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Longipinane Derivatives from *Stevia connata*

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The hexane extracts of the roots of *Stevia connata* afforded three new longipinene derivatives, longipinane-7β,8α,9α-triol-1-one 7-angelate-8-methylbutyrate (**1**), longipin-2-ene-7β,8α,9α-triol-1-one 8,9-diangelate (**6**), and longipin-2-ene-7β,8α,9α-triol-1-one 8-angelate-9-methylbutyrate (**8**), together with the known longipinane-7β,8α,9α-triol-1-one 8,9-diangelate (**2**), longipinane-7β,8α,9α-triol-1-one 7,9-diangelate (**3**), longipinane-7β,8α,9α-triol-1-one 7,8-diangelate (**4**), longipin-2-ene-7β,8α,9α-triol-1-one 7,8-diangelate (**5**), longipin-2-ene-7β,8α,9α-triol-1-one 7-angelate-8-methylbutyrate (**12**), and stigmasterol. The structures of the new compounds were determined by chemical transformations and spectral methods including 2D NMR measurements. Spontaneous intramolecular transesterifications starting from the 8-angelate-9-methylbutyrate **8** provided an equilibrated mixture of the 7-angelate-9-methylbutyrate **10**, the 7-angelate-8-methylbutyrate **12** and the starting material when stored in MeOH−H_2O solution, while the 8,9-diangelate **6** only provided a binary mixture of the 7,9-diangelate **7** and the starting material under the same conditions. The structures of **6**−**8**, **10**, and **12** and those of the nonisolable reaction intermediates **9**, **11**, and **14** were further evaluated by AM1 semiempirical calculations.

The New World genus *Stevia*, comprised by *ca.* 230 species, is distributed from southwestern United States to central Argentina. Although its chemistry is not very uniform, the main secondary metabolites of the genus are sesquiterpene lactones, diterpenes, and longipinenes, as recently reviewed.[1] Continuing the search for new substances from North American representatives of this genus,[2−9] we studied the hexane extracts of the roots of *Stevia connata* Lag. (Compositae), a wild shrub which grows in the mountains of the state of Michoacán, Mexico.

Results and Discussion

This study afforded the new substances **1**, **6**, and **8**. Compound **1** was isolated by HPLC. Its IR spectrum indicated the presence of a saturated ester carbonyl group (1733 cm^{-1}), an α,β-unsaturated ester group (1710 and 1648 cm^{-1}), and a ketone (1716 cm^{-1}). The mass spectrum showed $[M - H_2O]^+$ at *m/z* 416, consistent with the molecular formula $C_{25}H_{38}O_6$. The ^1H NMR spectrum showed signals characteristic of an angelate and a methylbutyrate ester residue. The signals for the protons geminal to the oxygen atoms appeared as a doublet at δ 5.50 (J = 11 Hz), a double doublet at δ 5.39 (J = 11 and 3 Hz), and a doublet at δ 3.75 (J = 3 Hz) and were ascribed to H-7, H-8, and H-9, respectively. The position of the individual ester groups at C-7 and C-8 was determined in a HMBC experiment. The signal at δ 166.5, which corresponds to the angelate carbonyl group, was correlated with the doublet at δ 5.50, corresponding to H-7. Similarly, the resonance at δ 175.1 which corresponds to the methylbutyrate carbonyl group was correlated with the double doublet at δ 5.39 which corresponds to H-8. Therefore, the angelate group was located at C-7 and the methylbutyrate group at C-8. The remaining ^1H and ^{13}C NMR data, given in the Experimental Section and in Table 1, respectively, indicated the presence of the longipinene moiety[2−9] in agreement with structure **1** (Chart 1).

Compound **6** was also isolated by HPLC. Its IR spectrum indicated the presence of α,β-unsaturated ester groups (1718 and 1648 cm^{-1}), and an α,β-unsaturated ketone (1674 and 1618 cm^{-1}). The mass spectrum showed $[M]^+$ at *m/z* 430, in agreement with the molecular formula $C_{25}H_{34}O_6$. The ^1H NMR spectrum showed signals characteristic of two angelate groups. In this case, the signals for the protons geminal to the oxygen atoms appeared as a double doublet at δ 3.93 (J = 11 and 4 Hz), which became a doublet upon addition of D_2O, a double doublet at δ 5.31 (J = 11 and 3 Hz), and a doublet at δ 5.60 (J = 3 Hz) and were assigned to H-7, H-8, and H-9, respectively. These chemical shifts, multiplicities and coupling constant values are indicative of a longipin-2-ene-7β,8α,9α-triol-1-one 8,9-diester, as it has been observed in related derivatives.[2,6,9] Therefore, this new natural product is **6**, in agreement with the remaining ^1H NMR and ^{13}C NMR spectral data given in the Experimental Section and Table 1, respectively.

Compound **8** was isolated by Si gel column chromatography. Its IR spectrum showed absorptions for a saturated ester carbonyl group (1730 cm^{-1}), an α,β-unsaturated ester group (1720 and 1646 cm^{-1}), and an α,β-unsaturated ketone (1674 and 1618 cm^{-1}). The mass spectrum showed $[M]^+$ at *m/z* 432, consistent with the molecular formula $C_{25}H_{36}O_6$. The ^1H NMR spectrum indicated the presence of an angelate and a methylbutyrate ester residue. The signals for the protons geminal to the oxygen atoms now appeared as a doublet at δ 3.91 (J = 11 Hz), a double doublet at δ 5.27 (J = 11 and 3 Hz), and a doublet at δ 5.50 (J = 3 Hz), corresponding to H-7, H-8, and H-9, respectively. These signals, together with the remaining ^1H NMR and ^{13}C NMR spectral data (see Experimental Section and Table 1, respectively), also indicate the presence of a longipin-2-ene-7β,8α,9α-triol-1-one 8,9-diester.

Positional assignment of the angelate and methylbutyrate groups in **8** was ascertained according to the

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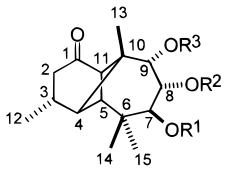
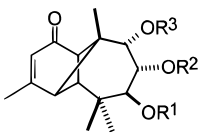
Table 1. ^{13}C NMR Data of Longipinene Derivatives **1**, **6**, and **8** (75.4 MHz, $CDCl_3$)

carbon	**1**	**6**[a]	**8**
1	211.7	202.6	202.6
2	42.0	122.8	122.7
3	26.8	170.1	170.2
4	44.3	48.1	48.2
5	46.4	65.8	65.9
6	35.3	36.9	36.9
7	70.4	71.0	70.6
8	71.2	71.5	71.6
9	75.3	74.0	73.9
10	45.8	54.9	54.9
11	51.6	53.4	53.2
12	19.7	23.3	23.3
13	20.5	21.0	21.0
14	20.1	18.7	18.7
15	27.1	26.7	26.6
	Ang	*Ang*	*Ang*
1	166.5	167.0	167.2
2	127.4	127.4	126.9
3	140.3	140.5	140.1
4	16.0	15.9	15.9
5	20.7	20.7	20.4
	Mebu	*Ang*	*Mebu*
1	175.1	166.7	175.5
2	41.2	126.9	41.3
3	26.3	139.3	26.7
4	11.6	15.9	11.7
5	16.3	20.3	16.6

[a] Pairs of ester signals may be interchanged.

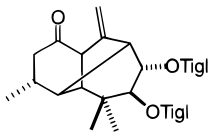
Chart 1



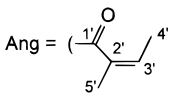


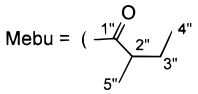
	R^1	R^2	R^3
1	Ang	Mebu	H
2	H	Ang	Ang
3	Ang	H	Ang
4	Ang	Ang	H

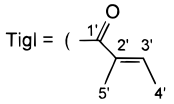
	R^1	R^2	R^3
5	Ang	Ang	H
6	H	Ang	Ang
7	Ang	H	Ang
8	H	Ang	Mebu
10	Ang	H	Mebu
12	Ang	Mebu	H



13







following findings. Compound **8**, was stable in solvents like hexane, $CHCl_3$ or EtOAc. However, when left in 3:2 MeOH−H_2O solution, it underwent intramolecular transesterification to afford **10**, **12**, and starting material **8**. Diester **12** was isolated by us from *Stevia porphyrea*, and the positional assignment of the ester residues confirmed[8] by selective alkaline hydrolysis of the methylbutyrate residue. Therefore, the structures of **8** and **10** follow from the transesterification process.

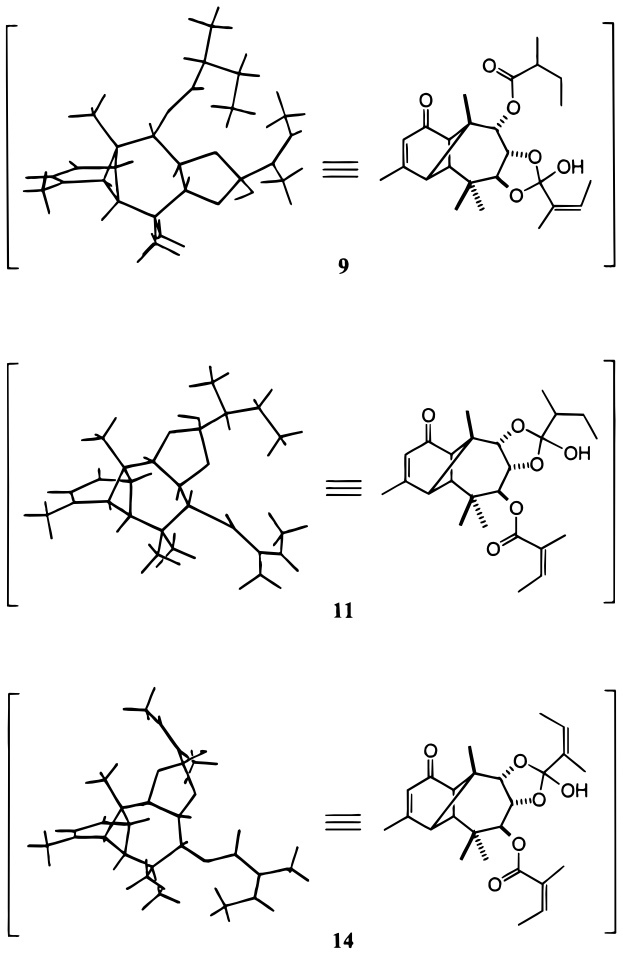


Figure 1. AM1 semiempirical structures of intermediates **9**, **11**, and **14**.

Compounds **10** and **12** were separated by HPLC and left in 3:2 MeOH−H_2O solution as was done with **8**. After 2 weeks, diester **8** yielded a mixture of **8** (58%), **10** (37%), and **12** (5%), while diester **10** gave a mixture of **8** (52%), **10** (38%), and **12** (10%), and diester **12** yielded a mixture of **8**, **10**, and **12** but in 18, 13, and 69%, respectively, indicating that in fact there is an equilibrium among the three diesters but that diester **12** takes longer to equilibrate than **8** or **10**.

In order to shed light on these findings, the geometry of **8**, **10**, and **12**, as well as that for nonisolable intermediates **9** and **11**, were minimized using the MMX[10] and SYBYL[11] force fields and then calculated at the AM1 semi-empirical level.[12,13] The minimized structures of the intermediates are depicted in Figure 1. The values for intermediates **9** ($\Delta H_f = -227.5$ kcal/mol) and **11** ($\Delta H_f = -226.6$ kcal/mol), in comparison with those for structures **8** ($\Delta H_f = -229.0$ kcal/mol), **10** ($\Delta H_f = -226.5$ kcal/mol) and **12** ($\Delta H_f = -225.6$ kcal/mol), predict that longipin-2-ene-7β,8α,9α-triol-1-one diesters can undergo intramolecular transesterification, and that the orientation the oxygen atoms at C-7 and C-8 is *pseudo*-equatorial while at C-9 it is *pseudo*-axial, in agreement with an early conformational analysis based on ^{1}H-NMR data of longipin-2-ene-7β,8α,9α-triol-1-one derivatives.[14] Therefore, ester migration from C-8 to C-7 involves a *trans*-diequatorial intermediate (**9**), while migration from C-9 to C-8 involves a *cis*-equatorial−axial intermediate (**11**) (Figure 1). The fact that diester **12** takes longer to equilibrate than **8** and **10** may be explained since formation

of the *cis*-equatorial−axial intermediate **11** is more difficult than formation of the *trans*-diequatorial intermediate **9**. This assumption is supported by the fact that longipinan-7β,8α,9α-triol-1-one yields only the *trans*-diequatorial 7β,8α-acetonide when dissolved in acetone in the presence of *p*-toluenesulfonic acid.[15]

We also explored transesterification reactions of longipinene **6** and its 2β,3β-dihydro derivative **2**. After 2 weeks at room temperature, the 8,9-diangelate **6** transformed into the 7,9-diangelate **7** in *ca*. 40% yield, while **2** gave **3**[6] in *ca*. 40% yield. In both cases, the proportion of 8,9-diester and 7,9-diester (60% and 40%) did not change during the following weeks and none of 7,8-diangelates could be detected. This can be explained since an angeloyl group is less susceptible to nucleophilic attack than a methylbutyroyl group and the corresponding *cis*-equatorial−axial intermediate **14** (Figure 1) is more difficult to form. According to the AM1 semiempirical calculations, the reaction intermediate **14** ($\Delta H_f = -200.7$ kcal/mol) (Figure 1), which would give the 7,8-diangelate **5**, is higher in energy by 25.9 kcal/mol than intermediate **11** ($\Delta H_f = -226.6$ kcal/mol), which can yield the 7-angelate-8-methylbutyrate **12**.

Alternatively, under acidic reaction conditions, transesterifications of 8,9-diangelates do yield 7,8-diesters. In this respect, we found that diangelate **2**, when subjected to *p*-toluenesulfonic acid treatment, affords the rearranged product **13**. This transformation might proceed by two successive transesterification reactions to yield rastevione (**4**) which is known[16] to afford **13** when treated with *p*-toluenesulfonic acid, or alternatively the angelate to tiglate isomerization might occur before ester migration.

Experimental Section

General Experimental Procedures. Column chromatography was carried out on Merck Si gel 60 (70−230 mesh ASTM) or (230−400 mesh ASTM). HPLC separations were done on a Varian Vista 5500 chromatograph using a reversed-phase Micropak MCH-5-N-CAP column, i.d. 4 mm, length 150 mm + 40 mm (pre-column), employing UV detection and a flow of 1 mL/min. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were measured on a Perkin-Elmer Lambda 12 spectrometer. IR spectra were obtained on a Perkin Elmer 16F PC FT spectrophotometer, and 300 MHz ^{1}H and 75.4 MHz ^{13}C NMR spectra were recorded on a Varian Associates XL-300GS spectrometer while HMBC measurements were determined on a Varian Associates Unity Plus 500 spectrometer. Mass spectra were recorded at 20 eV on a Hewlett-Packard 5989-A spectrometer.

Plant Material. Specimens of *S. connata* were collected at km 283 of the México-Morelia federal road No. 15, in the state of Michoacán, México, during October 1995. A voucher specimen is deposited at the herbarium of the Instituto de Ecología, A. C. Pátzcuaro, Michoacán, where Professor Jerzy Rzedowski identified the plant material.

Extraction and Isolation. Air-dried roots (1.5 kg) of *S. connata* were extracted with hexane to afford a yellow viscous oil (10 g). A 4 g portion was subjected to column chromatography. Fractions eluted with hexane yielded stigmasterol[17] (20 mg). The first fractions eluted with hexane−EtOAc (19:1) contained **1** and **4**, the intermediate fractions contained **2** and **3**, and the last fractions contained **6**. The first fractions eluted with hexane−EtOAc (9:1) contained **8**, and the last fractions contained **5** and **12**.

Compounds **1** and **4** were purified by HPLC injecting samples of 1.6 mg in MeOH, eluting with 3:2 MeOH−H_2O and using UV detection at 216 nm. Each run afforded 0.8 mg of rastevione (**4**) (t_R 52 min) and 0.7 mg of **1** (t_R 60 min). Compounds **2** and **3** were purified by column chromatography eluting with CH_2Cl_2−acetone (99:1) to yield **2** (30 mg) and **3** (15 mg) as white needles, which were recrystallized from acetone-hexane. Compounds **5** and **12** were purified by HPLC injecting samples of 1 mg in MeOH, eluting with 3:2 MeOH−H_2O and using UV detection at 254 nm. Each run afforded 0.5 mg of **5** (t_R 60 min) and 0.3 mg of **12** (t_R 40 min). Compound **6** was purified by injecting HPLC samples of 1.6 mg in MeOH, eluting with 3:2 MeOH−H_2O and using UV detection at 254 nm. Each run afforded 0.5 mg of **6** (t_R 19.6 min). Compound **8** was purified by column chromatography on Si gel eluting with 8:2 hexane−EtOAc to afford 16 mg.

Longipinane-7β,8α,9α-triol-1-one 7-angelate-8-methylbutyrate (1): colorless oil; $[\alpha]^{25}_{589}$ +4°, $[\alpha]^{25}_{578}$ +4°, $[\alpha]^{25}_{546}$ +3°, $[\alpha]^{25}_{436}$ +1°, $[\alpha]^{25}_{365}$ −17° (*c* 1.4, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 216 (3.94) nm; IR ($CHCl_3$) ν_{max} 3608, 1733, 1716, 1710, 1648 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.16 (1H, qq, J = 7.5 and 1.5 Hz, H-3 Ang), 5.50 (1H, d, $J_{7,8}$ = 11.3 Hz, H-7), 5.39 (1H, dd, $J_{7,8}$ = 11.3 and $J_{8,9}$ = 3 Hz, H-8), 3.75 (1H, d, $J_{8,9}$ = 3 Hz, H-9), 3.08 (1H, d, $J_{4,11}$ = 5.6 Hz, H-11), 2.58 (1H, dd, $J_{2\alpha,2\beta}$ = 19 and $J_{2\beta,3}$ = 8.5 Hz, H-2β), 2.34 (1H, m, H-3), 2.31 (1H, sext., J = 7 Hz, H-2 Mebu), 2.22 (1H, br d, $J_{4,11}$ = 5.6 Hz, H-4), 2.14 (1H, dd, $J_{2\alpha,2\beta}$ = 19 and $J_{2\alpha,3}$ = 6 Hz, H-2α), 2.04 (3H, dq, J = 7.5 and 1.5 Hz, Me-4 Ang), 1.88 (3H, dq, $J_d = J_q$ = 1.5 Hz, Me-5 Ang), 1.82 (1H, s, H-5), 1.64 (1H, m, H-3 Mebu), 1.38 (1H, m, H-3′ Mebu), 1.10 (3H, d, J = 7 Hz, Me-5 Mebu), 1.10 (3H, s, Me-14), 1.09 (3H, d, $J_{3,12}$ = 7 Hz, Me-12), 1.06 (3H, s, Me-13), 0.93 (3H, s, Me-15), 0.86 (3H, t, J = 7.5 Hz, Me-4 Mebu); ^{13}C NMR, see Table 1; EIMS *m/z* 416 $[M - H_2O]^+$ (0.2), 334 (1), 314 (2), 233 (24), 204 (10), 176 (4), 162 (12), 85 (47), 83 (100), 57 (56), 43 (13).

Longipin-2-ene-7β,8α,9α-triol-1-one 8,9-diangelate (6): colorless oil; $[\alpha]^{25}_{589}$ +122°, $[\alpha]^{25}_{578}$ +129°, $[\alpha]^{25}_{546}$ +148°, $[\alpha]^{25}_{436}$ +284°, $[\alpha]^{25}_{365}$ +706° (*c* 1.4, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 215 (4.32), 250 (3.86) nm; IR ($CHCl_3$) ν_{max} 3620, 1718, 1674, 1648, 1618 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.14 (1H, qq, J = 7.5 and 1.5 Hz, H-3 Ang), 6.12 (1H, qq, J = 7.5 and 1.5 Hz, H-3 Ang), 5.81 (1H, ddq, $J_{2,4} = J_{2,11} = J_{2,12}$ = 1.5 Hz, H-2), 5.60 (1H, d, $J_{8,9}$ = 3.3 Hz, H-9), 5.31 (1H, dd, $J_{7,8}$ = 11.2 and $J_{8,9}$ = 3.3 Hz, H-8), 3.93 (1H, dd, $J_{7,8}$ = 11.2 and $J_{7,OH}$ = 3.8 Hz, H-7), 3.03 (1H, dd, $J_{2,11}$ = 1.5 and $J_{4,11}$ = 7 Hz, H-11), 2.81 (1H, br d, $J_{4,11}$ = 7 Hz, H-4), 2.38 (1H, br s, H-5), 2.07 (3H, d, $J_{2,12}$ = 1.5 Hz, Me-12), 2.01 (3H, dq, J = 7.5 and 1.5 Hz, Me-4 Ang), 1.98 (3H, dq, J = 7.5 and 1.5 Hz, Me-4 Ang), 1.93 (3H, dq, $J_d = J_q$ = 1.5 Hz, Me-5 Ang), 1.82 (3H, dq, $J_d = J_q$ = 1.5 Hz, Me-5 Ang), 1.08 (3H, s, Me-15), 1.07 (3H, s, Me-14), 1.00 (3H, s, Me-13); ^{13}C NMR, see Table 1; EIMS *m/z* 430 $[M]^+$ (0.03), 330 (7), 230 (2), 201 (19), 187 (6), 83 (100), 55 (19).

Longipin-2-ene-7β,8α,9α-triol-1-one 8-angelate-9-methylbutyrate (8): colorless oil; $[\alpha]^{25}_{589}$ +58°, $[\alpha]^{25}_{578}$ +67°, $[\alpha]^{25}_{546}$ +79°, $[\alpha]^{25}_{436}$ +95°, $[\alpha]^{25}_{365}$ +313° (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 218 (3.89), 248 (3.47) nm; IR ($CHCl_3$) ν_{max} 3592, 1730, 1720, 1674, 1646, 1618 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.12 (1H, qq, J = 7.5 and 1.5 Hz, H-3 Ang), 5.82 (lH, ddq, $J_{2,4} = J_{2,11} = J_{2,12}$ = 1.5 Hz, H-2), 5.50 (1H, d, $J_{8,9}$ = 3 Hz, H-9), 5.27 (1H, dd, $J_{7,8}$ = 11.2 and $J_{8,9}$ = 3 Hz, H-8), 3.91 (1H, d, $J_{7,8}$ = 11.2 Hz, H-7), 3.04 (1H, br d, $J_{4,11}$ = 7 Hz, H-11), 2.80 (1H, br d, $J_{4,11}$ = 7 Hz, H-4), 2.42 (1H, sext., J = 7 Hz, H-2 Mebu), 2.38 (1H, br s, H-5), 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 Ang), 1.85 (3H, dq, $J_d = J_q$ = 1.5 Hz, Me-5 Ang), 1.70 (1H, m, H-3 Mebu), 1.50 (1H, m, H-3′ Mebu), 1.16 (3H, d, J = 7 Hz, Me-5 Mebu), 1.08 (3H, s, Me-15), 1.06 (3H, s, Me-14), 0.96 (3H, s, Me-13), 0.94 (3H, t, J = 7.5 Hz, Me-4 Mebu); ^{13}C NMR, see Table 1; EIMS *m/z* 432 $[M]^+$ (0.3), 416 (1), 330 (9), 230 (3), 201 (17), 187 (7), 135 (4), 83 (100), 57 (24).

General Procedure for the Transesterification of 2, 6, 8, 10, or 12. A solution of the longipinene diester (3 mg) in 3:2 MeOH−H_2O (50 mL) was stored at room temperature for 2 weeks, and the solvent was removed with a N_2 stream.

The ^{1}H NMR spectrum of the residue from **2** showed a mixture of **2** (60%) and **3** (40%), whose spectral data are in agreement with those reported.[6]

The ^{1}H NMR spectrum of the residue from **6** showed a mixture of **6** (60%) and **7** (40%). ^{1}H NMR of **7** (admixed with **6**) ($CDCl_3$, 300 MHz) δ 6.15 (1H, qq, J = 7.5 and 1.5 Hz, H-3 Ang), 6.12 (1H, qq, J = 7.5 and 1.5 Hz, H-3 Ang), 5.81 (1H,

ddq, $J_{2,4} = J_{2,11} = J_{2,12} = 1.5$ Hz, H-2), 5.56 (1H, d, $J_{8,9} = 3.3$ Hz, H-9), 5.21 (1H, d, $J_{7,8} = 11.2$ Hz, H-7), 4.14 (1H, ddd, $J_{7,8} = 11.2$, $J_{8,9} = 3.3$ and $J_{8,OH} = 4$ Hz, H-8), 3.09 (1H, br d, $J_{4,11} = 7$ Hz, H-11), 2.68 (1H, br d, $J_{4,11} = 7$ Hz, H-4), 2.36 (1H, br s, H-5), 2.07 (3H, d, $J_{2,12} = 1.5$ Hz, Me-12), 2.01 (3H, dq, $J =$ 7.5 and 1.5 Hz, Me-4 Ang), 1.98 (3H, dq, $J = 7.5$ and 1.5 Hz, Me-4 Ang), 1.93 (3H, dq, $J_d = J_q = 1.5$ Hz, Me-5 Ang), 1.82 (3H, dq, $J_d = J_q = 1.5$ Hz, Me-5 Ang), 1.11 (3H, s, Me-14), 1.05 (3H, s, Me-13), 0.96 (3H, s, Me-15).

The ^{1}H NMR spectrum of the residue from **8** indicated the presence of a mixture of **8** (58%), **10** (37%), and **12** (5%), that from diester **10** showed a mixture of **8** (52%), **10** (38%), and **12** (10%), and that from **12** showed a mixture of **8** (18%), **10** (13%), and **12** (69%). ^{1}H NMR of **10** (admixed with **8** and **12**) ($CDCl_3$, 300 MHz) δ 6.10 (1H, qq, $J = 7.5$ and 1.5 Hz, H-3 Ang), 5.81 (1H, ddq, $J_{2,4} = J_{2,11} = J_{2,12} = 1.5$ Hz, H-2), 5.45 (1H, d, $J_{8,9} = 3.5$ Hz, H-9), 5.22 (1H, d, $J_{7,8} = 11$ Hz, H-7), 4.10 (1H, m, H-8), 3.11 (1H, br d, $J_{4,11} = 7$ Hz, H-11), 2.68 (1H, br d, $J_{4,11} = 7$ Hz, H-4), 2.58 (1H, sext., $J = 7$ Hz, H-2 Mebu), 2.35 (1H, br s, H-5), 2.05 (3H, d, $J_{2,12} = 1.5$ Hz, Me-12), 1.98 (3H, dq, $J = 7.5$ and 1.5 Hz, Me-4 Ang), 1.92 (3H, dq, $J_d = J_q = 1.5$ Hz, Me-5 Ang), 1.80 (1H, m, H-3 Mebu), 1.60 (1H, m, H-3′ Mebu), 1.25 (3H, d, $J = 7$ Hz, Me-5 Mebu), 1.11 (3H, s, Me-15), 1.00 (3H, s, Me-14), 0.92 (3H, s, Me-13), 0.92 (3H, t, $J = 7.5$ Hz, Me-4 Mebu). The ^{1}H NMR spectrum of **12** was in agreement with that reported.[8]

Treatment of 2 with *p*-Toluenesulfonic Acid. A solution of **2** (44 mg) in C_6H_6 (10 mL) was treated with *p*-toluenesulfonic acid (9 mg). The reaction mixture was refluxed for 1 h using a Dean-Stark trap, concentrated to a small volume, diluted with H_2O, and extracted with EtOAc. The organic layer was washed with H_2O, dried, filtered, and evaporated giving **13** (30 mg, 71%) as a yellow oil, which was purified by silica gel chromatography. Its ^{1}H and ^{13}C NMR spectra were identical to those of an authentic sample.[16]

Molecular Modeling Calculations. Minimum energy structures were generated using MMX force-field calculations which is a derived version of the MM2 program[10] as implemented in the PCMODEL program V 6.00.[18] Conformational search for the ester side chains and the hydroxyl hydrogens were carried out by analysis of the rotational energy barrier plots in combination with E_{MMX} convergence, using the dihedral driver option. The π-system calculations were set for the restricted Hartree−Fock and full self-consistent field options. The structures were loaded into the PC Spartan plus software package,[19] reminimized using SYBYL force field[11] and submitted to the AM1 semiempirical calculation routine.[12,13]

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(18) PCMODEL Molecular Modeling Software is available from Serena Software, Box 3076, Bloomington, IN 47402-3076.
(19) PC Spartan Plus molecular modeling program is available from Wavefunction, Inc., 18401 Von Karman, Suite 370, Irvine, CA 92612.

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