Phytochemistry, Vol. 26, No. 5, pp. 1459–1461, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

LONGIPINANES AND A GERMACRANOLIDE CARBOXYLIC ACID FROM *STEVIA POTRERENSIS*

ROBERTO R. GIL, JUAN C. OBERTI, VIRGINIA E. SOSA* and WERNER HERZ*

Instituto Multidisciplinario de Biología Vegetal, CONICET and Facultad de Ciências Químicas, Universidad Nacional de Córdoba, 5016 Córdoba, Argentina; *Department of Chemistry, The Florida State University, Tallahassee, Florida 32306-3006, U.S.A.

(*Received* 12 *August* 1986)

Key Word Index—*Stevia potrerensis*; Eupatorieae; Compositae; longipinene derivatives; sesquiterpenes; germacranolide; sesquiterpene lactone.

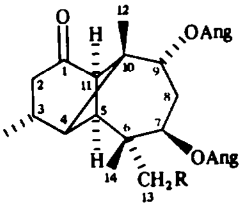
Abstract—The herb *Stevia potrerensis* afforded five longipinene derivatives, three of them new, and a new germacranolide carboxylic acid.

INTRODUCTION

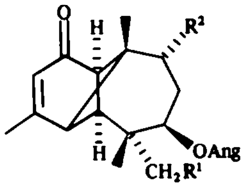
In continuation of our work on Argentinian *Stevia* species [1–4], we have isolated from the aerial parts of *Stevia potrerensis* Hieron five longipinene derivatives **1a**, **1b**, and **2a–c**, of which **1a**, **b** and **2b** are new, and the new grazielic acid analogue **3a**.

RESULTS AND DISCUSSION

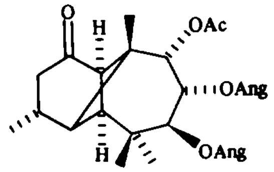
After initial confusion, the structure and stereochemistry of longipinenes from Compositae substituted in the 7-membered ring [5–14] was placed on a firm footing as the result of an X-ray analysis of rastevione acetate (**4**) [12]. Thus compound **2a**, originally isolated from the roots of *S. elatior* [8], was eventually [13] assigned the correct structure, as was **2c** [13], originally found as part of a mixture in the roots of *S. polycephala* [8] and *S. boliviensis* [10], and subsequently isolated in pure form from *S. salicifolia* and *S. lemmonii* [14]. Comparison of the ^{1}H NMR spectrum of **2b** (Table 1, all assignments confirmed by spin decoupling) with spectra of known compounds in the literature established that two of the three angelyloxy groups were 7β- and 9α-orientated and that



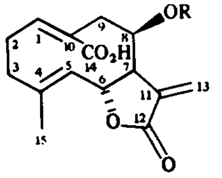
1a R = H
1b R = OAng



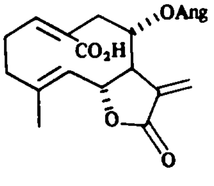
2a R¹ = OAng, R² = H
2b R¹, R² = OAng
2c R¹ = H, R² = OAng



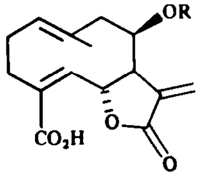
4



3a R = Ac
3b R = Ang
3c R = Tig
3d R = 4-OH-Tig



5



6

Table 1. ^1H NMR data for compounds **1a**, **b** and **2b** ($CDCl_3$, 270 MHz)

	1a	**1b**	**2b**
H-2a	2.55 *dd* (19, 8.5)	2.56 *dd*	5.76 *dddd* (1.5)†
H-2b	2.13 *dd* (19, 6)	2.13 *dd*	—
H-3	2.35 *dqdt* (8.5, 7, 6, 1.5)	2.35 *dqdt*	—
H-4	2.14 *dd* (6, 1.5)	2.20 *d*(*br*) (6)	2.68 *d*(*br*) (6.5)
H-5	1.80 *s*(*br*)	2.11 *s*(*br*)	2.57 *s*(*br*)
H-7	5.12 *dd* (12, 1.5)	5.43 *brdd* (12, 2)	5.56 *dd* (11.5, 2.5)
H-8a	2.21 *ddd* (15, 12, 3.5)	2.31 *ddd*	2.30 *ddd* (15, 11.5, 3)
H-8b	2.05 *ddd* (15, 3.5, 1.5)	2.14 *ddd* (15, 3.5, 2)	2.18 *ddd* (15, 3, 2.5)
H-9	5.02 *t* (3.5)	5.04 *t*	5.15 *t* (3)
H-11	3.04 *d*(*br*) (6)	3.20 *d*(*br*)	3.37 *d*(*br*) (6.5)
H-12*	0.94 *s*	0.90 *s*	1.00 (*br*)
H-13	0.90 *s**	4.01 *d* (11.5) 3.93 *d* (11.5)	4.05 *d* 3.82 *d*
H-14*	1.03 *s*	1.06 *s*(*br*)	1.08 *s*(*br*)
H-15*	1.11 *d* (7)	1.112 *d*	2.07 *d*(*br*) (1.5)
3′	6.09 (*br*) (7, 1.5)	6.11 *dq*	6.12 *dq*
3″	6.02 *q*(*br*)	6.07 *dq*	6.08 *dq*
3‴	—	6.01 *dq*	6.03 *dq*
4′*	2.02 *dq* (7, 1.5)	1.99 *dq*	1.95 *dq*
4″*	1.95 *dq*	1.99 *dq*	1.95 *dq*
4‴*	—	1.93 *dq*	1.93 *dq*
5′*	2.00 *s*(*br*)	1.99 *s*(*br*)	2.00 *s*(*br*)
5″*	1.88 (*s*)*br*	1.91 *s*(*br*)	1.93 *s*(*br*)
5‴*	—	1.85 *s*(*br*)	1.87 *s*(*br*)

*Intensity 3 protons.

†Coupled to H-4 and H-11 as well as to H-15.

Coupled to H-13.

Coupled to H-12.

Table 2. NOE difference spectrum of **2b**

Saturation	Observed NOE (%)
H-4	H-15 (7)
H-5	H-14 (8)
	H-11 (6)—neighbours
	H-13b (4)
	H-2 (8)
H-7	H-13a (2)
	H-11 (15)
H-9	H-12 (6)
H-12	H-2 (4)
	H-9 (5)
H-14	H-5 (10)

Table 3. ^1H NMR data for compound **3a** (270 MHz)

	$CDCl_3$	C_6D_6
H-1	5.90 *dd* (*br*)	5.15 *dd* (*br*)
H-2a	3.51 *m*	3.30 *m*
H-2b	2–2.5 *c*	1.96 *m*
H-3a		obsc.
H-3b		obsc.
H-5	5.02*	4.54 *d* (*br*)
H-6		4.03 *t* (*br*)
H-7	2.85 *m*	2.10 *dd* (*br*)
H-8	5.71 *d* (*br*)	5.52 *d* (*br*)
H-9q	3.51 *m*	3.41 *dd* (*br*)
H-9b	2–2.5 *c*	under H-15
H-13a	6.30 *d*	6.28 *d*
H-13b	5.63 *d*	5.32 *d* (*br*)
H-15	1.77 *s* (*br*)	1.52 *s* (*br*)
OAc	1.95 s	1.75 s
COOH	5.58 *s* (*br*)	6.22 *s*

*AB part of ABX system.

J (Hz): 1, 2a = 12; 1, 2b = 4; 1, 15 < 1; 2a, 2b = 14; 5, 6 = 6; 7 = 9.5; 5, 15 < 1–6, 15 < 1; 7, 8 < 1; 7, 13a = 3.5; 7, 13b = 3; 8, 9a = 6, 8, 9b < 1; 9a, 9b = 14.5.

the third was on C-13. The proposed stereochemistry was verified by the NOE difference spectrum (Table 2). Noteworthy is the chemical shift of H-11, which is lower than that of H-11 in any comparable longipinene derivative and is presumably due to the combined effect of the substituents on C-9 and C-13.

Compounds **1a** and **1b** were saturated derivatives of **2c** and **2b**, respectively, as shown by the ^1H NMR spectra (Table 1, all assignments confirmed by spin decoupling). The coupling constants involving H-3 show that the stereochemistry of the methyl group attached to C-3 is identical with that found in other similar compounds.

That compound **3a** was an 8β-acetoxy-1(10),4-germacradienolide in which one of the methyl groups on the 10-membered ring was oxidized to a carboxylic acid was shown by the MS, and the ^1H and ^{13}C NMR spectra (Tables 3 and 4). The chemical shift of H-1 and the existence of allylic (and homoallylic) coupling between H-5 and H-6, on the one hand, and H-15 on the other showed that the carboxyl group was on C-10; the very small value of $J_{7,8}$ showed that the acetoxy group was β-orientated. A comparison of the ^{13}C NMR spectrum with that reported for **5** [15], which in contrast to **3a** is conformationally flexible at room temperature, is instructive. Our assignments for C-6 and C-8 of **3a** are based on a comparison with compounds of type **6** where C-6 (at δ69.32) is shielded relative to C-8 (at δ74.98) [16]. Previously isolated ester analogues of **3a** are grazielia acid (**3b**) from *Grazielia intermedia* [17] as well as **3c** [18] and **3d**, both isolated recently from *S. amambayensis* [19].

While longipinene derivatives of the type isolated from *S. protrerensis* have been found in a number of other *Stevia* species [5, 6, 8, 10, 12, 14, 20] they do not seem to occur in all members of the genus (see, for example refs [1–4, 18, 21, 22]). Sesquiterpene lactones are fairly common also, although the present report is apparently the first one describing the isolation of both types of compounds from the same herb.

Table 4. ^{13}C NMR data for compound **3a** (20.15 MHz, $CDCl_3$)* and **5**

C	**3a**	**5** [15]
1	152.6 *d*	150.4
2	26.2 *t*	26.0
3	38.1 *t*	37.7
4	144.3 *s*	142.5
5	124.9 *d*	127.2
6	75.3 *d*	78.4
7	52.4 *d*	52.7
8	69.5 *d*	73.8
9	38.8 *t*	43 (very broad)
10	125.1 *s*	126.8
11	136.6 *s*	135.3
12	169.4 *s*†	166.9
13	120.9 *t*	124.1
14	172.9 *s*	172.3
15	16.8 *q*	17.1
1′	169.3 *q*†	170.0
2′	20.3 *q*	

*Multiplicities of signals were assigned by DEPT sequence.

†Assignments may be interchanged.

EXPERIMENTAL

Extraction of S. potrerensis. Aerial parts (320 g) of *S. potrerensis* Hieron., collected in Dique La Ciénaga, Jujuy Province, Argentina, in March 1985 and identified by Dr. Luis Ariza Espinar (voucher on deposit in Museo Botánico, Córdoba) were exhaustively extracted with $CHCl_3$. The usual work-up [23] yielded 6.7 g of crude gum which was adsorbed on 6 g of silica gel and chromatographed over 200 g of the same adsorbent packed in $CHCl_3$, 20 ml fractions being collected. The polarity of the eluent was increased by adding MeOH in the ratios 1:9 and 1:49.

Fractions 1–17 (470 mg) which had about the same composition on TLC (Bz–EtOAc, 4:1) were combined and rechromatographed over silica gel with $CHCl_3$. Fractions 1–4 of the rechromatogram contained mainly one component. Radial chromatography (1 mm plate, silica gel, gradient CH_2Cl_2–DEE, flow rate 4 ml/min) afforded 10 mg of **1a**. Fractions 6–15 of the rechromatogram were combined and purified by radial chromatography. Several runs yielded 13 mg **1b**, 29 mg **2b**, 13 mg **2a** [8] and 5 mg of a 2:1 mixture of **2c** [14] and **1b**. Fractions 48–56 from the original chromatogram gave 0.29 g of a yellow gum. Prep. TLC (silica gel, $CHCl_3$–MeOH, 9:1) of the combined material gave 35 mg **3a**.

3β(H)-7β,9α-Diangelyloxy-1-oxolongipinane (**1a**). Gum; IR $\nu^{\text{CM-1}}_{\text{CHCl}_3}$ 1725 (very broad). The positive CI MS failed to exhibit a peak for $[M+H]^+$, but had peaks at *m/z* (rel. int.) 317.3 (8.8) and 217.3 (28.8); ^{1}H NMR: Table 2.

3β(H)-1-Oxo-7β,9α,13-triangelyloxylongipinane (**1b**). Gum; IR $\nu^{\text{CM-1}}_{\text{CHCl}_3}$ 1735, 1730 (broad). The positive CI MS exhibited peaks at *m/z* (rel. int.) 515.3 $[M+1]^+$, (4.0), 415.3 (10.0) and 315.3 (21.0); ^{1}H NMR: Table 1.

1-Oxo-7β,9α,13-triangelyloxy-2-longipinene (**2b**). Also a gum; IR $\nu^{\text{CM-1}}_{\text{CHCl}_3}$1735, 1730 (broad), 1690. The positive CI MS exhibited peaks at *m/z* rel. int.) 513.3 $[M+1]^+$, (44.0) 413.3 (28.2) and 313.3 (12.0); ^{1}H NMR: Table 1.

Desacylgrazielia acid acetate (**3a**). Also a gum; IR $\nu^{\text{KBr}}_{\text{CM}^{-1}}$ 1765, 1740 (acetate), 1675 (conj. carboxyl), 1630 (C=C); MS *m/z* (rel. int.): 320 $[M]^+$, (3.0), 302 (6.0), 278 (1.7), 260 (13.5), 242 (14.3), 232 (3.9), 214 (17.4), 199 (5.6), 43 (100).

Acknowledgements—We wish to thank Dr. Manuel Gonzalez-Sierra for the ^{13}C NMR spectrum of **3a**. Work at the University of Córdoba was supported in part by grants from CONICET and CONICOR. R. R. G and V. E. S. thank CONICET for fellowships.

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. E., Oberti, J. C., Prasad, J. S. and W. Herz (1984) *Phytochemistry* **23**, 1515.
3. Sosa, V. E., Gil, R. R., Oberti, J. C., Kulanthaivel, P. and W. Herz (1985) *J. Nat. Prod.* **48**, 340.
4. Oberti, J. C., Gil, R. R., Sosa, V. E. and Herz, W. (1986) *Phytochemistry* **25**, 1479.
5. Bohlmann, F. and Zdero, C. (1975) *Chem. Ber.* **108**, 3543.
6. Bohlmann, F., Zdero, C. and Schöneweiss, S. (1976) *Chem. Ber.* **109**, 3366.
7. Bohlmann, F., Mahanta, P. K., Suwita, A., Natu, A. A., Zdero, C., Dorner, W., Ehlers, D. and Grenz (1977) *Phytochemistry* **16**, 1973.
8. Bohlmann, F., Suwita, A., Natu, A. A., Czerson, H. and Suwita, A. (1977) *Chem. Ber.* **110**, 3572.
9. Bohlmann, F., Jakupovic, J. and Zdero, C. (1978) *Phytochemistry* **17**, 2034.
10. Bohlmann, F., Dutta, L. N., Dorner, W., King, R. M. and Robinson, H. (1979) *Phytochemistry* **18**, 673.
11. Bohlmann, F., Ahmed, M., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 2027.
12. Roman, L. V., Del Rio, R. E., Hernandez, J. D., Joseph-Nathan, P., Zabel, V. and Watson, W. H. (1981) *Tetrahedron* 37, 2769.
13. Bohlmann, F., Ates (Gören), N., Jakupovic, J., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 2691.
14. Bohlmann, F. and Zdero, C. (1985) *Liebigs Ann.* 1764.
15. Bohlmann, F., Schmeda-Hirschmann, G., Jakupovic, J., King, R. M. and Robinson, H. (1984) *Phytochemistry* **23**, 1989.
16. Herz, W., deGroote, R., Murari, R., Kumar, N. and Blount, J. F. (1979) *J. Org. Chem.* **44**, 2784.
17. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 1069.
18. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1983) *Phytochemistry* **22**, 2860.
19. Schmeda-Hirschmann, G., Zdero, C. and Bohlmann, F. (1986) *Phytochemistry* **25**, 1755.
20. Bohlmann, F. and Czerson, H. (1979) *Phytochemistry* **18**, 115.
21. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 2021.
22. Bohlmann, F., Umemoto, K. and Jakupovic, J. (1985) *Phytochemistry* **24**, 1017.
23. Herz, W. and Högenauer, G. (1962) *J. Org. Chem.* **27**, 905.
24. Bohlmann, F. and Zdero, C. (1986) *Liebigs Ann.* 1764.

Note added in proof: Since submission of this manuscript a report on isolation of a guaianolide and several longipinanes from *Stevia boliviensis* has appeared [24].