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PREPARATION OF A NEW LONGIPINANE DERIVATIVE FROM *STEVIA SERRATA*

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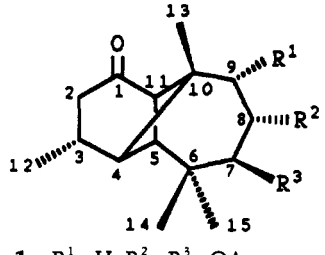
ABSTRACT.—The roots of *Stevia serrata* afforded the new longipinane derivative **1** whose structure and stereochemistry were elucidated from nmr spectral data and confirmed by chemical correlation with rastevione [**2**].

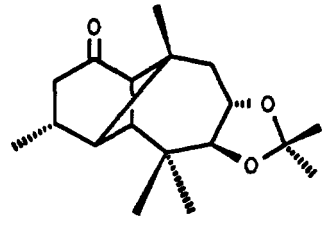
Longipinane derivatives are known as relevent secondary metabolites in many species of the genus *Stevia* (1–4). We described the X-ray structure and some aspects of the chemistry of rastevione [**2**], the main constituent of the roots of *Stevia serrata* Cav. (Compositae), a decade ago (5), and later we established the conformation and absolute configuration of **2** and related plant metabolites (6).

At present, we report the isolation of the minor new longipinane **1** from the roots of *S. serrata*, as well as a synthetic route for the conversion of rastevione [**2**] into **1**. This chemical correlation confirms the structure and stereochemistry of **1.**

RESULTS AND DISCUSSION

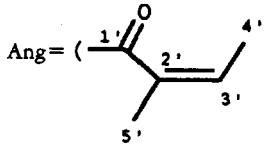
Hexane extracts of the roots of *S. serrata* contained the longipinane derivative **1**, which was isolated by hplc (7) from the mother liquors left after crystallization of rastevione [**2**]. Its ir spectrum indicated the presence of an α,β-unsaturated ester group (1712 and 1646 cm^{-1}) and a keto group (1710 cm^{-1}). The ^1H-nmr spectrum (Table 1) showed characteristic vinyl signals corresponding to angelate groups at 6.09 and 6.06 ppm. The protons geminal to ester groups appeared as a double double doublet at 5.43 ppm for H-8 and a doublet at 5.17 ppm for H-7. This coupling pattern corresponds to





1 R^1=H, R^2=R^3=OAng
2 R^1=OH, R^2=R^3=OAng
3 R^1=R^2=R^3=OH
10 R^1=H, R^2=OTigl, R^3=OH

9



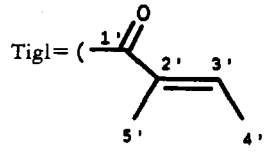


TABLE 1. ^{1}H-nmr Chemical Shifts,[a] Coupling Constants, and Multiplicity[b] for Longipinane Derivatives.

Proton	Compound					
	1[c]	**5**[d]	**6**[e]	**8**[f]	**9**[g]	**10**[h]
H-2α	2.12	2.16	1.72	2.10	2.11	2.11
H-2β	2.55	2.61	2.24	2.53	2.52	2.53
H-3	2.33	2.39	2.25	2.30	2.32	2.31
H-4	2.28	2.27	1.98	2.08	2.17	2.21
H-5	1.81	1.85	1.67	1.74	1.72	1.78
H-7	5.17	5.28	5.26	3.31	3.53	3.56
H-8	5.43	5.36	5.31	3.84	4.04	5.15
H-9α	1.84	—	—	1.59	1.63	1.65
H-9β	2.07	4.89	4.86	2.09	2.10	2.09
H-11	2.76	2.97	2.36	2.59	2.71	2.61
Me-12	1.09	1.11	0.98	1.07	1.07	1.08
Me-13	0.91	1.08	1.18	0.90	0.92	0.88
Me-14	0.94	0.95	0.99	0.96	0.98	1.01
Me-15	1.11	1.07	0.94	1.05	1.04	1.08

[a]In ppm at 300 MHz.

[b]Couplings are in Hz: H-2α for **1**, **5**, **8**, **9**, and **10** (dd, J=6, 19) and for **6** (complex m); H-2β for **1**, **5**, **8**, **9**, and **10** (dd, J=9, 19), and for **6** (complex m); H-3 (m), H-4 (br d, J=6), H-5 (br s), H-7 for **1**, **5**, **6**, and **10** (d, J=12) and for **8** and **9** (d, J=10); H-8 for **1**, **8–10** (ddd, J=5, 11, 11) and for **5** and **6** (dd, J=3, 11); H-9α for **1**, **8–10** (dd, J=11, 14); H-9β for **1**, **8–10** (dd, J=5, 14) and for **5** and **6** (d, J=3); H-11 (d, J=6); Me-12 (d, J=7); Me-13 (s); Me-14 (s); Me-15 (s).

[c]Angelates 6.09 (qq, J=1, 7), 6.06 (qq, J=1, 7), 1.96 (dq, J=1, 7), 1.95 (dq, J=1, 7), 1.84 (quintet, J=1), 1.76 (quintet, J=1).

[d]Methanesulphonate 3.21 (s); acetates 2.09 (s) and 2.08 (s).

[e]Ethyleneketal 3.87 (m); methanesulfonate 3.20 (s); acetates 2.08 and 2.07 (s).

[f]OH's 2.76 (br s)

[g]Acetonide 1.42 (s) and 1.40 (s).

[h]Tiglate 6.90 (qq, J=1, 7), 1.85 (quintet, J=1), 1.81 (dq, J=1, 7); OH 1.59 (br s).

the -Ċ-*CH*(OAng)-*CH*(OAng)-CH_2- moiety. Since the ^{13}C-nmr signals of the new compound **1** (Table 2) are in agreement with a longipinane structure closely related to rastevione [**2**], we assumed its structure and stereochemistry as depicted in **1**.

In order to corroborate this assumption, a chemical correlation was carried out. Alkaline hydrolysis of rastevione [**2**] afforded triolone **3** (6), which was acetylated selectively at C-7 and C-8 to give diacetate **4** (8). This substance **4** was the starting material for the chemical correlation shown in Scheme 1. Treatment of **4** with methanesulfonyl chloride in pyridine gave diacetate methanesulfonate **5**, which was treated with ethylene glycol and *p*-toluenesulfonic acid (9) to yield ethyleneketal diacetate methanesulfonate **6**. $LiAlH_4$ treatment of **6** afforded **7**, which was hydrolyzed using HCl in MeOH to give diolone **8**. When the same reaction was carried out in Me_2CO, acetonide **9** was formed, which was easily hydrolyzed to give **8**. The latter procedure facilitated the isolation of **8**. The ^{1}H-nmr spectrum of diolone **8** showed characteristic signals for the -Ċ-*CH*(OH)-*CH*(OH)-CH_2-Ċ- fragment, as can be seen in Table 1. In addition, the ^{13}C-nmr data were in agreement with structure **8**. This substance and a sample obtained by alkaline hydrolysis of the natural compound **1** were identical in all respects, including optical activity.

The final step in the reaction sequence was introduction of the angeloyl moieties at the hydroxyl groups of **8**; however, this reaction presented serious difficulties. When diolone **8** was treated with angeloyl chloride (10) in the presence of DMAP, only monotiglate **10** could be isolated. After several trials under various reaction conditions,

TABLE 2. ^{13}C-nmr Chemical Shifts[a] for Longipinane Derivatives.

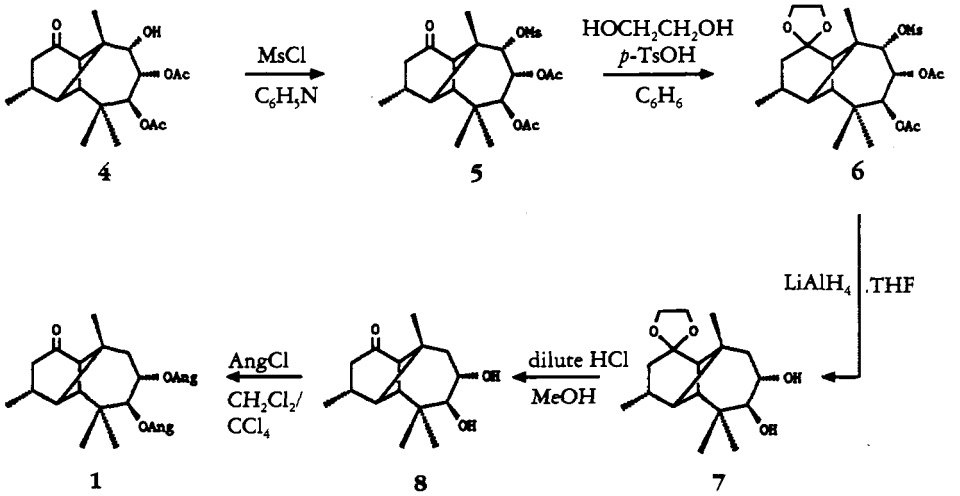
Carbon	Compound				
	1[b]	6[c]	8	9[d]	10[e]
C-1	212.1	113.4	213.5	212.6	212.8
C-2	41.9	39.4	41.9	42.0	41.9
C-3	26.9	28.5	26.9	27.0	26.9
C-4	45.3	44.2	45.1	45.7	44.9
C-5	46.1	47.7	46.1	46.5	46.1
C-6	35.5	34.4	35.5	32.5	35.7
C-7	76.3	71.6	78.9	85.0	77.0[f]
C-8	68.5	69.6	68.5	73.4	72.2
C-9	43.6	87.2	46.5	41.7	43.4
C-10	41.6	44.6	41.5	41.8	41.5
C-11	57.4	42.4	57.4	57.5	57.5
C-12	19.8	19.6	19.7	19.7	19.7
C-13	23.0	20.8	23.2	23.3	23.0
C-14	20.6	20.6	18.8	18.4	18.9
C-15	27.5	26.3	27.8	27.6	27.6

[a]In ppm at 75.4 MHz for $CDCl_3$ solutions.
[b]Angelates 166.8, 166.6, 139.2, 127.6, 127.5, 20.3, 20.3, and 15.7.
[c]Acetates 170.4, 169.4, 20.9, and 20.9; methanesulfonate 39.3; ethyleneketal 64.7 and 63.0.
[d]Acetonide 108.6, 27.4, and 27.0.
[e]Tiglate 167.8, 138.1, 128.5, 14.4, and 12.1.
[f]Overlapped with the $CDCl_3$ signal.

the introduction of the angeloyl groups was achieved by treatment of diolone **8** with angeloyl chloride in $CCl_4CH_2Cl_2$ (11). The reaction product and the natural compound isolated from *S. serrata* showed identical ^{1}H-nmr spectra, providing conclusive evidence for the structure and stereochemistry of the naturally occurring diangelate **1**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Uv spectra were obtained on a *Unicam SP-800 Spectrophotometer* in EtOH. Ir spectra were measured in $CHCl_3$ on a Nicolet MX-1 spectrophotometer. Specific rotations were determined in $CHCl_3$ on a Perkin-Elmer 241 polarimeter. Nmr



SCHEME 1. Reaction sequence to achieve the chemical correlation between rastevione [**2**] and diangelate [**1**].

measurements were performed at 300 MHz for ^1H and 75.4 MHz for ^{13}C from $CDCl_3$ solution containing TMS as the internal standard on a Varian Associates XL-300GS spectrometer. Cc was conducted on Merck Si gel 60 (70–230 mesh ASTM). Hplc separations were carried out on a Varian Associates Vista 5500 equipment using a Micropack MCH-5-N-CAP reversed-phase column, i.d. 4 mm, length 150 mm+40 mm (precolumn). Mass spectra were recorded at 70 eV on a Hewlett Packard 5989 A spectrometer. The microanalysis was performed by the Microanalytical Laboratory Elbach, Germany.

NATURAL DIANGELATE **1**.—*S. serrata* was collected in La Galera (km 65 Morelia-Mexico City highway), State of Michoacán, México in October 1988 and identified by Dr. Jerzy Rzedowski (Departmento Botánico, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City), where a sample (Voucher No. Román 1978) is deposited (5). The air-dried roots (1.2 kg) were processed as previously described (5) to afford a yellow viscous oil. Crystallization from $CHCl_3$/hexane gave 4.5 g of rastevione (5) The mother liquors were evaporated to dryness and chromatographed on a Si gel column. The fractions eluted with hexane-CH_2Cl_2 (1:1) were combined to give a yellow oil, which was dissolved in MeOH. Samples containing ca. 1 mg of the oil were separated by hplc eluting with MeOH-H_2O (8:2), with a flow rate of 1 ml/min. A uv detector operating at 250 nm was employed. Under these conditions, diangelate **1** had a retention time of 8.8 min. Each run afforded ca. 0.8 mg of **1** as a colorless oil: uv λ max 220 nm (log ε 4.1); ir ν max 1712 and 1646 (C=C-C=O) and 1710 cm^{-1} (C=O); $[\alpha]_{589}+1$, $[\alpha]_{578}+2$, $[\alpha]_{546}+4$, $[\alpha]_{436}+2$, $[\alpha]_{365}-1$ (c=0.84); ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (rel. int.) $[M]^+$ 416.4 (2), 316.3 (4), 217.3 (4), 162.3 (3), 83.2 (100), 55.1 (39).

DIACETATE METHANESULFONATE **5**.—A solution of **4** (8) (1 g) in pyridine (3 ml) was treated with methanesulfonyl chloride (1 ml). The reaction mixture was stored at 0° for 24 h, poured into ice-H_2O, and extracted with EtOAc. The organic layer was washed with diluted HCl, H_2O, aqueous $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was crystallized from EtOH to afford **5** (870 mg). Recrystallizations from EtOH provided the pure compound as white needles: mp 185–187°; ir ν max 1735 (C=O, acetates), 1715 (C=O, ketone), 1355 cm^{-1} (S-O); $[\alpha]_{589}$ −29, $[\alpha]_{578}$ −30, $[\alpha]_{546}$ −32, $[\alpha]_{436}$ −53, $[\alpha]_{365}$ −128 (c=1.50); ^1H nmr see Table 1. *Anal.* calcd for $C_{20}H_{30}O_8S$, C 55.80, H 7.02, O 29.73, S 7.44 (found C 55.75, H 6.90, O 29.60, S 7.60).

ETHYLENEKETAL **6**.—A solution of diacetate methanesulfonate **5** (1 g) in C_6H_6 (34 ml) was treated with ethylene glycol (10 ml) and *p*-toluenesulfonic acid (200 mg). The reaction mixture was refluxed for 4 h with a Dean-Stark trap, poured into ice-aqueous Na_2CO_3, and extracted with CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was chromatographed on a Si gel column. The fractions eluted with hexane-EtOAc (75:25) gave **6** as a white solid (825 mg), mp 165–167°. Recrystallization from hexane/EtOAc provided the pure compound as a white powder: mp 168–170°; ir ν max 1741 (C=O, acetate), 1174 (ketal), 1354 and 1035 (S=O), 914 cm^{-1} (S-O); $[\alpha]_{589}$ +5, $[\alpha]_{578}$ +8, $[\alpha]_{546}$ +9, $[\alpha]_{436}$ +12, $[\alpha]_{365}$ +15 (c=0.42); ^1H nmr see Table 1, ^{13}C nmr see Table 2.

DIOLONE **8**.—A solution of ethyleneketal **6** (1 g) in anhydrous THF (50 ml) was treated with $LiAlH_4$ (1 g). The reaction mixture was stirred at room temperature for 2 h, refluxed for 2 h, and concentrated. The solution was treated with 50 ml of EtOAc and 10 ml of H_2O and stirred at room temperature for 30 min. After filtration, the organic layer was separated, washed with H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue, diol **7**, was immediately treated with aqueous HCl (2.3 ml) in MeOH (50 ml). The reaction mixture was refluxed for 5 min, evaporated to one-half, poured into ice-H_2O, and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was chromatographed on Si gel. The fractions eluted with hexane-EtOAc (6:4) gave a white solid, which was recrystallized from CH_2Cl_2/hexane to afford **8** (98 mg) as white needles: mp 124–125°; ir ν max 3541 and 3445 (OH), 1701 cm^{-1} (C=O); $[\alpha]_{589}$ +42, $[\alpha]_{578}$ +44, $[\alpha]_{546}$ +49, $[\alpha]_{436}$ +77, $[\alpha]_{365}$ +87 (c=0.10); ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (rel. int.) $[M]^+$ 252.3 (24), 190.3 (13), 179.3 (15), 121.2 (28), 111.2 (88), 109.2 (46), 96.2 (86), 91.2 (44), 83.2 (40), 67.1 (20), 55.1 (71), 41.1 (100).

DIOLONE ACETONIDE **9**.—A solution of **7** (600 mg) in Me_2CO (50 ml) was treated with aqueous HCl (2.3 ml). The reaction mixture was refluxed for 5 min, concentrated, poured into ice-H_2O, and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was chromatographed on a Si gel column. The fractions eluted with hexane-EtOAc (8:2) gave **9** (140 mg) as white needles, mp 95–97°. Recrystallizations from Me_2CO/hexane provided the pure compound as white needles: mp 97–98°; ir ν max 1708 (C=O) cm^{-1} $[\alpha]_{589}$ +26, $[\alpha]_{578}$ +26, $[\alpha]_{546}$ +29, $[\alpha]_{436}$ +44, $[\alpha]_{365}$ +41 (c=0.14); ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (rel. int.) $[M-15]^+$ 277.3 (65), 217.3 (17), 133.3 (11), 121.2 (10), 107.2 (12), 93.1 (14), 69.1 (25), 55.0 (25), 43.0 (100), 41.0 (45).

HYDROLYSIS OF ACETONIDE **9**.—A solution of **9** (100 mg) in MeOH (15 ml) was treated with aqueous HCl (1 ml). The reaction mixture was refluxed for 15 min, concentrated, poured into ice-H_2O, and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was crystallized from CH_2Cl_2/hexane to give diolone **8** (72 mg) as white needles, mp 124–125°, identical in all respects with the compound reported above.

SYNTHETIC DIANGELATE **1**.—A solution of diolone **8** (50 mg) in CH_2Cl_2 (1.5 ml) was treated with a solution of angeloyl chloride (174 mg) in CCl_4 (5 ml). The reaction mixture was stored at room temperature for 48 h, evaporated to dryness, treated with aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was treated with MeOH (15 ml) and a solution of KOH (50 mg) in H_2O (2 ml) to remove a polymeric material formed during the reaction. The reaction mixture was stirred at room temperature for 1 h, neutralized with a solution of HCl (10%), concentrated, poured into ice-H_2O, and extracted with EtOAc. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was chromatographed on a Si gel column. The fractions eluted with hexane-EtOAc (9:1) gave diangelate **1** (7 mg) as a colorless oil, identical with the natural product.

MONOTIGLATE **10**.—A solution of diolone **8** (50 mg) in CH_2Cl_2 (1.5 ml) was treated with angeloyl chloride (96 mg) in the presence of DMAP (48 mg). The reaction mixture was stored at room temperature for 48 h, poured into ice-H_2O, and extracted with EtOAc. The organic layer was washed with a solution of HCl (10%), H_2O, aqueous $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was chromatographed using a Si gel column. The fractions eluted with hexane-EtOAc (7:3) gave monotiglate **10** as a white solid, mp 118–121°, which was recrystallized from CH_2Cl_2/hexane to give **10** (10 mg) as white needles: mp 121–123°; uv λ max 223 nm (log ε 3.9); ir ν max 3622 and 3420 (O-H), 1706 (C=O), 1706 and 1648 cm^{-1} (C=C-C=O); $[\alpha]_{589}$ +41, $[\alpha]_{578}$ +43, $[\alpha]_{546}$ +47, $[\alpha]_{436}$ +81, $[\alpha]_{365}$ +116 (*c*=0.14); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

HYDROLYSIS OF NATURAL DIANGELATE [**1**].—A solution of diangelate **1** (10 mg) in MeOH (2 ml) was treated with a solution of KOH (10 mg) in H_2O (0.2 ml). The reaction mixture was stirred at room temperature for 45 min, poured into ice-H_2O, and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue (5.5 mg) and synthetic **8** were identical.

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