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Longipinene derivatives from *Stevia porphyrea*

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Abstract

The new longipinene derivatives 8α-angeloyloxy-7β-hydroxy-9α-isovaleroyloxylongipin-2-en-1-one, 7β-angeloyloxy-8α,9α-dihydroxylongipin-2-en-1-one and 7β-angeloyloxy-9α-hydroxy-8α-isovaleroyloxylongipin-2-en-1-one together with five known longipinenes, friedelin and stigmasterol were isolated from the roots of *Stevia porphyrea*. The positional assignment of individual ester residues was done by HMBC experiments.

Keywords: *Stevia porphyrea*; Compositae; Sesquiterpenes; Longipinene derivatives

1. Introduction

Chemical studies revealed that sesquiterpene lactones, diterpenes and longipinene derivatives are the main secondary metabolites of the genus *Stevia*, as recently reviewed (Hernández, Catalán & Joseph-Nathan, 1998). In continuing our studies on the chemical constituents of *Stevia* species (Guerra-Ramírez, Cerda-García-Rojas, Puentes & Joseph-Nathan, 1998; Hernández, Catalán, Cerda-García-Rojas & Joseph-Nathan, 1995, 1996), we describe herein the isolation and structure elucidation of three new longipinene derivatives (**1**–**3**) from the hexane extracts of the roots of *S. porphyrea* McVaugh. These components were obtained together with stigmasterol (Wright et al., 1978), friedelin (Patra & Chaudhuri, 1987) and the known longipinenes, **4** (Bohlmann, Suwita, Natu, Czerson & Suwita, 1977b), **5** (Sánchez-Arreola, Cerda-García-Rojas, Joseph-Nathan, Román & Hernández, 1995), **6** (Bohlmann et al., 1977a), **7** (Amaro, Adrián, Cerda & Joseph-Nathan, 1988) and **8** (Román et al., 1981).

2. Results and discussion

Diester **1** was isolated as an oil by CC. Its IR spectrum showed typical absorptions for a hydroxyl group (3540 cm^{-1}), a saturated ester group (1732 cm^{-1}), an α,β-unsaturated ester group (1719 and 1648 cm^{-1}) and an α,β-unsaturated ketone (1674 and 1618 cm^{-1}). The mass spectrum of **1** showed a molecular ion, $[M]^+$ at m/z 432, in agreement with the molecular formula $C_{25}H_{36}O_6$. The ^1H NMR spectrum showed a vinylic proton at δ 6.14 and vinylic methyl groups at δ 2.02 and 1.84 characteristic of an angelate ester. The presence of an isovalerate ester was indicated by a multiplet at δ 2.24, a multiplet at δ 2.09 and a doublet for two methyl groups at δ 0.96 (J = 6.5 Hz). The signals for the protons geminal to oxygen atoms appeared as a doublet at δ 5.50 (J = 3.2 Hz, H-9), a double doublet at δ 5.27 (J = 3.2 and 11.2 Hz, H-8) and a double doublet at 3.91 (J = 4.2 and 11.2 Hz, H-7), which upon addition of D_2O collapsed to a doublet J = 11.2

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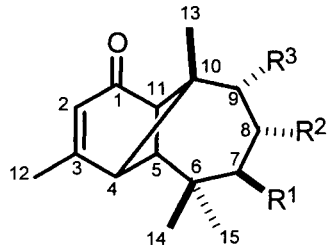

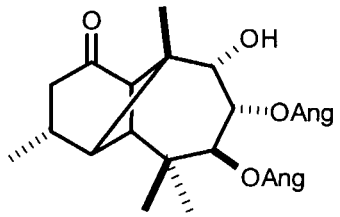

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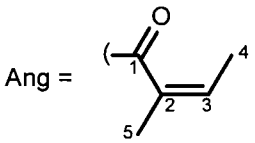
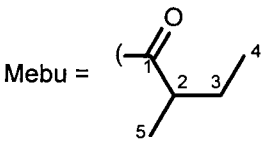
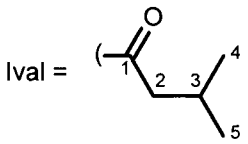
	R1	R2	R3
1	OH	OAng	OIval
2	OAng	OH	OH
3	OAng	OIval	OH
4	OAng	OMebu	OH
5	OAng	OAc	OAng
6	OAng	H	H
7	OAng	OAng	OH







Hz. The remaining ^{1}H NMR and ^{13}C NMR spectral data (see Section 3 and Table 1, respectively) revealed the presence of the longipinene moiety (Cerda-García-Rojas, Sáchez-Arreola, Joseph-Nathan, Román & Hernández, 1993; Joseph-Nathan, Cerda-García, Castrejón, Román & Hernández, 1991; Román, Hernández, Castañeda, Cerda & Joseph-Nathan, 1989).

In order to assign the positions of the angelate and isovalerate groups, a two- and three-bond heteronuclear correlation spectrum using the HMBC pulse sequence was obtained. This spectrum showed correlation of the ^{1}H signal at δ 1.84, assigned to Me-5 of the angelate group (Joseph-Nathan, Wesener & Günther, 1984), with the ^{13}C signal at δ 167.1 corresponding to the angelate carbonyl group. A correlation of the signal at δ 2.24, assigned to H-2 of isovalerate (Guerra-Ramírez et al., 1998), with the signal at δ 172.1, allowed the assignment of the isovalerate carbonyl group. Since the angelate carbonyl signal at δ 167.1 showed correlation with the double doublet at δ 5.27 assigned to H-8 and the isovalerate carbonyl signal at δ 172.1 showed correlation with the doublet at δ 5.50 due to H-9, the esters are located as in **1**.

Monoester **2** was isolated as an oil by HPLC. Its IR spectrum showed typical absorptions for hydroxyl groups (3506 cm^{-1}), an α,β-unsaturated ester group

Table 1
^{13}C NMR spectral data for longipinene derivatives (**1**–**4**)

C	1	2	3	4
1	202.6	202.9	202.7	202.7
2	122.8	122.9	122.9	122.9
3	170.2	169.9	169.8	169.7
4	48.2	48.0	48.1	48.0
5	65.8	65.6	65.5	65.5
6	36.9	36.5	36.5	36.6
7	70.6	73.7	70.1	69.9
8	71.5	70.2	71.2	71.1
9	74.1	77.2	75.2	75.2
10	54.7	55.6	55.4	55.4
11	53.1	52.1	52.3	52.3
12	23.3	23.3	23.3	23.3
13	21.0	22.0	21.8	21.8
14	18.7	19.9	20.0	20.1
15	26.6	26.8	26.5	26.5
	Ang	Ang	Ang	Ang
1	167.1	168.6	166.6	167.0
2	126.9	127.5	127.4	127.3
3	140.2	139.4	139.8	140.3
4	15.8	15.9	15.9	15.9
5	20.3	20.7	20.6	20.7
	Ival	–	Ival	Mebu
1	172.1	–	171.6	175.0
2	43.3	–	43.1	41.2
3	25.5	–	25.5	26.3
4	22.5	–	22.4	11.6
5	22.4	–	22.3	16.3

(1716 and 1646 cm^{-1}) and an α,β-unsaturated ketone (1674 and 1618 cm^{-1}). The mass spectrum showed $[M+1]^+$ at m/z 349 in agreement with molecular formula $C_{20}H_{28}O_5$. The ^1H NMR spectrum showed a vinylic signal for an angelate group at δ 6.14. The resonances for H-8 and H-9 appeared superimposed in the δ 3.96 to 3.90 range and the resonance for H-7 appeared as a complex signal shifted downfield (δ 5.24), indicating the presence of an ester group at C-7. The chemical shifts and coupling constants for this three-spin system were obtained after spin–spin simulation (Bothner-By & Castellano, 1968). The simulated spectrum fit satisfactorily with the experimental spectrum when using the parameters δ 5.24 for H-7, 3.93 for H-8, 3.93 for H-9, $J_{7,8}=11.1$ and $J_{8,9}=3.3$ Hz, which are the expected values for a 7β-acyloxy-8α,9α-dihydroxy-longipinene. The remaining ^1H NMR and ^{13}C NMR spectral data, as reported in the experimental section and Table 1, respectively, were in agreement with structure **2**.

Diester **3** was isolated as an oil by HPLC. Its IR spectrum showed absorptions for a hydroxyl group (3525 cm^{-1}), a saturated ester group (1731 cm^{-1}), an α,β-unsaturated ester group (1718 and 1647 cm^{-1}) and an α,β-unsaturated ketone (1674 and 1618 cm^{-1}). The mass spectrum showed $[M]^+$ at m/z 432, consistent with $C_{25}H_{36}O_6$. The ^1H NMR spectrum showed a vinylic proton signal at δ 6.15 and two vinylic methyl groups at δ 2.03 and 1.88 for an angelate. An isovaleric ester group was indicated by the signals at δ 2.15 and 2.00 and two doublets for the methyl groups at 0.92 and 0.93 ppm (J = 6.5 Hz). The signals for the protons geminal to the oxygen atoms appeared as a doublet at δ 5.50 (J = 11.1 Hz, H-7), a double doublet at δ 5.40 (J = 2.8 and 11.2 Hz, H-8) and a doublet at δ 3.86 (J = 2.8 Hz, H-9). The ester groups were also located by HMBC which showed correlation of the signal at δ 1.88 ppm, assigned to Me-5 of the angelate (Joseph-Nathan et al., 1984) with the signal at δ 166.6, corresponding to the angelate carbonyl group and of the signal at δ 2.15, assigned to H-2 of the isovalerate group, with the signal at δ 171.6 ascribed to the isovalerate carbonyl group. Since the angelate carbonyl signal at δ 166.6 showed correlation with the doublet at δ 5.50 (H-7) and the isovalerate carbonyl signal at 171.6 showed correlation with the double doublet at δ 5.40 (H-8), the ester groups are located as in **3**.

Diester **4** was also isolated as an oil. It corresponded to a substance which was isolated from *S. serrata* (Bohlmann et al., 1977b). However, the stereochemistry at C-7, C-8 and C-9 and the position of the ester groups needed revision according to its ^1H and ^{13}C NMR spectral data in comparison with those of rastevione (**8**). The stereostructure of **8** was determined by X-ray diffraction analysis (Román et al., 1981), and as a consequence the stereostructures and ester locations of other longipinane derivatives were also reformulated (Bohlmann, Ates, Jakupovic, King & Robinson, 1982). To confirm the structure of **4**, a correlation with monoester **2** was done by selective alkaline hydrolysis.

Previous studies (Hernández et al., 1998; de Hernández, Catalán, Hernández, Guerra-Ramírez & Joseph-Nathan, 1999) of the genus *Stevia* have shown that 18 species contain only sesquiterpene lactones, 6 contain only longipinanes, 7 contain only diterpenes, 7 contain sesquiterpene lactones along with longipinenes, 6 contain diterpenes along with longipinenes, only 4 contain simultaneously sesquiterpene lactones, longipinenes and diterpenes and in 6 species none of these metabolites are reported. Thus, the present study indicates that *S. porphyrea* belongs to those species which contain only longipinanes.

3. Experimental

3.1. General

UV spectra were obtained in EtOH. IR spectra were measured in $CHCl_3$. Specific rotations were determined in $CHCl_3$. NMR measurements were performed at 300 MHz for ^1H and 75.4 MHz for ^{13}C from $CDCl_3$ solns containing TMS as int. standard. HMBC experiments were performed at 500 MHz. CC was carried out on Merck silica gel 60 (230–400 mesh ASTM). HPLC sepns were done by using a reverse-phase Micropack MCH-5-N-CAP column, i.d. 4 mm, length 150 + 40 mm (pre-column) employing UV detection at 254 nm. Mass spectra were recorded at 70 eV.

3.2. Plant material

Specimens of *Stevia porphyrea* McVaugh. were collected at km 62 of the Morelia-Maravatío highway, in the state of Michoacán, Mexico, during October 1992. A voucher specimen is deposited at the herbarium of the Instituto de Ecología, A.C., Pátzcuaro, Michoacán, Mexico where Professor Jerzy Rzedowski identified the plant material.

3.3. Extraction and isolation

Air-dried roots (1 kg) of *S. porphyrea* were extracted (×3) with hexane under reflux for 4 h. The solvent was evaporated to dryness to afford a yellow viscous oil (10 g) which was chromatographed by CC. Frs eluted with hexane afforded stigmasterol (30 mg). Frs eluted with hexane–EtOAc (19:1) afforded friedelin (10 mg). Frs with hexane–EtOAc (9:1) contained **5**. The first frs eluted with hexane–EtOAc (8:2) contained **6**, followed by frs containing **1** and rastevione (**8**). The last frs eluted with hexane–EtOAc (8:2) contained a mixture

of **3**, **4** and dehydrorastevione (**7**). Finally, frs eluted with hexane–EtOAc (7:3) contained **2**. Compound **5** (5 mg) was purified by CC eluting with CH_2Cl_2–Me_2CO (19:1). Compound **6** (10 mg) was purified by CC eluting with hexane–EtOAc (9:1). Compound **1** (8 mg) was purified by CC eluting with hexane–EtOAc (8:2). Compounds **3**, **4** and **7** were purified by HPLC injecting samples of ca. 1.5 mg of the mixture in MeOH and eluting with MeOH–H_2O (65:35), with a flow rate of 1 ml min^{-1}. Each run afforded ca. 0.2 mg of **3** with a retention time of 30 min, 0.3 mg of **4** with a retention time of 27 min and 0.7 mg of **7** with a retention time of 24 min. Compound **2** was purified by HPLC injecting samples of ca. 1.5 mg in MeOH and eluting with MeOH–H_2O (55:45), with a flow rate of 1 ml min^{-1}. Each run afforded ca. 0.6 mg of **2**.

*3.4. 8α-Angeloyloxy-7β-hydroxy-9α-isovaleroyloxylongipin-2-en-1-one (**1**)*

Colorless oil. EIMS *m/z* (rel. int.): 432 $[M]^+$ (1), 330 (8), 201 (25), 187 (19), 135 (7), 109 (7), 83 (100), 57 (26); UV λ_{max} (EtOH) nm (log ε): 224 (3.83), 250 (3.63); IR ν_{max} ($CHCl_3$) cm^{-1}: 3540 (OH), 1732 (O=C), 1719, 1648 (O=C–C=C, angelate), 1674, 1618 (O=C–C=C, ketone).

[α] =	589	578	546	436	365	($CHCl_3$, c = 1.7)
	+95	+99	+115	+215	+497	

^{1}H NMR (300 MHz): δ 6.14 (1H, qq, J = 7.5 and 1.5 Hz, H-3 angelate), 5.81 (1H, ddq, $J_{2,4}=J_{2,11}=J_{2,12}=1.5$ Hz, H-2), 5.50 (1H, d, $J_{8,9}=3.2$ Hz, H-9), 5.27 (1H, dd, $J_{7,8}=11.2$ and $J_{8,9}=3.2$ Hz, H-8), 3.91 (1H, dd, $J_{7,8}=11.2$ and $J_{7,OH}=4.0$ Hz, H-7), 3.04 (1H, dd, $J_{2,11}=1.5$ and $J_{4,11}=6.9$ Hz, H-11), 2.79 (1H, br d, $J_{4,11}=6.9$ Hz, H-4), 2.37 (1H, br s, H-5), 2.24 (2H, m, CH_2-2 isovalerate), 2.09 (1H, m, H-3 isovalerate), 2.06 (3H, d, $J_{2,12}=1.5$ Hz, Me-12), 2.02 (3H, dq, J = 7.5 and 1.5 Hz, Me-4 angelate), 1.84 (3H, dq, $J_d=J_q=1.5$ Hz, Me-5 angelate), 1.08 (3H, s, Me-15), 1.05 (3H, s, Me-14), 0.97 (3H, s, Me-13), 0.96 (6H, d, J = 6.5 Hz, Me-4 and Me-5 isovalerate); ^{13}C NMR spectral data: see Table 1.

*3.5. 7β-Angeloyloxy-8α,9α-dihydroxylongipin-2-en-1-one (**2**)*

Colorless oil. EIMS *m/z* (rel. int.): 349 $[M+H]^+$ (0.2), 248 (1.0), 230 (2.1), 187 (6.4), 175 (7.7), 164 (1.8), 149 (5.0), 135 (10.9), 109 (9.5), 83 (100), 55 (30.1); UV λ_{max} (EtOH) nm (log ε): 217 (3.58), 250 (3.17); IR ν_{max} ($CHCl_3$) cm^{-1}: 3506 (OH), 1716, 1646 (O=C–C=C, angelate), 1674, 1618 (O=C–C=C, ketone).

[α] =	589	578	546	436	365	($CHCl_3$, c = 0.8)
	+21	+24	+30	+59	+190	

^{1}H NMR (300 MHz): δ 6.14 (1H, qq, J = 7.5 and 1.5 Hz, H-3 angelate), 5.81 (1H, ddq, $J_{2,4}=J_{2,11}=J_{2,12}=1.5$ Hz, H-2), 5.24 (1H, *complex signal*, H-7), 3.96-3.90 (2H, *complex signal* H-8 and H-9), 3.12 (1H, dd, $J_{2,11}=1.5$ and $J_{4,11}=6.5$ Hz, H-11), 2.70 (1 H, br d, J = 7.6 Hz, OH), 2.61 (1H, br s, OH), 2.58 (1H, d, $J_{4,11}=6.5$ Hz, H-4), 2.31 (1H, s, H-5), 2.05 (3H, d, $J_{2,12}=1.6$ Hz, Me-12), 2.03 (3H, dq, J = 7.5 and 1.5 Hz, Me-4 angelate), 1.96 (3H, dq, $J_d=J_q=1.5$ Hz, Me-5 angelate), 1.18 (3H, s, Me-13), 1.09 (3H, s, Me-14), 0.92 (3H, s, Me-15); ^{13}C NMR spectral data: see Table 1.

*3.6. 7β-Angeloyloxy-9α-hydroxy-8α-isovaleroyloxylongipin-2-en-1-one (**3**)*

Colorless oil. EIMS *m/z* (rel. int.): 432 $[M]^+$ (1), 330 (6), 248 (6), 201 (25), 187 (13), 175 (9), 109 (9), 83 (100), 57 (10); UV λ_{max} (EtOH) nm (log ε): 217 (3.88), 250 (3.46); IR ν_{max} ($CHCl_3$) cm^{-1}: 3525 (OH), 1731 (O=C), 1718, 1647 (O=C–C=C, angelate), 1674, 1618 (O=C–C=C, ketone).

[α] =	589	578	546	436	365	($CHCl_3$, c = 1.4)
	+18	+19	+23	+45	+154	

^{1}H NMR (300 MHz): δ 6.15 (1H, qq, J = 7.5 and 1.5 Hz, H-3 angelate), 5.81 (1H, ddq, $J_{2,4}=J_{2,11}=J_{2,12}=1.5$ Hz, H-2), 5.50 (1H, d, $J_{7,8}=11.1$ Hz, H-7), 5.40 (1H, dd, $J_{7,8}=11.1$ and $J_{8,9}=$ 2.7 Hz, H-8), 3.86 (1H, d, $J_{8,9}=2.7$ Hz, H-9), 3.19 (1H, *dd*, $J_{2,11}=1.5$ and $J_{4,11}=6.9$ Hz, H-11), 2.72 (1H, br d, $J_{4,11}=6.9$ Hz, H-4), 2.33 (1H, s, H-5), 2.15 (2H, m, CH_2-2 isovalerate), 2.05 (3H, d, $J_{2,12}=1.5$ Hz, Me-12), 2.03 (3H, dq, J = 7.5 and 1.5 Hz, Me-4 angelate), 2.00 (1H, m, H-3 isovalerate), 1.88 (3H, dq, $J_d=J_q=1.5$ Hz, Me-5 angelate), 1.16 (3H, s, Me-13), 1.13 (3H, s, Me-14), 0.91 (3H, s, Me-15), 0.93 (3H, d, J = 6.5 Hz, Me-5 isovalerate), 0.92 (3H, d, J = 6.5 Hz, Me-4 isovalerate); ^{13}C NMR spectral data: see Table 1.>

*3.7. 7β-Angeloyloxy-8α-methylbutyroyloxy-9α-hydroxylongipin-2-en-1-one (**4**)*

Colorless oil. UV λ_{max} (EtOH) nm (log ε): 217 (3.86), 250 (3.52); IR, MS and ^{1}H NMR in Bohlmann et al. (1977b); ^{13}C NMR: see Table 1.

[α] =	589	578	546	436	365	($CHCl_3$, c = 1.0)
	+28	+30	+34	+73	+270	

3.8. Selective alkaline hydrolysis of **4**

A solution of **4** (10 mg) in MeOH (1 ml) was treated with a solution (27 μl) of KOH (96 mg) in H_2O (0.5 ml). The reaction mixture was stirred at room temp. for 20 min and extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, filtered and evapd under vacuum. The residue was chromatographed on silica gel. Elution with hexane–EtOAc yielded **2** (4 mg, 50%).

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