# Grindelane Diterpenoids from *Stevia subpubescens*

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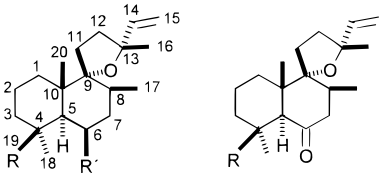
*Received May 5, 1999*

Four new 9*R*,13*R*-epoxylabdane diterpenes (**1**−**4**) and a known clerodane derivative, 3,4*β*-epoxy-5*β*,10*β*-*cis*-17α,20α-clerod-13(14)-en-15,16-olide, were isolated from the leaves of *Stevia subpubescens*. The structures, which correspond to the grindelane class of diterpenoids, were elucidated by NMR data, chemical correlation, and single-crystal X-ray diffraction analysis of monoacetate **5**. The absolute configuration of **1**−**4** is based on the optical activity of ketone **3** as compared with data of closely related substances.

*Stevia subpubescens* Lag. (Asteraceae) is a bush which grows in the hills near Pátzcuaro Lake in the state of Michoacán, Mexico. Previous studies of this plant yielded two longipinene derivatives from the hexane extract of the roots[1] and an *ent*-kaurane glycoside from the butanol-soluble fraction of the methanolic extract of the leaves.[2] As has been pointed out in a recent summary on *Stevia*,[3] it is possible to outline chemical subdivisions of the studied taxa according to the content of their secondary metabolites. A detailed knowledge of the chemistry of the genus might be useful for a more precise delimitation of their section as well as to establish chemotaxonomic relationships with other genera of the tribe Eupatorieae. Therefore, exhaustive chemical studies on each member of *Stevia* are desirable. Additionally, isolation of new diterpenes from *Stevia* becomes an interesting subject in connection with the significant sweet-tasting *ent*-kaurene glycosides extracted from *S. rebaudiana*.[4]

In the present work, we explored the ether-soluble part of the methanolic extracts of the leaves, which yielded the four new labdane derivatives **1**−**4** along with known 3,4*β*-epoxy-5*β*,10*β*-*cis*-17α,20α-clerod-13(14)-en-15,16-olide, previously isolated from *Solidago shortii*[5] and *Ageratina saltillensis*.[6]




**1**: R = $CH_2OH$; R´ = OH **3**: R = $CH_2OH$

**2**: R = $CH_2OH$; R´ = H **6**: R = COH

**4**: R = COOH; R´ = H **7**: R = COOH

**5**: R = $CH_2OAc$; R´ = OH **8**: R = $CH_2OAc$

## Results and Discussion

Diterpene **1** was isolated as white needles mp 108−110 °C, showing an optical rotation of +24, and a strong IR absorption at 3360 $cm^{-1}$ for hydroxyl groups. The mass spectrum showed $[M - H_2O]^+$ at *m*/*z* 304 consistent with the molecular formula $C_{20}H_{34}O_3$. The $^1H$ and $^{13}C$ NMR spectra listed in Tables 1 and 2, respectively, indicated a labdane-type diterpene. A typical AMX proton system at *δ* 6.04 (dd, *J* = 17.5 and 10.8 Hz), 5.14 (dd, *J* = 17.5 and 1.4 Hz) and 4.97 (dd, *J* = 10.8 and 1.4 Hz) indicated the presence of a vinyl group, located at C-13 which was in agreement with a labdane skeleton. A quartet at *δ* 4.35 (*J* = 3.0 Hz), attributed to an axially oriented secondary hydroxyl group, was placed at C-6 due to its coupling with H-5. An AB system at *δ* 4.28 and 3.15 ($J_{AB}$ = 11.2 Hz) revealed the presence of a hydroxymethylene group, which upon acetylation shifted to *δ* 4.59 and 4.40. These chemical shifts together with the $^{13}C$ NMR resonance at *δ* 68.8 imply an axial orientation,[7] for the hydroxyl group located at C-19. The $^1H$ NMR spectrum also showed the presence of three tertiary methyl groups at *δ* 1.38, 1.32 and 1.05 and a secondary methyl group at *δ* 1.36 (d, *J* = 7.2 Hz). Additional evidence, which confirmed the placement of the secondary hydroxyl group at C-6 in the natural product **1**, was found in the $^1H$ NMR spectra of keto aldehyde **6** and ketoacid **7**, obtained after chromium trioxide oxidation of **1**. The spectra of both **6** and **7** showed the signals for H-5 and H-7α shifted downfield with respect to those of **1**, as can be seen in Table 1. Moreover, the $^{13}C$ NMR signal of C-5 was also shifted downfield from *δ* 50.2 in diol **1** to *δ* 60.7 in keto aldehyde **6** and to *δ* 62.2 in ketoacid **7** (Table 2). The fact that acetylation of **1** under standard conditions afforded only the 19-monoacetate (**5**) is consistent with the axial orientation of the secondary hydroxy group at C-6.

The $^{13}C$ NMR spectra of **1** also showed resonances at *δ* 82.6 and 92.9 due to quaternary carbons bearing an oxygen atom, which indicate the presence of an oxygen bridge. The exceptionally low field resonance of the signal at *δ* 92.9 gave evidence that this carbon was located in a position adjacent to a quaternary carbon atom and therefore assigned to C-9. Consequently, the oxygen bridge was located between C-9 and C-13.

The relative stereochemistry at C-8, C-9, and C-13 was established after single-crystal X-ray diffraction analysis[8] of monoacetate **5**. A stereoview of the molecule is illustrated in Figure 1, showing the diterpene conformation in the solid state. As expected, both six-membered rings are in slightly distorted chair conformations with the acetyloxymethylene group at C-4, the hydroxy group at C-6 and the methyl

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10.1021/np990216i CCC: $19.00 
Published on Web 01/27/2000

**Table 1.** $^1$H NMR Data of Labdane Derivatives **1**−**8** (200 MHz, $CDCl_3$)[a]

| H | 1 | 2 | 3 | 4 | 5[b] | 6 | 7[c] | 8[d] |
|---|---|---|---|---|---|---|---|---|
| 5 | 1.73 d | *e* | 3.24 s | *e* | 1.70 d | 3.24 s | 3.27 s | 3.07 s |
| 6 | 4.35 q | *e* | – | *e* | 4.42 m[f] | – | – | – |
| 7α | 2.31 ddd | *e* | 3.29 dd | *e* | 2.30 ddd | 3.24 dd | 3.44 dd | 3.15 dd |
| 7β | 1.65 brd | *e* | 2.07 brd | *e* | 1.63 brd | 2.10 brd | 2.22 brd | 1.99 brd |
| 8 | *e* | *e* | 2.49 dq | *e* | *e* | 2.50 dq | 2.58 dq | 2.42 dq |
| 14 | 6.04 dd | 6.04 dd | 6.08 dd | 6.04 dd | 6.02 dd | 6.08 dd | 6.07 dd | 6.08 dd |
| $15_t$ | 5.14 dd | 5.16 dd | 5.22 dd | 5.13 dd | 5.12 dd | 5.21 dd | 5.19 dd | 5.22 dd |
| $15_c$ | 4.97 dd | 4.97 dd | 5.05 dd | 4.97 dd | 4.96 dd | 5.05 dd | 5.06 dd | 5.04 dd |
| 16 | 1.32 s[g] | 1.29 s | 1.38 s | 1.32 s | 1.30 s | 1.39 s | 1.40 s | 1.37 s |
| 17 | 1.36 d | 1.01 d | 1.06 d | 1.05 d | 1.32 d | 1.09 d | 1.09 d | 1.04 d |
| 18 | 1.05 s[g] | 0.97 s | 1.01 s | 0.87 s | 1.01 s | 0.85 s | 0.92 s | 0.94 s |
| 19 | 4.28 d | 3.75 d | 4.19 d | – | 4.59 d | 10.40 s | – | 4.77 d |
| 19′ | 3.15 d | 3.45 brd | 3.20 d | – | 4.40 d | – | – | 4.50 d |
| 20 | 1.38 s[g] | 0.90 s | 1.02 s | 1.24 s | 1.33 s | 1.11 s | 1.28 s | 1.03 s |

[a] *J*(Hz) for compounds **1**−**8**: 5,6 = 6,7α = 6,7β = 3.0; 14,$15_t$ = 17.5; 14,$15_c$ = 10.8; $15_t$,$15_c$ = 1.4; 19,19′ = 11.2. Compounds **1**, **5**: 7α,7β = 15.0; 7α,8 = 6.0. Compound **3**: 7α,7β = 12.0; 7α,8 = 8.0. Compounds **6**−**8**: 7α,7β =11.4; 7α,8 = 7.4. Compounds **1**, **4**, **6**, **7**: 8,17 = 7.2. Compounds **2**, **3**, **8**: 8,17 = 7.7. Compounds **5**: 8,17 = 6.5. [b] OAc: 2.08 s. [c] COOH: 13.00 br s. [d] OAc: 2.05 *s*. [e] Overlapped signal. [f] Partially overlapped with the H-19′ signal. [g] Confirmed by long-range HETCOR.

**Table 2.** $^{13}$C NMR Spectral Data of Labdane Derivatives **1**−**8** (50 MHz, $CDCl_3$)

| | 1 | 2 | 3 | 4 | 5[a] | 6 | 7 | 8[b] |
|---|---|---|---|---|---|---|---|---|
| 1 | 37.6 | 37.7 | 39.8 | 37.9 | 38.0 | 37.7 | 38.4 | 37.7 |
| 2 | 18.0 | 17.8 | 17.5 | 18.4 | 18.2 | 17.9 | 18.0 | 17.3 |
| 3 | 36.5 | 35.2 | 37.6 | 37.6 | 37.6 | 34.4 | 37.9 | 35.1 |
| 4 | 39.2 | 38.6 | 37.5 | 44.0 | 38.4 | 49.1 | 51.0 | 36.3 |
| 5 | 50.2 | 48.2 | 61.9 | 49.9 | 51.0 | 60.7 | 62.2 | 60.5 |
| 6 | 67.2 | 30.2 | 218.2 | 29.2 | 68.2 | 213.0 | 221.9 | 213.3 |
| 7 | 41.2 | 17.0 | 47.4 | 18.8 | 38.8 | 47.1 | 46.7 | 47.8 |
| 8 | 39.5 | 40.6 | 46.1 | 40.2 | 39.9 | 45.4 | 46.1 | 45.5 |
| 9 | 92.9 | 92.6 | 91.3 | 92.4 | 93.2 | 90.7 | 90.5 | 91.9 |
| 10 | 41.8 | 42.3 | 49.8 | 42.6 | 42.0 | 47.8 | 43.6 | 48.6 |
| 11 | 29.5 | 29.6 | 29.6 | 29.8 | 29.9 | 29.6 | 29.4 | 29.7 |
| 12 | 34.9 | 33.3 | 33.7 | 33.1 | 35.0 | 32.7 | 33.3 | 33.3 |
| 13 | 82.6 | 82.7 | 83.7 | 82.6 | 83.0 | 83.8 | 83.9 | 84.0 |
| 14 | 145.0 | 145.1 | 144.3 | 145.2 | 145.3 | 144.2 | 143.9 | 144.4 |
| 15 | 110.6 | 110.8 | 111.4 | 110.7 | 111.0 | 111.5 | 111.6 | 111.4 |
| 16 | 27.9 | 27.6 | 27.9 | 27.9 | 28.1 | 27.7 | 28.0 | 27.9 |
| 17 | 19.8 | 18.0 | 19.9 | 17.8 | 20.2 | 18.8 | 18.0 | 20.8 |
| 18 | 27.0 | 26.7 | 27.2 | 28.9 | 27.2 | 24.8 | 27.5 | 26.3 |
| 19 | 68.8 | 65.6 | 67.9 | 181.8 | 69.4 | 208.6 | 176.6 | 65.2 |
| 20 | 20.4 | 18.9 | 18.4 | 16.2 | 21.9 | 18.7 | 18.0 | 18.8 |

[a] OAc: 171.5 and 21.4. [b] OAc: 176.1 and 21.1.

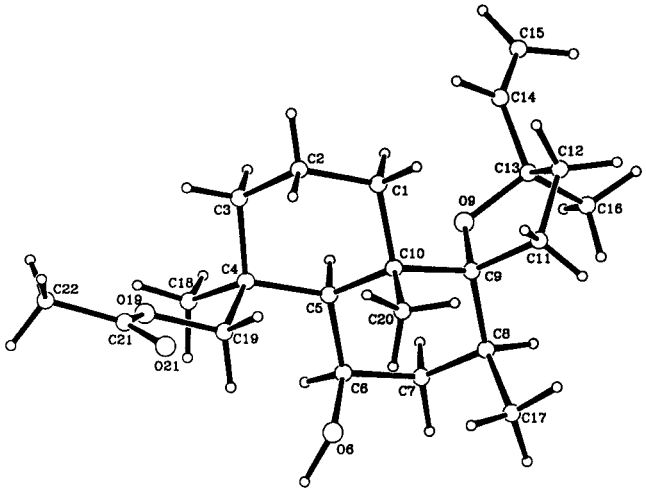


**Figure 1.** X-ray structure of monoacetate **5**.

groups at C-8 and C-10 remaining in axial positions. The moderate distortions of the C(10)−C(5)-C(6)−O(6) and C(17)−C(8)-C(9)−C(10) torsion angles (74.5 and −75.9°, respectively) are due to the repulsion of the three axial substituents attached to ring B. The five-membered ring exists in a flattened half-chair with conformational parameters[9] $\theta_m$ = 28.6° and *P* = 4.5°. The experimentally refined final fractional atomic coordinates are listed in a table included as Supporting Information, while crystal data, collection and refinement parameters are described in the Experimental Section.

Diterpene **2** showed an optical rotation of +23, and *m*/*z* at 306 $[M]^+$ which was in agreement with $C_{20}H_{34}O_2$. Its structure directly followed from the $^1$H NMR and $^{13}$C NMR spectra which were similar to those of **1** (Tables 1 and 2), except for the absence of the signals for H-6 at δ 4.35 and C-6 at δ 67.2 due to the secondary hydroxyl group at C-6 and by the presence of an additional methylene resonance at δ 30.2 in the $^{13}$C NMR spectrum.

Diterpene **3** showed $[\alpha]_{589}$ +35, and *m*/*z* at 302 $[M - H_2O]^+$, corresponding to $C_{20}H_{32}O_3$. Comparison of its $^1$H NMR spectrum with that of **1** indicated the presence of a carbonyl group at C-6, since the H-5 signal changed from a doublet at δ 1.73 (*J* = 3.0 Hz) in **1** to a singlet at δ 3.24 in **3**. Additionally, the proton signals for the axially oriented methyl groups Me-17 and Me-20 were shifted upperfield by ca. 0.3 ppm on going from **1** to **3** (Table 1) due to the shielding effect of the carbonyl group at C-6. These data together with the $^{13}$C NMR chemical shifts (Table 2) support structure **3**.

Diterpene acid **4** showed $[\alpha]_{589}$ +6, and *m*/*z* at 320 $[M]^+$, in agreement with $C_{20}H_{32}O_3$. The IR spectrum exhibited typical absorptions for a carboxyl group at 3640 and 1700 $cm^{-1}$. The $^1$H NMR spectrum demonstrated a lack of the AB system for the hydroxymethylene group found in **2**, while the remaining $^1$H NMR signals were consistent with the oxidation of a primary alcohol to a carboxylic acid. In the $^{13}$C NMR spectrum, a resonance at δ 181.8 confirmed the presence of a carboxyl group, which was located at C-19 by comparison with the $^{13}$C NMR spectrum of **1**.

After recognizing the close similarity of the $^{13}$C NMR spectra of **1**−**4** (Table 2), which were assigned with the aid of HETCOR experiments, and based on biogenetic considerations, we assume that all compounds possessed the same carbon skeleton, as well as the same absolute stereochemistry. Confirmative evidence of this assumption was achieved by oxidation of monoacetate **5** to give **8**, followed by alkaline hydrolysis, which afforded a substance identical in all respects with natural ketone **3**.

Regarding the absolute configuration of the new natural products **1**−**4**, we compared the positive optical rotations determined for ketone **3** at different wavelengths with a positive Δε 295 value for methyl 6-oxo-7,8-dihydrogrindelate[10] prepared from grindelic acid, whose absolute con-

figuration has been revised[11] and recently confirmed by enantiomeric synthesis[12] of *ent*-grindelic acid.

Diterpenes **1**−**4** are related to grindelic acid, which shows feeding deterrence toward aphids.[13] From the taxonomic point of view, it is interesting to note that, this is the first report on the isolation of grindelane class diterpenoids from the genus *Stevia*, although they have been isolated from several genus of the Asteraceae family, such as *Grindelia*,[14] *Chrysothamnus*,[15] *Haplopappus*,[16] *Erigeron*,[17] and *Austrobrickellia*.[18] The last two genera are notable in affording *ent*-grindelane diterpenes.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 341 polarimeter. IR spectra were recorded in $CHCl_3$ on a Perkin-Elmer 599B spectrophotometer. NMR spectra were recorded on a Varian Gemini 200 spectrometer at 200 MHz ($^1$H) and 50 MHz ($^{13}$C) in $CDCl_3$, using TMS as the internal reference. Si gel (Merck 60−200 $\mu$m) and neutral alumina (Merck 60−200 $\mu$m) were used for column chromatography. The X-ray diffraction analysis was performed on a Nicolet P3m four circle diffractometer interfaced to a DEC VAXstation 4000-60.

**Plant material.** *S. subpubescens* Lag. was collected near Lagunillas, in the state of Michoacán, México, in November 1994. A voucher specimen (JDHernández 34) is deposited at the Instituto de Ecología A. C., Pátzcuaro, Michoacán, México where taxonomic characterization was accomplished by Dr. Jerzy Rzedowski.

**Extraction and Isolation.** Fresh leaves (100 g) of *S. subpubescens* were extracted with MeOH (2.5 L) at room temperature for 3 days and concentrated under vacuum. The residue was suspended in $H_2O$ and extracted with ether. The ethereal extract (5.5 g) was chromatographed over a column containing alumina (15 g) on top of Si gel (90 g) eluting with mixtures of hexanes−EtOAc and collecting 30 mL fractions as follows: fractions 1−5 (9.5:0.5), 6−10 (9:1), 11−40 (8:2). Fractions 7−19 contained **1**−**4**, and fractions 20−28 yielded 3,4$\beta$-epoxy-5$\beta$,10$\beta$-*cis*-17α,20α-clerod-13(14)-en-15,16-olide (900 mg). Fractions 7−19 were rechromatographed on a column eluting with hexanes−EtOAc (9:1). Fractions 1′−6′ gave a mixture of **2**−**4**, and fractions 7′−12′ gave **1** (200 mg). The mixture of **2**−**4** was rechromatographed on a further column eluting with hexanes−EtOAc (95:5) to yield **2** (15 mg), **3** (10 mg), and **4** (5 mg).

**(4*S*,5*S*,6*R*,8*S*,9*R*,10*S*,13*R*)-9,13-Epoxy-14-labdene-6,19-diol (1).** Colorless needles from acetone−hexane: mp 108−110 °C; $[\alpha]_{589}$ +24, $[\alpha]_{578}$ +25, $[\alpha]_{546}$ +28, $[\alpha]_{436}$ +43, $[\alpha]_{365}$ +63 (*c* 0.20, $CHCl_3$ ); IR ($CHCl_3$) $\nu_{max}$ 3360 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; EIMS (20 eV) *m*/*z* 304 $[M - H_2O]^+$ (4), 303 (20), 207 (13), 189 (11), 151 (33), 121 (46), 109 (48), 96 (26), 81 (48), 69 (40), 43 (100).

**(4*S*,5*S*,8*S*,9*R*,10*S*,13*R*)-9,13-Epoxy-14-labden-19-ol (2).** Colorless oil; $[\alpha]_{589}$ +23, $[\alpha]_{578}$ +23, $[\alpha]_{546}$ +18, $[\alpha]_{436}$ +16, $[\alpha]_{365}$ +19 (*c* 0.03, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3640 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; EIMS (20 eV) *m*/*z* 306 $[M]^+$ (6), 276 (12), 165 (16), 151 (100), 138 (17), 123 (15), 109 (26), 96 (15), 81 (20), 69 (16), 43 (8).

**(4*S*,5*S*,8*S*,9*R*,10*S*,13*R*)-9,13-Epoxy-14-labden-19-ol-6-one (3).** Colorless oil; $[\alpha]_{589}$ +35, $[\alpha]_{578}$ +35, $[\alpha]_{546}$ +48, $[\alpha]_{436}$ + 69, $[\alpha]_{365}$ +145 (*c* 0.02, $CHCl_3$); IR $\nu_{max}$ ($CHCl_3$) 3350, 1695 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; EIMS (20 eV) *m*/*z* 302 $[M - H_2O]^+$ (25), 234 (44), 217 (73), 206 (69), 191 (43), 150 (29), 135 (32), 123 (199), 109 (26), 81 (22), 69 (29), 43 (18).

**(4*S*,5*R*,8*S*,9*R*,10*S*,13*R*)-9,13-Epoxy-14-labden-19-oic acid (4).** Gum; $[\alpha]_{589}$ +16, $[\alpha]_{578}$ +22, $[\alpha]_{546}$ +22, $[\alpha]_{436}$ +22, $[\alpha]_{365}$ +28 (*c* 0.03, $CHCl_3$); IR $\nu_{max}$ ($CHCl_3$) 3200−2840 and 1700 cm$^{-1}$; $^1$H- and $^{13}$C NMR, see Tables 1 and 2; EIMS (20 eV) *m*/*z* 320 $[M]^+$ (22), 177 (7), 165 (20), 151 (100), 138 (20), 109 (21), 83 (29), 71 (13), 43 (9).

**3,4$\beta$-Epoxy-5$\beta$,10$\beta$-*cis*-17α,20α-clerod-13(14)-en-15,16-olide.** Colorless solid, mp 89−90 °C (lit.,[6] mp 91 °C); $[\alpha]_{589}$ +14, $[\alpha]_{578}$ +15, $[\alpha]_{546}$ +16, $[\alpha]_{436}$ +26, $[\alpha]_{365}$ +42 (*c* 0.21, $CHCl_3$); $^1$H and $^{13}$C NMR identical with literature values.[6]

**Acetylation of 1.** A solution of **1** (50 mg) in pyridine (0.5 mL) was treated with $Ac_2O$ (0.5 mL). The reaction mixture was heated on a steam bath for 3 h, poured over ice−water, and extracted with EtOAc. The organic layer was washed with diluted HCl, $H_2O$, aqueous $NaHCO_3$, and water, dried over anhydrous $NaSO_4$, filtered, and evaporated. The residue was chromatographed on Si gel (10 g). Fractions 7−9, eluted with hexanes−EtOAc (9:1), gave recovered starting material **1** (10 mg), and fractions 11−13 yielded (4*S*,5*S*,6*R*,8*S*,9*R*,10*S*,13*R*)-9,13-epoxy-14-labdene-6,19-diol 19-acetate (**5**) (25 mg) as white needles from acetone−hexane: mp 157−159 °C; $[\alpha]_{589}$ −14, $[\alpha]_{578}$ −14, $[\alpha]_{546}$ −14, $[\alpha]_{436}$ −21, $[\alpha]_{365}$ −36 (*c* 0.10, $CHCl_3$); IR $\nu_{max}$ ($CHCl_3$) 3520, 1730 cm$^{-1}$; EIMS (20 eV) *m*/*z* 364 $[M]^+$ (2), 346 (20), 304 (2), 255 (2), 213 (12), 181 (2), 152 (100),138 (34),123 (18), 109 (20), 96 (34), 81 (10), 69 (6), 43 (3).

**Oxidation of 1.** A solution of **1** (100 mg) in AcOH (2 mL) was treated with a solution of $CrO_3$ (100 mg) in $H_2O$ (0.5 mL) and AcOH (2 mL) for 1 h at room temperature. The reaction mixture was diluted with ice−water and extracted with $Et_2O$. The organic layer was washed with $H_2O$, aqueous $NaHCO_3$ and $H_2O$, dried over $Na_2SO_4$, filtered and evaporated under vacuum. The residue was chromatographed on Si gel (14 g), collecting 20 mL fractions. Fractions 4−6 eluted with hexanes−EtOAc 9:1 yielded (4*S*,5*R*,8*S*,9*R*,10*S*,13*R*)-9,13-epoxy-6-oxo-14-labden-19-al (**6**) (40 mg) as needles from acetone−hexane: mp 155−157 °C; $[\alpha]_{589}$ +33, $[\alpha]_{578}$ +36, $[\alpha]_{546}$ +41, $[\alpha]_{436}$ +78, $[\alpha]_{365}$ + 140 (*c* 0.16, $CHCl_3$); IR $\nu_{max}$ ($CHCl_3$) 1710 cm$^{-1}$. Further elution with the same solvents provided (4*S*,5*R*,8*S*,9*R*,10*S*,13*R*)-9,13-epoxy-6-oxo-14-labden-19-oic acid (**7**) (40 mg) as an amorphous powder from acetone-hexane: mp 137−139 °C; $[\alpha]_{589}$ +30, $[\alpha]_{578}$ +32, $[\alpha]_{546}$ +43, $[\alpha]_{436}$ +87, $[\alpha]_{365}$ +203 (*c* 0.05, $CHCl_3$); IR $\nu_{max}$ ($CHCl_3$) 3200−2540, 1730, 1665 cm$^{-1}$; EIMS (20 eV) *m*/*z* 334 $[M]^+$ (6), 247 (5), 219 (7), 190 (34), 175 (13), 165 (5), 151 (100), 138 (23), 123 (16), 109 (50), 96 (40), 81 (15), 71 (13), 43 (12).

**Oxidation of 5.** A solution of **5** (40 mg) in AcOH (0.5 mL) was treated with a solution of $CrO_3$ (40 mg) in $H_2O$ (0.5 mL) and AcOH (0.5 mL). The reaction mixture was stored at room temperature during 1 h. Workup as in the case of **7** gave a residue which was chromatographed on Si gel (2 g). Fractions eluted with hexane: EtOAc 95:5 gave **8** (30 mg) as a white solid: mp 89−90 °C; $[\alpha]_{589}$ −12, $[\alpha]_{578}$ −14, $[\alpha]_{546}$ −14, $[\alpha]_{436}$ −14, $[\alpha]_{365}$ +26 (*c* 0.04, $CHCl_3$); IR $\nu_{max}$ ($CHCl_3$) 1734, 1702 cm$^{-1}$.

**Hydrolysis of 8.** A solution of **8** (30 mg) in MeOH (1 mL) was treated with a solution of KOH (70 mg) in $H_2O$ (0.5 mL). The mixture was refluxed for 30 min, concentrated to a small volume and extracted with EtOAc. The organic layer was washed with $H_2O$, dried over anhydrous $Na_2SO_4$, filtered, and evaporated under vacuum to yield **3** as an oil (10 mg) whose NMR spectra and optical rotation data were identical with those of natural ketone **3**.

**X-ray Structure Analysis of 5.**[8] Suitable crystals were grown by slow evaporation of chloroform-hexane solutions. A colorless crystal measuring 0.24 × 0.16 × 0.45 mm was mounted on a Nicolet P3 four-circle diffractometer interfaced to a DEC VAXstation 4000-60 operated with Crystal Logic, Inc. (Los Angeles, CA) software. The diffractometer was operated using Cu Kα graphite-monochromated radiation with $\lambda$ = 1.54178 Å. The final unit-cell parameters for an orthorhombic crystal of molecular formula $C_{22}H_{36}O_4$, MW = 364.52, $Z$ = 4, calculated density = 1.17 g cm$^{-3}$ were obtained by least-squares on the setting angles for 25 reflections as $a$ = 7.719(3) Å, $b$ = 15.835(5) Å, and $c$ = 16.923(5) Å, $V$ = 2068.04(1) Å$^3$, and $F(000)$ = 800 e$^-$. The crystal data were collected at 298 K in the $\theta$:2$\theta$ scan mode using a scan speed of 3.0 deg min$^{-1}$, a scan width of 1.3 deg below K$\alpha_1$ and of 1.6 deg above K$\alpha_2$ in the 3−110° range to obtain 1571 reflections during a 26.9 h period. From these, 1430 reflections were unique and 1202 were considered as observed using the $I \geq 3\alpha(I)$ criterion. The intensity of two standard reflections, measured every 98

reflections throughout the data collection, showed no significant variation and therefore the data were corrected for background, Lorentz and polarization effects, but not for crystal decay. The absorption coefficient $\mu = 5.89$ cm$^{-1}$ precluded the need of absorption corrections. The systematic absences of the observed reflections were consistent with the $P2_12_12_1$ space group and the structure was solved by direct methods using the SHELX86 software package provided by Crystal Logic, Inc. For the structural refinements the non-hydrogen atoms were treated anisotropically, the hydroxyl hydrogen became evident from a $\Delta F$ synthesis, and the hydrogen atoms bonded to carbons in calculated positions with C−H = 1.00 Å were refined isotropically. After removal of redundant reflections, a few reflections were excluded from the final refinement calculations to improve the fit. The 993 reflections used for the final refinement of 244 parameters gave $R = 5.8\%$ and $R_w = 6.6\%$. A final difference map showed a maximum residual density of 0.31 e Å$^{-3}$.

**Acknowledgment.** We are grateful to Dr. Jerzy Rzedowski for identifying the plant material and to QFB Isaías Tapia for technical assistance. Partial financial support from CoNaCyT, México, is also acknowledged.

**Supporting Information Available:** Table of experimentally refined final fractional atomic coordinates ($\times 10^4$) of compound **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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(8) Tables of fractional atomic coordinates, complete bond lengths and angles, anisotropic temperature factors, hydrogen coordinates with temperature factors, torsion angles and a listing of observed and calculated structure factors are in deposit with the Cambridge Crystallographic Data Center, University Chemical Laboratory, 12 Union Road, Cambridge, CB2 1EZ, U.K.
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NP990216I