

KUKULKANINS A AND B, NEW CHALCONES FROM
MIMOSA TENUFOLIA

XORGE A. DOMINGUEZ, SERGIO GARCIA G.,

Departamento de Química, Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM),
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

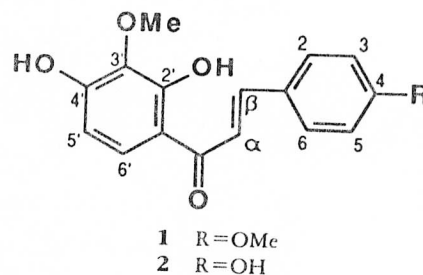
ABSTRACT.—Two new methoxychalcones, kukulkanins A (2',4'-dihydroxy-3',4'-dimethoxychalcone) and B (2',4',4'-trihydroxy-3'-methoxychalcone), were isolated by flash chromatography of extracts of small branches of *Mimosa tenuifolia*. The compounds were identified using ir, nmr, uv, and mass spectroscopy, and their structures were confirmed by X-ray crystallography.

Powdered bark of the plant *Mimosa tenuifolia* L. (= *Mimosa cabreria*) (Fabaceae), "tepescohuite," historically has been used in treatment of burns and prevention of inflammation. In Mexico, this material has recently been used to alleviate suffering of burn victims following the eruption of the volcano Chichonal in 1982, the 1985 earthquake, the San Juanico natural gas explosion in 1984, and the crash of an airliner in Toluca in 1986. Although no scientific literature is available on the subject, many accounts in the popular press and on television indicate that application of properly prepared tepescohuite bark powder to burned tissue facilitates skin regeneration and prevents scarring. We have undertaken a study of constituents of the plant extracts and report the first identifications of new compounds.

RESULTS AND DISCUSSION

Step gradient flash chromatography of extracts of the plant produced two bright yellow crystalline compounds. Mass spectra were similar in the low mass region, differing in molecular ion m/z values that were 300 for component A and 286 for component B, indicating possible molecular formulas of $C_{17}H_{16}O_5$ and $C_{16}H_{14}O_5$, respectively. Mass spec-

tral and uv data were consistent with methoxy chalcone structures. 1H -Nmr spectra were also in good agreement with the assignments of Mabry *et al.* (1) for substituted chalcones. Compound A exhibited methoxy singlets at δ 3.81 and 3.72 while one methoxy at δ 3.72 was found in B. Hydrogens in the α and β positions produced a doublet of doublets at δ 7.82 and 7.77 ($J = 15.3$ Hz) in A, but appeared as an apparent singlet in B at δ 7.75 (in MeOH- d_4 , the doublet of doublets was present). Peaks for hydrogens at the 3,5 and 2,6 positions appeared as 2H doublets of doublets at δ 7.01 and 7.86 ($J = 8.7$ Hz) for A and δ 6.83 and 7.75 ($J = 8.5$ Hz) for B, proving the presence of a single para substituent in one ring. Doublets attributed to hydrogens in the 6' and 5' positions were located at δ 7.96 and 6.47 ($J = 9$ Hz) in A and δ 7.95 and 6.47 ($J = 9$ Hz)



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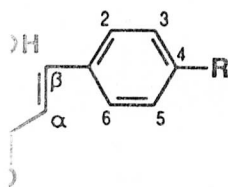
Monterrey (ITESM),

OTT,

Texas 77843

2,4-dihydroxy-3',4-dimethoxy-6-methylchalcone isolated by flash chromatography. The structures were identified and confirmed by X-ray

structures were consistent with the proposed structures. ¹H-Nmr spectra were in good agreement with assignments of Mabry et al. (1) for chalcones. Compound A showed singlets at δ 3.81 and 3.72 for methoxy protons in the α and β positions, respectively, and a doublet of doublets (J = 15.3 Hz) in A, which appeared as a singlet in B (due to H-d₄, the doublet of doublets). Peaks for hydrogens at the 2,6 positions appeared as doublets at δ 7.96 and 6.47 (J = 9 Hz) for A and δ 7.85 and 6.47 (J = 9 Hz) for B, providing evidence for a single para substitution pattern. Doublets attributed to protons at the 6' and 5' positions were observed at δ 7.96 and 6.47 (J = 9 Hz) for A and δ 7.85 and 6.47 (J = 9 Hz) for B, providing evidence for a single para substitution pattern.



R=OMe
R=OH

in B, indicating adjacent tetrasubstitution on the second ring. A 2,3,6 substitution pattern that would also produce a doublet of doublets for these two hydrogens was considered unlikely on both chemical shift and biosynthetic grounds. In view of the ¹H shift differential between the two compounds at the 3,5 positions, the additional methoxy of compound A was tentatively placed at the 4 position.

¹³C-Nmr spectra of both components exhibited carbonyl peaks (δ 191.4 for A, δ 192.1 for B) and methoxy peaks (δ 59.2 and 54.9 for A, δ 59.8 for B). Peaks attributed to the 2,6 and 3,5 positions were found at δ 130.5 and 113.9 in A and δ 131.3 and 115.9 in B (2). For both compounds, DEPT experiments produced 6 CH peaks. Major mass spectral fragmentations at 166 and 138 m/z for both components can be attributed to transfer of the 2' hydroxyl hydrogen to the α position to form a flavanone, followed by cleavage on either side of the carbonyl (3), again providing evidence for presence of the additional methoxy methyl in component A in the 4 position which is lost in this fragmentation. The uv spectra of both compounds were little changed on addition of H₃BO₃, indicating that no ortho free hydroxyls were present in either compound (1). The methoxy present on the larger fragment

in both compounds must therefore be in the 3' position.

Proposed structures were confirmed through X-ray crystallography.¹ The components are, thus, 2',4'-dihydroxy-3',4'-dimethoxychalcone and 2',4',4'-trihydroxy-3'-methoxychalcone; they are given the trivial names kukulkanins A and B, respectively, derived from the name given to the plant by the Indians who originally used it as a burn treatment. Clark-Lewis and Porter (4) isolated from the heartwood of *Acacia kempiana* a small quantity of a monomethyl chalcone that was tentatively identified as the latter component based on co-occurrence of a corresponding flavan-3,4-diol, but to our knowledge, the former compound has not previously been reported. Biological testing of the compounds is now in progress.

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 Fourier transform instrument in DMSO-d₆ solution. Tentative ¹³C assignments are based on selective proton decoupling studies and assignments of related compounds (2). Mass spectra were obtained on a VG Instruments VG-70 S instrument in ei mode at 70 eV. X-ray crystallography was performed on a Nicolet R3/M diffractometer.

PLANT MATERIAL.—*M. tenuifolia* was collected in Chiapas, Mexico during May 1987. Voucher specimen #8199 is deposited at the ITESM herbarium.

EXTRACTION AND FRACTIONATION PROCEDURES.—Small branches of "tepescohuite" plants (160 g) were extracted for 3 days in CH₂Cl₂-hexane-MeOH (1:1:1). Rotary evaporation gave 3.0 g of greenish residue that was subjected to flash chromatography on Si gel (Merck)

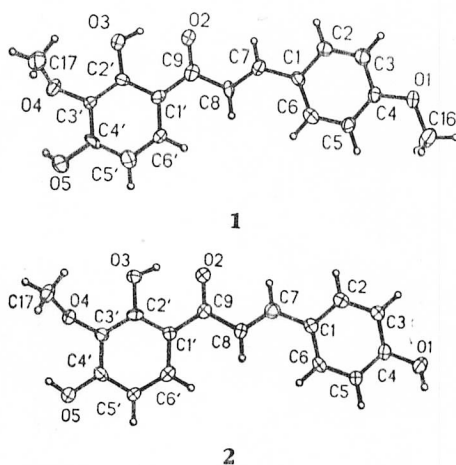


FIGURE 1. Thermal ellipsoid plots (50% probability) of kukulkanins A [1] and B [2].

¹Atomic coordinates for these structures 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.

using a hexane/Me₂CO step gradient. The hexane-Me₂CO (9:1) fraction gave 23 mg of yellow crystals of kukulkanin A [1], and hexane-Me₂CO (7:3) gave 45 mg of deep yellow crystals of kukulkanin B [2].

Kukulkanin A (2',4'-dihydroxy-3',4'-dimethoxy-chalcone) [1].—Mp 172°, ir cm⁻¹ 3432, 2925, 2854, 1635, 1608, 1563, 1512, 1439, 1428, 1356, 1323, 1309, 1262, 1227, 1176, 1163, 1120, 1036, 992, 984, 920, 856, 849, 828, 798, 706, 610; uv λ max nm (MeOH), 242 sh, 300 sh, 358; (NaOMe) 275 sh, 310 sh, 330 sh, 400; (AlCl₃) 250 sh, 310 sh, 328 sh, 402; (AlCl₃/HCl) 250 sh, 310 sh, 340 sh, 398; (NaOAc) 315 sh, 340 sh, 380; (NaOAc/H₃BO₃) 280 sh, 310 sh, 368; eims m/z (%) 301 (19.6), [M]⁺ 300 (100.0), [M-H-CH=CH-φ-OMe]⁺ 166 (76.7), [M-H-O=C-CH=CH-φ-OMe]⁺ 138 (64.9), [H+CH=CH-φ-OMe]⁺ 134 (99.3); ¹³C nmr δ 191.4 (C=O), 160.9 (C-3' or C-4' or C-4), 158.0 (C-3' or C-4' or C-4), 157.0 (C-3' or C-4' or C-4), 143.3 (C-β), 134.2 (C-2'), 130.5 (C-2, C-6), 126.7 (C-6'), 126.5 (C-1), 118.0 (C-α), 113.9 (C-3, C-5), 113.0 (C-1'), 107.5 (C-5'), 59.2 (3'-OMe), 54.9 (4-OMe); ¹H nmr (δ) 7.96 (1H, d, 9 Hz, H-6'), 7.86 (2H, d, 8.7 Hz, H-2, H-6), 7.82 (1H, d, 15.3 Hz, H-α), 7.77 (1H, d, 15.3 Hz, H-β), 7.01 (2H, d, 8.7 Hz, H-3, H-5), 6.47 (1H, d, 9 Hz, H-5'), 3.81 (3H, s, 3'-OMe), 3.72 (3H, s, 4-OMe).

Kukulkanin B (2',4',4'-trihydroxy-3'-methoxy-chalcone) [2].—Mp 215°, ir cm⁻¹ 3360, 3290, 1635, 1602, 1560, 1500, 1437, 1430, 1420, 1350, 1310, 1272, 1190, 1177, 1150, 1103, 1022, 905, 878, 822, 812, 772, 755, 720, 700; uv λ max nm (MeOH) 245 sh, 295 sh, 310 sh, 370; (NaOMe) 242 sh, 265 sh, 310 sh, 345 sh, 360 sh, 428; (AlCl₃) 245 sh, 310 sh, 330 sh, 400; (AlCl₃/HCl) 250 sh, 322 sh, 338 sh, 412; (NaOAc) 248 sh, 298 sh, 312 sh, 373; (NaOAc/H₃BO₃) 250 sh, 310 sh, 372; eims m/z (%) 287 (17.7), [M]⁺ 286 (100.0), [M-CH=CH-φ-OH]⁺ 167 (83.3), [M-H-CH=CH-φ-OH]⁺ 166 (84.0), 152 (22.3), [M-H-O=C-CH=CH-φ-OH]⁺ 138 (99.0), 137 (26.5), 123 (20.8), [H+CH=CH-φ-OH]⁺ 120 (40.1), 91 (22.2), 65 (22.6); ¹³C nmr δ 192.1 (C=O), 160.3 (C-3' or C-4' or C-4), 158.6 (C-3' or C-4' or C-4), 157.2 (C-3' or C-4' or C-4), 144.5 (C-β), 134.7 (C-2'), 131.3 (C-2, C-6), 127.0 (C-6'), 125.7 (C-1), 117.3 (C-α), 115.9 (C-3, C-5), 113.7 (C-1'), 107.9 (C-5'), 59.8 (3'-OMe); ¹H nmr δ 7.95 (1H, d, 9.0 Hz, H-6'), 7.75 (2H, d, 8.5 Hz, H-2, H-6), 7.75 (2H, ap. s, H-α, H-β), 6.83 (2H, d, 8.5 Hz, H-3, H-5), 6.47 (1H, d, 9.0 Hz, H-5'), 3.72 (3H, s, 3'-OMe).

X-RAY CRYSTALLOGRAPHY.—Examination and data collection for crystals from hexane/Me₂CO of kukulkanin A [1] (0.02 mm × 0.11 mm × 0.25 mm) and kukulkanin B [2] (0.04

mm × 0.14 mm × 0.48 mm) were performed on a Nicolet R3m/V diffractometer [MoKα (λ = 0.71073 Å) radiation]. Cell parameters for **1** were *a* = 21.954(6) Å, *b* = 3.9294(10) Å, *c* = 17.085(6) Å, β = 105.91(2)°, *V* = 1417.4(7) Å³, *D_x* = 1.407 g/cm³, μ = 0.097 mm⁻¹, *Z* = 4, *F*(000) = 632 e⁻, space group P2₁/c; for **2**, *a* = 12.370(12) Å, *b* = 5.397(4) Å, *c* = 20.120(17) Å, β = 98.11(7)°, *V* = 1330(2) Å³, *D_x* = 1.430 g/cm³, μ = 0.100 mm⁻¹, *Z* = 4, *F*(000) = 600 e⁻, space group P2₁/c. For **1**, data were collected for 4.0 ≤ 2θ ≤ 40.0, θ = 2θ scans, 0 ≤ *b* ≤ 21, -3 ≤ *k* ≤ 0, -16 ≤ *l* ≤ 15 at -80°. For **2**, data were collected for 4.0 ≤ 2θ ≤ 50.0, ω (Wyckoff) scans, -14 ≤ *b* ≤ 14, 0 ≤ *k* ≤ 6, 0 ≤ *l* ≤ 23 at -80°. The scan rate for **1** and **2** was 2.03 to 29.30°/min. For **1** and **2**, three control reflections every 97 reflections showed no significant trends. Lorentz and polarization corrections were applied to 1628 reflections for **1** and 2716 reflections for **2**. No absorption correction was applied to either data set. Reflection intensities for **1** were profiled employing a learnt profile technique (5). For **1**, 875 reflections with |*I*| ≤ 2.5σ(*F*) and for **2**, 1416 reflections with |*I*| ≤ 4.0σ(*F*) were used in further calculations. Structures were solved by direct methods [SHELXS, SHELXTL-PLUS program package (6)]. All nonhydrogen atoms for **1** and **2** were refined anisotropically, and hydrogen atoms were placed in idealized positions with isotropic thermal parameters fixed at 0.08. Full matrix least-squares refinement [quantity minimized Σw(*F_o* - *F_c*)²; w = (σ²*F* + *gF*²)⁻¹ where *g* = 0.0009 for **1** and *g* = 0.0001 for **2**] yielded for **1**, *R* = 0.0716, *wR* = 0.0680, *S* = 1.29 (largest Δ/*σ* = 0.001, mean Δ/*σ* = 0.0001, largest peak in the final Fourier difference map 0.32 e⁻/Å³, largest negative peak -0.31 e⁻/Å³) and for **2**, *R* = 0.0747, *wR* = 0.0676, *S* = 2.32 (largest Δ/*σ* = 0.001, mean Δ/*σ* = 0.0001, largest peak in the final Fourier difference map 0.40 e⁻/Å³, largest negative peak -0.40 e⁻/Å³) at convergence. Neutral scattering factors are taken from Cromer and Waber (7).

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were performed on a Siemens diffractometer [MoK α radiation]. Cell parameters for **1** were $a = 3.9294(10)$ Å, $b = 3.91(2)^\circ$, $V = 1417.4(7)$ Å³, $\mu = 0.097$ mm⁻¹, space group P2₁/c; for **2**, $a = 5.397(4)$ Å, $b = 8.11(7)^\circ$, $V = 1330(2)$ Å³, $\mu = 0.100$ mm⁻¹, space group P2₁/c. For **1**, $2\theta = 4.0 \leq 2\theta \leq 40.0$, $\theta = -20$ to 20 , $-16 \leq \phi \leq 15$ at -80° . For **2**, $2\theta = 4.0 \leq 2\theta \leq 50.0$, $\omega = 0 \leq \omega \leq 14$, $0 \leq k \leq 6$, $0 \leq l \leq 23$. For **1** and **2** was 2.03 to three control reflections. No significant trends were observed. Corrections were applied and 2716 reflections for **1** and 2716 reflections for **2**. Scale correction was applied to either **1** or **2**. Intensities for **1** were profiled by the χ^2 technique (5). For **1**, $2.5\sigma(F)$ and for **2**, $1416\sigma(F)$ were used in further refinement. Structures were solved by direct methods using the SHELXTL-PLUS program. Non-hydrogen atoms for **1** and **2** were refined anisotropically, and hydrogen atoms were placed in calculated positions with isotropic displacement parameters fixed at 0.08. Full matrix least-squares refinement [quantity minimized $\sum w(F_o - F_c)^2$] where $w = 1/\sigma^2(F_o)$ for **1** and $w = 1/\sigma^2(F_c)$ for **2**] yielded for **1**, $R = 0.080$, $S = 1.29$ (largest $\Delta\rho = 0.0001$, largest peak in difference map 0.32 e⁻/Å³, largest hole -0.31 e⁻/Å³) and for **2**, $R = 0.076$, $S = 2.32$ (largest $\Delta\rho = 0.0001$, largest peak in difference map 0.40 e⁻/Å³, largest hole -0.40 e⁻/Å³) at converging factors are taken as a measure of quality (7).

ACKNOWLEDGMENTS

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