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MELAMPOLIDES AND GERMACRANOLIDES FROM *STEVIA AMAMBAYENSIS*

G. SCHMEDA-HIRSCHMANN, C. ZDERO* and F. BOHLMANN*

Facultad de Ciencias Quimicas, Universidad Nacional de Asuncion, Paraguay; * Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, West Germany

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Key Word Index—*Stevia amambayensis*; Compositae; sesquiterpene lactones; germacranolides; melampolides; bisabolene derivatives.

Abstract—From the aerial parts of *Stevia amambayensis* in addition to known compounds three new sesquiterpene lactones, a grazielic acid derivative and two melampolides were isolated. The roots gave two new bisabolene derivatives.

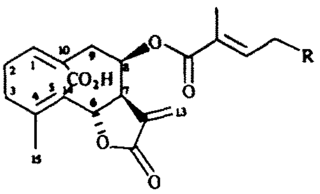
From the large genus *Stevia* (Compositae, tribe Eupatorieae, subtribe Piqueriinae) several species have been studied chemically [1]. Most species contain longipinene derivatives but these typical compounds are absent in a few species. We have investigated a species from Paraguay. The aerial parts of *Stevia amambayensis* B. L. Robinson gave, in addition to widespread compounds (see Experimental), desacetylgrazielic acid tiglate (**1**) [2] as the main constituent and three further sesquiterpene lactones, the grazielic acid derivative **2** and the isomeric aldehydes **3** and **4**.

The ^{1}H NMR spectrum (Table 1) of **2** was in part close to that of **1** [2]. However, the presence of a pair of broadend double doublets and an acetate methyl singlet indicated the replacement of the tiglate by a 4-acetoxytiglate residue. After addition of deuteriobenzene all signals could be assigned by spin decoupling. The chemical shifts showed as expected some differences if compared with those of **1**.

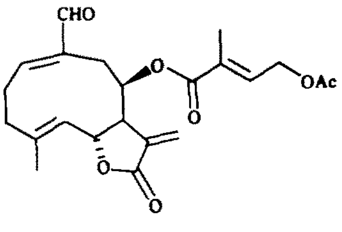
The ^{1}H NMR spectral data (Table 1) of **3** were close to those of the corresponding angelate [2]. The presence of the 4-acetoxy tiglate again followed from the typical ^{1}H NMR signals. The ^{1}H NMR spectrum of **4** (Table 1) indicated that we were most likely dealing with an isomer of **3**. The signals of the ester residue were similar in the spectra of **3** and **4** but all the others clearly differed though spin decoupling showed that the same sequences as in **3** had to be proposed. The presence of a *cis,cis*-germacranolide was deduced from the coupling of H-6 and from the chemical shifts of H-8 and H-15 which were close to those of known *cis,cis*-14-oxo-germacranolides [3–7], where the configuration of the double bond was established. In particular $J_{6,7}$ and the upfield shift of the H-8 signal in *cis,cis*-derivatives is typical when compared with the shifts of H-8 in 4*E*-isomers.

The roots gave β-bergamotene, the bisabolene derivative **5** [8] as well as two further ones, the keto angelate **6** and the corresponding epoxide **7**. The structure of **6** could be deduced from the ^{1}H NMR spectrum (see Experimental) which was close to that of the corresponding acetate [9]. The presence of the angelate residue followed from the typical ^{1}H NMR signals. Similarly, the structure of **7** was deduced from the ^{1}H NMR spectral data which were close to those of the desacyloxy derivative **8** [9]. As the chemical shifts and couplings were nearly identical in both compounds the configuration also was the same. The absolute configuration of **6** and **7** has not been determined.

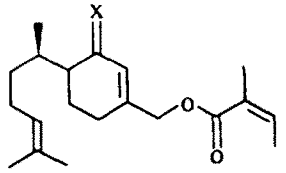
The chemistry of this species indicated that it belongs to a group of *Stevia* species where the typical longipinene derivatives are missing. The presence of several bisabolene derivatives could be an indication that these compounds, as proposed previously, may be precursors of the longipinenes.



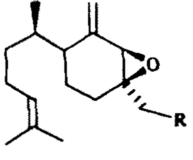
1 R = H
2 R = OAc



3
4 4Z



5 X = αOH,H
6 X = O



7 R = OAng
8 R = H

Table 1. ^{1}H NMR spectral data of compounds **2**–**4** (400 MHz $CDCl_3$, TMS as internal standard)

H	**2**	**3**	**4**
1	5.80 *m**	6.61 *ddd*	6.62 *br dd*
2	3.43 *dddd*	2.28 *br ddd*	2.78 *m*
2′	2.48 *br dd*	2.53 *m*	2.70 *m*
3	2.38 *br dd*	2.39 *br dd*	3.10 *ddd*
	2.30 *m*	2.10 *br dd*	2.26 *ddd*
5	4.96 *br d*	5.02 *br d*	4.74 *br d*
6	5.07 *t*	5.04 *t*	4.87 *dd*
7	2.90 *dddd*	2.50 *m*	2.64 *dddd*
8	5.80 *m**	6.42 *ddd*	5.98 *ddd*
9	3.49 *br dd*	2.78 *ddd*	3.00 *br dd*
9′	2.22 *br d*	1.94 *ddd*	2.57 *ddd*
13	6.30 *d*	6.20 *d*	6.32 *d*
13′	5.67 *d*	5.54 *d*	5.08 *d*
14	—	9.42 *d*	9.40 *d*
15	1.83 *br s*	1.84 *br s*	1.74 *d*
OAc	2.11 *s*	2.08 *s*	2.10 *s*
OCOR	6.54 *br t*	6.65 *br t*	6.63 *br t*
	4.72 *br dd*	4.74 *dq*	4.73 *br dd*
	4.64 *br dd*		4.48 *br dd*
	1.82 *br s*	1.82 *br s*	1.83 *d*

*After addition of C_6D_6 H-1 5.45 *dd*, H-8 5.51 *br d*.

J (Hz): compound **2**: 1,2 = 12: 1,2′ = 3.5; 2,2′ = 2,3′ = 3,3′ ~ 12; 2,3 = 5; 5,6 = 6,7 = 9.5; 7,8 ~ 1; 7,13 = 3.5; 7,13′ = 3; 8,9 = 5.5; 8,9′ ~ 0.5; 9,9′ = 14; 3′,4′ = 6; 4,4′ = 14; compound **3**: 1,2 = 10; 1,2′ = 7; 1,9 = 2; 2,2′ = 13; 2,3 ~ 2; 2,3′ = 12; 3,3′ = 12; 5,6 = 6,7 = 9; 7,8 = 1.5; 7,13 = 3.5; 7,13′ = 3; 8,9 = 7; 8,9′ = 10; 9,9′ = 14; 3′,4′ = 6; 4′,5′ ~ 1; compound **4**: 1,2 ~ 8; 1,2′ ~ 7; 1,9 = 1,9′ ~ 1; 2,3 = 7; 2′,3 = 11; 2,3′ = 3; 2′,3′ = 7; 3,3′ = 14; 5,6 = 9; 6,7 = 4; 7,8 = 2.3; 7,13 = 3.5; 7,13′ = 3; 8,9 = 7; 8,9′ = 10; 9,9′ = 14; 3′,4′ = 6; 4′,4′ = 14.

EXPERIMENTAL

The air dried plant material (voucher Schmeda 677, deposited in the US National Herbarium) was extracted with Et_2O–Et_2O (1:1) as reported elsewhere [10]. From the extract of the aerial parts 1.085 kg three CC (silica gel) fractions were taken; Fr. 1: petrol, Fr. 2: Et_2O–petrol (1:9) and Fr. 3: Et_2O and Et_2O–MeOH (9:1). TLC of fraction 1 (silica gel, PF 254, petrol) gave 50 mg germacrene D and 10 mg bicyclogermacrene. TLC of fraction 2 (Et_2O–petrol, 1:10) gave 20 mg lupeyl acetate, 15 mg 1β,10α-epoxycaryophyllene, 30 mg beyeren-19-oic acid and 20 mg ent-kaurenic acid. Medium pressure chromatography (silica gel, ϕ 30–60 μ, Et_2O–petrol, 1:10, 1:3, 1:1, 3:1, Et_2O and Et_2O–MeOH, 20:1) gave 65 fractions. Fractions 1–40 gave 3 g linoleic and linolenic acid, fractions 41–50 afforded 100 mg **1** and fractions 51–65 gave a mixture which was separated by TLC (Et_2O–petrol, 3:1) affording two bands (band 3/1 and band 3/2). TLC of band 3/1 (Et_2O–petrol, 3:1, two developments) gave 5 mg **4** (R_f 0.56) and 10 mg **3** (R_f 0.50). TLC of band 3/2 ($CHCl_3$–C_6H_6–Et_2O, 1:1:1) afforded 30 mg **3** (R_f 0.58) and 8 mg **2** (R_f 0.36). The extract of the roots (100 g) was separated by CC into three fractions (Fr. 1: petrol, Fr. 2: Et_2O–petrol, 1:3 and Fr. 3: Et_2O–petrol, 1:1 and Et_2O). TLC of fraction 1 (petrol) gave 10 mg β-bergamotene. TLC of fraction 2 (Et_2O–petrol, 1:9) gave 10 mg **5** (R_f 0.50) and a mixture (R_f 0.65) which was further separated by TLC (Et_2O–petrol, 1:9) affording 10 mg **7** (R_f 0.68) and 3 mg **6** (R_f 0.60). Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material.

Desacylgrazielic acid-[*4-acetoxytiglate*] (**2**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–2400, 1725 ($C{=}CCO_2H$, $C{=}CCO_2R$), 1760 (γ-lactone, OAc); MS *m/z* (rel. int.): 418 $[M]^+$ (0.4), 358.142 [M − HOAc] (calc. for $C_{20}H_{22}O_6$: 358.142) (1.6), 340 $[358 - H_2O]^+$ (0.6), 260 $[M - RCO_2H]^+$ (12), 242 $[260 - H_2O]^+$ (11), 99 $[C_4H_6(OH)CO]^+$ (100), 71 $[99 - CO]^+$ (37); $[\alpha]_D^{24} = -6.1°$ ($CHCl_3$; *c* 0.5).

8β-[*4-Acetoxytigloyloxy*]-14-*oxo-acanthospermolide* (**3**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2730, 1685, 1620 ($C{=}CCHO$), 1765 (γ-lactone, OAc), 1720 ($C{=}CCO_2R$); MS *m/z* (rel. int.): 402 $[M]^+$ (0.1), 342.147 [M − HOAc] (calc. for $C_{20}H_{22}O_5$: 342.147) (1.2), 244 $[M - RCO_2H]^+$ (3.5), 99 $[C_4H_6(OH)CO]^+$ (100); $[\alpha]_D^{24} = -38°$ ($CHCl_3$; *c* 0.8).

8β-[*4-Acetoxytigloyloxy*]-14-*oxo-4Z-acanthospermolide* (**4**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2720, 1695, 1640 ($C{=}CCHO$), 1775 (γ-lactone), 1750 (OAc), 1725 ($C{=}CCO_2R$); MS *m/z* (rel. int.): 402 $[M]^+$ (0.2), 342.147 [M − HOAc] (calc. for $C_{20}H_{22}O_5$: 342.147), 244 $[M - RCO_2H]^+$ (24), 99 $[C_4H_6(OH)CO]^+$ (100); $[\alpha]_D^{24} = -101°$ ($CHCl_3$; *c* 0.5).

15-*Angeloyloxy-bisabol*-1-*one* (**6**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1725, 1645 ($C{=}CCO_2R$), 1680 ($C{=}CC{=}O$); MS *m/z* (rel. int.): 318.219 $[M]^+$ (11) (calc. for $C_{20}H_{30}O_3$: 318.219), 218 $[M - RCO_2H]^+$ (22), 135 (58), 109 (70), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (78); ^{1}H NMR ($CDCl_3$): δ6.02 (*tt*, H-2), 2.33 (*m*, H-4), 2.25 (*ddd*, H-6), 2.33 (*m*, H-7), 1.30 (*dt*, H-8), 2.00 (*m*, H-9), 5.10 (*br t*, H-10), 1.68 (*br s*, H-12), 1.60 (*br s*, H-13), 0.81 (*d*, H-14), 4.74 (*br s*, H-15), 6.16 (*qq*), 2.01 (*dq*) and 1.94 (*dq*, OAng); [*J* (Hz): 2,4 = 2,15 = 1; 5,6 = 12; 5′,6 = 6,7 = 4; 7,14 = 7,8 = 8,9 = 9,10 = 3′,4′ ~ 7; 3′,5′ = 4′,5′ = 1.5].

15-*Angeloyloxy*-2,3-*epoxy-bisabol*-10-*en*-1-*one* (**7**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1715 ($C{=}CCO_2R$, $C{=}O$); MS *m/z* (rel. int.): 334.214 $[M]^+$ (3) (calc. for $C_{20}H_{30}O_4$: 334.214), 234 $[M - RCO_2H]^+$ (5), 109 (54), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (47); $[\alpha]_D^{24} = +103°$ ($CHCl_3$; *c* 0.24); ^{1}H NMR ($CDCl_3$): δ3.28 (*s*, H-2), 2.27 (*m*, H-7), 5.08 (*br t*, H-10), 1.68 (*br s*, H-12), 1.59 (*br s*, H-13), 0.80 (*d*, H-14), 4.41 and 4.19 (*d*, H-15), 6.14 (*qq*), 2.00 (*dq*) and 1.90 (*dq*, OAng); [*J* (Hz): 7,14 = 9,10 = 7; 15,15′ = 12; 3′,4′ = 7; 3′,5′ = 4′,5′ = 1.5].

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