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ISOLATION AND PREPARATION OF TWO LONGIPINENE DERIVATIVES FROM *STEVIA SUBPUBESCENS*

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Key Word Index—*Stevia subpubescens*; Compositae; sesquiterpenes; longipinene derivatives; isolation; preparation.

Abstract—The roots of *Stevia subpubescens* Lag. afforded longicyclene, longipin-2-ene-7β,9α-diol-1-one diangelate and longipin-2-ene-7β,9α-diol-1-one 7-angelate-9-seneciate. A synthetic pathway involving the protection of the C-7 hydroxyl group of longipin-2-ene-7β,9α-diol-1-one with *p*-nitrobenzoyl chloride allowed the preparation of the 7-angelate-9-seneciate, while the diangelate was prepared by direct esterification.

INTRODUCTION

Many diesters of longipin-2-ene-7β, 9α-diol-1-one (**1**) have been found as constituents of *Stevia* species [1, 2], although in most of the cases they appear as complex mixtures. This fact hinders their isolation and complete characterization [3, 4]. In contrast, good yields of diol **1** [5] are easily achieved by hydrolysis of natural ester mixtures. Thus, it seems attractive to develop methodology for the selective preparation of such diesters starting from **1**. The present paper reports the isolation of two of these longipinene derivatives (**2** and **7**) [4, 6] from the Mexican plant *S. subpubescens* Lag., as well as their preparation in substantial amounts which allowed their detailed characterization.

RESULTS AND DISCUSSION

Chromatography of the hexane extracts of the roots of *S. subpubescens* afforded longicyclene [7], longipin-2-ene-7β,9α-diol-1-one diangelate (**2**), and longipin-2-ene-7β,9α-diol-1-one 7-angelate-9-seneciate (**7**) [4, 6]. The preparation of the diangelate **2** was achieved by treatment of **1** with angeloyl chloride [8] in methyl cyanide. Under these conditions, tiglic esters, which are usual by-products in several preparations of angelic esters [9, 10], were not obtained. To prepare the 7-angelate-9-seneciate (**7**), a synthetic pathway for placing each ester residue at the required position was developed. Esterification of **1** with *p*-nitrobenzoyl chloride afforded the monoester **3** as the main product and the diester **4** in small amounts. The positional assignment of the *p*-nitrobenzoate group in **3** was deduced from the ^1H NMR chemical shifts and multiplicities of H-7 and H-9 by comparison with those of related monoesters [2, 5]. The difference in chemical reactivity between the hydroxyl groups at C-7 and C-9 is explained by the seven-membered ring conformation of **1** [11] in which the hydroxyl group at C-7 has a *pseudo*-equatorial orientation and that at C-9 has a *pseudo*-axial one. Treatment of **3** with senecioyl chloride yielded the

Table 1. ^{1}H NMR data of compounds **2** to **7**

H	2	3	4	5	6	7
2 (*qdd*)	5.81	5.82	5.90	5.85	5.79	5.80
4 (*dd*)	2.67	2.62	2.82	2.72	2.62	2.66
5 (*s*)	2.34	2.33	2.49	2.38	2.31	2.32
7 (*dd*)	5.11	5.38	5.40	5.32	3.84	5.12
8α (*ddd*)	2.11	2.7–2.0[a]	2.7–2.0[a]	2.7–2.0[a]	1.97	2.08
8β (*ddd*)	2.24				2.24	2.23
9 (*dd*)	5.13	3.93	5.40	5.10	5.07	5.06
11 (*dd*)	3.17	3.19	3.36	3.22	3.08	3.18
12 (*d*)	2.06	2.03	2.13	2.08	2.05	2.06
13 (*s*)	1.02	1.15	1.11	1.03	0.96	0.99
14 (*s*)	1.08	1.20*	1.27*	1.22*	0.96	1.07
15 (*s*)	0.93	0.96*	1.05*	1.00*	1.00	0.92
R^1 =	Ang	*p*NO_2Bz	*p*NO_2Bz	*p*NO_2Bz	H	Ang
2	—	8.21 (*m*)	8.15 (*m*)	8.20 (*m*)	"	"
3	6.03 (*qq*)	8.35 (*m*)	8.33 (*m*)	8.35 (*m*)	—	6.02 (*qq*)
4	1.95 (*dq*)	"	"	"	—	1.96 (*dq*)
5	1.87 (*dq*)	8.35 (*m*)	8.33 (*m*)	8.35 (*m*)	"	1.87 (*dq*)
6	"	8.21 (*m*)	8.15 (*m*)	8.20 (*m*)	—	"
O	"	"	—	"	1.67 (*br s*)	"
R^2 =	Ang	H	*p*NO_2Bz	Sen	Sen	Sen
2	"	"	8.42 (*br s*)	5.85 (*m*)	5.72 (*m*)	5.82 (*m*)
3	6.11 (*qq*)	—	8.42 (*br s*)	—	"	"
4	2.03 (*dq*)	—	—	2.20 (*d*)	2.18 (*d*)	2.19 (*d*)
5	2.01 (*br s*)	—	8.42 (*br s*)	1.95 (*d*)	1.92 (*d*)	1.93 (*d*)
6	—	—	8.42 *br s*)	—	—	—
O	—	2.10 (*br s*)	—	—	—	—

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. Ang: 3,4 = 7.5; 3,5 = 1.5. Sen: 2,4 = 2,5 = 1.0.

* Assignments may be interchanged; [a]Overlapped signals.

diester **5** which was converted by selective alkaline hydrolysis to the monoseneciate **6**, followed by esterification with angeloyl chloride [8] in CCl_4 to afford 7-angelate-9-seneciate (**7**) identical to the natural product.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra were measured from $CDCl_3$ solns containing TMS as the int. standard. CC were performed on activated alumina (80–200 mesh).

Plant material. Stevia subpubescens Lag. was collected at Km 59 of the Morelia-Zacapu road near Constitución, Michoacán, México in November 1981. A voucher specimen is deposited at the Herbarium of Departamento Botánico, ENCB-IPN, Mexico City, where Dr Jerzy Rzedowski identified the plant material.

Extraction and isolation. The air-dried roots (1 kg) of *S. subpubescens* were extracted twice with hexane under reflux for 4 hr. After evapn, the residue was precipitated with MeOH and then filtered. Evaporation of the MeOH soln gave a dark yellow syrup (6 g) which was chromatographed on alumina (60 g). Elution with petrol afforded longicyclene (100 mg) which was identified by comparison of its ^{1}H NMR spectra with that described [7]. Elution with hexane–C_6H_6 (3:1) provided **2** (250 mg) from the initial fractions followed by **7** (70 mg).

Alkaline hydrolysis of the extract of S. subpubescens. The hexane extracts of the roots (4 g) were hydrolysed as described for *S. salicifolia* Cav. [5]. Longipin-2-ene-7β,9α-diol-1-one (**1**) (1.2 g) was obtained as a white powder mp 184° [5].

Longipin-2-ene-7β,9α-diol-1-one diangelate (**2**). A soln of **1** (200 mg) in MeCN (15 ml) was treated with angeloyl chloride (600 mg) and stored at room temp. for 24 hr. The solvent and the unreacted angeloyl chloride were evapd by heating to 40° using a N_2 flow. The residue was dissolved in CH_2Cl_2 and chromatographed over alumina. Elution with CH_2Cl_2 provided the diangelate **2** (280 mg, 85%) as white prisms, mp 113–116°. Recrystallization from CH_2Cl_2–hexane provided the pure sample mp 115–117° (lit [6] 110°); $[\alpha]_D$ + 57° ($CHCl_3$; *c* 0.1); UV λ_{max}^{EtOH} nm (log ε): 219 (3.97), 248 (3.57); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 and 1649 (C=C–C=O, angelates), 1672 and 1619 (C=C–C=O, ketone); ^{1}H NMR (300 MHz) and ^{13}C NMR (75.4 MHz): Tables 1 and 2 respectively.

Reaction of **1** *with p-nitrobenzoyl chloride.* A soln of **1** (3 g) in dry pyridine (75 ml) was treated with *p*-nitrobenzoyl chloride (3 g), stirred under N_2 at 4° for 24 hr, poured over ice-H_2O, and extracted with EtOAc. The organic layer was washed with dil. HCl, H_2O, aq. $NaHCO_3$, and H_2O, dried over Na_2SO_4, filtered and evapd under vacuum. The yellow oily residue was chromatographed on alumina. Elution with CH_2Cl_2 afforded the diester **4** (700 mg, 11%) as slightly yellow needles mp 260–265°. Repeated recrystallization from CH_2Cl_2–MeOH provided the pure sample, mp 296–298°; $[\alpha]_D$ + 91° ($CHCl_3$; *c* 0.1); UV λ_{max}^{EtOH} nm (log ε): 212 (3.96), 256 (4.40); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1723 (C=O, ben-

Table 2. ^{13}C NMR data of compounds **2–7**

C	2	3	4	5	6	7
1	202.9	203.3	202.1	202.3	203.9	203.1
2	122.8	122.6	122.7	122.4	122.6	122.8
3	170.4	170.6	170.0	170.0	171.1	170.4
4	48.8	48.8	48.8	48.2	48.4	48.4
5	65.9	65.8	65.4	65.3	66.4	65.9
6	37.4	37.6	37.5	37.4	38.2	37.4
7	72.3	74.8	74.4	74.5	70.1	72.3
8	32.4	35.7	32.4	32.1	35.8	32.5
9	74.7	73.0	76.6	73.5	74.3	73.9
10	55.9	57.3	55.7	55.7	56.0	56.0
11	54.1	52.8	53.7	53.6	53.7	53.9
12	23.4	23.3	23.4	23.2	23.4	23.3
13	21.4	21.8	21.4	21.1	21.3	21.3
14	19.1	19.1	18.8	18.9	17.7	19.2
15	26.2	26.3	26.2	26.1	26.4	26.2
R^1 =	Ang	pNO$_2$Bz	pNO$_2$Bz	pNO$_2$Bz	H	Ang
1	166.8	135.7	135.3	135.5	—	166.9
2	128.0	130.4	130.4	130.3	—	128.0
3	137.8	123.4	123.4*	123.2	—	137.6
4	15.7	150.3	150.4	150.2	—	15.7
5	20.6	123.4	123.4*	123.2	—	20.6
6	—	130.4	130.4	130.3	—	—
C=O (benzoate)	—	163.9	164.1	163.4	—	—
R^2 =	Ang	H	pNO$_2$Bz	Sen	Sen	Sen
1	167.3	—	135.3	165.5	165.9	166.0
2	128.1	—	130.8	115.6	115.9	116.0
3	138.3	—	123.5*	157.3	157.8	157.3
4	15.8	—	150.4	20.2	20.3	20.3
5	20.6	—	123.5*	27.4	27.5	27.5
6	—	—	130.8	—	—	—
C=O (benzoate)	—	—	163.7	—	—	—

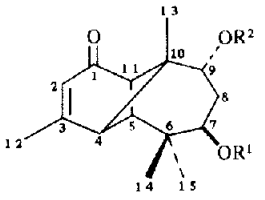
* Assignments may be interchanged.

zoates), 1675 and 1611 (C=C–C=O), 1530 and 1279 (NO_2); ^1H NMR (90 MHz) and ^{13}C NMR (25.1 MHz): Tables 1 and 2 respectively. Elution with $CHCl_3$–EtOH (99:1) yielded the monoester **3** (2.85 g, 60%) as a white powder mp 192–194°. Recrystallization from CH_2Cl_2–hexane provided the pure sample: mp 195–196°; $[\alpha]_D$ + 95° ($CHCl_3$; *c* 0.1); UV λ_{max}^{EtOH} nm (log ε): 212 (3.98), 255 (4.30); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3609 and 3466 (OH), 1722 (C=O, benzoate), 1674 and 1611 (C=C–C=O), 1530 and 1278 (NO_2); ^1H NMR (90 MHz) and ^{13}C NMR (25.1 MHz): Tables 1 and 2 respectively. Finally, elution with EtOAc afforded starting material (**1**) (350 mg, 12%).

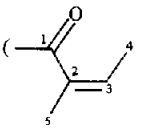
Longipin-2-ene-7β,9α-diol-1-one 7-p-nitrobenzoate-9-seneciate (**5**). A soln of **3** (800 mg) in CH_2Cl_2 (20 ml) was treated with senecioyl chloride (1 g), refluxed during 30 min, evapd to dryness and crystallized from CH_2Cl_2–hexane. Repeated recrystallizations of $CHCl_3$–hexane afforded **5** as slightly yellow needles mp 238–240°; $[\alpha]_D$ + 72° ($CHCl_3$; *c* 0.1); UV λ_{max}^{EtOH} nm (log ε): 221 (4.19), 255 (4.27); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (C=O, benzoate), 1720 and 1651 (C=C–C=O, seneciate), 1673 and 1616 (C=C–C=O, ketone), 1530 and 1277 (NO_2); ^1H NMR (90 MHz) and ^{13}C NMR (25.1 MHz): Tables 1 and 2 respectively.

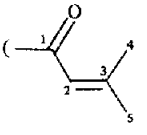
Longipin-2-ene-7β,9α-diol-1-one 9-seneciate (**6**). A soln of **5** (1 g) in MeOH–CH_2Cl_2 (5:1) (120 ml) was treated with a soln of KOH (1.34 g) in H_2O (3 ml), stored at room temp. for 30 min, neutralized with aq. HCl (20%), evapd to a small vol. and extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, filtered and evapd under vacuum. The residue was chromatographed on alumina. Elution with CH_2Cl_2 yielded **6** as white needles, mp 144–147°. Recrystallization from CH_2Cl_2–hexane provided the pure compound: mp 146–148°; $[\alpha]_D$ + 50° ($CHCl_3$; *c* 0.1); UV λ_{max}^{EtOH} nm (log ε): 220 (4.16), 250 (3.75); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610 and 3470 (OH), 1712 and 1651 (C=C–C=O, ester), 1671 and 1617 (C=C–C=O, ketone); ^1H NMR (300 MHz) and ^{13}C NMR (75.4 MHz): Tables 1 and 2 respectively.

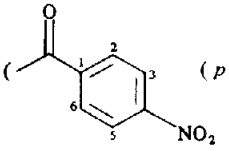
Longipin-2-ene-7β,9α-diol-1-one 7-angelate-9-seneciate (**7**). A soln of **6** (410 mg) in CCl_4 (10 ml) was treated with angeloyl chloride (600 mg), stored at room temp. for 24 hr, and worked-up as in the case for obtaining **2**. The above procedure afforded **7** (332 mg, 65%) as white needles mp 156–160°. Recrystallization from CH_2Cl_2–hexane provided the pure sample: mp 159–161° (lit [6] 150°); $[\alpha]_D$ + 72° ($CHCl_3$; *c* 0.1); UV λ_{max}^{EtOH} nm (log ε): 220



1 $R^1 = R^2 = H$
2 $R^1 = R^2 =$ Ang
3 $R^1 = pNO_2Bz$, $R^2 =$ OH
4 $R^1 = R^2 = pNO_2Bz$
5 $R^1 = pNO_2Bz$, $R^2 =$ Sen
6 $R^1 =$ OH, $R^2 =$ Sen
7 $R^1 =$ Ang, $R^2 =$ Sen

Ang =  (angeloyl)

Sen =  (senecioyl)

pNO_2Bz =  (*p*-nitrobenzoyl)

(4.40), 248 (3.87); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1711 and 1653 (C=C–C=O, esters), 1672 and 1617 (C=C–C=O, ketone); ^1H NMR (300 MHz) and ^{13}C NMR (75.4 MHz): Tables 1 and 2 respectively.

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SESQUITERPENE LACTONES FROM *PYRETHRUM SANTOLINOIDES*

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Key Word Index *Pyrethrum santolinoides*; Compositae; sesquiterpene lactones; germacranolides; heliangolides; eudesmanolide.

Abstract—A reinvestigation of the aerial parts of *Pyrethrum santolinoides* afforded, in addition to known compounds, four new sesquiterpene lactones, three germacranolides and an eudesmanolide. The structures were elucidated by high field ^1H NMR techniques.

INTRODUCTION

Pyrethrum santolinoides DC (= *Tanacetum sinaicum* Del. ex DC) has been studied previously. From the aerial parts some sesquiterpene lactones were reported [1] and from the roots various compounds, including unusual triterpenes, were isolated [2]. We have studied a sample of the aerial parts collected at Wadi Elarbaeen, Sanct Kathe-