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Xorge A. Dominguez, H. Sanchez V., Sergio Garcia G., Griselda Espinosa B., Howard J. Williams, Claudio Ortiz, A. Ian Scott, and Joseph H. Reibenspies

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ISOLATION AND IDENTIFICATION OF XOCHITLOLDIONE AND ISOXOCHITLOLONE FROM *CNIDOSCULUS URENS*

XORGE A. DOMINGUEZ,* H. SANCHEZ V., SERGIO GARCIA G., GRISELDA ESPINOSA B.,

Departamento de Quimica, Instituto Tecnologico y de Estudios Superiores de Monterrey, Sucursal de Correos "J," Monterrey, N.L. 64849, Mexico

HOWARD J. WILLIAMS,* CLAUDIO ORTIZ, A. IAN SCOTT,

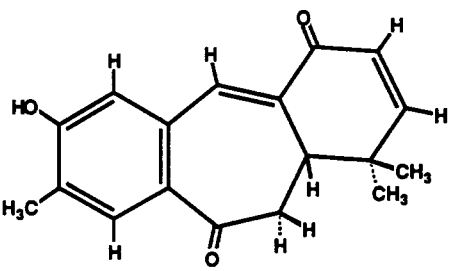
Center for Biological NMR, Department of Chemistry,

and JOSEPH H. REIBENSPIES

Department of Chemistry, Texas A&M University, College Station, Texas 77843

ABSTRACT.—Major components of MeOH extracts from the plant roots of *Cnidosculus urens* purified by cc and tlc and crystallization were lupeol acetate and the previously unreported compounds isoxochitlolone [**1**] and xochitloldione [**2**], which were identified through mass, ir, nmr, and uv spectroscopy and X-ray crystallography. In preliminary testing, isoxochitlolone has been found to be active against *Escherichia coli* and *Staphylococcus aureus*.

Extracts of roots of *Cnidosculus urens* L. (Euphorbiaceae) are used in folk medicine by the Huasteca Indians of central eastern Mexico. Dried ground roots of *C. urens* were extracted first with hexane and then with MeOH. The more polar MeOH components were purified by partitioning between CH_2Cl_2 and H_2O, and the organic soluble materials were separated chromatographically, giving lupeol acetate and the two previously unreported compounds isoxochitlolone [**1**] and xochitloldione [**2**], which were identified through nmr, uv, ir, mass spectral, and crystallographic data. The carbon skeleton of these compounds is identical to that of the recently reported compound xochitlolone [**3**], which was isolated from root extracts of the plant *Jatropha multiloba* (Euphorbiaceae) (1). Nmr and mass spectral data of this compound is included for comparison. To our knowledge, these are the only reported natural compounds containing this carbon skeleton. Their biosynthetic origin is at present undetermined, but may include acetate and terpenoid precursors. Preliminary bioassays of the compounds indicate that isoxochitolone is active against *Escherichia coli* and *Staphylococcus aureus*.



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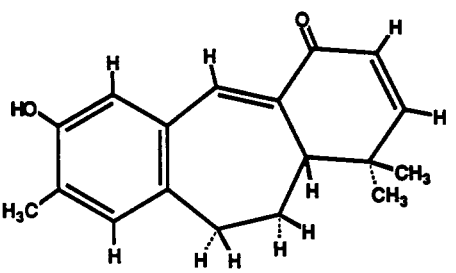

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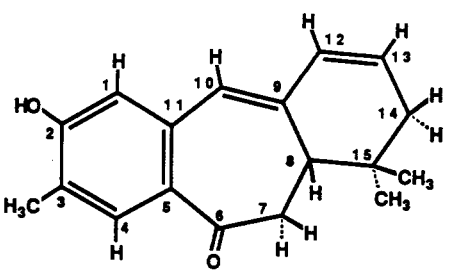

1

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. Uv spectra were obtained on a Perkin-Elmer Lambda 3 instrument in MeOH with added reagents as indicated. Ir spectra were obtained on a Beckman model 4244 instrument using KBr pellets. Nmr spectra were obtained on a Bruker AM 500 500 MHz Fourier transform instrument in MeOH-d_4 solution. ^{1}H- and ^{13}C-nmr assignments were made through COSY, DEPT, and selective proton carbon decoupling experiments and by analogy to related compounds. Mass spectra were obtained on a VG Instruments VG-70 C instrument in ei mode at 70 eV. X-ray crystallography was performed on a Nicolet R3/M diffractometer (oriented graphite monochromator; MoKα $\lambda = 0.71073$ Å radiation), and structures were solved by direct methods (2). Optical rotations were recorded on a Perkin-Elmer Model 141 polarimeter in EtOH.

PLANT MATERIAL.—Roots of *C. urens* were collected from the Huasteca region of Mexico on May 5, 1989. Voucher specimen 8390 has been placed in the herbarium at ITESM.

EXTRACTION AND PURIFICATION PROCEDURES.—Dried milled roots (2100 g) were extracted in a Soxhlet apparatus, first with hexane for 7 days and then with MeOH for 7 days. Evaporation of the MeOH extract gave 34.5 g of a brownish residue which was partitioned between CH_2Cl_2 and H_2O (3:1). On evaporation, the CH_2Cl_2 layer gave 19 g of brownish oil, which was flash chromatographed on Si gel using a hexane/CH_2Cl_2 step gradient solvent system. Among the pure compounds obtained were 200 mg of lupeol acetate, which was identified by comparison of its chromatographic and spectral properties with those of an authentic sample, 13 mg of a yellow powder, mp 236°, given the name isoxochitlolone [**1**], and 30 mg of yellow needles, mp 227°, given the name xochitloldione [**2**].

Isoxochitlolone [**1**].—Compound **1**: ms *m/z* (%) 269 (51.3), $[M]^+$ 268 (100.0), $[M-Me]^+$ 253 (26.8), 226 (26.2) $[M-Me_2C]^+$ 225 (49.4), 212 (12.0), $[M-Me-Me_2C]^+$ 211 (16.4), 200 (15.0), 197 (25.0), 165 (15.1); ^{13}C nmr δ 200.6 (s, C-6), 159.3 (s, C-2), 142.2 (s), 136.9 (s), 131.6 (d, C-4), 130.8 (d, C-10), 128.6 (d, C-12), 128.0 (d, C-13), 127.1 (s), 123.8 (s), 117.1 (d, C-1), 45.4 (d, C-8), 44.4 (t, C-7), 41.6 (t, C-14), 35.7 (s, C-15), 29.7 (q, Me-3), 22.5 (q, Me-15), 16.3 (q, Me-15); ^{1}H nmr 7.64 (1H, s, H-4), 6.62 (1H, s, H-1), 6.26 (1H, dd, $J = 2.3$, 6.6, H-12), 6.24 (1H, d, $J = 2.8$, H-10), 5.85 (1H, ddd, $J = 2.6$, 5.6, 6.6, H-13), 2.81 (1H, dd, $J = 5.1$, 14.8, H-7), 2.77 (1H, d, $J = 14.8$, H-7), 2.39 (1H, m, $J = 2.3$, 2.8, 5.1, H-8), 2.18 (3H, s, Me-3), 2.10 (1H, dt, $J = 2.6$, 17.5, H-14), 2.0 (1H, dd, $J = 5.6$, 17.5, H-14), 1.10 (3H, s, Me-15), 0.88 (3H, s, Me-15); ir (cm^{-1}) 3318, 3035, 2952, 2921, 1651, 1587, 1571, 1509, 1354, 1283, 1150, 1070, 985, 889, 730, 673, 559; uv (MeOH) λ max 360, 275 (MeOH + NaOme) 365, 300.

Xochitloldione [**2**].—Compound **2**: ms *m/z* (%) 283 (25.3), $[M]^+$ 282 (100), 267 (21.2), 239 (33.5), 211 (10.2), 200 (12.8), 187 (20.5), 186 (35.2), 96 (36.7), 43 (11.1); ^{13}C nmr δ 201.6 (s, C-6), 189.2 (s, C-12), 162.2 (d, C-14), 161.3 (s, C-2), 138.8 (d, C-10), 138.4 (s), 134.8 (s), 133.1 (d, C-4), 130.9 (s), 128.1 (s), 128.0 (d, C-13), 121.7 (d, C-1), 46.0 (d, C-8), 43.7 (t, C-7), 38.2 (s, C-15), 27.4 (q, 3-Me), 24.0 (q, 15-Me), 15.9 (q, 15-Me); ^{1}H nmr δ 7.62 (1H, s, H-4), 7.54 (1H, d, $J = 2.7$, H-10), 6.97 (1H, d, $J = 10$, H-14), 6.88 (1H, s, H-1), 6.10 (1H, d, $J = 10$, H-13), 3.11 (1H, t, $J = 11.8$, H-7), 2.90 (1H, dt, $J = 11.8$, 2.5, 2.7, H-8), 2.85 (1H, dd, $J = 11.8$, 2.5, H-7), 2.21 (3H, s, 3-Me), 1.31 (3H, s, 15-Me), 1.00 (3H, s, 15-Me); ir 3246, 1658, 1652; uv (EtOH) 245, 284, 310, 355; $[\alpha]_{23}$ (6.0 mg/ml EtOH) 589 (−193.8°), 578 (−211.2°), 546 (−264.2°), 430 (−753.3°).

Xochitlolone [**3**].—Compound **3**: ms *m/z* (%) 269 (26.8), 268 (100.0) $[M]^+$ 254 (11.7), $[M-Me]^+$ 253 (48.0), $[M-CO]^+$ 240 (18.5), 225 (25.5), 172 (28.6), 171 (17.1), 158 (10.4), 157 (8.6), 128 (10.0); ^{13}C nmr δ 192.1 (s, C-12), 164.4 (d, C-14), 154.7 (s, C-2), 139.0 (d, C-10), 137.4 (s), 136.5 (s), 133.2 (s), 132.1 (s), 132.1 (d, C-4), 127.7 (d, C-13), 127.5 (s), 120.6 (d, C-1), 53.5 (d, C-8), 39.1 (s, C-15), 34.0 (t, C-6), 29.3 (t, C-7), 28.1 (q, 3-Me), 22.2 (q, 15-Me), 16.0 (q, 15-Me); ^{1}H nmr δ 7.49 (1H, d, $J = 2.4$, H-10), 6.93 (1H, d, $J = 10$, H-14), 6.84 (1H, s, H-4), 6.75 (1H, s, H-1), 6.04 (1H, d, $J = 10$, H-13), 2.92 (1H, ddd, $J = 2.4$, 5.7, 12.1, H-8), 2.82 (1H, ddd, $J = 1.3$, 7.2, −14.8, H-6), 2.68 (1H, dd, $J = 9.9$, −14.8, H-6), 2.32 (1H, m, $J = 1.3$, 5.7, 9.9, −13.9, H-7), 2.16 (3H, s, 3-Me), 1.65 (1H, m, $J = 1$, 1.3, 9.9, 12.1, −13.9, H-7), 1.25 (3H, s, 15-Me), 0.93 (3H, s, 15-Me).

X-RAY CRYSTALLOGRAPHY.[1]—A light yellow crystal of **1** [0.12 mm × 0.30 mm × 0.32 mm] was mounted on a glass fiber with epoxy at room temperature (formula: $C_{18}H_{20}O_2$, formula weight = 268.3 AMU). Cell parameters [Monoclinic, $P2_1$, $a = 8.334$ (2) Å, $b = 6.9411$ (15) Å, $c = 12.973$ (4) Å, $\beta = 101.93$ (2)°, V = 734.2 (3)

[1]Atomic coordinates for **1** and **2** have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

$Å^3$, $D_x = 1.214$ g·cm^{-3}, $\mu = 0.072$ mm^{-1}, $Z = 2$, $F(000) = 288$ e$^-$] were calculated from the least-squares fitting of the setting angles for 25 reflections ($2\theta_{avg} = 19.6$). Data was collected for $4.0° \leq 2\theta \leq 50.0°$ [Wyckoff scans, $-9 \leq h \leq 9$, $0 \leq k \leq 8$, $0 \leq l \leq 15$] at 296° K. Scan range for the data collection was 1.20° plus Kα separation, with a variable scan rate of 1.50 to 15.00°/min. Lorentz and polarization corrections applied to 1486 reflections. No absorption correction was applied. A total of 979 unique observed reflections ($R_{int} = 0.03$), with $|I| \leq 2.0\ \sigma$ (I), were used in further calculations. The structure was solved by direct methods (2). Full-matrix least-squares anisotropic refinement for all non-hydrogen atoms [number of least-squares parameters = 180; quantity minimized $\Sigma w(F_o\text{-}F_c)^2$; $w = \sigma^2F + gF^2$, $g = 0.0008$] (3) yielded $R = 0.053$, $wR = 0.059$, and $S = 1.36$ at convergence [largest $\Delta/\sigma = 0.0017$; mean $\Delta/\sigma = 0.0001$; largest positive peak in the final Fourier difference map = 0.15e$^-$ $Å^3$; largest negative peak in the final Fourier difference map = −0.22e$^-$ $Å^3$] (2). Hydrogen atoms were placed in idealized positions with isotropic thermal parameters fixed at 0.08. Neutral atom scattering factors were taken from Cromer and Weber.

A light yellow needle of **2** (0.20 mm × 0.24 mm × 0.50 mm) was mounted on a glass fiber with vacuum grease at room temperature and cooled to 193° K in an N_2 cold stream (formula: $C_{18}H_{18}O_3$, formula weight = 282.3 AMU). Cell parameters [triclinic, *P1*, $a = 6.000$ (2) Å, $b = 7.954$ (3) Å, $c = 8.371$ (3) Å, $\alpha = 102.812°$, $\beta = 101.21$ (2)°, $\gamma = 106.24$ (3)°, $V = 359.7$ (2) $Å^3$, $D_x = 1.303$ g·cm^{-3}, $\mu = 0.082$ mm^{-1}, $Z = 1$, $F(000) = 150e^-$] were calculated from the least-squares fitting of the setting angles for 25 reflections ($2\theta_{avg} = 24.2$). Data was collected for $4.0° \leq 2\theta \leq 50.0°$ (Wyckoff scans, $-7 \leq h \leq 7$, $-9 \leq k \leq 9$, $-9 \leq l \leq 0$) at 193° K. Scan range for the data collection was 2.00° plus Kα separation, with a variable scan rate of 1.50 to 15.00°/min. Lorentz and polarization corrections applied to 1362 reflections. No absorption correction was applied. The structure was solved by direct methods (2). A total of 1322 unique observed reflections ($R_{int} = 0.004$), with $|I| \leq 1.5\ \sigma$ (I), were used in further calculations. Full-matrix least-squares anisotropic refinement for all non-hydrogen atoms [number of least-squares parameters = 187; quantity minimized $\Sigma w(F_o - F_c)^2$; $w = \sigma^2F + gF^2$, $g = 0.0010$] (3) yielded $R = 0.039$, $wR = 0.052$ and $S = 1.46$ at convergence (largest $\Delta/\sigma = 0.0012$; mean $\Delta/\sigma = -0.0001$; largest positive peak in the final Fourier difference map = 0.26e$^-$ $Å^3$; largest negative peak in the final Fourier difference

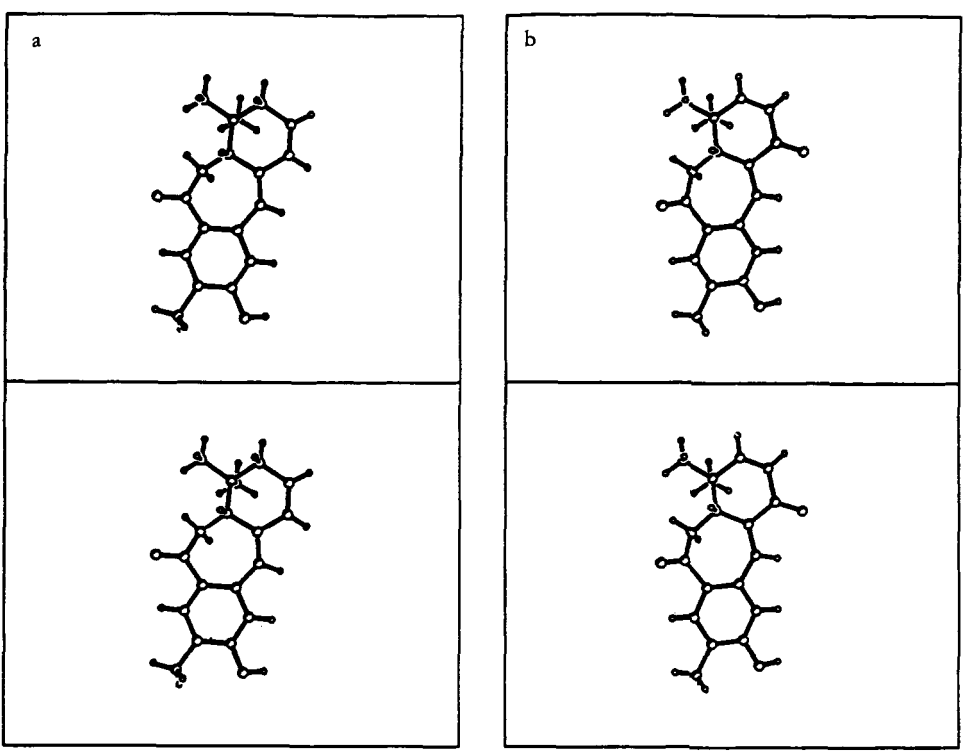

FIGURE 1. Stereoviews of isoxochitlolone [**1**] (a) and xochitloldione [**2**] (b). Hydrogens are in idealized positions.

map = $-0.25e^-$ Å^3). Hydrogen atoms were placed in idealized positions with isotropic thermal parameters fixed at 0.08 (Figure 1). Neutral atom scattering factors were taken from Cromer and Weber (3).

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