Oxidative Transformations of Guaia-1(10)-en-12,8-olides into Xanthanolides[§]

Mariano Martínez-Vázquez,[†] Jorge Cárdenas,[†] Lucas Godoy,[†] Martha Martínez-Bahena,[†] René Miranda,[‡] and Manuel Salmón*[,†]

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria Coyoacán 04510, México D. F., México, and Departamento de Ciencias Químicas FES-C, Universidad Nacional Autónoma de México

Received December 18, 1998

Dihydropsuedoivalin (**1**) was isolated from *Stevia tomentosa*, which, when treated with base, afforded epidihydropseudoivalin (**2**). The stereochemistry of **1** and **2** was established by crystallographic X-ray studies of the two derivatives of epidihydropseudoivalin. Treatment of **1** and **2** with Jones's reagent afforded the xanthanolides **3** and **4**, respectively.

Biogenetic hypothesis proposes that germacranolides and their epoxide derivatives are the precursors of guaianolides and xanthanolides, an argument that is supported by in vitro cyclizations of germacranolide-4-epoxides into guaianolides and xanthanolides.[1] For instance, BF_3-initiated Markovnikov-type trans-annular cyclization of the germacranolide parthenolide provided 4α-hydroxy-guai-1(10)-en-12,6*β*-olide and the xanthanolide 2-desoxy-11*β*,13-dihydro-6-epiparthenolide.[2] Regardless of their common biogenetic origin, to our knowledge only one transformation of a guaianolide into a xanthanolide has been reported. Thus, guaian-1(10)-en-6,12-olide was transformed by a peroxy-acid-mediated oxidation to 4,5-dioxo-11,13α-xath-1(10)-en-12,6α-olide.[3]

Continuing with our systematic study of the *Stevia* genus,[4−6] we now investigate *Stevia tomentosa* H. B. K. (Asteraceae). Chromatography of methanolic extracts of the plant material led to the isolation of **1**. Treatment of **1** with sodium methoxide yielded its C-11 epimer, epidihydropseudoivalin (**2**).[7] A survey of the literature showed that *Iva microcephala* is the only known source of **1** and **2**; both structures were reported without established stereochemistry.[7] These results prompted us to determine the stereochemistry of **1** and **2**. In this work we also report the transformation of the guaianolides dihydropseudoivalin (**1**) and epidihydropseudoivalin (**2**) by Jones's reagent to the 4,5-dioxo-xanthanolides **3** and **4**, respectively.

The only difference between **1** and pseudoivalin (**5**) is the presence in the latter of a double bond at C-11/C-13. Because the absolute stereochemistry of **5** has been established by an X-ray diffraction analysis of its bromoacetate derivative,[8] the stereochemistry at C-4, C-5, C-7, and C-8 of **1** was easily assigned. Only the configuration between C-11 and the corresponding C-11 of epimer **2** remains to be distinguished.

Transformation of **1** to **2** in basic conditions suggests that **2** corresponds to 4α-hydroxy-guai-1(10)-en-13α-methyl-12,8*β*-olide, whereas **1** corresponds to the 13*β*-methyl isomer. One way to assess the correct structures of **1** and **2** is by the coupling constants between H-7/H-11. However, the ^{1}H NMR (300 MHz) spectra of **1** and **2** showed the H-3, H-5, H-7, and H-11 signals overlapped, resulting in a complex signal centered at *δ* 2.32. The problem was solved by analysis of the X-ray diffraction of 4α-acetyl-guaia-1(10)-en-13α-methyl-12,8*β*-olide (**6**) and of 4α-acetyl-guaia-1(10)α-epoxy-13α-methyl-12,8*β*-olide (**7**), both prepared by standard reactions from **2**. The stereostructures (Figures 1 and 2) showed that the C-13 methyl group is α oriented. Consequently **2** is 4α-hydroxy-guai-1(10)-en-13α-methyl-12,8*β*-olide, while **1** is the 13*β*-methyl isomer.

Treatment of the guaianolides **1** and **2** separately with Jones's reagent afforded the epimeric xanthanolides **3** and **4**, respectively. The EIMS of xanthanolide **3** exhibited the molecular ion at *m*/*z* 264 [M^+] and major peaks at *m*/*z* 221 [$M - MeCO^+$] and 206 [$M - C_3H_6O^+$]. The latter two fragments, together with an IR absorption at 1715 cm^{-1}, a three-proton ^{1}H NMR signal at *δ* 2.09, and a ^{13}C NMR resonance at *δ* 208, supported a methyl ketone moiety. Evidence for an additional α,*β*-unsaturated carbonyl system was provided by an IR absorption at 1660 cm^{-1}, ^{13}C NMR signals at *δ* 201.5, 143.5, and 138.8 (carbons of an α,*β*-unsaturated ketone system), and a three-proton signal in the ^{1}H NMR at *δ* 2.04, indicating a methyl group at the *β*-carbon. The MS fragmentation of **4** was similar to that of **3** with respect to peaks at *m*/*z* 264 [M^+], 221 [$M^+ -$ MeCO] and 43 [$MeCO^+$]. The 2D ^{1}H NMR COSY spectra of **3** and **4** established the relationships between the series of protons. The ^{13}C NMR assignments were achieved by HETCOR experiments and by comparison of their spectral data with those published for similar compounds.[9]

A previous report claims the isolation of **4** from *Dittrichia graveolens*;[10] however, comparison of the reported ^{1}H NMR data with those of **4** clearly shows that they are different. Therefore, the compound isolated from *D. graveolens* corresponds to the 12,8α-olide isomer.

Cleavage products are commonly found in chromic acid oxidation.[11−14] For instance, it has been demonstrated that oxidation of a tricyclic diterpenoid with Jones's reagent affords a ring B-opened, substituted *p*-benzoquinone.[13] A possible route leading to the formation of **3** and **4** is shown in Scheme 1.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher−Johns melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-360 digital polarimeter. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. EIMS data were determined on a JEOL JMS-AX505HA mass spectrometer at 70 eV. The UV spectra were obtained on a Shimadzu 160 UV spectrometer in MeOH solutions. All homonuclear and heteronuclear 1D and 2D NMR spectra were recorded on a Varian Unity 300 spectrometer at room temperature using standard pulse programs of the Varian library. Chemical shifts are given

* To whom correspondence should be addressed. Tel.: (525) 6-224405. Fax: (525) 6-162217.
† Instituto de Química, UNAM.
‡ FES−C, UNAM.
§ Contribution no. 1690 of the Instituto de Química, UNAM.

10.1021/np980571t CCC: $18.00
Published on Web 05/01/1999

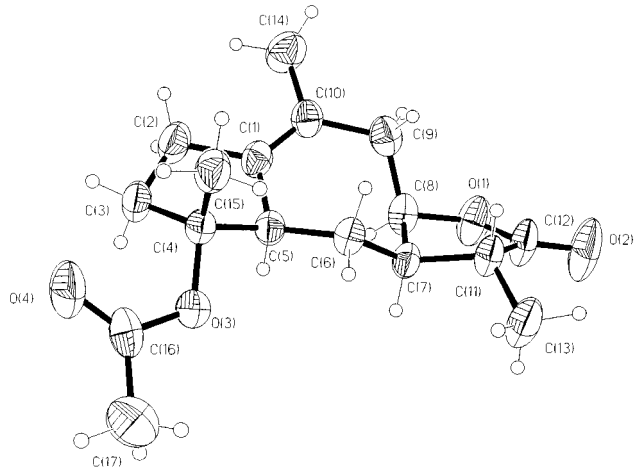


Figure 1. ORTEP-like view of 4α-acetyl-guaia-1(10)-en-13α-methyl-12,8*β*-olide. Thermal ellipsoids at 30% probability level.

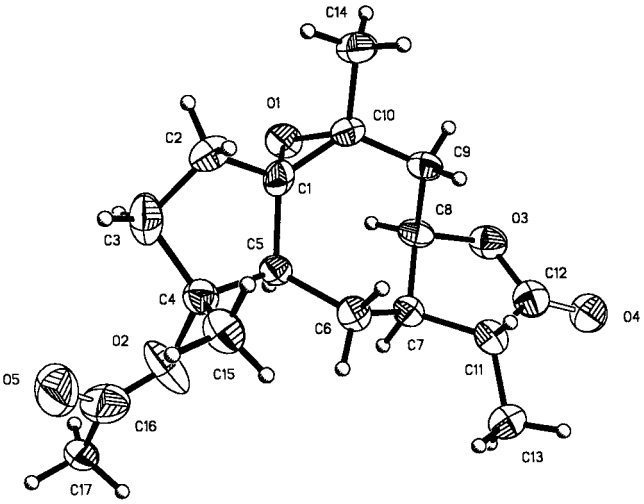
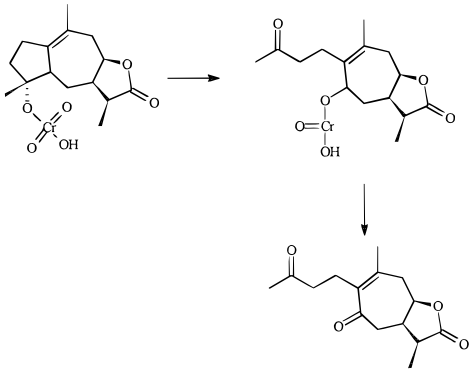


Figure 2. ORTEP-like view of 4α-acetyl-guaia-1(10) α-epoxy-13α-methyl-12,8*β*-olide. Thermal ellipsoids at 30% probability level.

Scheme 1



in parts per million (*δ*), referred to TMS. ^{13}C NMR spectra, including APT and HETCOR data were measured in $CDCl_3$. Column chromatographies were carried out on Kieselgel G (Merck, Darmstadt, Germany). TLC was performed on Si gel 60 (Merck).

Plant Material. *Stevia tomentosa* was collected near Tepeji del Rio, Querétaro, México, in August 1996. A voucher specimen (MEXU 561026) was deposited at the Herbarium of Instituto de Biología, UNAM, Coyoacán, D. F. México.

Extraction and Isolation. Dried and powdered aerial parts (2.6 kg) were extracted with MeOH (7 L) at reflux for 4 h. The MeOH extract was concentrated to ca. 2 L in vacuo, then H_2O was added (500 mL) and extracted with $CHCl_3$ (3 × 2 L). After concentration in vacuo, the chloroformic portion (21.8 g) was chromatographed on Si gel (750 g, 1:4 C_6H_6−EtOAc). A material (10.1 g), obtained from the first 20 fractions was rechromatographed on Si gel and eluted with the same solvent system, yielding dihydropseudoivalin (**1**) (2 g).

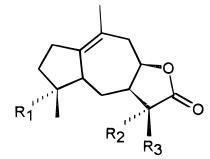
Dihydropseudoivalin (1). reddish oil $[\alpha]_D$ 145° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.55) nm; IR (film) ν_{max} 3450, 1755 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.61 (1H, m, H-8), 1.58 (3H, s, H-14), 1.20 (3H, d, J = 7 Hz, H-13), 1.04 (3H, s, H-15), ^{13}C NMR ($CDCl_3$, 75.5 MHz,) δ 180.5 (C-12), 138.2 (C-10), 122.2 (C-1), 80.6 (C-8), 79.5 (C-4), 52.8 (C-5), 40.5 (C-7), 38.7 (C-2), 38.2(C-11), 37.2(C-9), 27.7 (C-3), 22.7 (C-6), 21.5 (C-14), 21.4 (C-15), 12.9 (C-13); EIMS *m*/*z* 250 [M]$^+$ (10), 232 (5), 217 (10); *anal.* C 71.00%, H 8.81%, calcd for $C_{15}H_{22}O_3$, C 72.00%, H 8.80%.

Epidihydropseudoivalin (2). Treatment of **1** with sodium methoxide in MeOH, as previously reported[7] afforded **2** as white crystals ($CHCl_3$): mp 137−138 °C; $[\alpha]_D$ −74.5° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (3.51) nm; IR (film) ν_{max} 3470, 1760 cm^{-1}; ^{1}H NMR ($CDCl_3$ 300 MHz) δ 4.61 (1H, ddd, J = 2, 5.3, 8 Hz H-8), 2.61 (1H, dd, J = 8, 10 Hz H-9), 2.11 (1H, dd, J = 8, 10 Hz H-9′), 1.61 (3H, s, H-14), 1.24 (3H, d, J = 6 Hz, H-13), 0.99 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 179.3 (C-12), 137.3 (C-10), 123.7 (C-1), 79.5 (C-8), 79.3 (C-4), 52.7 (C-5), 45.5 (C-7), 39.1 (C-2) and (C-11), 35.3 (C-9), 28.0 (C-3), 26.3 (C-6), 21.6 (C-14), 21.0 (C-15), 13.5 (C-13); EIMS *m*/*z* 250 [M]$^+$ (30), 232 (10), 217 (15); *anal.* C 71.71%, H 8.85%, calcd for $C_{15}H_{22}O_3$, C 72.00%, H 8.80%.

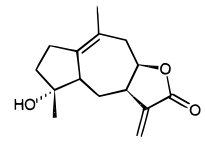
4α-Acetyl-guaia-1(10)-en-13α-methyl-12,8*β*-ol (6). A solution of **2** (600 mg) in isopropenyl acetate (18 mL) and catalytic amounts of *p*-toluensulfonic acid was heated at 50 °C for 40 min. Then the reaction mixture was poured over ice and extracted with EtOAc. The organic layer was washed with H_2O, dried, and evaporated under vacuum. The solid residue was chromatographed on a Si gel column (100 g) and eluted with hexane−EtOAc (7:3). Fractions 20−35 (5 mL each) gave **6**, which was crystallized from hexane−EtOAc to give white needless (595 mg, 98%): mp 154−155 °C $[\alpha]_D$ −102° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (3.51) nm; IR (film) ν_{max} 1770, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.64 (1H, ddd, J = 2, 5, 8 Hz H-8), 2.02 (3H, s, *CH*$_3$COO), 1.65 (3H, s, H-14), 1.30 (3H, d, J = 6 Hz, H-13), 1.23 (3H, s, H-15), ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 179.3 (C-12), 170.3 (*CO*CH$_3$), 137.3 (C-10), 123.7 (C-1), 87.5 (C-4), 79.5 (C-8), 52.7 (C-5), 45.5 (C-7), 39.1 (C-2), (C-11), 35.3 (C-9), 28.0 (C-3), 26.3 (C-6), 22.0 (CO*CH*$_3$), 21.8 (C-14), 17.4 (C-15), 13.5 (C-13); EIMS *m*/*z* 250 [M]$^+$ (3), 232 (61), 217 (5) 43 (53); *anal.* C 69.66%, H 8.24%, calcd for $C_{17}H_{24}O_4$, C 69.86%, H 8.21%.

X-ray Analysis of 6. Crystals obtained from CH_2Cl_2 were used for X-ray crystallography. Space group: trigonal, $P3_2$; cell parameters: a = 11.243(3) Å, c = 11.122(3) Å, Z = 3; D = 1.196 mg/m^3; R = 0.0432 for 1931 reflections [$F > 4.0\sigma(F)$]. The intensity data were collected on a Siemens P4 diffractometer, and the structure was solved using Siemens SHELXTL Plus (PC Version) software. Atomic coordinates, distances, angles, and torsional angles for **6** and **7** have been deposited at the Cambridge Crystallographic Data Centre.

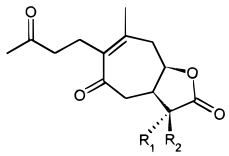
4α-Acetyl-guaia-1(10)-α-epoxy-13α-methyl-12,8*β*-olide (7). A mixture of **6** (430 mg) and *m*-CPBA (250 mg as 80% purity) in dry CH_2Cl_2 (20 mL) was stirred for 1 h at room temperature. The solution was diluted with CH_2Cl_2, washed with $NaHCO_3$ after H_2O, and dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was chromatographed on Si gel column (100 g) and eluted with hexane−EtOAc (7:3). Fractions 7−11 (5 mL each) gave **7**, which was crystallized from hexane to give white crystals (320 mg):mp 132−133 °C, $[\alpha]_D$ −101°, ^{1}H NMR ($CDCl_3$ 300 MHz) δ 4.64 (1H, ddd, J = 2, 5, 8 Hz H-8), 2.02 (3H, s, *CH*$_3$COO), 1.42 (3H, s, H-14), 1.28 (3H, d, J = 6 Hz, H-13), 1.30 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 178.6 (C-12), 137.3 (C-10), 123.7 (C-1), 88.1 (C-4), 75.4 (C-8), 50.9 (C-5), 44.3 (C-7), 35.7 (C-2), 39.5 (C-11), 34.9 (C-9), 30.8 (C-3), 26.6 (C-6), 22.0 (C-14), 21.7 (C-15), 13.8 (C-13); *anal.* C 69.85%, H 8.31%, calcd for $C_{17}H_{24}O_4$, C 69.86%, H 8.21%.



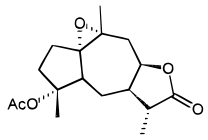
1 R_1=OH, R_2=H, R_3=Me
2 R_1=OH, R_2=Me, R_3=H
6 R_1=OAC, R_2=Me, R_3=H



5



3 R_1=Me, R_2=H
4 R_1=H, R_2=Me



7

X-ray Analysis of 7. Crystals obtained from CH_2Cl_2 were used for X-ray crystallography. Space group: orthorhombic, $P_{2,2,2}$ cell parameters: $a = 8.689(2)$ Å, $b = 13.125(3)$ $c = 14.445$ Å, $Z = 4$; $D = 1.196$ mg/m^3; $R = 0.096$ for 1015 reflections [$F > 3.0\sigma(F)$]. The intensity data were collected on a Siemens P3/F diffractometer, and the structure was solved using Siemens SHELXTL Plus (PC Version) software.

4,5-Dioxo-xanth-1(10)-en-13*β*-methyl-12,8*β*-olide (3). A solution of **1** (100 mg) in Me_2CO (5 mL) was treated with Jones's reagent at 0 °C for 15 min. Then H_2O was added and the reaction mixture extracted with EtOAc, washed with brine, dried, and the solvent removed under vacuum. Chromatography of the residue on Si gel (100 g) using C_6H_6−EtOAc (1:1) as developing solvent led to isolation of **3** as white crystals: mp 62 °C, $[\alpha]_D$ 133° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.99) nm; IR (film) ν_{max} 1760, 1715, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$ 300 MHz) δ 4.66 (1H, dt, $J = 7.2$, 8 Hz H-8), 2.87 (1H, qd, $J = 8$, 7.2 Hz, H-11) 2.71 (1H, m, H-7) 2.09 (3H, s, H-15), 2.01 (3H, s, H-14), 1.18 (3H, d, $J = 7.2$ Hz, H-13); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 208.2 (C-4), 201.5 (C-5), 177.9 (C-12), 143.5 (C-10), 138.8 (C-1), 76.3 (C-8), 42.4 (C-3), 39.1 (C-6), 38.2 (C-11), 37.7 (C-9), 36.9 (C-7), 29.7 (C-14), 23.5 (C-15), 23.1 (C-2), 10.3 (C-13); EIMS *m/z* 264 [M]$^+$ (3), 221 (10), 206 (33), 43 (100); *anal.* C 68.31%, H 7.66%, calcd for $C_{15}H_{20}O_4$, C 68.18%, H 7.57%.

4,5-Dioxo-xanth-1(10)-en-13α-methyl-12,8*β*-olide (4). A solution of **2** (100 mg) in Me_2CO (5 mL) was treated with Jones's reagent as described for **3**, yielding **4** as white crystals mp 82 °C, $[\alpha]_D$ −78.5° (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 245, (3.876); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.62 (1H, dt, $J =$ 6, 7.2, 8.5 Hz H-8), 2.50 (1H, q, $J = 7.5$ Hz, H-11) 2.75 (1H, d, $J = 8$ Hz, H-7) 2.12 (3H, s, H-15), 2.08 (3H,s, H-14), 1.30 (3H, d, $J = 7.5$ Hz, H-13); ^{13}C NMR ($CDCl_3$ 75.5 MHz) δ 207.9 (C-4), 199.1 (C-5), 178.0 (C-12), 147.8 (C-10), 139.5 (C-1), 77.6 (C-8), 44.4 (C-6), 42.5 (C-3), 40.5 (C-11), 39.9 (C-7), 38.8 (C-9), 29.7 (C-14) 24.4 (C-15), 23.3 (C-2), 14.8 (C-13); EIMS *m/z* 264 [M]$^+$ (93), 246 (15), 221 (15), 43 (100); *anal.* C 67.82%, H 7.61%, calcd for $C_{15}H_{20}O_4$, C 68.18%, H 7.57%.

Acknowledgment. We are indebted to Rubén Gaviño, Hector Ríos, Rocio Patiño, and Javier Pérez for technical assistance. We also wish to thank M.S. Georgina Espinosa and Dr. Rubén A. Toscano for X-ray determinations. Partial financial support from UNAM (DGAPA IN500597) is acknowledged.

References and Notes

(1) Fischer, N. H.; Olivier, E. J.; Fischer, H. D. In *Progress in the Chemistry of Organic Natural Products*; Herz, W.; Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: New York, 1979; Vol. 38, pp 47−388.
(2) Parodi, F. J.; Fischer, N. H. *J. Chem. Soc. Chem. Commun.* **1986**, 1405−1405.
(3) Parodi, F. J.; Fronczeck, F. R.; Fischer, N. H. *J. Nat. Prod.* **1989**, *52*, 554−566.
(4) Angeles, E.; Fotting, K.; Griecco, P. A.; Hoffman, J.; Miranda, R.; Salmón, M. *Phytochemistry* **1982**, *21*, 1804−1806.
(5) Salmón, M.; Díaz, E.; Ortega, A. *J. Org. Chem.* **1973**, *38*, 1759−1762.
(6) Martínez-Vázquez, M.; Gallegos, R. E.; Joseph-Nathan, P. *Phytochemistry* **1990**, *29*, 1689−1690.
(7) Herz, W.; Romo de Vivar, A.; Lakshmikanthan, M. V. *J. Org. Chem.* **1965**, 118−122.
(8) Gitany, R.; Anderson, G. D.; McEwen, R. S. *Acta Crystallogr.* **1974**, *B30*, 1900−1904.
(9) Budesinsky, M.; Saman, D. *Annu. Rep. NMR Spectrosc.* **1995**, *30*, 408−411.
(10) Rustaiyan, A.; Jakupovic, J.; ChanThi, T. V.; Bohlman, F.; Sadjadi, *Phytochemistry* **1987**, *26*, 2603−2606.
(11) Wiberg, K. B.; Mukherjee, S. K. *J. Am. Chem. Soc.* **1974**, *96*, 1884−1888.
(12) Cawley, J. J.; Spaziano, V. T. *Tetrahedron Lett.* **1973**, 4719−4722.
(13) Beckett, S. K.; Bendall, J. G.; Cambie, R. C.; Rutledge, P. S.; Walker, M. F.; Woodgate, P. D. *Aust. J. Chem.* **1997**, *50*, 933−937.
(14) Liu H. *Can. J. Chem.* **1976**, *54*, 3113−3115.

NP980571T