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Ent-kaurane derivatives from the root cortex of yacon and other three *Smallanthus* species (Heliantheae, Asteraceae)

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ABSTRACT

The metabolites produced by the secretory canals of the root cortex from four *Smallanthus* species belonging to the yacon group were identified as *ent*-kaurane-type diterpenes. The dichloromethane root cortex extracts of the four species were treated with diazomethane and analyzed comparatively by GC–MS using a simple and rapid procedure which is very sensitive and reproducible permitting detection of minor components. In all cases, *ent*-16-kauren-19-oic acid (kaurenoic acid) methyl ester was the main component, differences being observed only in the minor components. The minor components identified were grandiflorenic acid methyl ester, ent-16-kauren-19-al, 16α,17-epoxy-15α-angeloyloxy-kauran-19-oic acid methyl ester and several O-acyl derivatives at C-15 or C-18 of kaurenoic acid. One of the minor components, 18-isobutyroyloxy-*ent*-kaur-16-en-19-oic acid is a new kaurenoic acid derivative. Grandiflorenic acid and 15-α-angeloyloxy-16,17-α-epoxy-*ent*-16-kauren-19-oic acid were present only in *Smallanthus sonchifolius* and *Smallanthus siegesbeckius* which showed very similar GC traces. The different GC profile of RC diterpenes from *Smallanthus connatus* and *Smallanthus macroscyphus* supports the view that they are different taxa. Some chemotaxonomic aspects of the genus *Smallanthus* and the subtribe Milleriinae are briefly discussed.

1. Introduction

The genus *Smallanthus* (subtribe Milleriinae, Tribe Heliantheae, Family Asteraceae) comprises 21 species of shrubs and small trees distributed from southern United States to northern Argentina. *Smallanthus sonchifolius*,"yacon", an ancient Andean crop is the most relevant member of the genus. Yacon tuberous roots are rich in fructooligosacharides (FOS) and phenolic compounds being consumed raw as 'fruits' since pre-Columbian times in many South American countries (Grau and Rea, 1997; Genta et al., 2009; Lachman et al., 2003). In the past two decades yacon was introduced into several Asian and European countries. The Asian dispersal began in Japan, where the use of its leaves, emerged, apparently for the first time, as an anti-diabetic herbal tea (Valentová et al., 2005). Anti-diabetic properties have been recently demonstrated for decoctions of yacon leaves (Aybar et al., 2001; Baroni et al., 2008) where enhydrin, its main sesquiterpene lactone, along with several caffeic acid derivatives have been shown to be the active principles (Genta et al., 2010; Terada et al., 2009). Farmers from local communities in northern Argentina and southern Bolivia feed parturient cattle with yacon root cortex just before and after birth.

Considering geographical distribution, growth habit and morphology of the aerial parts, the following six species appear to be close to *S. sonchifolius* forming the so-called "yacon group", i.e. *Smallanthus macroscyphus*, *Smallanthus connatus*,

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Smallanthus riparius, Smallanthus suffruticosus, Smallanthus meridensis and *Smallanthus siegesbeckius* (Grau and Rea, 1997). In this paper we report the metabolites produced by the secretory canals of the root cortex from four species belonging to the yacon group, i.e. *S. sonchifolius*,*S. macroscyphus*, *S. connatus* and *S. siegesbeckius*. The anatomical structure of the tuberous roots from *S. sonchifolius* (Machado et al., 2004), *S. macroscyphus* (Coll Aráoz et al., 2008) and *S. siegesbeckius* (Mercado et al., 2009) has been reported. The root cortex of all the four species investigated here showed secretory canals containing an amber coloured substance. Secretory canals associated to the endodermis are a common character in the Asteraceae, and although there are several studies reporting that the secretions contained in the canals are terpenoids (Appezzato-da-Glória et al., 2008; Cury and Appezzato-da-Glória, 2009; Luque Arias and Estrada Sanchez, 2004; Lotocka and Geszprych, 2004), the precise type of terpenoids involved has not been described.

Melampolide-type sesquiterpene lactones and *ent*-kaurane diterpenes are characteristic metabolites of *Smallanthus. Ent*-kauranes have been reported in the leaves of *S. sonchifolius* (Kakuta et al., 1992; Dou et al., 2010), *S. connatus* (Bach et al., 2007), *Smallanthus macvaughii* (Castro et al., 1989) and *Smallanthus maculatus* (Le Van and Fischer, 1979); in the roots and leaves of *Smallanthus uvedalius* (Bohlmann et al., 1980a), *Smallanthus fruticosus* (Bohlmann et al., 1980b), *Smallanthus pyramidalis* (Bohlmann and Zdero, 1977) and *S. siegesbeckius* (Bohlmann et al., 1979); and in the roots of *S. riparius* (Calle et al., 1988). Recently, acyclic diterpenes (Dou et al., 2008) and polyhydroxy *ent*-kaurane derivatives have been isolated from yacon leaves (Dou et al., 2010).

We have found that the amber substance contained in the secretory canals of the root cortex (RC) is diterpenes that can be easily extracted with methylene chloride. The diterpenes of the RC from the four species investigated were analyzed comparatively by GC–MS using a simple and rapid procedure which is very sensitive and reproducible permitting detection of minor components. In all the four species investigated, the RC extract was found to be constituted mainly by *ent*-16-kauren-19-oic acid (kaurenoic acid) (**1**) accompanied by minor amounts of several derivatives. In order to facilitate GC–MS analysis, the RC extracts were treated with an ethereal solution of diazomethane to obtain the corresponding methyl esters.

2. Materials and methods

2.1. Plant material

Fresh roots of *S. sonchifolius* (Poepp. & Endl.) H. Robinson (1 kg), *S. siegesbeckius* (DC.) H. Robinson (1.5 kg), *S. connatus* (Spreng) H. Robinson (0.7 kg) and *S. macroscyphus* (Backer ex Martius) Grau (0.7 kg) were collected in September 2007 from experimental plots cultivated at Centro Universitario Horco Molle, Tucumán, Argentina 26°47′S 65°19′W 547 m.a.s.l. Roots were randomly selected from at least four plants of each species.

The following additional samples were also analyzed: roots from *S. siegesbeckius* (four plants) collected in August 2007 and August 2008, and roots from *S. macroscyphus* (five plants), *S. connatus* (five plants) and *S. sonchifolius* (three plants) collected in August 2006 and again in September 2008. The results were essentially identical to those obtained with the corresponding roots collected in September 2007. Plant species were identified by Prof. Alfredo Grau. Voucher specimens were deposited at the Herbarium of the Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina: *S. sonchifolius* LIL607173, *S. connatus* LIL607374, *S. macroscyphus* LIL607375 and *S. siegesbeckius* LIL607376.

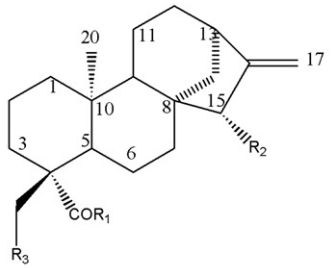
2.2. Extraction and isolation of constituents

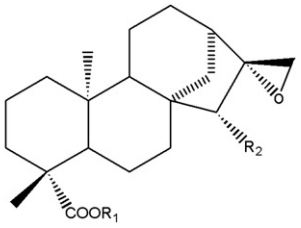
Fresh cortex from root was obtained by peeling the tuberous roots with a knife. The cortex was chopped into small pieces and extracted twice with dichloromethane for 24 h at room temperature. The extracts were filtered and the solvent was evaporated under vacuum to give the respective RC extracts.

A standard procedure of isolation is exemplified with *S. siegesbeckius*. The RC of *S. siegesbeckius* possesses big secretory canals (diameter: 70–250 μm, occasionally up to 500 μm). Fresh tuberous roots of *S. siegesbeckius* (1500 g) afforded 467 g of fresh RC. After maceration for 24 h with dichloromethane (x 2), filtering and solvent evaporation, 2.328 g of RC extract (0.16% yield based on fresh root weight) was obtained, a portion of which (0.821 g) was column chromathographed over Si gel (Merck, 230–400 Mesh, ASTM) using hexane/ethyl acetate 12:1 to obtain 50 fractions which were reunited according to their profile on TLC. Fractions 5–6 (228 mg) showed to be essentially pure ent-16-kauren-19-oic acid (kaurenoic acid) (**1**) containing minor amounts of grandiflorenic acid (**10**). Frs. 7–11 (156 mg) were a mixture of **1** (major) and **3** (minor). Frs. 12–24 (109.3 mg) and Frs. 25–29 (32.8 mg) were mixtures of **1**, **3** and **4**. Frs. 30–34 (60 mg) were a mixture of **5** (89%) accompanied by minor amounts **3** and **4**. Frs. 35–39 (56.3 mg) were a 2:1 mixture of **5** and **6**. Frs. 40–44 (34.4 mg) and 45–50 (57.5 mg) were a mixture of **5**, **6**, **7**, **8** and **9**. The structures of the compounds are shown in Fig. 1.

Pure compounds were obtained by semipreparative HPLC of Frs. 25–29, 35–39, 40–44 and 45–50. Compounds **3** (8 mg) and **4** (10 mg) were isolated from Frs. 25–29. Frs. 35–39 yielded **5** (9.5 mg) and a 5:4 mixture of **5** and **6** (15.7 mg). Frs. 40–44 and Frs. 45–50 gave a 4:1 mixture of **5** + **6** (22.1 mg), **7** (6.8 mg), **8** (6.4 mg) and **9** (4.4 mg). HPLC separations were performed using a Refractive Index Detector and a Rheodyne injector with a 2.0 mL loop. The column employed was a semipreparative Zorbax CN column (9.4 mm × 250 mm) and n-hexane containing 1.3% isopropanol at 1.5 mL/min as eluant. The peaks were collected separately and rechromatographed when necessary.

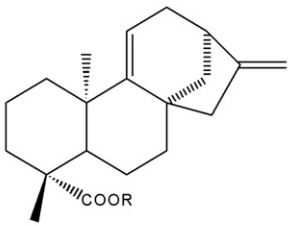
The isolated compounds were identified by their ^{1}H NMR and ^{13}C NMR spectra and comparison with data reported in the literature (Piozzi et al., 1971; Calle et al., 1988; Bohlmann et al., 1979, 1980a,b; Kakuta et al., 1992; Peixoto et al., 2008). NMR





	R_1	R_2	R_3
1	OH	H	H
1a	OMe	H	H
2	H	H	H
3	OH	OAng	H
3a	OMe	OAng	H
4	OH	OiBu	H
4a	OMe	OiBu	H
5	OH	H	OAng
5a	OMe	H	OAng
6	OH	H	OiVal
6a	OMe	H	OiVal
7	OH	H	OSen
7a	OMe	H	OSen
8	OH	H	OiBu
8a	OMe	H	OiBu

	R_1	R_2
9	H	OAng
9a	Me	OAng



	R
10	H
10a	Me

Fig. 1. Kaurenoic acid derivatives identified in *Smallanthus* spp.

measurements were recorded on a Bruker AC 200 operating at 200 MHz for ^{1}H and at 50 MHz for ^{13}C using 5 mm sample tubes and deuterated chloroform as solvent.

Fresh roots of *S. sonchifolius* (1.0 kg), *S. connatus* (0.7 kg) and *S. macroscyphus* (0.7 kg) afforded 513 g, 272 g and 318 g of fresh cortex respectively which were extracted as above to yield, after solvent evaporation, respectively 0.740 g (0.07% yield), 0.454 g (0.06% yield) and 1.347 g (0.19%) of residue. Yields are based on fresh root weight.

2.3. Analysis by GC–MS

Small portions of RC extracts of *S. siegesbeckius* (20 mg), *S. sonchifolius* (184 mg), *S. macroscyphus* (35 mg) and *S. connatus* (40 mg) were dissolved in a small amount of ether and treated separately with an ethereal solution of diazomethane. Also, the underivatized pure diterpenes isolated from *S. siegesbeckius* by HPLC were esterified with diazomethane. After solvent evaporation, all the four methylated RC extracts along with the reference pure diterpenes isolated from *S. siegesbeckius*were analyzed by GC/MS.

Gas chromatographic analysis and mass spectra were recorded using a Hewlett–Packard 5973 selective mass detector coupled to a Hewlett–Packard 6890 Gas Chromatograph (GC–MS system) fitted with an HP-5MS (5% phenylmethylsiloxane) capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness); ionization energy, 70 eV. The injector, GC–MS interphase, ion source and selective mass detector temperatures were maintained at 220, 280, 230 and 150 °C respectively. Carrier gas: Helium with a flow rate of 1.1 mL min^{-1}. The oven temperature was programmed as follows: 180–300 °C at 2.0 °C min^{-1} and then held at 300 °C for 10 min. The samples were injected as a 1% solution in methylene chloride (1.0 μL; split mode). Percentages are the mean of three runs and were calculated from TIC (Total Ion Chromatogram) by the computer. Identification of the individual components was based on: (a) computer matching with commercial mass spectra libraries (NBS 75 K; NIST, 1999; Mc Lafferty and Stauffer, 1994) and mass spectra reported in the literature; (b) comparison with the mass spectra of the methylated diterpenes isolated from *S. siegesbeckius*; and (c) co-injection with authentic samples whenever available.

2.4. Identification of diterpene ***8***

The molecular formula of **8** followed from the HRMS that showed $[M]^{+}$ at *m/z* 388.2620 (calcd. for $[C_{24}H_{36}O_4]$ 388.2614). The structure of **8** was evident after comparing its ^{1}H NMR spectrum with that of **5** (Calle et al., 1988). Thus, the ^{1}H and ^{13}C NMR data of **8** essentially duplicated those of **5** excepting the signals corresponding to the angeloyl residue at C-18 which

are now replaced by those of a 2-methylpropanoyl (*iso*-butyroyl) moiety, i.e. δ 2.56 (1H, hept, $J = 7$ Hz, H-2′), δ 1.17 (3H, d, $J = 7$ Hz, H-3′) and δ 1.15 (3H, d, $J = 7$ Hz, H-4') in the ^{1}H NMR spectrum; and δ 175.9 (s, C-1'), 34.1 (d, C-2′), 19.1 (q, C-3′), 18.9 (q, C-4') in the ^{13}C NMR spectrum.

2.4.1. Preparation of methyl ester ***8a***

To a solution of **8** (4 mg) in diethyl ether (0.5 mL), an ethereal solution of diazomethane was added dropwise until persistence of yellow colour. The solution was set aside for 15 min, the excess of diazomethane was destroyed by slowly adding dropwise a 5% ethereal solution of acetic acid (yellow colour disappearance) and the solvent evaporated to yield **8a**.

*2.4.2. 18-isobutyroyloxy-ent-kaur-16-en-19-oic acid (**8**)*

Solid; $C_{24}H_{36}O_4$. HRMS 388.2620 (calcd. for [$C_{24}H_{36}O_4$] 388.22614). EIMS (70 eV) of methyl ester **8a** (obtained by GC–MS): *m/z* (rel. int. %) 402 ([M]$^+$, 1), 314 (51), 299 (12), 286 (4), 271 (9), 255 (22), 239 (17), 225 (3), 211 (19), 199 (8), 187 (10), 174 (36), 159 (15), 145 (16), 131 (29), 119 (28), 105 (38), 91 (51), 79 (38), 71 (49), 55 (16), 43 (100). ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.80 (1H, brs, H-17a), 4.75 (1H, brs, H-17b), 4.43 (1H, d, $J = 10.5$ Hz, H-18a), 3.96 (1H, d, $J = 10.5$ Hz, H-18b), 2.64 (1H, brs, H-13), 2.56 (1H, hept, $J = 7$ Hz, H-2′), 1.16 (3H, d, $J = 7$ Hz, H-3′), 1.15 (3H, d, $J = 7$ Hz, H-4'), 0.98 (3H, s). ^{13}C NMR ($CDCl_3$): δ 181.8 (s, C-19), 175.9 (s, C-1'), 155.5 (s, C-16), 103.2 (t, C-17), 72.0 (t, C-18), 55.0 (d, C-5), 52.2 (d, C-9), 48.8 (t, C-15), 47.5 (s, C-4), 43.9 (s, C-8), 43.7 (d, C-13), 40.7 (t, C-1), 40.1 (t, C-7), 39.8 (t, C-14), 39.4 (s, C-10), 34.1 (d, C-2′), 33.0 (t, C-3), 32.3 (t, C-12), 21.7 (t, C-6), 19.1 (q, C-3′), 18.9 (q, C-4'), 18.35 (t, C-2), 18.30 (t, C-11), 15.4 (q, C-20).

2.5. GM/MS data of ***1a***, ***2***, ***3a***, ***4a***, ***5a***, ***6a***, ***7a***, ***9a*** *and* ***10a***

*2.5.1. Kaurenoic acid, methyl ester (**1a**), $C_{21}H_{32}O_2$*

EIMS (70 eV): *m/z* (rel. int. %) 316 ([M]$^+$, 23), 301 (15), 273 (30), 257 (53), 241 (49), 213 (24), 199 (11), 187 (18), 173 (12), 159 (23), 145 (23), 131 (53), 121 (73), 105 (70), 91 (100), 79 (69), 67 (42), 55 (48), 41 (50).

*2.5.2. Grandiflorenic acid, methyl ester (**10a**), $C_{21}H_{30}O_2$*

EIMS (70 eV): *m/z* (rel. int. %) 314 ([M]$^+$, 17), 299 (62), 285 (2), 267 (4), 255 (28), 239 (100), 225 (5), 211 (9), 197 (17), 183 (26), 173 (15), 155 (29), 143 (31), 129 (37), 117 (29), 105 (33), 91 (67), 79 (29), 69 (12), 55 (21), 41 (28).

2.5.3. Ent-*16-kauren-19-al (**2**), $C_{20}H_{30}O$*

EIMS (70 eV): *m/z* (rel. int. %) 286 ([M]$^+$, 23), 271 (10), 257 (18), 243 (30), 225 (17), 215 (14), 199 (17), 187 (30), 171 (9), 161 (24), 147 (25), 133 (33), 123 (47), 105 (62), 91 (100), 77 (59), 67 (46), 55 (55), 41 (38).

*2.5.4. 15-α-Angeloyloxy-ent-16-kauren-19-oic acid, methyl ester (**3a**), $C_{26}H_{38}O_4$*

EIMS (70 eV): *m/z* (rel. int. %) 414 ([M]$^+$, 2), 356 (1), 332 (1), 314 (13), 299 (10), 286 (1), 271 (3), 255 (9), 239 (6), 225 (1), 211 (6), 199 (1), 185 (2), 171 (2), 159 (4), 147 (5), 133 (5), 121 (13), 107 (13), 91 (16), 83 (100), 55 (35), 41 (8).

*2.5.5. 15-α-Isobutiroyloxy-ent-16-kauren-19-oic acid, methyl ester (**4a**), $C_{25}H_{38}O_4$*

EIMS (70 eV): *m/z* (rel. int. %) 402 ([M]$^+$, 2), 387 (1), 332 (11), 314 (27), 299 (27), 286 (5), 271 (6), 255 (29), 239 (20), 226 (3), 211 (9), 197 (6), 185 (9), 173 (5), 159 (9), 147 (19), 133 (13), 121 (28), 105 (24), 91 (36), 71 (69), 43 (100).

*2.5.6. 18-Angeloyloxy-ent-16-kauren-19-oic acid, methyl ester (**5a**), $C_{26}H_{38}O_4$*

EIMS (70 eV): *m/z* (rel. int. %) 414 ([M]$^+$, 0.1), 354 (1), 331 (1), 314 (35), 299 (7), 286 (4), 271 (7), 255 (11), 239 (8), 226 (2), 211 (10), 199 (4), 187 (9), 174 (34), 159 (11), 145 (11), 131 (19), 119 (20), 105 (27), 83 (100), 67 (11), 41 (10).

*2.5.7. 18-Isovaleroyloxy-*ent-*16-kauren-19-oic acid, methyl ester (**6a**), $C_{26}H_{40}O_4$*

EIMS (70 eV): *m/z* (rel. int. %) 416 ([M]$^+$, 1), 398 (1), 384 (1), 356 (1), 341 (2), 314 (61), 299 (13), 286 (5), 271 (13), 255 (30), 239 (22), 226 (5), 211 (25), 199 (9), 187 (19), 174 (54), 159 (23), 145 (25), 131 (37), 119 (41), 105 (53), 85 (76), 57 (100), 41 (40).

*2.5.8. 18-Senecioyloxy-*ent-*16-kauren-19-oic acid methyl ester (**7a**), $C_{26}H_{38}O_4$*

EIMS (70 eV): *m/z* (rel. int. %) 414 ([M]$^+$, 1), 314 (16), 299 (2), 285 (1), 271 (3), 255 (4), 239 (4), 225 (1), 211 (4), 199 (2), 187 (6), 174 (17), 159 (5), 145 (7), 131 (8), 119 (9), 105 (12), 83 (100), 55 (19), 41 (5).

*2.5.9. 15-α-Angeloyloxy-16,17-α-epoxy-*ent-*16-kauren-19-oic acid, methyl ester (**9a**), $C_{26}H_{38}O_5$*

EIMS (70 eV): *m/z* (rel. int. %) 430 ([M]$^+$, 1), 397 (1), 347 (2), 330 (7), 315 (7), 297 (6), 271 (9), 253 (6), 237 (5), 212 (3), 197 (2), 181 (3), 163 (4), 145 (7), 121 (20), 107 (16), 83 (100), 55 (48), 41 (7).

3. Results

The structure of the isolated compounds is shown in Fig. 1. GC/MS analysis revealed a similar profile for the RC extract of the four species (Fig. 2). The percentages of the compounds identified in the four species are summarized in Table 1. As can

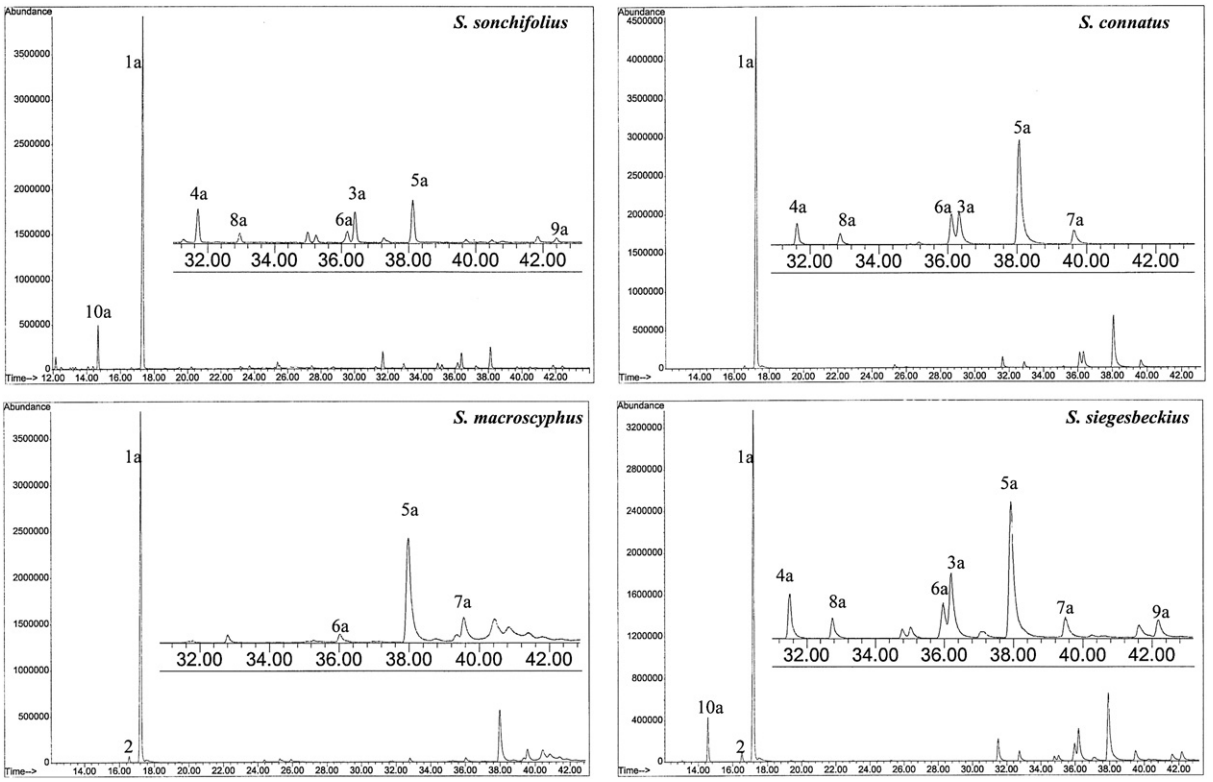


Fig. 2. GC profiles of the RC extracts from four *Smallanthus* spp.

Table 1
Kaurenoic acid derivatives identified in the RC of *Smallanthus* spp.

	%1a	%2	%3a	%4a	%5a	%6a	%7a	%8a	%9a	%10a	%Total
S. sonchifolius	74.0	–	3.6	3.6	5.2	1.5	–	0.7	trace	7.3	95.9
S. siegesbeckius	51.0	1.4	8.4	4.5	17.0	3.9	1.0	1.7	2.0	5.7	96.6
S. connatus	71.0	–	4.2	2.3	15.8	3.7	1.4	1.2	–	–	99.6
S. macroscyphus	72.7	1.2	–	–	18.2	–	4.0	–	–	–	96.1

Percentages are the average of three separate extractions and were obtained from electronic integration measurements using selective mass detector.

be seen in Fig. 2 and Table 1, kaurenoic acid methyl ester (**1a**) was the predominant diterpene in the RC of the four studied species, representing 51% of the RC extract from *S. siegesbeckius* and a little bit more than 70% in *S. connatus, S. macroscyphus* and *S. sonchifolius*.

4. Discussion and conclusion

Diterpenes **1**, **2**, **5** and **7** have been isolated from the RC of *S. macroscyphus*. Only kaurenoic acid (**1**) had been reported in the leaves of *S. connatus* (Bach et al., 2007); in addition to **1**, we have now identified kaurenoic acid derivatives **3**, **4**, **5**, **6**, **7** and **8** in the RC extract. Diterpenes **1**, **3**, **5** and **9** were previously reported for *S. sonchifolius* (Kakuta et al., 1992); in addition we have identified **4**, **6** , **8** and **10**. Diterpenes **1**, **3** and **5** were reported for *S. siegesbeckius* (Bohlmann et al., 1979); additionally we have isolated **2**, **4**, **6**, **7**, **8**, **9** and **10**. Compound **8** appears to be a new natural product. Diterpene **2** was previously reported for *Espeletia grandiflora* (Piozzi et al., 1971), **4** was reported for *Mikania* spp. (Bohlmann et al., 1978), **6** was isolated from *Wedelia* spp. (Bohlmann and Le Van, 1977), and **7** from *S. uvealius* (Bohlmann et al., 1980a). It is interesting to note that the GC profile of the RC extract from the wild species *S. siegesbeckius* is very similar to that from yacon. *S. macroscyphus* and S. *connatus* were treated as synonymous by Wells (1965) and Robinson (1978). However, this synonymy has not been accepted by South American taxonomists (Cabrera, 1978; Zardini, 1991; Grau and Rea, 1997) who consider that they are different species, with the slight pubescence on both surfaces of the blade and the lack of auricles at the base of the leaves (Cabrera, 1978) as distinctive characters of *S. macroscyphus*. Furthermore, the species occupy separated distribution areas, *S. connatus* in South-eastern Brazil, Paraguay, Uruguay and North-eastern Argentina, while *S. macroscyphus* grows in Southern Bolivia and

North-western Argentina (Grau and Rea, 1997; Zuluaga and Morrone, 1999). The different GC profile of RC diterpenes for *S. connatus* and *S. macroscyphus* supports the view that they are different taxa in agreement with a previous study based on sesquiterpene lactone chemistry (Bach et al., 2007). Kauranes are an important class of diterpenes possessing a rigid tetracyclic skeleton and exhibiting a wide variety of biological activities such as antitumor, anti-HIV, trypanocidal and antimicrobial (Ghisalberti, 1997; Alves et al., 1995; Da Costa et al., 1996), antifungal (Boeck et al., 2005; Sartori et al., 2003), hypoglycemic (Bresciani et al., 2004) and anti-inflammatory (Paiva et al., 2002), among others. Kaurenoic acid, an intermediate in the biosynthesis of numerous plants and fungal secondary metabolites, including gibberellins (Ghisalberti, 1997), was also reported to exert cytotoxic effects (Costa-Lotufo et al., 2002). In nature kauranes exhibit antifeedant properties (Hanson, 1999; Bruno et al., 2001) and canal systems secreting kauranes located in the root cortex could act as defence against herbivores.

The overall picture of the chemistry of the genus *Smallanthus* shows that melampolide-type sesquiterpene lactones and *ent*-kaurane derivatives are characteristic metabolites which are also widespread in all the subtribe Milleriinae. Melampolides, kaurenoic acid derivatives and geranylnerol derivatives (Dou et al., 2008) similar to those reported in *Smallanthus* have been reported in *Ichtyothere* species (Stefani and Da Costa, 2006; Bohlmann et al., 1981, 1982) suggesting a close relationship between them. *Milleria* and *Siegesbeckia* show a clear relationship by the nature of the sesquiterpene lactones and by the co-occurrence of ent-pimaranes and *ent*-kauranes (Kim et al., 1979; Jakupovic et al., 1987; Xiang et al., 2004; Wang and Hu, 2006; Wang et al., 2009). On the other hand, a labdane diterpene (Fujimoto et al., 1990) and an eudesmanolide (Zdero et al., 1991) have been isolated from *Guizotia scabra* raising questions about the placement of this genus in Milleriinae (Anderberg et al., 2007). It would be of interest to know about the chemistry of the other genera placed in the Milleriinae, i.e. *Axiniphyllum, Micractis, Rumfordia, Stachycephalum, Trigonospermum* and *Unxia*.

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