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BIOLOGICALLY ACTIVE FLAVONES FROM *GUTIERREZIA RESINOSA*

MAGALIS BITTNER, MARIO SILVA, JULIO VARGAS and FERDINAND BOHLMANN*

Laboratorio de Quimica de Productos Naturales, Departamento de Botánica, Facultad de Ciencias Biológicas y de Recursos Naturales, Universidad de Concepción, Concepción, Chile; *Institut for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, West Germany

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Key Word Index—*Gutierrezia resinosa*; Compositae; 5,4'-dihydroxy-3,6,7,8,3'-pentamethoxyflavone; 5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone.

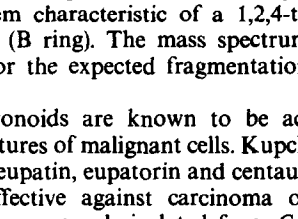
Abstract—The aerial parts of *Gutierrezia resinosa* afforded two highly oxygenated flavones 5,4'-dihydroxy-3,6,7,8,3'-pentamethoxyflavone and 5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone. The structures were elucidated by spectroscopic methods and confirmed by X-ray diffraction techniques. These flavones showed significant inhibition *in vitro* against the cells derived from human epidermoid carcinoma of the nasopharinx (KB).

Gutierrezia resinosa (Hook. et Arn.) Blake (Compositae, tribe Astereae) is an endemic plant of central Chile. Few species of the genus *Gutierrezia* have been investigated chemically and diterpenes [1, 2], triterpenes [2], acetylenes [3] and flavonoids [4] have been reported.

A phytochemical examination of the aerial parts of *Gutierrezia resinosa* was undertaken because no work has been carried out before on this plant. During the present study a new flavone, **2**, was isolated which showed significant activity (KB) against human carcinoma of the nasopharinx. We report the isolation and identification of two flavones 5,4'-dihydroxy-3,6,7,8,3'-pentamethoxyflavone (**1**) and 5,3',4'-trihydroxy-3,6,7,8-tetramethoxyflavone (**2**).

These two flavones were isolated from the ethyl acetate extract by chromatography and crystallization. Their structures were established by spectral data and confirmed by X-ray diffraction [Watson, W., personal communication]. The isolated compounds showed typical flavonoid bands in the IR (3400, 3200, 1590, 1275, 1220, 1160, 1040, 810 cm^{-1}). The bathochromic shift induced in the UV spectra by aluminium chloride, sodium acetate and sodium methoxide led us to conclude that in the flavonoid **1** there are only two free hydroxyls attached at C-5 and C-4'. The ^1H NMR spectra of **1** and **2** acetylated showed an ABX system characteristic of a 1,2,4-trisubstituted aromatic ring (B ring). The mass spectrum was easily rationalized for the expected fragmentation pattern of flavones.

Several flavonoids are known to be active against laboratory cultures of malignant cells. Kupchan *et al.* [5] reported that eupatin, eupatorin and centaureidin are all moderately effective against carcinoma of the nasopharynx. The compounds isolated from *Gutierrezia resinosa* were tested against (KB) human carcinoma of the nasopharynx and showed significant activity on this system [6, 7]. The minimal required activity is 25. Compound **1** gave 2.0×10^{-1} and **2** gave 4.4×10^{-1}.



1 R = Me
2 R = H

EXPERIMENTAL

Mps are uncorr. ^1H NMR spectra were determined at 270 MHz in $CDCl_3$ (TMS as int. standard). MS were obtained at 70 eV, direct inlet. CC was carried out with Si gel (Merck) and TLC with Si gel GF_{254} (Merck).

Extraction and isolation. The dry powered leaves and stems (3 kg) of *Gutierrezia resinosa* collected near Coquimbo, Chile, were exhaustively extracted with EtOH. The alcoholic extract (120 g) was treated with C_6H_6 and EtOAc successively. The EtOAc extract (30 g) was chromatographed on Kieselgel and two flavones were obtained.

Compound **1**. 5,4'-Dihydroxy-3,6,7,8,3'-pentamethoxyflavone (120 mg), yellow crystals: mp 174–176°. UV λ_{max}^{MeOH} nm: 260, 278, 350; λ_{max}^{NaOMe} nm: 275, 305sh, 396; $\lambda_{max}^{AlCl_3}$ nm: 273, 286, 305sh, 374; $\lambda_{max}^{AlCl_3-HCl}$ nm: 269, 289, 305sh, 368, 410; λ_{max}^{NaOAc} nm: 260, 278, 348; $\lambda_{max}^{NaOAc-H_3BO_3}$ 260, 278, 354. IR ν_{max}^{Nujol} cm^{-1}: 3420 (OH), 1650, 1600 (flavone) 1270, 1220, 1080, 1050, 1020, 1000, 980, 900, 860, 810, 760, 725. ^1H NMR ($CDCl_3$) (diacetate): δ8.07 (1H, *dd*, J = 9.0 Hz, H-6'), 7.89 (1H, *d*, J = 1.5 Hz, H-2'), 7.10 (1H,*d*,J = 9 Hz, H-5'), 4.10 (3H, *s*, OMe), 4.02 (3H, *s*, OMe), 3.93 (3H, *s*, OMe), 3.89 (3H, *s*, OMe), 3.82 (3H, *s*, OMe), 2.51 (3H, *s*, OAc), 2.37 (3H, *s*, OAc). MS 70 eV, *m/z* (rel. int.): 404 $[M]^+$ ($C_{20}H_{20}O_9$), 389 (100%), 374, 359, 202, 84.

Compound **2**. 5,3',4'-Trihydroxy-3,6,7,8-tetramethoxyflavone (170 mg), yellow crystals: mp 165–168°. UV λ_{max}^{MeOH} nm: 262, 275, 360; λ_{max}^{NaOMe} nm: 273, 412 (dec); $\lambda_{max}^{AlCl_3}$ nm: 284, 305 sh, 364, 454. $\lambda_{max}^{AlCl_3-HCl}$ nm: 273, 285, 305 sh, 370; λ_{max}^{NaOAc} nm: 271, 416. $\lambda_{max}^{NaOAc-H_3BO_3}$ nm: 268, 388. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1650, 1590 (flavone) 1270, 1220, 1160, 1045, 810, 760. ^1H NMR ($CDCl_3$) (triacetate): δ8.06 (1H, *dd*, J = 9.0 Hz, H-6'), 8.04 (1H, *d*, J = 1.5 Hz, H-2'), 7.36 (1H, *d*, J = 9.0 Hz, H-5'), 4.10 (3H, *s*, OMe) 4.01 (3H, *s*, OMe), 3.89 (3H, *s*, OMe), 3.84 (3H, *s*, OMe), 2.51 (3H, *s*, OAc), 2.36 (3H, *s*, OAc), 2.35 (3H, *s*, OAc). MS 70 eV, *m/z* (rel. int.): 390 $[M]^+$ ($C_{19}H_{18}O_9$) 375 (100%), 360, 345, 279 167, 149.

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A NEOFLAVONOID FROM *COUTAREA HEXANDRA* (RUBIACEAE)

G. REHER, LJ. KRAUS, V. SINNWELL* and W. A. KÖNIG*

Lehrstuhl für Pharmakognosie der Universität Hamburg; Hamburg, West Germany; *Institut für Organische Chemie und Biochemie der Universität Hamburg, Hamburg, West Germany

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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.

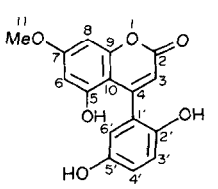
INTRODUCTION

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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