A** and **5B** supported the proposed structures. Thus, the chemical shifts of the aromatic system 4-hydroxyphenethyl moiety carbons are similar for both isomers ($\delta c = 116.5$ for $\delta_H = 6.31$ or 6.67 and $\delta c = 131.0$ for δ_H 6.98 or 7.05). The presence of the α,β-unsaturated carboxamide groups and the chemical shifts of the protons in the vicinal AB system, belonging to a *cis–trans* substituted system with different coupling constants ($J = 12.7$ and 15.7 Hz) were easily correlated by HMBC. Diagnostic resonances were δ_C 170.7 ($\delta_H = 5.80$, 6.60) and δ_c 169.5 ($\delta_H = 6.38$, 7.43). Additional correlations in the HMBC diagram allow the differentiation of the signals at $\delta_C = 131.0$ (C-4′) and (quaternary C-3′) $\delta_C = 131.5$ with the aromatic protons at $\delta_H = 2.67$ (**5A**) and 2.75 (**5B**), and $\delta_C = 131.5$ with the H-1′ protons at $\delta_H = 3.37$ (**5A**) and 3.43 (**5B**). On the basis of the correlations of the C-2′ signals at δ_C 36.0 with δ_H 6.98 (**5A**) and at δ_C 36.3 with $\delta_H = 7.05$ (**5B**), it was possible to identify the signals in the ^1H NMR spectrum arising from each isomer, even though they were very similar. Remaining signals are shown in Table 3.

With NOE difference spectra it was possible to allocate the methoxyl groups for both isomers. Thus, the OMes belonging to the *trans*-isomer have a different chemical shift and correlation by HMBC. The carbon atom resonating at $\delta_C = 149.7$ couples to the aromatic protons $\delta_H = 7.10$ and $\delta_H = 6.78$ (Table 4) and the OMe protons at $\delta_H = 3.88$, while the remaining carbons in the ring ($\delta_C = 150.5$) have an additional correlation with the protons at $\delta_H = 6.78$, 7.10 and 7.03. An unsaturated amide structurally related to **5A** and **5B** has been isolated from an African Rutaceae species, but

Table 4. ^1H NMR and ^{13}C NMR spectral data of compounds **5A** and **5B**

Position (*cis*/*trans*)	*CH_n	δ_c†		δ_H‡		J_{HH}		δ_H§	
1	C	170, 7	169, 5					6.60, 5.80, 3.37	7.43, 6.38, 3.43
6′	C	157, 5	157, 5					6.98, 6.31	7.05, 6.67
7/8 7	C	149, 0	150, 5					7.35, 6.92, 6.73, 3.83	7.10, 7.03, 6.78
6	C		149, 7						7.10, 6.78, 3.88
3	CH	138, 7	142, 5	6, 60	7, 43	*d*, 12.7	*d*, 15.7	7.35, 6.92, 5.80	7.10, 7.03
3′	C	131, 5	131, 5					6.31, 3.37, 2.67	6.67, 3.43, 2.75
4	C	129, 0	128, 5					6.73, 5.80	7.43, 6.78, 6.38
4′	CH	131, 0	131, 0	6, 98	7, 05				
9	CH	125, 5	123, 5	6, 92	7, 03	*dd* 8.0/2.0		7.35, 6.60	7.43, 7.10
2	CH	122, 0	119, 0	5, 80	6, 38	*d*, 12.7	*d*, 15.7	6, 60	
8	CH	116, 3	117, 0	6, 73	6, 78		*d*, 8.0		
5	CH	114, 5	112, 0	7, 35	7, 10	*d*, 2.0		6.92, 6.60	7.43, 7.03
5′	CH	116, 5	116, 5	6, 31	6, 67	*s*			
10	CH_3	56, 5	56, 5	3, 83	3, 88		*s*		
1′	CH_2	42, 5	43, 0	3, 37	3, 43	*t*, 7.0	*t*, 7.0	2, 67	2, 75
2′	CH_2	36, 0	36, 3	2, 67	2, 75	*t*, 7.0	*t*, 7.0	6.98, 3.37	7.05, 3.43

*Multiplicity (DEPT).
†Broad band decoupling.
‡Correlation HMQC.
§Long range correlation HMBC.

differs in its substitution pattern [13]. We do not have a clear justification for the existence of these amides in *Schizanthus* and to the best of our knowledge this is the first report of their isolation in a species belonging to the Solanaceae.

EXPERIMENTAL

Plant material. Schizanthus litoralis (Phil.) was collected in November 1990 in the Quinta Región, Valparaíso, Chile. Voucher specimens are kept at the Faculty of Sciences, Universidad de Chile Herbarium.

General. CC was carried out using silica gel 60 G (Merck 7734). TLC was performed on silica gel GF254 (Merck 5554) with i) Me_2CO–NH_4OH (20:1), ii) $CHCl_3$–Me_2CO (1:2), iii) $CHCl_3$–Me_2CO–NH_4OH (18:5:2) and on aluminium oxide 60 F254 with iv) Et_2O–EtOH (95:2). Spots were detected by UV light or Dragendorff's reagent. Prep. TLC was performed on 2-mm thick silica gel F_{254} plates (Merck 7731). HPLC: Lichro Prep. Si 60 (5 μm, 250 × 8 mm) elution with $CHCl_3$–MeOH (19:1) and Kieselgel RP-18 (5 μm, 250 × 8 mm) elution with MeCN–H_2O (30:7); flow rate 3 ml min^{-1}; UV detector at 228 nm. ^1H and ^{13}C NMR were recorded in CD_3OD or $CDCl_3$ at 200 and 500 MHz for ^1H and 50 and 125 MHz for ^{13}C; chemical shifts δ relative to int. standard TMS. 1D (^1H, ^{13}C) and 2D (COSY, HMQC, HMBC) expts were performed using standard Brucker microprograms.

Isolation of alkaloids. Ground aerial parts (10.1 kg) were extracted with EtOH at room temp. for 1 week and processed by the general method previously described [4]. The basic material (1 kg) was subjected to repeated CC or silica gel (5–40 μm) and alumina using mixts of hexane–EtOAc–MeOH and hexane–EtOAc, respectively, to afford the following products: **1** (2.5 mg) and **4** (12.2 mg). The fr. eluted with hexane–EtOAc–MeOH was further purified by CC over silica gel-60, eluted with $CHCl_3$–MeOH and then purified by prep. HPLC (Lichro Prep. $CHCl_3$–MeOH 19:1 and RP-18 MeCN–H_2O (3:7) yielding **2** (1.5 mg), **3** (1.0 mg) and **5A**, **5B** (2 mg).

Compound (**1**). Oil (25 mg). $[\alpha]_D = -10°$ (EtOH *c* 0.091). IR ν_{max}^{KBe} cm^{-1}: 1705, 1160. ^1H NMR (Table 1). ^{13}C NMR (Table 2). EIMS (70 eV, direct inlet) *m/z* (rel. int.): 474, 276 ($C_{26}H_{38}N_2O_6$ calc. 474.2631) $[M]^+$ (29), 375, 2431 ($C_{21}H_{31}N_2O_2$ calc. 375.2130) (3), 54, 1303 ($C_{13}H_{20}NO_4$ calc. 254.1310 (14) 223, 1565 ($C_{13}H_{21}NO_2$ calc. 223.1573 (8) 222, 1509 ($C_{13}H_{20}NO_2$ calc. 222.1495 (44) 140, 100 ($C_8H_{14}NO$ calc. 140.1076 (25) 138, 0934 ($C_8H_{12}NO$ calc. 138.0919 (37) 125 (29), 124 (100), 123 (21) 122 (83), 110 (9), 96 (86), 95 (100), 94 (100). *Hydrolysis of* **1**. An H_2O–EtOH (1:1) soln (15 ml) of **1** (20 mg) and $Ba(OH)_2$ (100 mg) was refluxed for 15 hr and worked up as described in ref. [4] to yield, after sublimation, (+)-(3*R*, 6*R*)-tropan-3α, 6β-diol (12.5 mg). $[\alpha]_D$ +19.1° (EtOH, *c*, 0.051) (lit. +24.0) [8].

(−)-6β-*Senecioyloxytropan*-3α-*methylmesaconate* (**2**). Brown oil (1.5 mg). IR ν_{max}^{KBr} cm^{-1}: 3422, 2927, 1718, 1649, 1437, 1265, 1147. $[\alpha]_D^{25}$ −18.7 ($CHCl_3$, *c*, 0.15). UV λ_{max}^{EtOH} nm: 216 (4.09). ^1H NMR (Table 1). ^{13}C NMR (Table 2). EI-MS 70 eV direct inlet *m/z* (rel. int): 365.1841 ($C_{19}H_{19}NO_6$) $[M]^+$ (18), 266 (5), 238 (20), 222 (23), 138 (20), 122 (42), 95 (100), 94 (100).

(−)-6β-*Senecioyloxyropan*-3α-*ol* (**4**). Oil IR ν_{max}^{KBr} cm^{-1} 3650, 3100, 1690, 1.170. $[\alpha]_D$ −23.0 (EtOH, *c*, 0.31). ^1H NMR (Table 1). ^{13}C NMR (Table 2). EI-MS (70 eV direct inlet) *m/z* (rel. int.): (239.1525 $C_{13}H_{21}NO_3$) calc. 239.1521) $[M]^+$ (10), 156 (8), 140 (13), 138 (10), 122 (19), 114 (20), 113 (100), 112 (42), 96 (100).

Cis–trans N-(4-*hydroxyphenethyl*) *ferulamides* (**5A**/**B**). Solid. UV λ_{max}^{EtOH} nm: 204 (4.16) 287 (3.78), 316 (3.78), 375 (2.0). ^1H NMR (Table 4). ^{13}C NMR (Table 4). EI-MS (70 eV, direct inlet) *m/z* (rel. int.): 313 $[M]^+$ (23), 194 (34), 193 (34), 193 (70), 192 (62), 177 (100), 162 (4), 145 (35), 134 (8), 120 (30), 107 (18), 89 (18), 77 (18).

REFERENCES

1. Grau, J. and Gronbach, E. (1984) *Mitt. Bot. München* **20**, 111.
2. Grau, J. and Zizca, G. (eds) (1992) in *Flora Silvestre de Chile*, p. 122. Palmengarten, Frankfurt.
3. Muñoz, O., Hartmann, R. and Breitmaier, E. (1991) *J. Nat. Prod.* **54**, 1094.
4. San Martín, A., Labbé, C., Muñoz, O., Castillo, M., Reina, M., de la Fuente, G. and González, A. (1987) *Phytochemistry* **26**, 819.
5. Muñoz, O., Schneider, Ch. and Breitmaier, E. (1994) *Liebigs Ann. Chem.*, 521.
6. Hartmann, R., San Martín, A., Muñoz, O. and Breitmaier, E. (1990) *Angew. Chem. Int. Ed. Engl.* **29**, 385.
7. Gambaro, V., Labbé, C. and Castillo, M. (1983) *Phytochemistry* **22**, 1838.
8. San Martín, A., Rovirosa, J., Gambaro, V. and Castillo, M. (1980) *Phytochemistry* **19**, 2007.
9. Lounasmaa, M. (1988) in *The Alkaloids* (Brossi, A., ed.) pp. 1–81. Academic Press, New York.
10. De La Fuente, G., Reina, M., Muñoz, O., San Martin, A. and Girault, J. P. (1988) *Heterocycles* **27**, 1887.
11. Ripperger, H. (1979) *Phytochemistry* **18**, 713.
12. Al-Said, M., Evans, W. C. and Grout, R. J. (1989) *Phytochemistry* **28**, 3211.
13. Adesina, K. S. and Reisch, J. (1989) *Phytochemistry* **28**, 842.
14. Hellwig, V. (1994) Dipl. Thesis, Universität Bonn, Germany.