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Melampolides from *Smallanthus macroscyphus*

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1. Subject and source

Aerial parts of *Smallanthus macroscyphus* (Baker ex Martius) A. Grau were collected on May 12, 2000 on the road to Tiraxi at 1800 m in the Departamento Manuel Belgrano, Jujuy Province, Argentina. A voucher specimen (Alfredo Grau s/n, LIL 600 980) is on deposit in the herbarium of the Instituto Miguel Lillo, Tucumán, Argentina.

2. Previous work

In his treatment of the genus *Polymnia* within subtribe Melampodiinae of the Heliantheae Wells (1965) treated *P. macroscyphus* as a synonym of *P. connatus* Spreng., a view which was adopted by H. Robinson (1978) who included only *P. connatus* in his reestablishment of the genus *Smallanthus* as *S. connatus* (Spreng.) H. Robinson (for an addition to the genus see Turner, 1988). However the synonymy has not been recognized by Argentine taxonomists who distinguish between these two species which occur in overlapping areas of southeastern South America

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(Cabrera, 1978; Zardini, 1991; Grau and Rea, 1997). Articles have appeared on the chemistry of nine *Smallanthus* species, frequently under the old *Polymnia* label (Herz and Bhat, 1970, 1973; Bohlmann and Zdero, 1977; Bohlmann et al., 1979; LeVan and Fischer, 1979; Bohlmann et al., 1980; Bohlmann et al., 1980; Bohlmann et al., 1980; Bohlmann et al., 1985; Malcolm and Fischer, 1987; Dominguez et al., 1988; Castro et al., 1989; Kakuta et al., 1992; Inoue et al., 1995). Characteristic constituents are sesquiterpene lactones of the melampolide type, such as **1b** and **2a** with kauranes being isolated mainly from the roots, but there are no reports on the chemistry of either *S. connatus* or *S. macroscyphus*.

3. Present work

3.1. General procedures

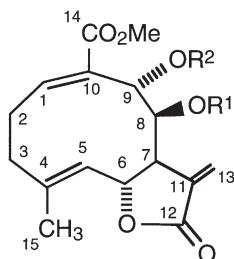
For separation of mixtures HPLC with a differential refractometer was used. The columns employed were a Beckman Ultrasphere C 18 (5 μ , 10 \times 250 nm) (column A) and a Phenomenex Maxsil 10 C8 (10 μ , 10 \times 250 nm) (column B). Retention times were measured from the solvent peak. ^1H NMR spectra were recorded in CDCl_3 at 500 MHz on a Varian Inova spectrometer. Mass spectra were run on a JEOL MS route 600 H instrument.

3.2. Extraction and isolation of constituents

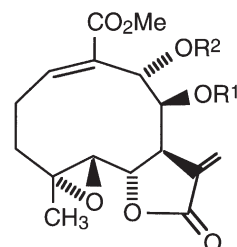
Flowers and leaves (390 g) of *Smallanthus macroscyphus* were extracted with CHCl_3 (2 \times 2.5 l) at room temperature for five days. The extract was evaporated at reduced pressure (40 $^\circ\text{C}$) to give 44.60 g of crude residue which was suspended in EtOH (388 ml) at 40 $^\circ\text{C}$, diluted with H_2O (317 ml) and extracted successively with hexane (3 \times 500 ml) and CHCl_3 (3 \times 500 ml). Evaporation of the hexane fraction at red press gave 26.73 g of residue which was not studied further. Evaporation of the CHCl_3 extract at red press gave 12.42 g of residue which was chromatographed by CC over Si gel (355 g) using CHCl_3 with increasing amounts of EtOAc (0–100%), 112 frs being collected. These were grouped according to their TLC profiles and monitored by IR spectrometry. Fractions showing γ -lactone absorption were processed by RP-HPLC. Frs. 18–21 (170 mg) were combined and a portion (24 mg) was processed by HPLC using column B (MeOH- H_2O 4:1, 1.5 ml min^{-1}) to give three peaks which were rechromatographed using the same column to give **1a** (Bardón et al., 2001, 1 mg, rt 5.1 min, (MeOH- H_2O 83:17, 1.8 ml min^{-1}), **1b** (polymatin A, Bohlmann et al., 1979; LeVan and Fischer, 1979) (3 mg, rt 6.1 min, (MeOH- H_2O 73:27, 2.0 ml min^{-1}) and 1 mg of a mixture (rt 2.5, MeOH- H_2O 7:3, 2.0 ml min^{-1}).

Frs 22–25 (480 mg) were combined; a portion (145 mg) was processed by RP-HPLC using column B (MeOH- H_2O 3:1, 1.5 ml min^{-1}) to give several peaks which were collected and rechromatographed using column B (MeOH- H_2O 7:3, 2.0 ml min^{-1}) to give **2b** (longipilin, Seaman and Fischer, 1978) containing some **2c** as judged by NMR (10.3 mg, rt 3.2 min), **2d** (4.6 mg, rt 5.1 min), **2d** containing some **2b** (4.0

mg, rt 3.8 min), two fractions of **1c**, each slightly contaminated by **1a** (11.3 mg, rt 4.9 min and 5.0 mg, rt 7.6 min), two fractions of polymatin A (**1b**) (2.9 mg, rt 5.0 min and 14.4 mg, rt 6.5 min), **1c** (12.1 mg, rt 7.3 min) and **1d** (6.5 mg, rt 10.6 min). Frs 60–62 (540 mg) were combined; chromatography of a portion (133 mg) on column A (MeOH-H₂O 13:7, 2.8 ml min⁻¹) yielded several peaks which on rechromatography over column A and column B yielded small amounts of mixtures of melampolides as judged by NMR analysis. Known compounds **1a**, **1b** and **2b** were identified by ¹H NMR spectrometry and MS.



- 1a** R¹ = MeAcr, R² = H
b R¹ = Ang, R² = H
c R¹ = H, R² = MeAcr
d R¹ = H, R² = Ang



- 2a** R¹ = MeAcr, R² = H
b R¹ = Ang, R² = H
c R¹ = H, R² = MeAcr
d R¹ = H, R² = Ang

3.3. Identification of new constituents

Lactones **1c** and **1d** were previously unknown 9-acylated analogs of 8-acylated **1a** from *Enydra anagallis* (Bardón et al., 2001) and **1b** (polymatin A) from *Smallanthus maculatus* (LeVan and Fischer, 1979), *S. riparius* and *siegesbeckia* (Bohlmann et al., 1979), *S. macvaughii* (Castro et al., 1989) and *S. sonchifolius* (Inoue et al., 1995) as shown by a change in the chemical shifts of H-8 and H-9 (Table 1), H-8 experiencing an upfield shift of ca. 1 ppm and H-9 a downfield shift of 1.2 ppm. Signals of the remaining protons were affected only slightly with the exception of H-7 whose signal experienced an upfield shift of ca. 0.2 ppm. Lactones **2c** and **2d** were new 8-hydroxy-9-acylated analogs of 8-acylated-9-hydroxy **2b** (longipilin, Seaman and Fischer, 1978) from *Melampodium longipilum* as again shown by a change in the chemical shifts of H-8 and H-9 (Table 1) with H-8 experiencing the expected upfield shift of ca. 1 ppm and H-9 a downfield shift of 0.8 ppm. In these instances the H-7 signal was affected only slightly.

(6*R**, 7*S**, 8*S**, 9*S**)-8-Hydroxy-9-methacryloxy-10-carbomethoxymelampolide(10),4,11(13)-trien-6,12-olide(**1c**). Gum; MS EI 376(5), 290 (12), 258 (35), 69 (100); MS CI (isobutane) 377.1593 (100); calcd for C₂₀H₂₄O₇ + H 377.1600; ¹H NMR spectrum in Table 1.

(6*R**, 7*S**, 8*S**, 9*S**)-8-Hydroxy-9-angeloxy-10-carbomethoxymelampolide(10),4,11(13)-trien-6,12-olide(**1d**). Gum; MS EI 390(5), 290 (11, M-100), 258 (17), 83 (100), 55 (24); HRMS + FAB (Na) 413.1576; C₂₁H₂₆O₇ + Na requires 413.1596; ¹H NMR spectrum in Table 1.

Table 1

¹H NMR spectra of compounds **1c**, **1d**, **2c** and **2d** (500 MHz, CDCl₃)

H	1c	1d	2c^a	2d^b
1	6.97dd(10,7.5)	6.96dd	7.10dd(11,7)	7.10dd(10,7.5)
2 α	2.43ddd(14,7.5,6,2)	2.44ddd	2.48 ddd(13.5,7,6,2)	2.43ddd
2 β	2.76ddd(14,13,11.5,2)	2.77ddd	2.55ddd(13.5,13,11,2)	3.11ddd
3 α	2.01ddd(13.5,13.5,2)	2.01ddd	1.20ddd(13.5,13,2)	1.19brt(13,13,2)
3 β	2.35ddd(13,6,2)	2.35ddd	2.29ddd(13,6,2)	2.31ddd
5	4.91brd(10)	4.91brd	2.66d(10)	2.63d(9.5)
6	5.22t(10)	5.19dd(10.5,10)	4.25t(10)	4.43t(9.5)
7	2.56brddd(10.5,3.5,3,1)	2.58ddd(10.5,3.5,3,1.5)	2.86ddd(10,3.5,3,1)	2.79ddd(9.5,3,2.5,1)
8	5.21brs	5.19dd(9,1.5)	6.36dd(8,1)	5.70dd(8,1)
9	5.21brs	5.25d(9)	4.43d(8)	5.24brd(8)
13a	6.32d(3.5)	6.32d(3)	6.31d(3.5)	6.39d(3)
13b	5.60d(3)	5.59d(3)	5.77d(3)	5.60d(2.5)
15 ^c	1.95brs	1.96brs	1.55s	1.67s
-OCH ₃ ^c	3.73s	3.72s	3.82s	3.75s
3' α	6.2brs	6.10qq(7.5,1.5)	6.12qq(7.5,1.5)	6.15qq
3' β	5.59brs			
4' ^c	1.92brs	1.95dq(7.5,1.5)	1.97dq	1.97dq
5' ^c		1.86 quint(1.5)	1.83 quint	1.89 quint

^a From mixture with **2b** (longipilin) which was the major component.^b Contaminated by a very small amount of **2b**.^c Intensity three protons.

(4*S**, 5*S**, 6*R**, 7*R**, 8*S**, 9*S**)-4,5-Epoxy-8-hydroxy-9-angeloxy-10-carbomethoxy-melampo-1(10),11(13)-dien-6,12-olide(**2d**). Gum; MS + FAB (Na) 429.1576; C₂₁H₂₆O₈ + Na requires 429.1576; ¹H NMR spectrum in Table 1.

4. Chemotaxonomic significance

S. macroscyphus represents a slight departure from previously studied *Smallanthus* species in that it contains a relatively large proportion of the previously unknown class of 8-hydroxy-9-acylmelampolides but otherwise fits the chemical profile of the genus overall. In a recent study of the molecular phylogenetics of the *Espeletia* complex of Melampodiinae Rauscher (Rauscher, 2002), having examined 13 out of Wells' (Wells, 1965) and Robinson's (Robinson, 1978) 20 *Smallanthus* species recognized four lineages or clades, one of which consisted of *S. connatus*, *S. fruticosus* and *S. siegesbeckius*. From a chemical point of view, at least, there seems to be somewhat less of an overlap between *S. fruticosus* (Bohlmann and Zdero, 1977; Bohlmann et al., 1980) and *S. siegesbeckius* (Bohlmann et al., 1979) on the one hand and our *S. macroscyphus* on the other than between *S. macroscyphus* and some *Smallanthus* species which according to Rauscher belong to other clades such as *S. fruticosus* (Bohlmann and Zdero, 1977; Bohlmann et al., 1980), *S. maculatus* (Herz

and Bhat 1973 as *Polymnia maculata*, LeVan and Fischer, 1979; Malcolm and Fischer, 1987), *S. riparius* (Bohlmann et al., 1979), *S. sonchifolius* (Inoue et al., 1995) and *S. uvedalius* (Herz and Bhat as *P. uvedalia* 1970; Bohlmann et al., 1980). A chemical study of authentic *S. connatus* would be of considerable interest.

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