Pergamon

PII: S0031-9422(97)00793-0

Phytochemistry, Vol. 48, No. 1, pp. 151–154, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

LONGIPINENE DIESTERS FROM *STEVIA LUCIDA*

DIANA GUERRA-RAMÍREZ, CARLOS M. CERDA-GARCÍA-ROJAS, AURA M. PUENTES† and PEDRO JOSEPH-NATHAN*

Departamento de Química, Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, Apartado 14-740, México, D.F. 07000, México; † Departamento de Quimica, Facultad de Ciencias, Universidad Nacional de Colombia, A.A. 14490, Santafé de Bogotá, D.C., Colombia

(*Received* 21 *July* 1997)

Key Word Index—*Stevia lucida*; Compositae; sesquiterpenes; longipinene diesters; isolation; preparation.

Abstract—The complex mixture of longipinene diesters from the roots of *Stevia lucida* was studied by HPLC, followed by ^{1}H NMR analysis and comparison of the spectral data of the fractions with those of a series of semi-synthetic longipinenes, thus allowing detection of two new substances. Their structures, which were confirmed by partial synthesis, corresponded to longipin-2-ene-7β,9α-diol-1-one-7-tiglate-9-isovalerate and longipin-2-ene-7β,9α-diol-1-one-7-seneciate-9-isovalerate. In addition, 13 known longipin-2-ene-7β,9α-diol-1-one diesters and 3β-friedelinol were isolated.

INTRODUCTION

Stevia lucida is a wild shrub widely distributed in some regions of Colombia [1], Venezuela [2] and Mexico [3]. Previous studies on the aerial parts of this species afforded kaurene [4] and labdane derivatives [3, 5], flavonoids [6] and a longipinene diester [2]. Longipinene esters have been found in several species of *Stevia* [2, 7–17], although in most cases they appear as complex mixtures hindering their isolation and complete characterization [8, 9]. The present paper describes the results of an exhaustive analysis of the chemical components of the roots of *S. lucida* collected in Colombia, which afforded two new longipin-2-ene-7β,9α-diol-1-one diesters by using the methodology that we previously developed to fully analyze and characterize this kind of component [10].

The methodology essentially consists of the separation of small quantities of each component by HPLC, determination of the ^{1}H NMR spectrum of each fraction and comparison of the spectra with those of reference diesters which we prepared in a previous work [11]. When a compound seems to be new, its preparation can easily be achieved from diol **1**, which is obtained in good yields by alkaline hydrolysis of the original diester mixture [12].

RESULTS AND DISCUSSION

The hexane extracts of the roots of *Stevia lucida* were subjected to column chromatography followed by reverse phase HPLC, which provided 15 longipinene diesters (**2**–**16**). Their retention times and approximate percentage are listed in Table 1. Compounds **14** and **15**, which contain an isovalerate residue at C-9, were unknown up to now. Therefore, it was desirable to carry out their preparation to allow their full characterization. Esterification of **1** [12] with

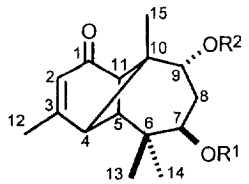
Table 1. Retention time, percentage composition and references of longipinene diesters from *Stevia lucida*

Compound	R_t (min)	(%)	References
2	39	25	[2, 13]
3	56	1	[9]*
4	63	4	[9, 13, 14]*
5	72	2	[9]*
6	77	2	[9]*
7	80	2	[9]*
8	85	3†	[9]*
9	85	5†	[9, 15]*
10	85	3†	[9, 15]*
11	90	12	[9, 13, 16]*
12	96	4	[10]
13	102	18	[7, 9, 13, 14, 16, 17]*
14	118	4	Present work
15	134	3	Present work
16	144	12	[13]

* The stereostructure reported in references prior to 1982 was reassigned in Román *et al.* [18] and Bohlmann *et al.* [14].

† Measured in the ^{1}H NMR spectrum of the ternary mixture.

* Author to whom correspondence should be addressed.



1 $R^1 = R^2 = OH$
2 R^1 = Ang, R^2 = Ac
3 R^1 = Meacr, R^2 = Sen
4 R^1 = Meacr, R^2 = Ang
5 R^1 = Tigl, R^2 = Tigl
6 R^1 = Tigl, R^2 = Sen
7 R^1 = Sen, R^2 = Tigl
8 R^1 = Sen, R^2 = Sen
9 R^1 = Ang, R^2 = Tigl
10 R^1 = Tigl, R^2 = Ang
11 R^1 = Ang, R^2 = Sen
12 R^1 = Sen, R^2 = Ang
13 R^1 = Ang, R^2 = Ang
14 R^1 = Tigl, R^2 = i-Val
15 R^1 = Sen, R^2 = i-Val
16 R^1 = Ang, R^2 = i-Val
17 R^1 = i-Val, R^2 = i-Val
18 R^1 = H, R^2 = i-Val
19 R^1 = i-Val, R^2 = H
20 R^1 = i-Val, R^2 = Tigl
21 R^1 = i-Val, R^2 = Sen

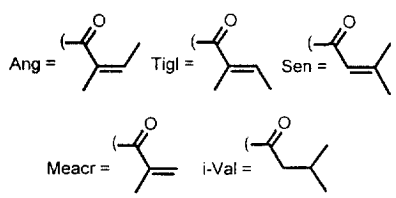


Table 2. ^{1}H NMR 2 data of longipinenes (300 MHz, $CDCl_3$)

H	14	15	17	18	19	20	21
2(*qdd*)	5.80	5.79	5.81	5.78	5.78	5.80	5.81
4(*dd*)	2.65	2.62	2.65	2.61	2.56	2.63	2.62
5(*s*)	2.31	2.30	2.32	2.31	2.26	2.32	2.30
7(*dd*)	5.05	5.05	5.03	3.80	5.06	5.03	5.02
8α(*ddd*)	1.96	2.01	2.00	1.96	1.92	1.99	2.01
8β(*ddd*)	2.17	2.19	2.18	2.22	2.24	2.20	2.18
9(*t*)	5.02	5.00	5.02	5.06	3.86	5.08	5.03
11(*dd*)	3.16	3.16	3.17	3.09	3.11	3.18	3.13
12(*d*)	2.05	2.05	2.07	2.04	2.04	2.05	2.04
13(*s*)	0.97	1.00	1.00	0.96	1.11	0.98	0.97
14(*s*)	1.07	1.03	1.05	0.97	1.02	1.04	1.03
15(*s*)	0.90	0.89	0.92	0.99	0.89	0.91	0.90
R^1 =	Tigl	Sen	*i*-Val	H*	*i*-Val	*i*-Val	*i*-Val
2	—	5.63	2.17†	·	2.19†	2.14†	2.14†
3	6.80	—	2.10†	·	2.11†	2.10†	2.09†
4	1.78	2.14	0.97	—	0.98	0.95	0.95
5	1.90	1.88	0.96	·	0.96	0.92	0.93
R^2 =	*i*-Val	*i*-Val	*i*-Val	*i*-Val	H‡	Tigl	Sen
2	2.33†	2.36†	2.33†	2.22†	—	—	5.80
3	2.17†	2.17†	2.16†	2.11†	—	6.94	—
4	0.99	0.99	0.99	0.98	—	1.82	2.18
5	0.98	0.97	0.98	0.97	—	1.89	1.91

J (Hz): 2,4 = 1.5; 2,11 = 1.5; 2,12 = 1.5; 4,11 = 7.0; 7,8α = 2.0; 7,8β = 11.5; 8α,8β = 15.0; 8α,9 = 4.0; 8β,9 = 3.0. Tigl: 3,4 = 7; 3,5 = 1.5; 4,5 = 1.0. Sen: 2,4 = 2,5 = 1.0. *i*-Val: 3,4 = 3,5 = 6.5.

*OH: δ 1.70 (*br s*).

†Complex signals.

‡OH: δ 2.43 (*br s*).

isovaleryl chloride afforded a mixture of diester **17** and monoesters **18** and **19**, which were easily separated by column chromatography. The presence of the isovaleryl group at C-9 in **18** was deduced from its ^{1}H NMR spectrum, where the signal for H-9 appeared at δ 5.06 as a triplet, while the signal for H-7 appeared at δ 3.80 as a double doublet, as it is characteristic for the longipinene C-9 monoesters [12]. Similarly, the isovaleryl group present in **19** was located at C-7 from the ^{1}H NMR signals of the protons geminal to oxygen, since the signal for H-7 appeared at δ 5.06 as a double doublet and that for H-9 appeared δ 3.86 as a triplet. Treatment of **18** with tigloyl or senecioyl chloride yielded diesters **14** and **15**, respectively, whose ^{1}H NMR spectra were identical to those of the natural products isolated from *S. lucida*. Diesters **20** and **21** were also prepared by esterification of monoester **19** with tigloyl or senecioyl chloride, respectively. The new compounds (**14**, **15**, and **17**–**21**) were fully characterized by physical methods including ^{1}H and ^{13}C NMR for which it was necessary to resort to ^{1}H/^{13}C heteronuclear correlation diagrams.

EXPERIMENTAL

General

Mps: uncorr. ^{1}H (Table 2) and ^{13}C NMR spectra (Table 3) were measured at 300 and 75.4 MHz, respectively, from $CDCl_3$ solutions with TMS as the int. standard. CC were performed on activated alumina (70–290 mesh) or silica gel (70–230 mesh).

Plant material

Stevia lucida Lag. was collected at Vereda El Charquito, Soacha Municipality, Department of Cundinamarca, Colombia in April 1996. A voucher specimen is deposited at the Herbario Nacional Colombiano (COL 388247) where Dr Santiago Díaz Piedrahita, from Instituto de Ciencias Naturales de la Universidad Nacional de Colombia, kindly identified the plant material.

Extraction and isolation

The air-dried roots (400 g) of *Stevia lucida* were extracted exhaustively (×4) with hexane at room

Table 3. ^{13}C NMR data of longipinenes (75.4 MHz, $CDCl_3$)

C	14	15	17	18	19	20	21
1	203.1	203.1	203.0	203.5	203.6	203.0	203.1
2	122.8	122.7	122.8	122.7	122.8	122.8	122.8
3	170.3	170.3	170.3	170.3	170.6	170.3	170.3
4	48.5	48.5	48.4	48.4	48.8	48.5	48.9
5	65.9	65.9	65.8	66.2	66.0	65.9	65.9
6	37.5	37.3	37.2	38.2	37.2	37.3	37.3
7	72.4	71.3	72.2	70.3	72.5	72.3	72.3
8	32.3	32.4	32.4	35.7	35.7	32.7	32.5
9	74.8	74.8	74.7	75.1	73.3	74.7	73.8
10	55.7	55.6	55.6	55.7	57.3	56.0	56.0
11	53.8	53.8	53.7	53.6	52.9	53.9	53.8
12	23.3	23.3	23.3	23.4	23.3	23.3	23.3
13	21.3	21.3	21.7	21.3	21.7	21.3	21.3
14	19.0	18.9	18.9	17.7	18.9	18.9	18.9
15	26.2	26.1	26.1	26.4	26.2	26.2	26.2
R^1 =	Tigl*	Sen	*i*-Val	H	*i*-Val	*i*-Val	*i*-Val
1	167.0	165.5	172.7	—	172.8	172.0	172.1
2	129.0	116.0	43.8		43.9	43.9	43.9
3	137.0	156.7	25.6	—	25.7	25.7	25.7
4	14.3	20.1	22.5	—	22.5	22.5	22.5
5	12.1	27.3	22.4		22.4	22.4	22.4
R^2 =	*i*-Val	*i*-Val	*i*-Val	*i*-Val	H	Tigl*	Sen
1	172.8	172.8	172.0	172.4	—	167.4	166.0
2	43.5	43.5	43.5	43.7	—	128.5	116.0
3	25.5	25.5	25.7	25.8	—	138.1	157.5
4	22.4	22.4	22.5	22.4		14.5	20.4
5	22.4	22.4	22.4	22.4	—	12.1	27.5

* Assigned according to data described in Ref. [18].

temp. for 1 week each time. Filtration and evaporation of the extract afforded a dark yellow syrup (20 g) which was subjected to CC on alumina (60 g). Elution with toluene provided nine fractions totaling 14 g. Fr 3 (6.5 g) was processed by reverse phase HPLC. The optimal chromatographic conditions were as follows: 4.75 mg of sample in 10 μl of MeOH–H_2O (8:2) injected to C-18 reverse phase column (i.d. 4 mm, length 150 mm + 40 mm pre-column), using EtOH–H_2O (46:54) as the mobile phase at 0.9 ml min^{-1} and a UV detector operated at 254 nm. Thirteen frs were collected during 12 successive runs. Each fr was analyzed by 300 MHz ^1H NMR spectroscopy. The spectra revealed the presence of one component per peak (**2**–**7** and **11**–**16**), excepting fr 7 which contained three substances (**8**–**10**). This mixture was further processed by HPLC using similar conditions, but with MeOH–H_2O (60:40) as the mobile phase. Compound **8** was obtained in pure form. Although compounds **9** and **10** remained as a mixture, its identification was easy by comparison of the ^1H NMR spectra of the two semi-synthetic constituents with that of the mixture. Fr 8 (5 g) of the original chromatography on alumina was rechromatographed on silica gel (60 g). Elution with petrol afforded friedelinol (65 mg).

Reaction of **1** *with isovaleryl chloride*

A soln of **1** [12] (1.0 g) in CH_2Cl_2 (50 ml) was treated with isovaleryl chloride (1.5 ml), stirred at room temp. for 15 min, treated with aq. $NaHCO_3$ 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 CH_2Cl_2 afforded diester **17**, followed by monoester **18**. Further elution with CH_2Cl_2–MeOH (95:5) gave **19**, followed by **1** (122 mg, 12%). Crystallization from CH_2Cl_2–hexane afforded pure **17** (268 mg, 10%), **18** (147 mg, 11%) and **19** (214 mg, 16%).

Longipin-2-ene-7β,9α-diol-1-one 7,9-diisovalerate (**17**). White powder mp 54–55°; $[\alpha]_{589}$ +46°, $[\alpha]_{578}$ +49°, $[\alpha]_{546}$ +59°, $[\alpha]_{436}$ +124°, $[\alpha]_{365}$ +376° ($CHCl_3$; c 1.25). EIMS 20 eV, *m/z* (rel. int.): 418 $[M]^+$ (5), 232 (61), 214 (51), 173 (46), 122 (70), 85 (100), 57 (93). UV λ_{max}^{EtOH} nm (log ε): 249 (3.82); IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 1728 (isovalerate), 1724 (isovalerate), 1674 and 1616 (C=C—C=O).

Longipin-2-ene-7β,9α-diol-1-one 9-isovalerate (**18**). White powder mp 114–116°; $[\alpha]_{589}$ +75°, $[\alpha]_{578}$ +79°, $[\alpha]_{546}$ +93°, $[\alpha]_{436}$ +195°, $[\alpha]_{365}$ +609° ($CHCl_3$; c 0.1). EIMS 20 eV, *m/z* (rel. int.): 335 $[M+H]^+$ (1), 250 (24), 232 (22), 189 (36), 173 (41), 135 (39), 122 (100), 109 (37), 57 (8). UV λ_{max}^{EtOH} nm (log ε): 201 (3.61), 250 (3.86); IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 3684 and 3614 (OH), 1727 (isovalerate), 1672 and 1618 (C=C—C=O).

Longipin-2-ene-7β,9α-diol-1-one 7-isovalerate (**19**). White needles mp 121–123°; $[\alpha]_{589}$ +52°, $[\alpha]_{578}$ +57°, $[\alpha]_{546}$ +66°, $[\alpha]_{436}$ +136°, $[\alpha]_{365}$ +473° ($CHCl_3$; c 0.1). EIMS 20 eV, *m/z* (rel. int.): 335 $[M+H]^+$ (5), 232 (16), 189 (25), 173 (26), 135 (74), 122 (100), 109 (25), 85 (45), 57 (66). UV λ_{max}^{EtOH} nm (log ε): 202 (3.65), 251 (3.91); IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 3684 and 3620 (OH), 1726 (isovalerate), 1672 and 1616 (C=C—C=O).

Longipin-2-ene-7β,9α-diol-1-one 7-tiglate-9-isovalerate (**14**). A soln of **18** (40 mg) in CH_2Cl_2 (15 ml) was treated with tigloyl chloride (30 μl), stirred at room temp. for 24 h, evapd to a small volume, treated with aq. $NaHCO_3$ 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 CH_2Cl_2 yielded **14** (39 mg, 78%) as a colorless oil; $[\alpha]_{589}$ +38°, $[\alpha]_{578}$ +41°, $[\alpha]_{546}$ +48°, $[\alpha]_{436}$ +106°, $[\alpha]_{365}$ +321° ($CHCl_3$; c 0.17). EIMS 20 eV, *m/z* (rel. int.): 416 $[M]^+$ (4), 333 (12), 232 (52), 214 (49), 173 (20), 135 (21), 122 (33), 83 (100), 57 (14). UV λ_{max}^{EtOH} nm (log ε): 215 (4.05), 252 (3.81); IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 1726 and 1618 (tiglate), 1726 (isovalerate), 1672 and 1618 (C=C—C=O).

Longipin-2-ene-7β,9α-diol-1-one 7-seneciate-9-isovalerate (**15**). A soln of **18** (40 mg) in CH_2Cl_2 (15 ml) was treated with senecioyl chloride (30 μl), stirred at room temp. for 24 h, evapd to a small volume, treated with aq. $NaHCO_3$ 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 CH_2Cl_2 yielded **15** (38 mg, 76%) as a colorless oil; $[\alpha]_{589}$ +47°, $[\alpha]_{578}$ +50°, $[\alpha]_{546}$ +59°, $[\alpha]_{436}$ +126°, $[\alpha]_{365}$ +389° ($CHCl_3$; c 0.22). EIMS 20 eV, *m/z* (rel. int.): 416 $[M]^+$ (1), 334 (17), 232 (33), 214 (28), 173 (10), 135 (12), 122 (16), 83 (100), 57 (7). UV λ_{max}^{EtOH} nm (log ε): 217 (4.19), 251.6 (3.80); IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 1710 and 1616 (seneciate), 1718 (isovalerate), 1672 and 1616 (C=C—C=O).

Longipin-2-ene-7β,9α-diol-1-one 7-isovalerate-9-tiglate (**20**). A soln of **19** (100 mg) in CH_2Cl_2 (25 ml) was treated with tigloyl chloride (100 μl), stirred at room temp. for 24 h, evapd to a small volume, treated with aq. $NaHCO_3$ 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 CH_2Cl_2 yielded **20** as a white solid. Recrystallization from CH_2Cl_2–hexane afforded the pure compound (112 mg, 90%) as white needles mp 114–117°; $[\alpha]_{589}$ +78°, $[\alpha]_{578}$ +83°, $[\alpha]_{546}$ +96°, $[\alpha]_{436}$ +191°, $[\alpha]_{365}$ +505° ($CHCl_3$; c 0.1). EIMS 20 eV, *m/z* (rel. int.): 416 $[M]^+$ (3), 332 (29), 232 (28), 214 (36), 173 (30), 135 (11), 122 (24), 83 (100), 57 (18). UV λ_{max}^{EtOH} nm (log ε): 216 (4.12), 250 (3.84); IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 1724 (isovalerate) 1716 and 1618 (tiglate), 1674 and 1618 (C=C—C=O).

Longipin-2-ene-7,β,9α-diol-1-one 7-isovalerate-9-seneciate (**21**). A soln of **19** (100 mg) in CH_2Cl_2 (25 ml) was treated with senecioyl chloride (100 μl), stirred at room temp. for 24 h, evapd to a small volume, treated with aq. $NaHCO_3$ 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 CH_2Cl_2 yielded **21** as a white solid. Recrystallization from CH_2Cl_2–hexane afforded the pure compound (110 mg, 88%) as white needles mp 116–117°; $[\alpha]_{589}$ +76°, $[\alpha]_{578}$ +81°, $[\alpha]_{546}$ +94°, $[\alpha]_{436}$ +194°, $[\alpha]_{365}$ +532° ($CHCl_3$; c 0.1). EIMS 20 eV, *m/z* (rel. int.): 416 $[M]^+$ (2), 332 (10), 232 (22), 214 (24), 173 (16), 135 (7), 122 (18), 83 (100), 57 (10). UV λ_{max}^{EtOH} nm (log ε): 219 (4.32), 252 (3.99); IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 1727 (isovalerate), 1714 and 1618 (seneciate), 1672 and 1618 (C=C—C=O)

Acknowledgments—We are grateful to Dr Santiago Díaz Piedrahita (Instituto de Ciencias Naturales, Universidad Nacional de Colombia) for the identification of the plant material. We also thank COLCIENCIAS-Colombia, CYTED (Spain) and CoNaCyT (México) for stimulating support.

REFERENCES

1. Robinson, B. L., *Contributions from the Gray Herbarium of Harvard University*, 1931, **96**, 28.
2. Amaro, J. M., Adrián, M., Cerda, C. M. and Joseph-Nathan P., *Phytochemistry*, 1988, **27**, 1409.
3. Salmón, M., Ortega, A., García de la Mora, G. and Angeles, E., *Phytochemistry*, 1983, **22**, 1512.
4. Amaro-Luis, J. M., *Phytochemistry*, 1993, **32**, 1611.
5. Amaro-Luis, J. M. and Hung, M., *Acta Científica Venezolana*, 1988, **39**, 21.
6. Amaro, J. M. and Hung, M., *Revista de la Facultad de Farmacia, Universidad de Los Andes*, 1985, **25**, 125.
7. Gil, R. R., Oberti, J. C., Sosa, V. E. and Herz, W., *Phytochemistry*, 1987, **26**, 1459.
8. Bohlmann, F., Ates, N., Jakupovic, J., King, R. M. and Robinson, H., *Phytochemistry*, 1982, **21**, 2691.
9. Bohlmann, F., Suwita, A., Natu, A. A. and Czerson, H. and Suwita, A., *Chemische Berichte*, 1977, **110**, 3572.
10. Joseph-Nathan, P., Cerda-García-Rojas, C. M., Castrejón, S., Román, L. U. and Hernández, J. D., *Phytochemical Analysis*, 1991, **2**, 77.
11. Joseph-Natan, P., Cerda, C. M., Román, L. U. and Hernández, J. D., *Journal of Natural Products*, 1989, **52**, 481.
12. Román, L. U., del Río, R. E., Hernández, J. D., Cerda, C. M., Cervantes, D., Castañeda, R. and Joseph-Nathan, P., *Journal of Organic Chemistry*, 1985, **50**, 3965.
13. Bohlmann, F. and Zdero, C., *Liebigs Annalen der Chemie*, 1985, 1764.
14. Bohlmann, F., Dutta, L. N., Dorner, W., King, R. M. and Robinson, H., *Phytochemistry*, 1979, **18**, 673.
15. Bohlman, F., Zdero, C. and Schöneweiss, S., *Chemische Berichte*, 1976, **109**, 3366.
16. Román, L. U., Hernández, J. D., Castañeda, R., Cerda, C. M. and Joseph-Nathan, P., *Phytochemistry*, 1989, **28**, 265.
17. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H., *Liebigs Annalen der Chemie*, 1986, 799.
18. Joseph-Nathan, P., Wesener, J. R. and Günther, H., *Organic Magnetic Resonance*, 1984, **22**, 190.