

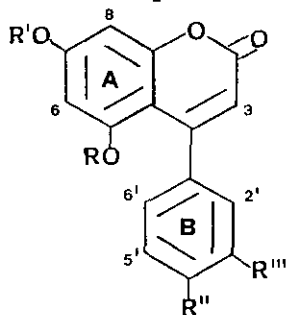
A NEW NEOFLAVANOID FROM COUTAREA HEXANDRA

Giuliano Delle Monache\*, Bruno Botta, Vittorio Vinciguerra,  
and Eszter Gacs-Baitz<sup>1</sup>

Centro Chimica dei Recettori del C.N.R., Istituto di Chimica,  
U.C.S.C., Largo F. Vito 1, 00168 Roma, Italy

Abstract - A new neoflavone was isolated from Coutarea hexandra and assigned to 7,3',4'-trihydroxy-5-methoxy-4-phenylcoumarin.

In our continuing investigation for Coutarea hexandra<sup>2-4</sup> (Rubiaceae), we isolated a new neoflavonoid (1), C<sub>16</sub>H<sub>12</sub>O<sub>6</sub>, containing three hydroxy and one methoxy groups. The latter, giving a relatively high field signal ( $\delta$  3.53) in the <sup>1</sup>H nmr spectrum<sup>5</sup>, requires its location at C-5. While the substitution pattern of the A ring was evident from the spectral data, the pattern of the hydroxyl functions in the B ring could not be immediately assigned. Based on previously isolated neoflavanoids<sup>2-4</sup>, substitution at the C-3' and C-4' positions seemed the most likely, but the alternative of substitution at C-2' and C-5' could not be excluded. On methylation with CH<sub>2</sub>N<sub>2</sub> compound 1 gave 5,7,3',4'-tetramethoxy-4-phenylcoumarin, identical with the synthetic one<sup>6</sup>; moreover 7-hydroxy-5-methoxy-3',4'-methylenedioxy-4-phenylcoumarin (5) was prepared from 5,7-dihydroxy-3',4'-methylenedioxy-4-phenylcoumarin and led to compound 1 by treatment with BCl<sub>3</sub>; thus the 3',4'-substitution was confirmed. Finally, the natural product was identical with 7,3',4'-trihydroxy-5-methoxy-4-phenylcoumarin synthesized by Iinuma *et al.*<sup>7</sup> on tlc.



	R	R'	R''	R'''
1	CH <sub>3</sub>	H	OH	OH
2	H	CH <sub>3</sub>	OH	OH
3	H	H	O-CH <sub>2</sub> O	
4	H	CH <sub>3</sub>	O-CH <sub>2</sub> O	
5	CH <sub>3</sub>	H	O-CH <sub>2</sub> O	

Since the <sup>1</sup>H nmr data proved to be solvent dependent and the <sup>13</sup>C nmr data were sometimes ambiguous, we also run hetcor and long range hetcor spectra of compound 1 and the results are summarised in the table.

From these findings the assignments of the C-2 to C-8a resonances of the neoflavones reported in the literatures<sup>8,9</sup> need mostly to be revised.

An interesting diagnostic feature was noted in the uv spectra of 7-hydroxyneoflavones, which only showed a bathochromic shift after addition of fused NaOAc.

Conversely compounds 2 and 4 showed with NaOMe larger bathochromic shifts (83 and 78 nm, respectively) than those of 1 and 5 (48 and 53 nm, respectively).

TABLE: Carbon and proton resonances assignment of compound 1

c		linked H ( $J^1$ )	long range coupled H ( $J^2$ and $J^3$ )
2	160.7		H-3
3	112.1	5.80 (1H, s)	
4	156.7		H-2', H-6'
4a	103.4		H-3, H-6, H-8
5	159.9		H-6, OMe
6	97.0	6.36 (1H, d)	
7	162.6		H-6, H-8
8	96.7	6.43 (1H, d)	
8a	158.2		H-8
1'	132.7		H-3, H-5'
2'	115.8	6.83 (1H, d)	H-6'
3'	145.0		H-2', H-5'
4'	146.1		H-2', H-6'
5'	115.1	6.85 (1H, d)	
6'	120.1	6.68 (1H, dd)	H-2'
OMe	56.0	3.53 (3H, s)	

Coupling constants (Hz):  $J_{6,8} = 2.5$ ;  $J_{2',6'} = 2.0$ ;  $J_{5',6'} = 8.0$

#### EXPERIMENTAL

Extraction and isolation. Collection of Coutarea hexandra (Jacq.) Schumm, extraction of the caulis with  $C_6H_6$  and partial separation have been previously reported<sup>2</sup>. An extended chromatography gave 7,3',4'-trihydroxy-5-methoxyneoflavone (1, 24 mg), mp 269-70°C (MeOH); uv  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 259 (4.43), 332 (4.77);  $\lambda_{max}^{NaOAc}$ : 257, 272sh, 332sh, 378;  $\lambda_{max}^{NaOMe}$ : 245, 271, 381;  $^1H$  nmr and  $^{13}C$  nmr ( $Me_2CO-d_6$ ) in the table; ms  $m/z$  (%): 300 ( $M^+$ , 100), 272 ( $M^+-CO$ , 100), 257 ( $M^+-MeCO$ , 12), 239 ( $M^+-43-H_2O$ , 30), 229 ( $M^+-43-CO$ , 8), 211 (12), 150 ( $M^+/2$ , 6), 136 ( $M^+-CO/2$ , 4); calcd for  $C_{15}H_{12}O_6$ , C 64.0%, H 4.0%; found: C 63.8%, H 4.1%.

Methylation of 1 (8 mg) with  $CH_2N_2$  in MeOH (3 ml) gave 5,7,3',4'-tetramethoxyneoflavone (10 mg), identical with the synthetic sample<sup>6</sup> (mp 170-1°C).

Methylation of 5,7-dihydroxy-3',4'-methylenedioxy-4-phenylcoumarin (3). To a solution of compound 3<sup>6</sup> (600 mg) in  $Et_2O$  (55 ml) and MeOH (5 ml) a saturated solution of  $CH_2N_2$  in  $Et_2O$  (20 ml) was added dropwise. The mixture of methyl

derivatives was separated from the starting product by silica gel column chromatography (with  $\text{CHCl}_3/\text{MeOH}$ , 98:2). The unreacted material was subsequently treated with  $\text{CH}_2\text{N}_2$  under the same conditions. The reaction mixtures gave three methylated neoflavones: 5,7-dimethoxy-3',4'-methylenedioxyneoflavone<sup>3,6</sup> (280 mg); 5-hydroxy-7-methoxy-3',4'-methylenedioxyneoflavone (4, 193 mg),  $\text{C}_{17}\text{H}_{12}\text{O}_6$ , mp 266-8°C; uv  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 251 (4.08), 260sh (4.04), 325 (4.18);  $\lambda_{\text{max}}^{\text{NaOAc}}$ : 251, 282, 327;  $\lambda_{\text{max}}^{\text{NaOMe}}$ : 278, 328, 403;  $^1\text{H}$  nmr ( $\text{DMSO}-d_6$ ):  $\delta$  3.78 (OMe); ms m/z (%): 312 ( $\text{M}^+$ , 100), 284 (88), 269 (9), 241 (3); 7-hydroxy-5-methoxy-3',4'-methylenedioxyneoflavone (5, 75 mg),  $\text{C}_{17}\text{H}_{12}\text{O}_6$ , mp 260-2°C; uv  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 249 (4.06), 258sh (4.0), 330 (4.17);  $\lambda_{\text{max}}^{\text{NaOAc}}$ : 249, 272, 320sh, 382;  $\lambda_{\text{max}}^{\text{NaOMe}}$ : 249, 272, 320sh, 383;  $^1\text{H}$  nmr ( $\text{DMSO}-d_6$ ):  $\delta$  3.44 (OMe); ms m/z (%): 312 ( $\text{M}^+$ , 100), 284 (98), 269 (4), 241 (25).

**Demethylenation.**  $\text{BCl}_3$  (1.0M) in  $\text{CH}_2\text{Cl}_2$  (5 ml) was added to a cooled soln of 5 (50 mg) in diglyme (2 ml). After 2 days the mixture was evaporated and added with MeOH. The residue was chromatographed on silica gel with  $\text{CHCl}_3$ -MeOH (95:5) to give 7,3',4'-trihydroxy-5-methoxyneoflavone (22 mg: mp 268-70°C), identical with the natural compound (1) and with the synthetic sample<sup>7</sup> from Iinuma on tlc. Under the same conditions 4 (50 mg) gave 5,3',4'-trihydroxy-7-methoxy-4-phenylcoumarin (2, 18 mg), identical with coutareagenin<sup>8,10</sup> on tlc.

#### ACKNOWLEDGMENTS

We thank Dr. G. Reher (Hamburg, F.R.G.) for a sample of coutareagenin and Dr. M. Iinuma (Gifu, Japan) for synthetic 7,3',4'-trihydroxy-5-methoxyneoflavone.

#### REFERENCE AND NOTES

1. Permanent address: Central Research Institute of Chemistry, P.O. Box 17, H-1525, Budapest (Hungary).
2. G. Delle Monache, B. Botta, A. Serafim Neto, and R. Alves de Lima, Phytochemistry, 1983, 22, 1657.
3. G. Delle Monache, B. Botta, and R. Alves de Lima, Phytochemistry, 1984, 23, 1813.
4. G. Delle Monache, B. Botta, F. Menichini, and R.M. Pinheiro, Bull. Chem. Soc. Ethiop., 1987, 1, 65.
5. F. Sánchez-Viesca, Phytochemistry, 1969, 8, 1821.
6. G. Delle Monache, B. Botta, F. Delle Monache, and M. Botta, Phytochemistry, 1985, 24, 1355.
7. M. Iinuma, T. Tanaka, K. Hamada, M. Mizuno, and F. Asai, Chem. Pharm. Bull., 1987, 35, 3909.
8. G. Reher, Lj. Kraus, V. Sinnwell, and W. A. König, Phytochemistry, 1983, 22, 1524.
9. A. Ulubelen, R. R. Kerr, and T.J. Mabry, Phytochemistry, 1981, 21, 1145.
10. M. Iinuma, T. Tanaka, K. Hamada, M. Mizuno, F. Asai, G. Reher, and Lj. Kraus, Phytochemistry, 1987, 26, 3096.