PERGAMON

Phytochemistry 51 (1999) 1125–1128

PHYTOCHEMISTRY

Three flavonoids from *Licania densiflora*

A. Braca[a,*], A.R. Bilia[b], J. Mendez[c], I. Morelli[a]

[a]*Dipartimento di Chimica Bioorganica, Università di Pisa, via Bonanno 33, 56126, Pisa, Italy*
[b]*Dipartimento di Scienze Farmaceutiche, Università di Firenze, via G. Capponi 9, 50133, Firenze, Italy*
[c]*Universidad Central de Venezuela, Fac. Ciencias, Esc. Quimica, P.O. Box 47102, Caracas, 1020-A, Venezuela*

Received 29 July 1998; received in revised form 11 March 1999; accepted 11 March 1999

Abstract

Three new flavonoids were isolated from the dried leaves of *Licania densiflora* (synonymous *Licania kanukuensis*). Their structures were elucidated as 8-hydroxy-naringenin-4′-methyl ether, myricetin 3′,4′-dimethyl ether 3-*O*-*β*-D-glucopyranoside, and myricetin 3-*O*-*α*-L-(2″-*O*-*α*-L-rhamnopyranosyl)-rhamnopyranoside by means of spectral data. © 1999 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Licania densiflora*; Chrysobalanaceae; Flavonoids; Naringenin 8-hydroxy-4′-methyl ether; Myricetin 3′,4′-dimethyl ether 3-*O*-*β*-D-glucopyranoside; Myricetin 3-*O*-*α*-L-(2″-*O*-*α*-L-rhamnopyranosyl)-rhamnopyranoside

1. Introduction

In our continuing research on the Chrysobalanaceae family, particularly on *Licania* genus (Mendez, Bilia & Morelli, 1995; Bilia, Mendez & Morelli, 1996a; Bilia, Ciampi, Mendez & Morelli, 1996b; Bilia & Morelli, 1996), we now report the phytochemical investigation of *Licania densiflora* Kleinhoonte, synonymous *L. kanukuensis* Standley. The plant is a tree 30 m high, growing in forests and hills of Venezuela, Brazil, and Guyanes. Expecially in Venezuela it grows in Bolivar and Delta Amacuro regions; its common names are ''Guanay'', ''Hierrito'' and ''Merecurillo'' (Toledo, Kubitzi & Prance, 1982). Here we report the isolation and structural determination of three new flavonoids (compounds **1**–**3**), obtained from the $CHCl_3$–MeOH 9:1 and MeOH extracts of the plant's dried leaves.

2. Results and discussion

Compound **1** was purified by Sephadex LH-20 column from the chloroformic-methanolic extract of the leaves of *L. densiflora*. Compounds **2**–**3** were obtained similarly by using Sephadex LH-20 column and Lobar Lichroprep RP 8 chromatography.

The structures and molecular formulas of compounds **1**–**3** were determined by positive ion FAB MS and UV spectra, 1D and 2D-^{1}H, ^{13}C, and ^{13}C DEPT NMR data. Furthermore, the absolute configurations of the sugar moieties have been confirmed referring to the $[\alpha]_D^{25}$ after hydrolysis of the glycosides.

Compound **1** had molecular formula $C_{16}H_{14}O_6$. Mass spectrometry, ^{1}H, and ^{13}C NMR analysis indicated its flavanoidic structures and particularly it was evidenced from the ^{1}H-NMR spectrum (DMSO) for the presence of the ABX spin system: H-2 (X part of the system) appeared as a *dd* at δ 5.49 ($J_{BX} = 3.4$ Hz and $J_{AX} = 13.0$ Hz), while H_2-3, (AB part), were represented by two *dd*, one at δ 2.89 and one at δ 2.72 ($J_{AB} = 17.2$ Hz). The presence of one singlet at δ 5.83, attributed to H-6, determined the unusual 5,7,8-trihydroxy substitution of ring A. The presence of a derivative of 8-hydroxy naringenin was also confirmed by the evaluation of the resonances of C2–C10 of γ-pyronic ring C in the ^{13}C NMR spectrum if compared with literature data (Bilia et al., 1996b). Furthermore, the ^{1}H NMR data revealed also one signal at δ 3.75 (3H, *s*) ascribable to one methoxyl group that resonated at

* Corresponding author. Fax: +39 (50) 43321.
E-mail address: braca@farm.unipi.it (A. Braca)

0031-9422/99/$ - see front matter © 1999 Published by Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(99)00187-9

Table 1
^{13}C NMR data of compounds **1**–**2**

Carbon	DEPT	**1** (DMSO-d_6)	DEPT	**2** (CD_3OD)
2	CH	78.0	C	157.4
3	CH_2	42.0	C	136.0
4	C	196.4	C	178.2
5	C	156.8	C	163.9
6	CH	95.3	CH	99.8
7	C	160.8	C	165.9
8	C	128.2	CH	94.7
9	C	154.3	C	158.5
10	C	103.4	C	104.5
1′	C	130.4	C	121.8
2′	CH	128.4	CH	109.5
3′	CH	114.6	C	146.9
4′	C	162.4	C	137.3
5′	CH	114.6	C	146.9
6′	CH	128.4	CH	109.5
OCH_3	CH_3	56.1	—	—
1″	—	—	CH	103.8
2″	—	—	CH	79.1
3″	—	—	CH	71.8
4″	—	—	CH	72.0
5″	—	—	CH	70.3
6″	—	—	CH_3	17.8
1‴	—	—	CH	102.4
2‴	—	—	CH	72.2
3‴	—	—	CH	73.6
4‴	—	—	CH	73.9
5‴	—	—	CH	70.8
6‴	—	—	CH_3	17.8

56.1 ppm in the ^{13}C NMR spectrum. The exact position of methoxyl group was established by typical methoxylation shift observed in the ^{13}C NMR spectrum with respect to 8-hydroxy naringenin: downfield shift of C-4′ (ca. 5 ppm) and upfield shifts of C-3′ (ca. 1.3 ppm), C-5′ (ca. 1.3 ppm), and C-1′ (ca. 0.5 ppm) suggested the presence of the methoxyl group at C-4′. This was confirmed by bidimensional NOESY experiment that showed a correlation between the signal at δ 3.75 with the one at δ 6.95 (H-3′ and H-5′). Compound **1** was so identified as the new natural metabolite naringenin 8-hydroxy-4′-methyl ether.

Compound **2** had 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 (CD_3OD) the chemical shifts and the proton coupling constants indicated a 5,7-dihydroxylated pattern for ring A (two *meta*-coupled doublet at δ 6.21 and 6.42, $J=2.0$ Hz) and a 3′,4′,5′-trihydroxylation for ring B (two-proton singlet at δ 6.95), permitting to recognize the aglycon as myricetin. Furthermore two anomeric protons, resonating at δ 4.96 ($J=1.8$ Hz) and 5.45 ($J=1.8$ Hz), were easily identified in the ^{1}H NMR spectrum. Mass spectra evidenced the presence of two flanked deoxyhexose moieties due to the presence of a molecular peak at 611 m/z and two prominent peaks were evidenced at 465 m/z $[(M+H)-146]^+$ due to the loss of a deoxyhexose unit and at 319 m/z $[(M+H)-(146+146)]^+$ due to the loss of another deoxyhexose unit; actually they were identified by ^{13}C NMR data (Table 1) as two rhamnopyranosyl moieties with α-configuration at the anomeric carbon (Agrawal & Bansal, 1989). The position of the disaccaridic moiety at C-3 was determined by typical glycosylation shift observed in the ^{13}C NMR spectrum with respect to the aglycon myricetin: upfield shifts of C-2 and C-4 (about 5.0 and 3.7 ppm, respectively), and a downfield shift of C-3 (about 3.5 ppm).

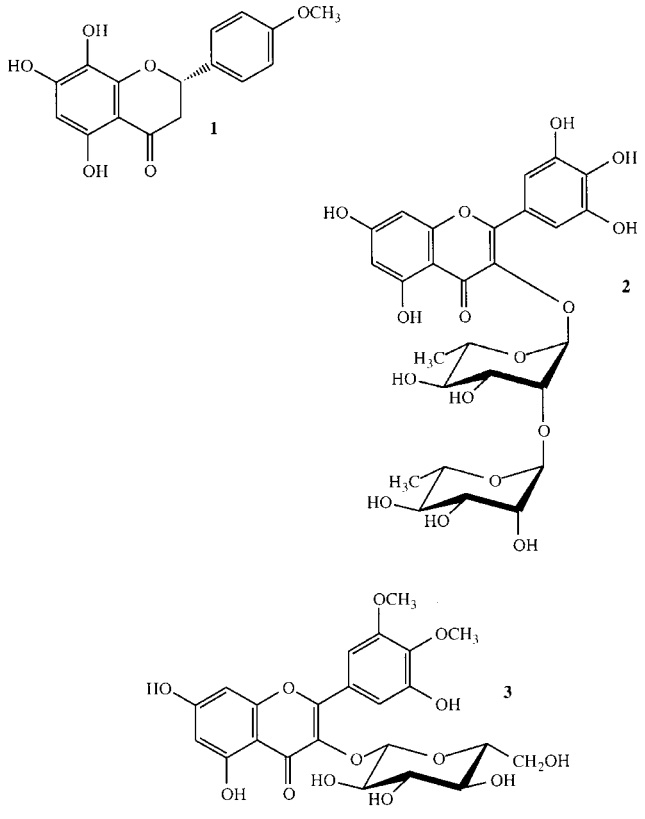
Actually myricetin 3-dirhamnoside was previously isolated from *Azara microphylla* leaves (Sagareishvili, Alaniya & Kemertelidze, 1983), but the authors did not specify sugar interlinkage. So from high digital resolution ^{1}H NMR spectral data and from ^{13}C NMR, we determined, with no doubt, this linkage that was confirmed by bidimensional COSY and HETCOR experiments. In the ^{1}H NMR the H-2 signal of one rhamnose unit resonated at a δ value (4.27) having a downfield shift with respect to H-2 of a terminal rhamnose unit. Similarly ^{13}C NMR data showed a signal at 79.1 ppm, ascribable at C-2 of a rhamnose unit, having a downfield shift (ca. 6.9 ppm) compared with C-2 of a terminal rhamnose moiety. The linkage between the two rhamnose units was so established as 1→2. After hydrolysis L-rhamnose was identified by TLC and $[\alpha]_D^{25}$ value by comparison their data with those of authentic sample; thus the structure of compound **2** is myricetin 3-*O*-α-L-(2″-*O*-α-L-rhamnopyranosyl)-rhamnopyranoside.

Finally compound **3** had molecular formula $C_{23}H_{24}O_{13}$. In the positive ion FAB MS spectrum molecular peak was observed at 509 m/z and two prominent peaks were evidenced at 479 m/z $[(M+H)-30]^+$ due to the loss of two methyl groups and at 347 m/z $[(M+H)-162]^+$ due to the loss of a hexose unit. Comparing spectral data of compound **3** with those of **2**, close similarities were observed between signal's values of the aglycon of both compounds, while sugar moiety provided the point of difference. Except myricetin signals, ^{1}H NMR spectrum revealed the presence of one-proton doublet at δ 5.27, $J=7.5$ Hz, representative of one anomeric proton, and two singlets at δ 3.93 (3H), and 3.95 (3H), ascribable to two methoxyl groups. The sugar was identified as β-D-glucopyranoside from absolute values of the coupling constants in the ^{1}H-NMR spectrum and the evaluation of TLC and $[\alpha]_D^{25}$ of the sugar moiety after hydrolysis of the glycoside by comparison with an authentic sample. Relative positions of β-D-glucopyranoside and of the methoxyl groups were determined by UV spectra registered with

$AlCl_3$, $AlCl_3$ + HCl, and NaOMe establishing respectively the absence of two *ortho* OH residues and substituted 3, 3′ and 4′ positions. Compound **3** is therefore 3′,4′-dimethylmyricetin-3-*O*-*β*-D-glucopyranoside.

In our previous investigations on *Licania* species (Chrysobalanaceae) we pointed out that the presence of myricetin derivatives suggested that chemical/phytogeographic correlations between and within genera of this family are probably more complex than those reported for the taxa of this family (Mendez *etal.*, 1995; Bilia et al., 1996a,b). So the presence of the aglycone myricetin also in *L. densiflora* encourage our previous suggestions.

On the other hand, methoxylated flavonoid glycosides are quite unusual and often play an important role of protection from attacks by herbivores or pathogens, so the biosynthesis of compounds **1** and **3** could be related to local environmental factors (Wollenweber, 1990)



.

3. Experimental

3.1. NMR

CD_3OD, DMSO-d_6, Bruker AC-200 Spectrospin spectrometer operating at 200 MHz for the proton and 50 MHz for the ^{13}C; chemical shifts are expressed in δ (ppm) referring to solvent peaks: δ_H 3.31 and δ_C 49.0 for CD_3OD; δ_H 2.39 and δ_C 49.5 for DMSO-d_6. Optical rotations were measured on a Perkin–Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. Positive FAB MS were recorded with a VG ZAB instrument spectrometer.

3.2. Plant material

Leaves of *L. densiflora* were collected in Amazonic forest near Cataniapo river, 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.

3.3. Extraction and isolation

Powdered, dried leaves of *L. densiflora* (500 g) were defatted with *n*-hexane and then extracted in a Soxhlet apparatus with $CHCl_3$ and $CHCl_3$–MeOH (9:1) each solvent for 24 h to give 5.0 and 11.9 g of residues, respectively. The plant material was then extracted at room temperature for 4 weeks with MeOH (27 g). $CHCl_3$–MeOH (9:1) and MeOH extracts were fractionated by Sephadex LH-20® (Pharmacia) column chromatography using MeOH–H_2O (4:1) as eluent to give fractions of 15 ml, eighteen (I–XVIII) from the first and twenty-two (I–XXII) from the last residue. All fractions were combined according to TLC separation [silica gel plates in EtOAc–AcOH–HCOOH–H_2O (100:11:11:27) and $CHCl_3$–MeOH–H_2O (70:30:3)]. 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). Fraction VII of chloroformic–methanolic 9:1 residue gave compound **1** (14 mg) that was crystallized from MeOH. From the methanolic extract were obtained compounds **2** (18 mg) and **3** (13 mg) respectively from fraction IX and XI chromatographated using Lobar Lichroprep RP 8 column with MeOH 50% (100 ml) and 60% (80 ml), respectively as eluents. Fractions from Lobar Lichroprep RP 8 chromatography were monitored by TLC separation [RP 8 plates in MeOH–H_2O (7:3), and MeOH–H_2O (3:2)].

3.4. Acid hydrolysis of **2–3**

A mixture containing 1 ml of 1 N HCl, 1 ml of dioxane and 10 mg of glycoside was heated in a sealed tube at 90°C for 4 h, then 5 ml of water were added and the aglycone was removed by extracting with 10 ml of $CHCl_3$. The aqueous layer was neutralized with Amberlite IRA 400 (OH^- type) and evaporated to dry-

ness. The sugar samples were identified by TLC by comparison with authentic samples and by evaluating their optical rotations.

*3.5.1. Compound **1***

Positive FAB MS ($C_{16}H_{14}O_6$): *m/z* 303 $[M+H]^+$; 288 $[M+H\text{-}15]^+$; $[\alpha]_D^{25} = -72^\circ$ (ca. 0.05, EtOH); UV (MeOH) λ_{max} nm: 225.9, 290.2, 354.7, +NaOMe: 225.3 sh, 255.4 sh, 291.0, 336.5, $+AlCl_3$ 255.3 sh, 314.8, 361.0, $+AlCl_3$ /HCl 311.0, 361.0; ^{1}H-NMR (DMSO-d_6): δ 2.72 (1H, *dd, J* = 3.4 and 17.2 Hz, H-3a), 2.89 (1H, *dd, J* = 13.0 and 17.2 Hz, H-3b), 3.75 (3H, *s*, OCH_3), 5.49 (1H, *dd, J* = 3.4 and 13.0 Hz, H-2), 5.83 (1H, *s*, H-6), 6.95 (2H, *d, J* = 8.7 Hz, H-3′ and H-5′), 7.43 (2H, *d, J* = 8.7 Hz, H-2′ and H-6′).^{13}C-NMR (DMSO-d_6): see Table 1.

*3.5.2. Compound **2***

Positive FAB MS ($C_{27}H_{30}O_{16}$): *m/z* 611 $[M+H]^+$, 465 $[(M+H)-146]^+$, 319 $[(M+H)-292]^+$; ^{1}H-NMR (CD_3OD): δ 0.97 (3H, *d, J* = 6.0 Hz, H-6′′′), 1.20 (3H, *d, J* = 6.1 Hz, H-6″), 3.32 (1H, *m*, H-4′′′), 3.39 (1H, *m*, H-4″), 3.49 (1H, *m*, H-5′′′), 3.53 (1H, *m*, H-5″), 3.62 (1H, *m*, H-3′′′), 3.84 (1H, *m*, H-3″), 3.94 (1H, *m*, H-2′′′), 4.27 (1H, *m*, H-2″), 4.96 (1H, *d, J* = 1.8 Hz, H-1′′′), 5.45 (1H, *d, J* = 1.8 Hz, H-1″), 6.21 (1H, *d, J* = 2.0 Hz, H-6), 6.42 (1H, *d, J* = 2.0 Hz, H-8), 6.95 (2H, *s*, H-2′ and H-6′); ^{13}C-NMR (CD_3OD): see Table 1.

*3.5.3. Compound **3***

Positive FAB MS ($C_{23}H_{24}O_{13}$): *m/z* 509 $[M+H]^+$, 479 $[M+H\text{-}30]^+$, 347 $[M+H\text{-}162]^+$; UV (MeOH) λ_{max} nm: 254.5, 355.1, +NaOMe 270.9, 417.8, $+AlCl_3$ 231.3 sh, 273.4 sh, 312.6, 358.9, $+AlCl_3$/HCl 231.5 sh, 273.4 sh, 312.6, 360.7; ^{1}H-NMR (CD_3OD): δ 3.44–3.88 (6H, *m*, sugar protons), 3.93 (3H, *s*, OCH_3), 3.95 (3H, *s*, OCH_3), 5.27 (1H, *d, J* = 7.5 Hz, H-1″), 6.22 (1H, *d, J* = 1.9 Hz, H-6), 6.45 (1H, *d, J* = 1.9 Hz, H-8), 7.53- 7.56 (2H, *m*, H-2′ and H-6′).

References

Agrawal, P. K., & Bansal, M. C. (1989). *Carbon-13 NMR of flavonoids*. Amsterdam: Elsevier.

Bilia, A. R., Mendez, J., & Morelli, I. (1996a) *Pharm. Acta Helvetiae, 71,* 191.

Bilia, A. R., Ciampi, L., Mendez, J., & Morelli, I. (1996b) *Pharm. Acta Helvetiae, 71,*199.

Bilia, A. R., & Morelli, I. (1996). *J. Nat. Prod*, *59*, 297.

Mendez, J., Bilia, A. R., & Morelli, I. (1995). *Pharm. Acta Helvetiae*, *70*, 223.

Sagareishvili, T. G., Alaniya, M. D., & Kemertelidze, E. P. (1983). *Khim. Prir. Soedin*, *3*, 289.

Toledo, C. L., Kubitzi, K., & Prance, G. H. (1982). In *Flora de Venezuela*, *IV*. Caracas, Ediciones Fundacion Educacion Ambiental.

Wagner, H., Bladt, S., & Zgainski, E. M. (1983). *Drogenanalyse. Dünnschichtchromatographische Analyse von Arzneidrogen*. Berlin: Springer.

Wollenweber, E. (1990). In J. B. Harborne, *The Flavonoids: Advances in research since 1986*. London: Chapman & Hall.