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Three flavonoids from *Licania heteromorpha*

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Abstract

Three new flavonoids were isolated from the methanolic extract of the aerial parts of *Licania heteromorpha* var. *heteromorpha*. Their structures were elucidated as myricetin 3,4′-di-*O*-α-L-rhamnopyranoside, myricetin 7-methyl ether 3,4′-di-*O*-α-L-rhamnopyranoside and myricetin 4′-methyl ether 3-*O*-β-D-galactopyranoside by means of spectral data. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Flavonoidic compounds have a very important role in the chemotaxonomy of the Chrysobalanaceae family (Coradin, Giannasi, & Prance, 1985). In our continuing study on *Licania* genus (Mendez, Bilia, & Morelli, 1995; Bilia, Mendez, & Morelli, 1996; Bilia, Ciampi, Mendez, & Morelli, 1996; Bilia, & Morelli, 1996) we now examined the chemical components of *L. heteromorpha* Bentham, var. *heteromorpha*, a tree from the Amazon forest (South America). Here we report the isolation and structural determination of three new flavonol glycosides with myricetin as aglycon (compounds **1**–**3**) from the methanolic extract.

2. Results and discussion

Compounds **1**–**3** were purified by Sephadex LH-20 column and RP-HPLC from the methanolic extract of the aerial part of *L. heteromorpha*. After spraying the TLC with Naturstoff reagent, they gave red spots typical of 3′,4′,5′-trihydroxyflavonols showing to have all the same aglycon.

The structure and molecular formulae of compound **1**–**3** were determined by negative ion ESI-MS spectra, 1-D and 2-D ^{1}H, ^{13}C and ^{13}C DEPT NMR data.

Compound **1** was assigned molecular formula $C_{27}H_{30}O_{16}$. Mass spectrometry, ^{13}C, and ^{13}C DEPT NMR analysis indicated its flavonoidic nature, and in particular 15 carbon atoms ascribable to the aglycon and 12 to the sugar moieties. In the ^{1}H NMR spectrum the chemical shifts and the coupling constants of protons indicated a 5,7-dihydroxylated pattern for ring A (two *meta*-coupled doublet at δ 6.22 and 6.36, $J = 1.8$ Hz) and a 3′,4′,5′-trihydroxylation for ring B (two-proton singlet at δ 6.94), permitting to recognize the aglycon as myricetin. Two anomeric protons were easily identified in the ^{1}H NMR spectrum. They resonated at δ 5.31 ($J = 1.5$ Hz) and 5.57 ($J = 1.5$ Hz), and correlated respectively with 103.9 and 103.1 ppm in HSQC spectra. Chemical shifts, multiplicity of the signal, absolute values of the coupling constant and the magnitude in the ^{1}H NMR spectrum as well as ^{13}C NMR data (Table 1) indicated the presence of two rhamnopyranosyl moieties with α-configuration at the

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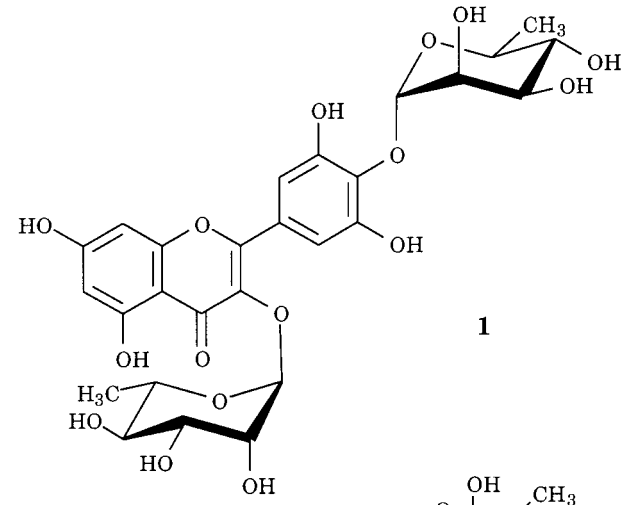

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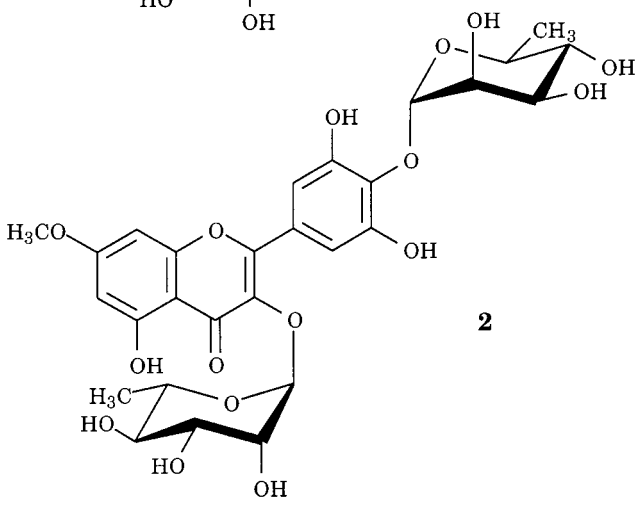

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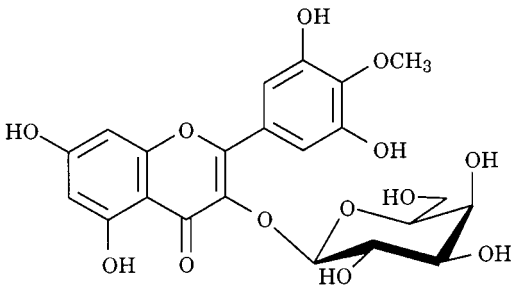

3

anomeric carbon. The exact position of rhamnose units was determined by typical glycosilation shifts observed in the ^{13}C NMR spectrum with respect to the aglycon myricetin: upfield shifts of C-2 (ca. 5.0 ppm) and C-4 (ca. 3.7 ppm), and downfield shift of C-3 (ca. 3.5 ppm) suggested the presence of a rhamnose unit at C-3; in the same way upfield shift of C-4′ (ca. 2.0 ppm) and downfield shifts of C-3′ and C-5′ (ca. 0.4 ppm) implied the position of the other α-L-rhamnose at C-4′ (Agrawal, & Bansal, 1989). This was confirmed by HMBC experiment correlations (δ 5.31 with 136.8 ppm (C-3) and δ 5.57 with 151.9 ppm (C-4′)). Therefore the structure of compound **1** is myricetin 3,4′-di-*O*-α-L-rhamnopyranoside.

Compound **2** had molecular formula $C_{28}H_{32}O_{16}$. Its ^1H NMR and ^{13}C spectra compared with those of **1** revealed each one more signal respectively at δ 3.76, (3H, s) and 51.9 ppm assigned to one methoxyl group. Its position is established from HMBC correlation data ((δ 3.76 with 165.6 ppm (C-7)). The methoxylation shift observed at C-6 and C-8 supported the position of the methoxyl group at C-7. Therefore compound **2** is myricetin 7-methyl ether 3,4′-di-*O*-α-L-rhamnopyranoside.

Finally, compound **3** was assigned molecular formula $C_{22}H_{22}O_{13}$. When **1** is used as reference compound in the spectral analysis of compound **3**, close similarities are observed between spectral data of the aglycon of both compound, while sugar moiety provided the point of difference. Except for aglycon signals, ^1H NMR spectrum revealed the presence of one-proton doublet at δ 5.24, $J=7.5$ Hz representative of one anomeric proton of a hexose unit, and one singlet at δ 3.92 (3H) again ascribable to one methoxyl group. Selected 1D-TOCSY obtained irradiating anomeric proton signal (δ 5.24) yielded the subspectrum of sugar residue with high digital resolution. The result of 1D-TOCSY compared with those of ^{13}C NMR experiment allowed the identification of sugar as galactopyranoside; its β-configuration at anomeric position is derived combining ^1H NMR and ^{13}C NMR data Table 1. Relative position of β-D-galactopyranose and of the methoxyl group is established from HMBC experiment correlations (δ 5.24 with 136.7 ppm (C-3), δ 3.92 with 151.4 ppm (C-4′)). Compound **3** is therefore myricetin 4′-methyl ether 3-*O*-β-D-galactopyranoside.

According to these results we can affirm that *L. heteromorpha* is unusual for its biosynthesis of methoxylated flavonoids glycosides that could be the response to local environmental factors, as evidenced by the role methoxylated flavonoids play in protecting plants from attacks by pathogens or herbivores (Wollenweber, 1986). It could also have an important chemotaxonomy value.

3. Experimental

NMR: CD_3OD, Bruker DRX-600 spectrometer; HSQC and HMBC (Martin, & Crouch, 1991) experiments were performed using the UXNMR software package; 1-D TOCSY (Davis, & Bax, 1985) were acquired using waveform generator-based GAUSS shaped pulse, mixing time ranging from 80 to 100 ms and a MLEV-17 spin-lock field of 10 Hz preceded by a 2 ms trim pulse; chemical shifts are expressed in δ (ppm) referring to solvent peaks: δ_H 3.34 and δ_C 49.0 for CD_3OD. Optical rotations were measured on a

Table 1
^{13}C NMR data (600 MHz, CD_3OD) of compounds **1**–**3**

Carbon	DEPT	1	2	3
2	C	158.6	158.5	157.8
3	C	136.8	136.5	136.7
4	C	179.5	179.3	179.1
5	C	162.8	162.8	162.8
6	CH	100.5	99.5	100.8
7	C	167.7	165.6	165.6
8	CH	95.1	94.4	95.5
9	C	158.6	158.2	158.0
10	C	105.6	105.56	104.4
1′	C	127.3	126.8	126.9
2′	CH	109.9	109.6	110.1
3′	C	136.3	136.0	138.0
4′	C	152.3	151.9	151.4
5′	C	136.3	136.0	138.0
6′	CH	109.9	109.6	110.1
Rha at C-3				
1″	CH	103.9	103.9	105.5
2″	CH	73.8	73.4	73.2
3″	CH	72.2	71.8	75.1
4″	CH	72.1	71.7	70.1
5″	CH	71.9	71.0	77.3
6″	CH_3	18.0	17.6	–
	CH_2	–	–	62.0
Rha at C-4′				
1‴	CH	103.1	102.7	–
2‴	CH	73.3	72.9	–
3‴	CH	72.1	71.7	–
4‴	CH	72.0	71.5	–
5‴	CH	71.3	70.6	–
6‴	CH_3	17.8	17.4	–
OCH_3	CH_3	–	51.9	60.8

Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. MS were recorded with a Hewlett Packward HP-1100 MSD spectrometer in the electrospray ionization (ESI) negative mode.

3.1. Plant material

Aerial parts of *L. heteromorpha* var. *heteromorpha* were collected in Puerto Ayacucho, Venezuela, in July 1996 and identified by Dr. A. Castillo. A voucher specimen is deposited in the herbarium of Facultad de Agronomia, Universidad Central de Venezuela, Maracay, Edo. Aragua.

3.2. Extraction and isolation

The powdered dried aerial parts of *L. heteromorpha* (480 g) were defatted with *n*-hexane and then extracted in a Soxhlet apparatus with $CHCl_3$ and $CHCl_3$–MeOH (9:1) each for 24 h to give 5.0 and 13.0 g of residues, respectively. The plant material was then extracted at room temperature for 4 weeks with MeOH to give 26.0 g of residue, which was partitioned between *n*-BuOH and H_2O. Part of the dried BuOH extract (2.2 g) was chromatographed on a Sephadex LH-20 column using MeOH as eluent and collecting fractions of 7 ml. All fractions were combined according to TLC (Silica gel plates in, *n*-BuOH–AcOH–H_2O (12:3:5) and $CHCl_3$–MeOH–H_2O (40:9:1)) composition. Chromatograms obtained were sprayed with a 1% solution of 2-aminoethyl diphenylborinate in MeOH (Naturstoff reagent, Roth), followed by a 5% solution of Polyethylene glycol 4000 (Fluka Chemie AC) in EtOH (Wagner, Bladt, & Zgainski, 1983). Fractions 34–38 (60 mg) and 43–47 (40 mg), containing the crude flavonoidic mixture were submitted to reversed-phase HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) using, respectively, MeOH–H_2O (2:3) and (1:1) as eluent to yield pure compound **1** (15.0 mg, $R_t = 14$ min) and **2** (10.0 mg, $R_t = 18$ min) from the first fraction and compound **3** (13.0 mg, $R_t = 10$ min) from the last.

3.3. Compound *1*

$[\alpha]_D^{25} = -160^\circ$ (MeOH, *c* 0.18); negative ESI-MS ($C_{27}H_{30}O_{16}$): *m/z* 609 $[M-H]^-$, 463 $[M-H\text{-}146]^-$; ^1H NMR (CD_3OD): δ 0.99 (3H, d, $J = 4.4$ Hz, H-6″), 1.31 (3H, d, $J = 4.4$ Hz, H-6‴), 5.31 (1H, d, $J = 1.5$ Hz, C-3–Rha H-1), 5.57 (1H, d, $J = 1.5$ Hz, C-4′–Rha H-1), 6.22 (1H, d, $J = 1.8$ Hz, H-6), 6.36 (1H, d, $J = 1.8$ Hz, H-8), 6.94 (2H, s, H-2′, H-6′). ^{13}C NMR: see Table 1.

3.4. Compound *2*

$[\alpha]_D^{25} = -150^\circ$ (MeOH, *c* 0.18); negative ESI-MS ($C_{28}H_{32}O_{16}$): *m/z* 623 $[M-H]^-$; ^1H NMR (CD_3OD): δ 0.95 (3H, d, $J = 4.4$ Hz, H-6″), 1.25 (3H, d, $J = 4.4$ Hz, H-6‴), 3.76 (3H, s, OCH_3), 5.28 (1H, d, $J = 1.5$ Hz, C-3–Rha H-1), 5.54 (1H, d, $J = 1.5$ Hz, C-4′–Rha H-1), 6.20 (1H, d, $J = 1.8$ Hz, H-6), 6.36 (1H, d, $J = 1.8$ Hz, H-8), 7.03 (2H, s, H-2′, H-6′). ^{13}C NMR: see Table 1.

3.5. Compound *3*

$[\alpha]_D^{25} = -25^\circ$(MeOH, *c* 0.1); negative ESI-MS ($C_{22}H_{22}O_{13}$): *m/z* 493 $[M-H]^-$; ^1H NMR (CD_3OD): δ 3.55 (1H, m, H-5″), 3.61 (1H, dd, $J = 9.0$, 4.0 Hz, H-3″), 3.66 (1H, dd, $J = 12.0$, 5.0 Hz, H-6″ a), 3.70 (1H, dd, $J = 9.0$, 7.5 Hz, H-2″), 3.75 (1H, dd, $J = 12.0$, 2.0 Hz H-6″b), 3.88 (1H, dd, $J = 4.0$, 2.5 Hz, H-4″), 3.92 (3H, s, OCH_3), 5.24 (1H, d, $J = 7.5$ Hz, H-1″), 6.20 (1H, d, $J = 1.8$ Hz, H-6), 6.36 (1H, d, $J = 1.8$ Hz, H-8), 6.91 (2H, s, H-2′, H-6′). ^{13}C NMR: see Table 1.

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