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SESQUITERPENE LACTONES AND OTHER CONSTITUENTS OF *STEVIA MAIMARENSIS* AND *SYNEDRELLOPSIS GRISEBACHII*

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Key Word Index—*Stevia maimarensis*; Eupatorieae; *Synedrellopsis grisebachii*; Heliantheae; Compositae; sesquiterpene lactones; germacradienolides; eudesmanolides; lignan; vomifoliol derivative.

Abstract—Aerial parts of *Stevia maimarensis* afforded two known and six new germacradienolides as well as one known and two new eudesmanolides. Aerial parts of *Synedrellopsis grisebachii* furnished only previously known compounds and no sesquiterpene lactones.

INTRODUCTION

In continuation of our work on the *Stevia* species of northern Argentina [1–13] we have examined a collection of *Stevia maimarensis* (Hieron.) Cabrera whose distribution is limited to the Quebrada de Humahuaca, Jujuy Province, Argentina [14]. Isolated from the aerial parts were the germacradienolides eupatoriopicrin (**1a**) [15], the lactone present in largest amount, desacetylovatifolin (**1b**) [16], the new analogues **1c–1g** and **2**, the eudesmanolide **3** [13] and the new analogues **4a** and **4b** as well as the flavonoids **5a–c**.

Synedrellopsis is a monotypic genus generally placed in subtribe Ecliptinae of Heliantheae [17, 18]. Its sole representative, *S. grisebachii* Hieron. *et* O. Kuntze, is limited to southern Bolivia and Paraguay and northern Argentina [14]. Aerial parts of a collection from near Tucumán furnished only lignan **6a** previously found in *Tinaspora cordifolia* [19] and *Chrysolaena verbascifolia* [19], the epoxydiol **9** earlier encountered in *Lessingianthus rubricaulis* [20] and common plant constituents (see Experimental).

RESULTS AND DISCUSSION

The structures of lactones **1c–1g** and **2**, with **1e** the minor component of a fraction containing mainly **1d** and the latter also obtained pure, were clear from the ^{1}H NMR spectra and extensive decoupling (Table 1.) H-1 was allylically coupled with the protons of the -CH_2OH group; thus, the hydroxyl group could be placed on C-14 rather than on C-15. In the case of **1g** esterification by the tiglate moiety moved the -CH_2O- signals downfield while the H-8 signal moved upfield. In the case of **1d–1g** the coupling constants involving H-3 showed that the functional group on C-3, hydroxyl in **1d**, **1e** and **1g**, acetate in **1f**, was β-orientated, while in the case of **2** the C-3 hydroxyl was α. The structures of **4a** and **4b** were evident from the ^{1}H NMR spectra (Table 2).

Stevia maimarensis closely resembles *S. satureifolia* (Lam.) Sch. Bip. and *S. mercedensis* Hieron, but was raised to species rank by Cabrera [14] because of its very restricted distribution and because it differs from *S. mercedensis* in having very thin leaves and a larger number of awns on the pappus, while *S. satureifolia* has somewhat larger heads and an involucral pubescence consisting of small curved hairs: The chemistry also appears to differentiate *S. maimarensis* from *S. satureifolia* and *S. mercedensis*, both of which produce a series of identical guaianolides, but not germacradienolides of type **1** or **2** or eudesmanolides of type **4** [2, 21].

EXPERIMENTAL

General. For sepn of mixts HPLC with a differential refractometer was used. The column employed was a Beckman C-18 (5 μm, 10 × 250 mm). R_t values were measured from the solvent peak.

Plant material. Stevia maimarensis (Hieron.) Cabrera was collected at the flowering stage on 12 March 1994 near Arroyo Perchel on Highway 9, Jujuy Province, Argentina; voucher specimen Hernández 324. Aerial parts of *S. grisebachii* Hieron. *et* O. Kuntze were collected in August 1994 in S. M. de Tucumán near the railway station; voucher specimen LIL 599941. Vou-

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Table 1. ^{1}H NMR spectra of compounds **1c–1g** and **2** (500 MHz, $CDCl_3$)

H	1c	1d	1e*	1f	1g	2
1	5.07 *brdd*	5.07 *brdd*	5.05 *m*	5.28 *brdd*	5.13 *brdd*	5.34 *brdd*
2a	2.47-	2.53 *dd*	obsc.	2.64 *dd*	2.60 *ddd*	2.58 *ddd*
2b	2.37 *m* (2H)	2.40 *ddd*	2.38 *m*	2.46 *ddd*	2.44 *ddd*	2.40 *brd*
3a	2.28 *m*	4.37 *dd*	4.36 *m*	5.10 *dd*	4.42 *dd*	4.56 *brt*
3b	2.16 *ddd*	—	—	—	—	—
5	4.85 *brd*	4.90 *brd*	4.84 *brd*	5.01 *brd*	4.93 *brd*	5.48 *brd*
6	5.11 *dd*	5.15 *dd*	5.11 *dd*	5.12 *dd*	5.31 *dd*	5.19 *dd*
7	2.93 *dddd*	2.91 *dddd*	2.95 *dddd*	2.93 *dddd*	2.77 *dddd*	3.03 *ddd*
8	5.81 *brd*	5.78 *brd*	5.81 *brd*	5.80 *dd*	4.58 *brd*	5.82 *brd*
9a	3.32 *brdd*	3.29 *brdd*	3.33 *brdd*	3.30 *dd*	2.91 *brdd*	3.29 *brdd*
9b	2.20 *brd*	2.13 *dd*	2.17 *dd*	2.19 *dd*	2.15 *brd*	2.23 *dd*
13a	6.30 *d*	6.31 *d*	6.30 *d*	6.33 *d*	6.38 *d*	6.32 *d*
13b	5.63 *d*	5.65 *d*	5.62 *d*	5.65 *d*	5.59 *d*	5.65 *d*
14a	4.22 *d*	4.25 *d*	4.24 *d*	4.25 *d*	4.95 *brd*	4.19 *brd*
14b	3.79 *d*	3.80 *d*	3.78 *d*	3.82 *d*	4.64 *brd*	3.80 *brd*
15†	1.69 *d*	1.71 *brd*	1.68 *brd*	1.71 *brs*	1.79 *brs*	1.64 *brs*
3′	6.80 *qq*	6.89 *qq*	6.73 *tq*	6.80 *qq*	6.80 *qq*	6.80 *qq*
4′†	1.81 *brd*	1.81 *brd*	4.31 *brd*‡	1.81 *brd*	1.81 *brd*	1.81 *brd*
5′	1.82 *brs*	1.82 *brs*	1.81 *brs*	1.82 *brs*	1.82 *brs*	1.82 *brs*
Ac†				2.12 *s*		

*From mixture with **1d**.

†Intensity three protons.

‡Intensity two protons.

J(Hz): Compounds **1c–1g**: 1,2a = 4.5; 1,2b = 13; 1,9 = 1,14 = 7,8 ~ 1′; 2a, 2b = 14a, 14b = 12; 2a,3 = 6; 2a,3 = 6; 2b, 3 = 5,6 = 10; 5,15 = 1.5; 6,7 = 9; 7,13a = 3.5; 7,13b = 3; 8,9a = 5; 8,9b ~ 2s; 9a,9b = 14.5. Compound **2**: 1,2a = 2a,2b = 13; 1,2b = 2a,3 = 2b,3 = ~3; 6,7 = 8.5.

chers are deposited in the herbarium of the Instituto Miguel Lillo, Tucumán.

Extraction and isolation. (*a*) Flowers and leaves of *S. maimarensis* (300 g) were extracted with $CHCl_3$ (2 × 3 l) at room temp. for 4 days to give 28.3 g of crude extract which was suspended in EtOH (240 ml) at 60°, diluted with H_2O (180 ml) and extracted with petrol (3 × 300 ml) and then with $CHCl_3$ (3 × 300 ml). Evapn of the $CHCl_3$ extracts at red. pres. furnished 11.5 g of residue which was chromatographed over silica gel (400 g) using mixts of $CHCl_3$–EtOAc (3:2) containing increasing amounts of MeOH (0–10%), 134 frs being collected and monitored by TLC. Frs 38–56 (100 mg) were combined and recrystallized from MeOH to give 60 mg of a mixt. of **5b** and **5c** identified by ^{1}H NMR spectrometry. Frs 80–89 (200 mg) were combined and processed by HPLC (MeOH–H_2O, 3:2, 2 ml min^{-1}) to give 2.5 mg of a complex mixt. (R_t 24.5 min) and 13.2 mg **5a** (R_t 41.5 min). A 200 mg portion of frs 104–109 (870 mg) was processed by HPLC (MeOH–H_2O, 11:9, 2 ml min^{-1}) to give 3.6 mg **1g** (R_t 22.0 min) and 90 mg **1f** (R_t 26.5 min). Frs 110–113 (897 mg) were combined and recrystallized from heptane–EtOAc (1:1) to give 445 mg **1a**, mp 158–160°. Evap of the mother liquors gave a residue (450 mg), a portion of which (200 mg) was processed by HPLC (MeOH–H_2O, 1:1, 2 ml min^{-1}) to afford 9.3 mg **1b**, (R_t 16.5 min), 9.6 mg **1f** (R_t 33 min), 1.7 mg **3** (R_t 31 min) and 90 mg **1a** (R_t 42.2 min). Recrystallization of frs 114–117 (808 mg) from heptane–EtOAc afforded an additional 737 mg **1a**. Frs 118–125 (690 mg) were combined. A portion (200 mg) was processed by HPLC (MeOH–H_2O, 5:6, 2 ml min^{-1}) to yield 21.5 mg of a mixt. containing **1d** as the major and **1e** as the minor constituent, whose ^{1}H NMR spectrum is listed in Table 1, 7.7 mg **2** (R_t 16.5 min), 5 mg **4a** (R_t 21.2 min), 3.1 mg unidentified material (R_t 27.5 min) and an additional 86 mg **1a**. Frs 126–134 (998 mg) were combined. A portion (500 mg) was rechromatographed over silica gel to give 250 mg **1d**, 12.1 mg unidentified material and 5.7 mg sitosterol glucoside.

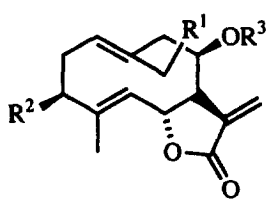
Table 2. ^{1}H NMR spectra of compounds **4a** and **4b** ($CDCl_3$, 500 MHz)

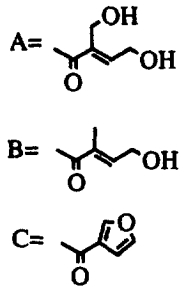
H	4a	4b
1	3.53 *dd* (11, 4.5)	3.55 *dd* (11.5, 5)
2a	obsc.	1.84 *m*
2b	1.60 *m*	1.60 *m*
3a	2.36 *ddd* (14, 4.5, 1.5)	2.37 *ddd* (14, 4.5, 1.5)
3b	2.15 *m*	2.18 *ddd* (14, 14, 5.5)
5	2.27 *brd* (11)	2.29 *brd* (11)
6	4.54 *t* (11)	4.57 *t* (11)
7	2.86 *dddd* (11, 3, 3, 3)	2.89 *dddd* (11, 3, 3, 3)
8	5.81 *q* (3)	5.92 *ddd* (4, 3, 2.5)
9a	2.40 *dd* (15, 2.5)	2.46 *dd* (15, 2.5)
9b	1.62 *dd* (15, 3)	1.66 *dd* (15, 4)
13a	6.15 *d* (3)	6.16 *d* (3)
13b	5.46 *d* (3)	5.56 *d* (3)
14*	0.98 *s*	1.02 *s*
15a	5.04 *brs*	5.05 *brs*
15*b*	4.97 *brs*	4.99 *brs*
3′	6.78 *tq* (6, 1.5)	7.96 *brd* (1.5)‡
4′	4.38 *brd* (6)†	6.70 *brd* (1.5)
5′	1.83 *brs**	7.41 *t* (1.5)

*Intensity three protons.

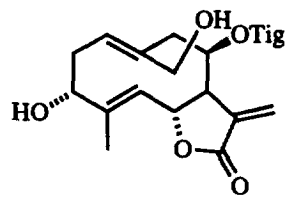
†Intensity two protons.

‡H-2′.





1 a R^1R^2=H, R^3=A
b R^1=OH, R^2, R^3=H
c R^1=OH, R^2=H, R^3=Tig
d R^1, R^2=OH, R^3=Tig
e R^1,R^2=OH, R^3=B
f R^1=OH, R^2=OAc, R^3=Tig
g R^1=OTig, R^2=OH, R^3=H



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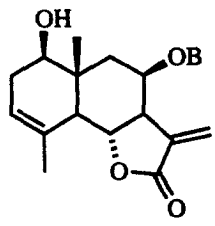

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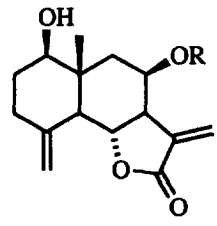
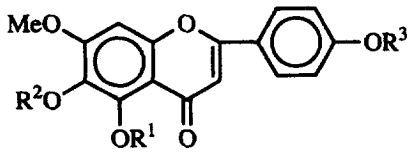
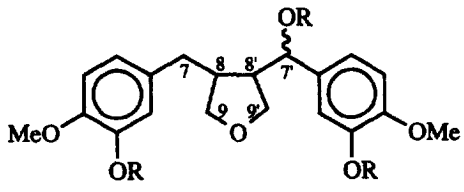


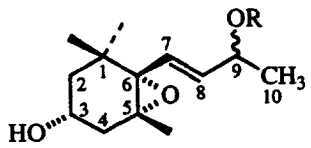
4a R=B
b R=C



5 a R^1, R^3=H, R^2=Me
b R^1, R^3=Me, R^2=H
c R^1=H, R^2, R^3=Me



6a R=H
b R=Ac



7a R=H
b R=A

(*b*) Leaves of *S. grisebachii* (770 g) were extracted with $CHCl_3$ (2 × 3 l) at room temp. for 7 days to give 23.5 g crude extract which was suspended in EtOH (200 ml) at 60°, diluted with H_2O (150 ml) and extracted successively with hexane (3 × 250 ml) and $CHCl_3$ (3 × 250 ml). Evap at red. pres. of the two extracts gave 13.3 g resp. 3.9 g of residue. Chromatography of the material from hexane (13 g) on silica gel (300 g) using mixts of hexane with increasing amounts of EtOAc (0–30%, 120 frs). Frs 59–63 furnished 193 mg crude β-amyrin; recrystallization from EtOH gave 110 mg of the pure triterpene. The residue from the $CHCl_3$ extract (3.9 g) was subjected to CC (silica gel, 120 g) using $CHCl_3$ with increasing amounts of EtOAc (0–60%, 167 frs.). Frs 48–54 (32 mg) were combined and processed by HPLC (MeOH–H_2O, 4:3, 2 ml min^{-1}) to give 3.6 mg stigmasterol (R_t 47 min) and 4.1 mg sitosterol (R_t 55 min). Frs 55–58 (22 mg)

on HPLC afforded unidentified mixts. Frs 95–110 (100 mg) were combined; a portion (61 mg) was processed by HPLC to produce several frs identified as loliolide (*ca* 9 mg). Frs 125–140 (89 mg) were combined and acetylated with Ac_2O–pyridine at room temp. overnight. After the usual work-up, flash chromatography and HPLC of the several frs gave 1.3 mg vomifoliol acetate (R_t 5 min), 1.1 mg triacetate **6b** and 0.8 mg acetate **7b**. Triacetate **6b** exhibited ^{1}H NMR signals (500 MHz, $CDCl_3$) at δ 6.99 (*d*, $J = 8$ *Hz*); 6.95 (*d*, $J = 1.5$ Hz) and 6.87 *dd* ($J = 8$, 1.5 Hz) (H-2, H-3, H-6 of aromatic ring A), 6.95 (*d*, $J = 8$ Hz) and 6.76 (*d*, $J = 2$ Hz) (H-2, H-3, H-6 of aromatic ring B), 4.85 (*d*, $J = 6$ Hz, H-7′), 4.38 (*dd*, $J = 11$, 7 Hz, H-9′a), 4.21 (*dd*, $J = 11$, 7 Hz, H-9′b) 4.09 (*dd*, $J = 9$, 6.5 Hz, H-9a), 3.84, 3.82 (each *s*, 3H, OMe's), 3.76 (*dd*, $J = 9$, 7.5 Hz, H-9b) 2.87 (*dd*, $J = 13.5$, 4.5, H-7a), 2.73 (*m*, H-8), 2.56 (*dd*, $J = 13.5$, 11 Hz, H-7b), 2.56 (*m*, H-8′) 2.31, 2.30 (each *s*, 3H, Ac), 2.04 (*s*, 3H, Ac), identical with the spectrum reported earlier [19]; MS PCI (isobutane) *m/z* (rel. int.): 503 $[M + H]^+$ (100). Acetate **7b** exhibited ^{1}H NMR signals ($CDCl_3$, 500 MHz) at δ 5.91 (*dd*, $J = 16$, 1 Hz, H-7), 5.66 (*dd*, $J = 16$, 6.5 Hz, H-8), 3.88 (*dddd*, $J = 10.5$, 8, 7, 4.5 Hz, H-3ax), 2.35 (*ddd*, $J = 14$, 5, 2 Hz, H-4 eq), 2.04 (*s*, 3H, Ac), 1.61 (*dd*, $J = 14$, 8.5 Hz, H-4ax), H-2eq (obsc.), 1.31 (*d*, $J = 6$ Hz, 3H, H-10), 1.21 (*dd*, $J =$ 12.5, 10.5 Hz, H-2ax), 1.17, 1.12, 0.95 (each *s* and 3H, H-11, 12, 13); MS PCI (isobutane) *m/z* (rel. int.): 269 $[M + H]^+$ (14.6), 209 (79.2), 191 (38.8), 117 (93.9), 75 (100).

(6R*, 7R*, 8R*)-14-*Hydroxy*-8-*tiglyloxygermacra*-1(10),4,11(13)-*trien*-6,12-*olide* (**1c**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 347 $[M + H]^+$ (100), 329 (25.0). ^{1}H NMR: Table 1.

(3S*, 6R*, 7R*, 8R*)-3,14-*Dihydroxy*-8-*tiglyloxygermacra*-1(10),4,11(13)-*trien*-6,12-*olide* (**1d**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 363 $[M + H]^+$ (36.1), 345 (37.8), 101 (100). IR ν_{max}^{film} cm^{-1}: 3400, 3010, 1755, 1650. ^{1}H NMR: Table 1.

(3S*, 6R*, 7R*, 8R*)-3-*Acetoxy*-14-*hydroxy*-8-*tiglyloxygermacra* - 1(10),4,11(13) - *trien* - 6,12 - *olide* (**1f**). Mp 80–82° (not recrystallized); MS PCI (isobutane), *m/z* (rel. int.): 405 $[M + H]^+$ (100) 345 (78.1), 261 (77.1). IR ν_{max} cm^{-1}: 3455, 1735, 1710, 1650. ^{1}H NMR: Table 1.

(3S*, 6R*, 7R*, 8R*)-3,8-*Dihydroxy*-14-*tiglyloxygermacra*-1(10),4,11(13)-*trien*-6,12-*olide* (**1g**). Mp 71–73° (not recrystallized) MS PCI (isobutane) *m/z* (rel. int.): 363 $[M + H]^+$ (27.5), 345 (28.9), 331 (100). ^{1}H NMR: Table 1.

(3R*, 6R*, 7R*, 8R*)-3,14-*Dihydroxy*-8-*tiglyloxygermacra*-1(10),4,11(13)-*trien*-6,12-*olide* (**2**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 363 $[M + H]^+$ (73.3), 345 (100). ^{1}H NMR: Table 1.

(1R*, 5S*, 6R*, 7R*, 8R*, 10R*)-1-*Hydroxy*-8-*sarracenyloxyeudesma* - 4(15),11(13) - *dien* - 6,12 - *olide* (**4a**). Gum; MS PCI (isobutane) *m/z* (rel. int.) 363]M + H]$^+$ (100), 247 (39.,3). ^{1}H NMR: Table 2.

(1R*, 5S*, 6R*, 7R*, 8R*, 10R*)-1-*Hydroxy*-8-(3-*furoyloxy*) - *eudesma* - 4(15),11(13) - *dien* - 6,12 - *olide* (**4b**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 359 $[M + H]^+$ (62.9), 247 (100). ^{1}H NMR: Table 2.

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REFERENCES

1. Oberti, J. C., Sosa, V. E., Herz, W., Prasad, J. S. and Goedken, V. L. (1983) *J. Org. Chem.* **48**, 4038.
2. Sosa, V. F., Oberti, J. C., Prasad, J. S. and Herz, W. (1984) *Phytochemistry* **23**, 1515.
3. Sosa, V. F., Gil, R. R., Oberti, J. C., Kulanthaivel, P. and Herz, W. (1985) *J. Nat. Prod.* **48**, 340.
4. Oberti, J. C., Gil, R. R., Sosa, V. E. and Herz, W. (1986) *Phytochemistry* **25**, 1479.
5. Gil, R. R., Oberti, J. C., Sosa, V. E. and Herz, W. (1987) *Phytochemistry* **26**, 1459.
6. Sosa, V. E., Oberti, J. C., Gil, R. R., Rúveda, E. A., Goedken, V. L., Gutiérrez, A. B. and Herz, W. (1989) *Phytochemistry* **28**, 1925.
7. DeHeluani, C. S., de Lampasona, M. P., Catalán, C. A. N., Goedken, V. L., Gutiérrez, A. B. and Herz, W. (1989) *Phytochemistry* **28**, 1931.
8. Gil, R. R., Pastoriza, J. A., Oberti, J. C., Gutiérrez, A. B., and Herz, W. (1989) *Phytochemistry* **28**, 2841.
9. Gil, R. R., Oberti, J. C., Gutiérrez, A. B. and Herz, W. (1990) *Phytochemistry* **29**, 3881.
10. Sigstad, E. E., Catalán, C. A. N., Gutiérrez, A. B., Díaz, J. G. Goedken, V. L. and Herz, W. (1991) *Phytochemistry* **30**, 1933.
11. Gil, R. R., Pacciaroni, A. del V., Oberti, J. C., Díaz, J. G. and Herz, W. (1992) *Phytochemistry* **31**, 593.
12. De Gutiérrez, A. N., Catalán, C. A. N., Díaz, J. G. and Herz, W. (1992) *Phytochemistry* **31**, 1818.
13. Hernández, L. R., Catalán, C. A. N., Cerda-García-Rojas, C. M. and Joseph-Nathan, P. (1994) *Phytochemistry* **37**, 1331.
14. Cabrera, A. L. (1978) *Flora de la Provincia de Jujuy*, Parte X. Collection Cientifica del INTA, Buenos Aires.
15. Drozdz, B., Grabarczyk, H., Samek, Z., Holub, M., Heróut, V. and Sorm, F. (1972) *Collect. Czech. Chem. Commun.* **37**, 1546.
16. Hoeneisen, M., Silva, M. and Bohlmann, F. (1980) *Phytochemistry* **19**, 2765.
17. Stuessy, T. F. (1977) 'Heliantheae—Systematic Review' *The Biology and Chemistry of the Compositae* (Heywood, V. H., Harborne J. B. and Turner B. L., eds), pp. 621–672, Academic Press, London.
18. Robinson, H. (1981) *Smithsonian Contrib. Botany* **51**, pp. 1–102.
19. Hanuman, J. B., Mishra, A. K. and Sabata, B. (1986) *J. Chem. Soc., Perkin 1* 1181.
20. Bardón, A., Montanaro, S., Catalán, C. A. N., Díaz, J. G. and Herz, W. (1993) *Phytochemistry* **34**, 253.
21. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1986) *Liebigs Ann. Chem.* 799.