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A NEOFLAVONOID FROM *COUTAREA HEXANDRA* (RUBIACEAE)

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Key Word Index—*Coutarea hexandra*; Rubiaceae; neoflavonoid; 5-hydroxy-7-methoxy-4-(2,5-dihydroxyphenyl)-2*H*-1-benzopyran-2-one.

Abstract—From the stem bark of *Coutarea hexandra* 5-hydroxy-7-methoxy-4-(2,5-dihydroxyphenyl)-2*H*-1-benzopyran-2-one, a neoflavonoid, was isolated. The structure was assigned by spectroscopic methods.

INTRODUCTION

Some species of the genera *Coutarea* and *Exostema* (Rubiaceae), trees native to Central and South America, are reputed to show antimalarial [1] or antidiabetic activity [2]. The stem barks of these species are called 'Copalchi'. In our studies of several different Copalchi [3], the stem bark of *Coutarea hexandra* Jacq. afforded a compound, the structure of which was characterized by spectroscopic methods. Phytochemical investigation of this species has not been reported before.

RESULTS AND DISCUSSION

On examination by TLC the methanolic extract of the stem bark of *Coutarea hexandra* showed the presence of a compound which in UV 366 nm light appeared as a characteristic brownish-yellow spot. This compound could be further enriched by extraction of the hydrolysed methanolic extract with ethyl acetate. The isolation was achieved by CC on Si gel.

The molecular formula was determined by high resolution mass spectrometry (M^+ observed 300.0630; $C_{16}H_{12}O_6$ requires 300.0638). The IR spectrum presented strong absorption bands for associated hydroxyl (*ca* 3350, 1085 cm^{-1}), carbonyl (1675 cm^{-1}), $>C=C<$ (1630 cm^{-1}) and C–O–C (1165/1175 cm^{-1}). TMS-derivatization revealed the presence of three hydroxyl functions. The characteristic maxima in the UV spectrum (260, 328 nm) indicated a coumarin-like structure; the flavonoid-like chromatographic behaviour led to the assumption of a 4-aryl-substituted coumarin. Several structural features could be ascertained from the ^1H and ^{13}C NMR spectra. The signal at δ 5.83 (*s*) in the ^1H NMR spectrum could be assigned to a vinylic proton combined with carbonyl, $=CH-C=O$. In the ^1H NMR spectrum the appearance of a signal at δ 3.87 (*s*) with an integral corresponding to three protons suggested a methoxy function which was confirmed by the signal at δ 56.1 in the ^{13}C NMR spectrum. The ^1H NMR spectrum showed five signals [δ 6.32 (*d*, $J = 2.6$ Hz), 6.48 (*d*, $J = 2.6$ Hz), 6.78 (*dd*, $J = 2.1$, 8.0 Hz), 6.87 (*d*, $J = 8.0$ Hz), 6.90 (*d*, $J = 2.1$ Hz)] which could be assigned to five aromatic protons. A

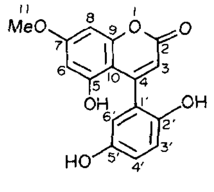

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Table 1. ^{13}C NMR chemical shifts (δ, ppm from TMS) for compound 1; solvent DMSO-d_6–CDCl$_3$ (7:3), upper; solvent Me$_2$CO-d_6, lower

^{13}C No.	2	3	4	5	6	7	8	9	10	11
Chemical shift (δ)	162.6	110.6	157.0	156.8	92.9	159.8	98.2	156.1	102.1	55.5
	164.1	112.5	158.2	157.3	94.4	160.6	99.4	156.3	103.1	56.1
^{13}C No.	1′	2′	3′	4′	5′	6′				
Chemical shift (δ)	130.4	144.1	114.6	115.5	145.6	118.8				
	131.7	145.3	115.6	116.0	146.6	120.3				

detailed study of the ^{13}C NMR spectrum suggested formula 1. The signals of the noise-decoupled ^{13}C NMR spectrum could be unequivocally assigned based on their chemical shifts, calculated by increment estimating and on their multiplicities found in the off-resonance decoupled and gated decoupling spectrum. Table 1 presents the ^{13}C NMR data of the compound and their assignments. Since the calculated shifts, partly based on reported data for 7-methoxy-4-phenylcoumarin [4], were in such a good agreement with the observed shifts, the isolated compound could be designated as 5-hydroxy-7-methoxy-4-(2,5-dihydroxyphenyl)-2*H*-1-benzopyran-2-one. As a substituted 4-phenylcoumarin, this constituent belongs to the group of neoflavonoids.

It is interesting that this compound isolated by us was found to be identical with an unidentified compound (identification by comparing their IR spectra) isolated by Bastien [5] 21 years ago from the stem bark of *Coutarea latiflora*. Bastien succeeded in isolating another constituent which after hydrolysis yielded glucose together with this compound; the author thus named the isolated aglycone 'coutareagenin' and the glycoside 'coutareosid'. We also could demonstrate, by hydrolysis of a TLC-separated glycoside, that the characterized neoflavonoid occurs not only as an aglycone but also as a glycoside. Up to now we could identify the sugar moieties glucose and rhamnose only by TLC but further investigation is in progress.

EXPERIMENTAL

Plant material. Plant material was collected in San Salvador in May 1981 and identified by Mrs. Lic. M.-L. Reyna de Aguilar, Ministerio de Agricultura y Ganaderia, San Salvador. A voucher specimen is deposited at Lehrstuhl für Pharmakognosie, Hamburg.

Extract. The powdered air-dried stem bark (200 g) of *Coutarea hexandra* was refluxed with MeOH (1.25 l.) for 20 min. The extract was concd under red. pres. and then hydrolysed by refluxing with HCl 12.5% (350 g) for 1 hr. The hydrolysed mixture was extracted with EtOAc. After concentrating under red. pres. the resulting EtOAc extracts were separated by CC on Si gel (Lobar® column, size B, Merck), using CHCl$_3$–MeOH–HCOOH (17:2:1). The yellowish compound was recrystallized from CHCl$_3$; yield 107 mg. TLC was performed on Si gel using toluene–EtOAc–HCOOH (8:2:1) detection UV 254 and 366 nm; R_f 0.23.

Physical data. Mp 208–210° (uncorr.), IR spectrum in KBr disc, UV spectrum in MeOH. MS were run on a Varian MAT CH 7, 70 eV, ion source temp. 250°, direct inlet; or high resolution: Varian MAT 311 A, 70 eV, ion source temp. 200° (peak matching) m/z (%): 300 (87) $[M]^+$, 299 (11), 273 (16), 272 (100) $[M-CO]^+$ (H.R.), 257 (25) $[M-(CO+Me)]^+$ (H.R.), 243 (9), 137 (9). TMS derivatization: 0.5 mg sample + 100 μl MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) 30 min at 100°. The ^{1}H NMR spectra were taken with TMS as int. standard using a Bruker WM 400 (400 MHz). Chemical shifts in δ (ppm) scale (Me$_2$CO-d_6 soln) 5.83 (H-3), 6.32 (H-8), 6.48 (H-6), 6.78 (H-4′), 6.87 (H-3′), 6.90 (H-6′). ^{13}C NMR spectra were determined with TMS as int. standard using a Bruker WM 400 (100.6 MHz). Chemical shifts in δ (ppm) scale, Me$_2$CO-d_6 or DMSO-d_6–CDCl$_3$ (7:3) soln.

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