Furanonaphthoquinones from *Tabebuia ochracea* ssp. *neochrysanta*

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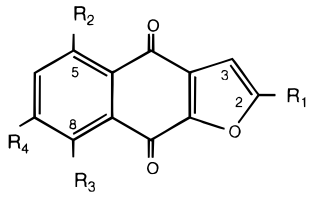
Activity-directed fractionation of the inner stem bark of *Tabebuia ochracea* ssp. *neochrysanta* led to the isolation of two new naphtho[2,3-*b*]furan-4,9-diones, the 5,8-dihydroxy-2-(1′-hydroxyethyl) and 2-acetyl-7-methoxy-8-hydroxy derivatives, **1** and **2**, together with four known naphthofurandiones.

The stem bark of *Tabebuia ochracea* ssp. *neochrysanta* (A. Gentry) A. Gentry (Bignoniaceae) has been used for many years by Tukuna indians of the Colombian Amazon as an antimalarial and for its healing action on ulcers.[1,2] This plant is native to tropical America, from El Salvador to northwest of Venezuela and Colombia and known in folk medicine as "To hua ri", "Vero", and "Cañaguate". Zani et al.[3] isolated β-sitosterol, cycloolivil, lapachol, and seven furanonaphthoquinones from the trunkwood of this plant. We found that the chloroform extract of *T. ochracea* ssp. *neochrysanta* showed cytotoxicity against melanoma B16 cells and antimalarial activity, *in vitro*, against strains of *Plasmodium berghei*. We report in this paper the structure elucidation of two new furanonaphthoquinones, **1** and **2**, which were isolated along with the known 2-acetylnaphtho[2,3-*b*]furan-4,9-dione, 7-methoxy-8-hydroxy-2-(1′-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione, 2-acetyl-7-methoxynaphtho[2,3-*b*]furan-4,9-dione, and 7-methoxy-2-(1′-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione.



(**1**) R_1=CH(OH)-CH_3; R_2= R_3= OH; R_4= H

(**2**) R_1= $COCH_3$; R_2= H; R_3= OH; R_4= OCH_3

Column chromatography (CC) on silica gel of the $CHCl_3$ extract of the stem inner bark of *T. ochracea* ssp. *neochrysanta*, using CH_2Cl_2/MeOH (gradient) as eluent, resulted in 16 fractions. Subsequent separation by silica gel column and repeated preparative thin-layer chromatography of the fractions which showed activity in the brine shrimp lethality test led to the isolation of six compounds whose UV and IR spectra of all compounds displayed absorption maxima typical of a naphthoquinone structure.[4] The known naphthofurandiones were identified from their respective ^{1}H-NMR and EIMS data and by comparison with values reported in the literature.[3,5,6]

The mass spectrum of 5,8-dihydroxy-2-(1′-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione, **1**, revealed a molecular ion at *m*/*z* 274, and its ^{1}H-NMR spectrum showed two singlets (1 H each) at 12.69 and 12.52 ppm, which disappeared on D_2O exchange and were assigned to two OH groups chelated with the carbonyl groups of the quinoid structure. According to the empirical rule formulated by Wagner *et al.*,[7] the signal at 12.69 ppm is attributed to OH-5. A singlet (2H) observed at 7.25 ppm could be readily assigned to H-6 and H-7, while a doublet (1H, J = 0.54 Hz) at 6.86 ppm was assigned to the furanoid H-3 coupled to H-1′ of a hydroxyethyl group at C2. The presence of this group was indicated by a one-proton broad quartet (J = 6.85 Hz) at 5.02 ppm coupled to a doublet (3H, J = 6.6 Hz) at 1.63 ppm and a broad singlet at 2.34 ppm (which disappeared on D_2O exchange). The furanoid proton cannot be placed at C-2 because it would produce a larger chemical shift.[5]

Compound **2**, 2-acetyl-7-methoxy-8-hydroxynaphtho-[2,3-*b*]furan-4,9-dione, was isolated as orange needles which revealed by EIMS a molecular ion at 286 amu. Its ^{1}H-NMR showed a singlet (1H) at 12.37 ppm which could be assigned to a chelated OH group, most likely at C-8, according with the relatively high field where it appears. The aromatic zone shows an AB pattern at 7.78 ppm (1 H, J = 8.39 Hz) and 7.56 ppm (1 H, J = 8.40 Hz). A singlet at 4.00 ppm (3H) due to an aromatic methoxy group accounts for the other substituent on the aromatic ring. A deshielded furanoid proton singlet at 7.56 ppm and a three-proton singlet at 2.63 ppm indicated the presence of an acetyl group at C-2 of the structure.

Experimental Section

General Procedures. Melting points are uncorrected. Column chromatography (CC): silica gel 60 Merck (70−230 mesh). Preparative TLC: precoated TLC plates, silica gel 60 F_{254} (2 mm, Merck). UV: spectrophotometer Milton Roy Spectronic 3000 Array; 1 mg/100 mL MeOH. FT-IR spectra (KBr): spectrophotometer Nicolet 5 DXD. ^{1}H-NMR spectra: Bruker AM 300 (300 MHz) in $CDCl_3$. EIMS: Kratos MS25RFA.

Plant Material. The stem bark of *T. ochracea* ssp. *neochrysanta* was collected in Cartagena de Indias, Colombia, in April 1993 and was taxonomically identified by H. Cuadros (Botanical Garden "Guillermo Piñeres", Cartagena, Colombia). A voucher specimen (no. 5153) is deposited at the above-mentioned Botanical Garden.

Extraction and Isolation. The powdered inner stem bark (1214 g) was extracted with $CHCl_3$ in a soxhlet apparatus. The crude $CHCl_3$ extract (3.4 g) showed important activity in the brine shrimp lethality test[8] (LC_{50} 85.3 μg/mL) and was fractionated by CC on

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a 6.5 × 80 cm column (silica gel; CH_2Cl_2/MeOH gradient). Sixteen fractions were collected. Fractions 4 and 5−9 (combined) showed significant lethality (LC_{50} 37.7 μg/mL) and were further purified by CC and repeated PLC ($CHCl_3$). Fraction 4 afforded 2-acetylnaphtho[2,3-*b*]furan-4,9-dione (35 mg) along with a mixture of three compounds whose mass spectra exhibited $[M]^+$ peaks at *m/z* 258, 256, and 220; their structures are still under study. Combined fractions 5−9 (424 mg) were rechromatographed (silica gel 60; hexane/MeOH gradient) to afford **1** (19 mg), **2** (3.3 mg), 7-methoxy-8-hydroxy-2-(1′-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione (13.1 mg), 2-acetyl-7-methoxynaphtho[2,3-*b*]furan-4,9-dione (5.7 mg), and 7-methoxy-2-(1′-hydroxyethyl)naphtho[2,3-*b*]-furan-4,9-dione (14.2 mg).

5,8-Dihydroxy-2-(1′-hydroxyethyl)naphtho[2,3-*b*]-furan-4,9-dione (1): yellow-orange needles; mp 132−135 °C; UV (MeOH) λ max (log ϵ) 239 (4.11), 492 (3.37) nm; FT-IR ν max 3600, 3200, 1638, 1623, 1534, 1458, 1348, 1172, 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) 12.69 (1H, s OH-C-5), 12.52 (1H, s, OH-C-8), 7.25 (2H, s, H-6,7), 6.86 (1H, d, J = 0.54 Hz, H-3), 5.02 (1H, c, J = 6.85 Hz, H-10), 2.34 (1H, bs, *H*O-1′), 1.65 (3H, d, J = 6.6 Hz, 3H-2′), ppm; EIMS (70 eV) *m/z* (rel int) $[M]^+$ 274 (100), 259 $[M - 15]^+$ (88), 231 $[M - MeCO]^+$ (11), 43 $[MeCO]^+$.

8-Hydroxy-7-methoxy-2-acetylnaphtho[2,3-*b*]furan-4,9-dione (2): orange needles; mp 206−210 °C; UV λ max (log ϵ) 255 (4.00), 284 (3.99), 399 (3.22), 450 (3.26) nm; FT-IR ν max 3400, 1671, 1641, 1566, 1469, 1285, 1256, 1237 cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) 12.37 (1H, s, OH-8), 7.78 (1H, d, J = 8.4 Hz, H-5), 7.56 (1H, s, H-3), 7.09 (1H, d, J = 8.4 Hz, H-6), 4.00 (3H, s, OMe), 2.64 (s, 3H-2′); EIMS (70 eV) *m/z* (rel int) $[M]^+$ 286, 271 $[M - 15]^+$ (3), 243 $[M - MeCO]^+$ (20), 215 $[M - MeCO - CO]^+$ (10), 187 $[M - MeCO - CO - CO]^+$ (10), 43 $[MeCO]^+$ (64).

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