

## FLAVONOIDS FROM VIROLA CALOPHYLLOIDEA

JUAN C. MARTINEZ V.\* and LUIS E. CUCA S.

Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia.  
AA 14490, Bogotá, Colombia

ABSTRACT.—The C<sub>6</sub>H<sub>6</sub> extract of the trunk wood of *Virola calophylloidea* (Myristicaceae) yielded three flavonoids: (±)-α,2'-dihydroxy-4,4'-dimethoxydihydrochalcone [**1a**], (±)-4'-hydroxy-3',7-dimethoxyflavan [**2a**], and 1-(2'-hydroxy-4'-methoxyphenyl)-3-(4"-hydroxy-3"-methoxyphenyl)-propane [**3a**]. Their structures were established by spectroscopic methods, preparation of derivatives, and also by data correlations with known compounds.

*Virola calophylloidea* Markgraf (Myristicaceae) grows in the southeastern area of Colombia (Vaupés, Amazonas, and Caquetá). According to the region it is known by different names such as "Bojorique," "Cumala," "Ko-ga," "Yakii," or "Parrika." In previous papers we reported the presence of some neolignans (1) and steroids (2) in the leaves and bark of this species. The present investigation of the C<sub>6</sub>H<sub>6</sub> extract of the wood led to the isolation of the α-hydroxydihydrochalcone **1a**, previously isolated from *Pterocarpus angolensis* DC. (3), together with the two novel natural compounds **2a** and **3a**.

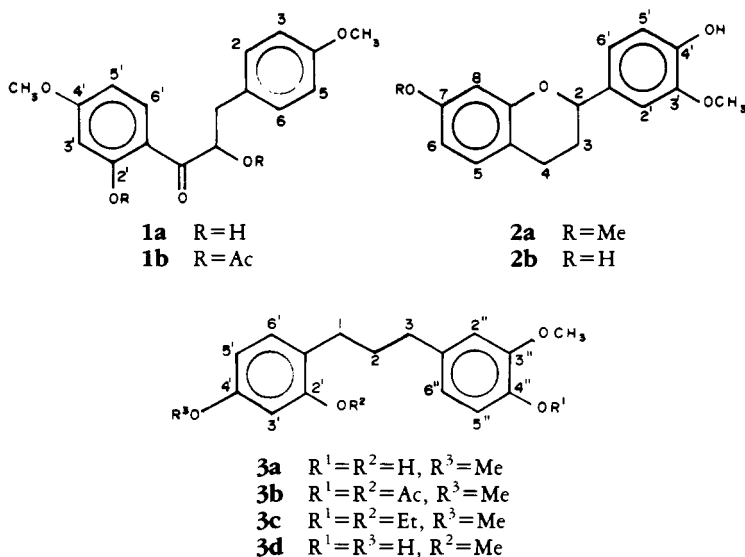
A review of the chemical composition of Myristicaceae quoted the occurrence of **3a** and **3d** in *Iryanthera elliptica* as unpublished results (4). A detailed analysis of this species confirmed the presence of **3d** (5). The presence of **3a** was not confirmed, and this compound described in the present article is, thus, a new natural product.

### RESULTS AND DISCUSSION

The α-hydroxydihydrochalcone **1a** was identified by comparison of the spectroscopic data with those reported in the literature (3). The use of different solvents accounts for the small differences in <sup>1</sup>H-nmr data. The ir and <sup>1</sup>H-nmr spectral data of the previously unknown di-acetate **1b** confirmed the presence of an alcoholic hydroxyl (1750 cm<sup>-1</sup> C=O and δ 2.05 CH<sub>3</sub>CO) and a phenolic hydroxyl (1775 cm<sup>-1</sup> C=O and δ 2.30 CH<sub>3</sub>CO). The hrms of compound **2a** was consistent with the formula C<sub>17</sub>H<sub>18</sub>O<sub>4</sub>. The presence of a phenolic hydroxyl was detected by uv (bathochromic shift of maxima in alkaline medium), ir (3340 cm<sup>-1</sup>), and <sup>1</sup>H-nmr (annulment of the peak at δ 5.6 by addition of D<sub>2</sub>O) spectral data. The mass spectrum showed two intense peaks at *m/z* 150 (100%) and 137 (90%), the former corresponding to a flavan B ring with -OH and -OCH<sub>3</sub> substituents and the latter to the A ring with a -OCH<sub>3</sub> substituent. This is in accordance with a retro-Diels-Alder type fragmentation which is characteristic for this carbon skeleton (6). The <sup>1</sup>H-nmr spectrum exhibited a double doublet at δ 4.93 (*J*=9 and 4 Hz), due to an oxymethine proton coupled to two protons whose signals appear as a multiplet at δ 1.9-2.3. The other multiplet at δ 2.6-2.9 is attributed to a benzylic methylene.

With respect to substitution in the B ring, it was concluded that the -OH group is located on C-4' because of a negative Gibbs test, and the -OCH<sub>3</sub> group is located on C-3' due to biogenetic considerations. The location of the other -OCH<sub>3</sub> group on C-7 (A ring) was deduced on the basis of the coupling systems seen in the <sup>1</sup>H-nmr spectrum: a doublet at δ 7.01 (*J*=7 Hz, *ortho* coupling), a double doublet at δ 6.45 (*J*=7 and 3 Hz, *ortho* and *meta* coupling), and a doublet at δ 6.50 (*J*=3 Hz, *meta*-coupling). Product **2a** is a racemic mixture ([α]<sub>D</sub>=0°). The known flavans **2b** (5) and **2a** show similar spectroscopic data for the basic skeleton. Flavans are uncommon natural products. They have been found in plants belonging to the genus *Iryanthera* (5-8) but seemed to be absent from the species of the closely related genus *Virola*.

The hrms of compound **3a** showed a molecular weight of 288.1084, which is in agreement with the condensed formula  $C_{17}H_{20}O_4$ . The basic structure of a 1,3-diarylpropane was deduced on the basis of the  $^{13}C$ - and  $^1H$ -nmr spectral analyses. The completely uncoupled  $^{13}C$ -nmr spectrum revealed two aromatic rings (12 peaks between 100 and 160). The signals at  $\delta$  28.93, 31.92, and 35.34, which appear as triplets in the off-resonance spectrum, are associated with a trimethylene chain. This fact was confirmed by comparison of ir, uv, ms, and  $^1H$ -nmr spectral data with those reported for the synthetic compound (9). The  $^{13}C$ -nmr chemical shifts of the acetyl derivative **3b** with respect to **3a** ( $\Delta\delta$ : C-1" +3.8, C-2" +1.1, C-3" +3.8, C-4" -4.2, C-5" +5.5, C-6" +0.5) confirmed a hydroxyl group on C-4" (B ring). A similar compound, **3d** (with inverted substituents on the A ring), was isolated from *I. elliptica* (5) and *Iryanthera coriacea* (10).



## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Melting points were taken on a Kofler hot plate and are uncorrected. Spectra were recorded on the following instruments:  $^1H$  nmr, Varian T-60 or Perkin-Elmer R-12B (60 MHz);  $^{13}C$  nmr, Varian FT-80 (20 MHz); ms, Shimadzu GEMS-9020-DF (70 eV); optical rotations on a digital polarimeter Perkin-Elmer 241; ir, Perkin-Elmer 467; uv, Beckmann 25. Si gel was used for all chromatographic separations.

**PLANT MATERIAL.**—The wood of *V. calophylloidea* was collected in the Vaupés region on the shores of the Piraparaná River, 2 miles away from the Sonaña, at 200 feet above sea level. Plant identification was done by Professor R. Jaramillo M. from the Instituto de Ciencias Naturales, Universidad Nacional de Colombia. A voucher specimen has been deposited in the Herbario Nacional Colombiano and registered under the number COL 231562.

**ISOLATION OF THE CONSTITUENTS.**—The dry and ground wood (6060 g) was extracted with  $C_6H_6$  in a Soxhlet apparatus. Evaporation of the solvent in vacuo led to a residue (12.6 g). This was fractionated by column chromatography (cc). Elution with mixtures of  $C_6H_6$ -EtOAc of increasing polarity gave five fractions: A (19:1), B (9:1), C (17:3), D (4:1), and E (7:3). Fraction A (2.086 g) consisted of aliphatic compounds. Purification of fraction B (0.719 g) by successive cc and preparative tlc ( $CHCl_3$ - $Me_2CO$ , 9:1) afforded **2a** (41 mg). Purification of fraction G (0.386 g) by cc and preparative tlc (light petrol-EtOAc, 7:3) yielded **1a** (80 mg). Purification of fraction D (2.895 g) by cc (light petrol-EtOAc, 7:3) gave **3a** (1.560 g) and a steroidal fraction (107 mg). Fraction E (4.070 g), found to contain polar substances, was not processed. Acetylation ( $Ac_2O$ /pyridine) of **1a** and **3a** gave **1b** and **3b**, respectively. The ethyl derivative **3c** was obtained from **3a** by using diethylsulfate and  $Na_2CO_3$ .

(±)- $\alpha$ ,2'-DIHYDROXY-4,4'-DIMETHOXYDIHYDROCHALCONE [**1a**].— $C_{17}H_{18}O_5$ , mp 85° (cy-

clohexane-Me<sub>2</sub>CO, 1:1) [lit. (3) 89.5° (EtOH)]; [ $\alpha$ ]<sup>20</sup><sub>D</sub> = 0 ( $c$  = 0.324, MeOH); uv  $\lambda$  max (MeOH) nm 320 ( $\epsilon$  5670), 281 (12240), 230 (12840), 222 (16120), 216 (16270);  $\lambda$  max (MeOH+NaOMe) nm 352 ( $\epsilon$  5670), 281 (9250), 246 (11640), 230 (19100), effect reversible by HCl;  $\lambda$  max (MeOH+AlCl<sub>3</sub>) nm 364 ( $\epsilon$  5820), 308 (14780), 228 (16720), 218 (16570), which do not change by addition of HCl; ir (film) cm<sup>-1</sup> 3480, 2920, 1630 (C=O), 1585, 1515, 1250; <sup>1</sup>H nmr (acetone-*d*<sub>6</sub>, TMS)  $\delta$  2.90-3.80 (m, 2H- $\beta$ ), 3.75 (s, CH<sub>3</sub>O-4'), 3.82 (s, CH<sub>3</sub>O-4), 5.00-5.40 (m, 1H- $\alpha$ ), 6.50 (dd,  $J$  = 9 and 3 Hz, 1H-5'), 6.50 (d,  $J$  = 3 Hz, 1H-3'), 6.78 (d,  $J$  = 9 Hz, 2H-3,5), 7.08 (d,  $J$  = 9 Hz, 2H-2,6), 7.60 (d,  $J$  = 9 Hz, 1H-6'), 12.2 (s; D<sub>2</sub>O, 1-OH-2'); ms (70 eV)  $m/z$  (rel. int.) 302 (1.0, M<sup>+</sup>), 284 (40), 181 (8), 151 (30), 121 (100).

$\alpha$ ,2'-DIACETOXY-4,4'-DIMETHOXYDIHYDROCHALCONE [1b].—C<sub>21</sub>H<sub>22</sub>O<sub>7</sub>, yellow oil; ir (film) cm<sup>-1</sup> 2920, 1775 (C=O), 1750 (C=O), 1700 (C=O), 1610, 1597, 1515, 1375, 1220; <sup>1</sup>H nmr (CDCl<sub>3</sub>, TMS)  $\delta$  2.05 (s, CH<sub>3</sub>CO- $\alpha$ ), 2.30 (s, CH<sub>3</sub>CO-2'), 2.70-3.20 (m, 2H- $\beta$ ), 3.80 (s, CH<sub>3</sub>O-4'), 3.85 (s, CH<sub>3</sub>O-4), 5.75-6.20 (m, 1H- $\alpha$ ), 6.50-7.30 (m, 6H-3',5',2,3,5,6), 7.70 (d,  $J$  = 8 Hz, 1H-6'); ms (70 eV)  $m/z$  (rel. int.) 386 (1, M<sup>+</sup>), 327 (5), 326 (23), 284 (29), 283 (17), 193 (18), 152 (12), 151 (100), 134 (10), 121 (68).

( $\pm$ )-4'-HYDROXY-3',7'-DIMETHOXYFLAVAN [2a].—Mp 104-105° (Me<sub>2</sub>CO), (M<sup>+</sup> found 286.1126, C<sub>17</sub>H<sub>18</sub>O<sub>4</sub> requires 286.1206); uv  $\lambda$  max (MeOH) nm 285 ( $\epsilon$  2200), 231 (6000), 214 (7900);  $\lambda$  max (MeOH+NaOMe) nm 292 ( $\epsilon$  2600), 286 (2470), 252 (3750), effect reversible by HCl; ir (film) cm<sup>-1</sup> 3340, 3000, 1615, 1590, 1445, 1340; <sup>1</sup>H nmr (CDCl<sub>3</sub>, TMS)  $\delta$  1.90-2.30 (m, 2H-3), 2.60-2.90 (m, 2H-4), 3.78 (s, CH<sub>3</sub>O-7), 3.92 (s, CH<sub>3</sub>O-3'), 4.93 (dd,  $J$  = 9 and 4 Hz, 1H-2), 5.60 (s, ex. D<sub>2</sub>O, 1-OH-4'), 6.45 (dd,  $J$  = 7 and 3 Hz, 1H-6), 6.50 (d,  $J$  = 3 Hz, 1H-8), 6.9 (br s, 3H-2',5',6'), 7.01 (d,  $J$  = 7 Hz, 1H-5); ms (70 eV)  $m/z$  (rel. int.) 287 (14, M+1), 286 (80, M<sup>+</sup>), 271 (6), 150 (100), 137 (90).

1-(2'-HYDROXY-4'-METHOXYPHENYL)-3-(4"-HYDROXY-3"-METHOXYPHENYL)-PROPANE [3a].—Mp 87-88° (MeOH-Me<sub>2</sub>CO, 1:1) [lit. (9) 72-74° (C<sub>6</sub>H<sub>6</sub>)]; M<sup>+</sup> found 288.1148, C<sub>17</sub>H<sub>20</sub>O<sub>4</sub> requires 288.1302; uv, ir, ms, and <sup>1</sup>H nmr in lit. (9); <sup>13</sup>C nmr (CDCl<sub>3</sub>, TMS)  $\delta$  159.31 (C-4'), 154.60 (C-2'), 146.73 (C-3"), 144.84 (C-4"), 134.52 (C-1"), 130.73 (C-6'), 121.39 (C-6"), 120.84 (C-1'), 114.62 (C-5"), 111.44 (C-2"), 106.26 (C-5'), 102.23 (C-3'), 55.96 (CH<sub>3</sub>O-4'), 55.29 (CH<sub>3</sub>O-3"), 35.34 (C-3), 31.92 (C-1), 28.93 (C-2).

1-(2'-ACETOXY-4'-METHOXYPHENYL)-3-(4"-ACETOXY-3"-METHOXYPHENYL)-PROPANE [3b].—C<sub>21</sub>H<sub>24</sub>O<sub>6</sub>, yellow oil; ir, ms, and <sup>1</sup>H nmr in lit. (9); <sup>13</sup>C nmr (CDCl<sub>3</sub>, TMS)  $\delta$  168.84 (OCOCH<sub>3</sub>-2'), 168.59 (OCOCH<sub>3</sub>-4"), 158.23 (C-4'), 150.48 (C-3"), 149.13 (C-2'), 140.65 (C-4"), 138.33 (C-1"), 130.09 (C-6'), 125.39 (C-1'), 121.98 (C-6"), 120.08 (C-5"), 112.54 (C-2"), 111.54 (C-5'), 107.73 (C-3'), 55.53 (CH<sub>3</sub>O-3"), 54.89 (CH<sub>3</sub>O-4'), 34.98 (C-3), 31.16 (C-1), 28.40 (C-2), 20.12 (CH<sub>3</sub>COO).

1-(2'-ETHOXY-4'-METHOXYPHENYL)-3-(4"-ETHOXY-3"-METHOXYPHENYL)-PROPANE [3c].—C<sub>21</sub>H<sub>28</sub>O<sub>3</sub>, yellow oil; ir (film) cm<sup>-1</sup> 2930, 1615, 1590, 1510, 1450; <sup>1</sup>H nmr (CDCl<sub>3</sub>, TMS)  $\delta$  1.30 (t,  $J$  = 7 Hz, 6H-OCH<sub>2</sub>CH<sub>3</sub>), 1.60-2.10 (m, 2H-2), 2.65 (t,  $J$  = 8 Hz, 4H-1,3), 3.60 (s, CH<sub>3</sub>O-3"), 3.68 (s, CH<sub>3</sub>O-4'), 3.92 (q, 4H-OCH<sub>2</sub>CH<sub>3</sub>), 6.15-7.00 (m, 6H-3',5',6',2',5",6"); ms (70 eV)  $m/z$  (rel. int.) 346 (6, M+2), 345 (45, M+1), 344 (100, M<sup>+</sup>), 192 (33), 179 (74), 178 (20), 165 (100), 166 (100), 151 (42), 138 (50), 137 (100).

#### ACKNOWLEDGMENTS

We are indebted to the Chemistry Department of the Universidad del Valle (Colombia) for <sup>1</sup>H-nmr spectra, to Doctor Masayoshi Yoshida of the Chemistry Institute of the Universidade de Sao Paulo (Brazil) for <sup>13</sup>C-nmr spectra, and to the Analysis Section of the Instituto Nacional de Salud (Colombia) for optical rotation measurements. Financial support for this work was provided by CINDEC-UN (PI-1303-118), COLCIENCIAS (PI-10000-1-13581), and OAS (Multinational Project in Chemistry).

#### LITERATURE CITED

1. J.C. Martínez, L.E. Cuca, M. Yoshida, and O.R. Gottlieb, *Phytochemistry*, **24**, 1867 (1985).
2. J.C. Martínez and L.E. Cuca, *Rev. Colomb. Quim.*, **13**(1), 109 (1984).
3. B.C.B. Bezuidenhoudt, E.V. Brandt, and D.G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 263 (1981).
4. O.R. Gottlieb, *Isr. J. Chem.*, **16**, 45 (1977).
5. R. Braz-Filho, P.P. Diaz, and O.R. Gottlieb, *Phytochemistry*, **19**, 455 (1980).
6. N.C. Franca, P.P. Diaz, O.R. Gottlieb, and B. de P. Rosa, *Phytochemistry*, **13**, 1631 (1974).
7. R. Braz-Filho, O.R. Gottlieb, and M.S. Silva, *Phytochemistry*, **19**, 1195 (1980).
8. N.C. Franca, O.R. Gottlieb, and B. de P. Rosa, *Phytochemistry*, **14**, 590 (1975).
9. A. Morais, R. Braz-Filho, and O.R. Gottlieb, *Phytochemistry*, **24**, 3023 (1985).
10. R. Alves de Lima, N.C. Franca, P.P. Diaz, and O.R. Gottlieb, *Phytochemistry*, **14**, 1831 (1975).