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A NEW NEOFLAVANOID FROM COUTAREA HEXANDRA

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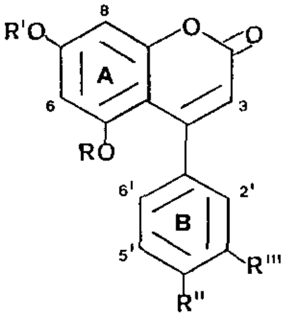
Abstract - A new neoflavone was isolated from Coutarea hexandra and assigned to 7,3',4'-trihydroxy-5-methoxy-4-phenylcoumarin.

In our continuing investigation for Coutarea hexandra[2-4] (Rubiaceae), we isolated a new neoflavonoid (1), $C_{16}H_{12}O_6$, containing three hydroxy and one methoxy groups. The latter, giving a relatively high field signal (δ 3.53) in the ^1H nmr spectrum[5], 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[2-4], 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_2N_2 compound 1 gave 5,7,3',4'-tetramethoxy-4-phenylcoumarin, identical with the synthetic one[6]; 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_3; 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.[7] on tlc.



	R	R'	R''	R'''
1	CH_3	H	OH	OH
2	H	CH_3	OH	OH
3	H	H	O-CH_2-O	
4	H	CH_3	O-CH_2-O	
5	CH_3	H	O-CH_2-O	

Since the ^1H nmr data proved to be solvent dependent and the ^{13}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[8,9] 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[2]. An extended chromatography gave 7,3',4'-trihydroxy-5-methoxyneoflavone (1, 24 mg), mp 269-70°C (MeOH); uv λ_{max}^{MeOH} nm (log ε): 259 (4.43), 332 (4.77); λ_{max}^{NaOAc}: 257, 272sh, 332sh, 378; λ_{max}^{NaOMe}: 245, 271, 381; ^{1}H 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 $C_{16}H_{12}O_6$, 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[6] (mmp 170-1°C).

Methylation of 5,7-dihydroxy-3',4'-methylenedioxy-4-phenylcoumarin (3). To a solution of compound 3 [6] (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 $CHCl_3$/MeOH, 98:2). The unreacted material was subsequently treated with CH_2N_2 under the same conditions. The reaction mixtures gave three methylated neoflavones: 5,7-dimethoxy-3',4'-methylenedioxyneoflavone[3,6] (280 mg); 5-hydroxy-7-methoxy-3',4'-methylenedioxyneoflavone (<u>4</u>, 193 mg), $C_{17}H_{12}O_6$, mp 266-8°C; uv λ_{max}^{MeOH} nm (log ε): 251 (4.08), 260sh (4.04), 325 (4.18); λ_{max}^{NaOAc}: 251, 282, 327; λ_{max}^{NaOMe}: 278, 328, 403; ^1H nmr (DMSO-d_6): δ 3.78 (OMe); ms m/z (%): 312 (M^+, 100), 284 (88), 269 (9), 241 (3); 7-hydroxy-5-methoxy-3',4'-methylenedioxyneoflavone (<u>5</u>,75 mg), $C_{17}H_{12}O_6$,mp 260-2°C; uv λ_{max}^{MeOH} nm (log ε): 249 (4.06), 258sh (4.0), 330 (4.17); λ_{max}^{NaOAc}: 249, 272, 320sh, 382; λ_{max}^{NaOMe}: 249, 272, 320sh, 383; ^1H nmr (DMSO-d_6): δ 3.44 (OMe); ms <u>m/z</u> (%): 312 (M^+, 100), 284 (98), 269 (4), 241 (25).

<u>Demethylenation</u>. BCl_3 (1.0M) in CH_2Cl_2 (5 ml) was added to a cooled soln of <u>5</u> (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 $CHCl_3$-MeOH (95:5) to give 7,3',4'-trihydroxy-5-methoxyneoflavone (22 mg: mp 268-70°C), identical with the natural compound (<u>1</u>) and with the synthetic sample[7] from Iinuma on tlc.

Under the same conditions <u>4</u> (50 mg) gave 5,3',4'-trihydroxy-7-methoxy-4-phenylcoumarin (<u>2</u>, 18 mg), identical with coutareagenin[8,10] on tlc.

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REFERENCE AND NOTES

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