PERGAMON

PHYTOCHEMISTRY

Phytochemistry 50 (1999) 883–886

Dibenzylbutane lignans from *Virola sebifera* leaves[1]

Juan C. Martínez V.*, Martha I. Aldana J., Luis E. Cuca S.

Departamento de Química, Universidad Nacional de Colombia, A.A. 14490, Santafe de Bogota, Colombia

Received 21 April 1998; accepted 18 August 1998

Abstract

Two new dibenzylbutane lignans, *rac*-(8α,8′β)-4,4′-dihydroxy-3,3′-dimethoxylignan-9,9′-diyl diacetate and *rac*-(8α,8′β)-4-hydroxy-3-methoxy-3′,4′-methylenedioxylignan-9,9′-diyl diacetate were isolated from the toluene extract of *Virola sebifera* leaves, in addition to the previously described lignan, (−)-haplomyrfolin. Their structures were established by spectral analysis.

Keywords: Virola sebifera; Myristicaceae; Leaves; Lignans

1. Introduction

Virola sebifera is one of the most common and abundant Myristicaceae species in America; it is found from Nicaragua to the south of Brazil. It has industrial use, especially in the manufacture of toilet soaps due to its high content of trimiristine and laurodimiristine. Its fat is used in Venezuela (Shultes & Holmstedt, 1971) to cure rheumatism. *V. sebifera* is the most widely studied species of the Myristicaceae family. The bark was studied in Venezuela (Corothie & Nakano, 1969) and Japan (Kawanishi, Uhara, & Hashimoto, 1985; Kawanishi & Hashimoto, 1987) and six alkaloids, one terpene, two lignans and some fatty acid derivatives were identified. The fruit was studied in Brazil and a large number of metabolites (Lopes, Yoshida, & Gottlieb, 1982; Lopes, Yoshida, & Gottlieb, 1983; Lopes, Yoshida, & Gottlieb, 1984a; Lopes, Yoshida, & Gottlieb, 1984b; Kato, Lopes, Paulino-Filho, Yoshida, & Gottlieb, 1985) including 23 lignans, one dimeric lignan and four polyketides were identified. Bark, wood and leaves were studied in Colombia (Martínez, Cuca, & Martínez, 1985; Von Rothz, Cuca, & Martínez, 1990; Von Rothz, Cuca, & Martínez, 1987) and three lignans and three 1,3-diarylpropanes have been identified. This paper describes, besides the known compound (−)-haplomyrfolin (**2a**) (Evcim, Gözler, Freyer, & Shamma, 1986), the isolation and structural elucidation of two new lignans (**1a**, **1b**) from the leaves of *V. sebifera* collected in the Chocó region of Colombia, grown under very different environmental conditions from those where the leaves were collected for the reported studies (Von Rothz et al., 1987).

2. Results and discussion

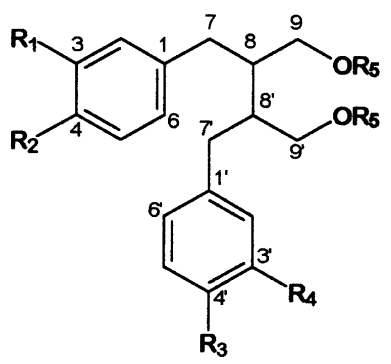
Compounds **1a** and **1b** exhibited IR absorptions at ca. 1735 cm^{-1} and UV maxima around 232 and 285 nm. The ^1H NMR spectrum indicated the presence of two acetyl groups (δ 2.06, s, 6H) and downfield signals at ca. δ 4.0–4.2 (4H) which were consistent with two acetylated CH_2–OH groups; these assignments were supported by the ^{13}C NMR signals at ca. δ 171, 64 and 21 ppm. The analysis of the ^1H and ^{13}C NMR spectra indicated that the two compounds contained two benzylic methylene groups (signals at ca. ^1H, δ 2.61; ^{13}C, δ 34.8) and two methine groups (signals at ca. ^1H, δ 2.05; ^{13}C, δ 39.5), characteristic of the dibenzylbutane lignans.

Compound **1a** exhibited a molecular formula of $C_{24}H_{30}O_8$ calculated from the low resolution MS (M^+ 446 (30%), $[M+1]^+$ (8.2%), $[M+2]^+$ (1.6%)) and from the ^1H and ^{13}C NMR spectral analysis. In the ^{13}C NMR spectrum, only 12 signals including those for acetyl, benzylic and methine groups were observed, suggesting that lignan **1a** had a magnetically symmetrical structure. The ^1H NMR spectrum showed two methoxyl groups at δ 3.75 (6H) and two phenolic hydroxyl groups at δ 5.40 (s br, 2H) confirmed by the IR (3428 cm^{-1}) and the UV absorptions (bathochromic shift on addition of MeONa). In the aromatic region of the ^1H NMR spectrum, two pairs of ABX protons [δ 6.40 (2H, d, $J=2.0$ Hz), 6.50

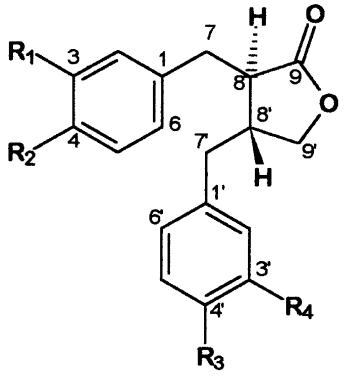
[1] Part 14 in the series "The Chemistry of Colombian Myristicaceae". For part 13 see Martínez and Torres (1997).

* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(98)00615-3



	R1	R2	R3	R4	R5
1a	OMe	OH	OH	OMe	Ac
1b	OMe	OH	O-CH2-O		Ac
1c	O-CH2-O		O-CH2-O		Ac
1d	OMe	OAc	OAc	OMe	Ac
1e	OMe	OH	OH	OMe	COR



	R1	R2	R3	R4
2a	O-CH2-O		OH	OMe
2b	OMe	OH	O-CH2-O	

Structure 1.

(2H, dd, $J=2.0$, 8.0 Hz) and 6.72 (2H, d, $J=8.0$ Hz)] were observed. Irradiation of the OMe at δ 3.75 enhanced the signal at δ 6.40 and gave clear evidence for the presence of the 4-hydroxy-3-methoxyphenyl moiety. On the basis of the above data, structure **1a** was concluded to be 4,4′-dihydroxy-3,3′-dimethoxylignan-9,9′-diyl diacetate, a new natural product. This structure was supported by its ^{13}C NMR data (Table 1) and MS fragmentation pattern (m/z 137 (100%)). Acetylation of compound **1a** gave tetraacetate **1d** with the same spectroscopical data reported in the literature (Fonseca, Campello, Barata, & Ruveda, 1978).

Compound **1b** had a molecular formula of $C_{24}H_{28}O_8$, that was deduced from its low resolution MS (M^+ 444) and from the ^{1}H and ^{13}C NMR spectral analysis. Compound **1b** had 2 amu lower than that of **1a** and this decrease in mass corresponds to the existence of one methylenedioxy group instead of one hydroxyl and one methoxyl groups, as indicated from its ^{1}H NMR spectrum which showed signals for one methylenedioxyl group (2H, δ 5.92) and for only one methoxyl group (3H, δ 3.82). The ^{1}H NMR spectrum of **1b** also showed two pairs of ABX aromatic protons (δ 6.40–6.62 (4H, m), 6.70 (1H, d, $J=8.4$ Hz) and 6.81 (1H, d, $J=8.8$ Hz)). The above data and the ^{13}C NMR data (Table 1) gave clear evidence of the presence of both 3,4-methylenedioxyphenyl and 4-hydroxy-3-methoxyphenyl moieties and the MS fragments [m/z 135 (100%) and 137 (94%)] confirmed the presence of these substructures in **1b**. Based on this evidence, **1b** was concluded to be 4-hydroxy-3-methoxy-3′,4′-methylenedioxylignan-9,9′-diyl diacetate, a new natural product.

Compounds **1a** and **1b** did not show specific rotation and, therefore, they are either the *meso* configuration (8α, 8′α) or racemates of the corresponding chiral isomers (8α, 8′β). Comparison of the chemical shifts and coupling constants of the 7, 8, 9-protons in the ^{1}H NMR spectra of **1a** and **1b** with those of the *meso-seco*isolariciresinol tetraacetate (*meso*-**1d**) (Agrawal & Rastogi, 1982), (−)-*seco*isolariciresinol tetraacetate (−)-**1d** (Agrawal & Rastogi, 1982) and lignan diesters (−)-**1e** (Rodrigues-Fo, Fernandes, Vieira, & Da Silva das G.F., 1992) (Table 2), establishes that **1a** and **1b** correspond to the racemate. This was corroborated in the ^{13}C NMR of **1a** and **1b** because the chemical shifts of 7, 8, 9-carbons are alike with those of the chiral **1c**, **1d** and **1e** (Table 1), and the ^{13}C NMR data of the diacetate of **1a** are superimposable with literature data of the tetraacetate (−)-**1d** (Fonseca et al., 1978). The complete assignment of the ^{13}C NMR signals for **1a** and **1b** (Table 1) was done using DEPT experiments and by comparison with those of analogous compounds **1c**, **1d** and **1e**.

Compound **2a**, $C_{20}H_{20}O_6$, showed a carbonyl absorption (1776 cm^{-1}) in the IR spectrum, and in its ^{1}H and ^{13}C NMR spectrum, a characteristic signal pattern of a 2,3-dibenzylbutyrolactone with both 3,4-methylenedioxyphenyl and 4-hydroxy-3-methoxyphenyl groups. From a comparison of the spectroscopic data (^{1}H NMR and EIMS) with reported values for **2b** (Corrie, Green, Ritchie, & Taylor, 1970) and for (−)-haplomyrfolin (Evcim et al., 1986), **2a** was identified as (8*R*,8′*R*)-4′-hydroxy-3′-methoxy-3,4-methylenedioxylignan-9,9′-olide, which had been previously isolated from *Haplophyllum myrtifolium* (Evcim et al., 1986).

Table 1
^{13}C NMR spectral data for compounds **1a**–**1e**

Carbons	**1a** DEPT	**1b** DEPT	**1c** (Hernandez, Roman, Espiñeira & Nathan, 1983)	**1d** (Fonseca et al., 1978)	**1e** (Rodrigues-Fo et al., 1992)
1	131.2 C	131.4 C	133.2	137.9	131.5
2	111.1 CH	111.3 CH	108.9	111.7	111.2
3	146.4 C	146.5 C	147.5	150.8	146.4
4	143.7 C	144.0 C	145.7	138.4	143.5
5	114.1 CH	114.3 CH	107.9	122.4	114.1
6	121.4 CH	121.6 CH	121.6	120.8	121.6
7	34.7 CH_2	34.9 CH_2	34.9	35.2	35.0
8	39.3 CH	39.8 CH	40.0	39.5	39.8
9	64.2 CH_2	64.4 CH_2	64.1	64.1	64.1
1′	131.2 C	133.5 C	133.2	137.9	131.5
2′	111.1 CH	108.1 CH	108.9	112.7	111.2
3′	146.4 C	147.7 C	147.5	150.8	146.4
4′	143.7 C	146.5 C	145.7	138.4	143.5
5′	114.1 CH	109.2 CH	107.9	122.4	114.1
6′	121.4 CH	121.8 CH	121.6	120.8	121.6
7′	34.7 CH_2	34.9 CH_2	[34.9]	35.2	35.0
8′	39.3 CH	39.8 CH	40.0	39.5	39.8
9′	64.2 CH_2	64.4 CH_2	64.1	64.1	64.1
CH_3O–	55.4 CH_3	55.8 CH_3	–	55.7	55.6
–OCH_2O–	–	100.9 CH_2	100.7	–	–
CH_3CO–	170.9 C	171.0 C	170.5	170.7 168.2	173.6
CH_3CO–	20.7 CH_3	21.0 CH_3	20.7	20.8 [20.6]	–

3. Experimental

3.1. General

M.p.s: uncorr. ^1H NMR: 90 MHz in $CDCl_3$ with TMS as int. standard. ^{13}C NMR: 22.4 MHz. EI-MS: direct inlet system at 70 eV. UV spectra in MeOH, except where noted. CC: silica gel (Kieselgel 60, Merck). TLC: silica gel 60 HF_{254}. TLC spots were visualized by UV and exposure of the plates to I_2 vapour.

3.2. Plant material

Leaves of *Virola sebifera* Aublet were collected from Quibdó (Chocó region) in Colombia, by J.C.M.V. and L.E.C.S. and authenticated by Dr. Roberto Jaramillo

Table 2
^1H NMR Chemical shifts of H-7,7′, H-8,8′ and H-9,9′ for compounds **1a**, **1b**, *meso*-**1d**, (−)-**1d** and (−)-**1e** in $CDCl_3$[a]

Compound	H-7,7′	H-8,8′	H-9,9′
1a	2.61 (d, $J=7.2$)	1.90–2.15 (m)	3.95 (dd, $J=11.4$ and 5.6), 4.23 (dd, $J=11.4$ and 5.6)
1b	2.62 (d, $J=6.6$)	2.00–2.20 (m)	4.00 (dd, $J=11.2$ and 5.3), 4.17 (dd, $J=11.2$ and 5.3)
(*meso*-**1d**) (Agrawal & Rastogi, 1982)	2.70 (d, $J=7.0$)	2.18 (m)	4.12 (d, $J=5$), 4.20 (d, $J=5$)
(−)-**1d** (Agrawal & Rastogi, 1982)	2.67 (d, $J=7.5$)	2.17 (m)	4.02 (dd, $J=11$ and 5), 4.25 (dd, $J=11$ and 5)
(−)-**1e** (Rodrigues-Fo et al., 1992)	2.60 (d, $J=7$)	na	4.00 (dd, $J=11$ and 6), 4.22 (dd, $J=11$ and 6)

[a] δ in ppm, J in Hz.

(Instituto de Ciencias Naturales, Universidad Nacional de Colombia). A voucher specimen is deposited in the Herbarium Nacional Colombiano and registered under the number COL 364808.

3.3. Extraction and isolation

Dried and powdered leaves (2.17 kg) were washed with petrol and exhaustively extracted with toluene at room temperature. The toluene extracts were concentrated at reduced pressure to yield a dark green residue (124 g). A portion of this extract (25 g), submitted to CC using solvents of increasing gradient of polarity (toluene and EtOAc), gave different groups of frs (1–7). Frs 1 (7950 mg) and 2 (3130 mg) contained a mixture of non-polar materials and were not further investigated. Frs 3 (330 mg), 4 (320 mg) and 6 (3100 mg) were purified separately by repeated CC on silica gel and eluted with petrol–EtOAc (7:3) and $CHCl_3$–MeOH (49:1) to give **1a** (352 mg), **1b** (16 mg) and **2a** (44 mg). Frs 5 (1600 mg) and 7 (5750 mg) contained chlorophylls and other substances and were not further investigated.

3.4. rac-(8α,8′β)-4,4′-Dihydroxy-3,3′-dimethoxylignan-9,9′-diyl diacetate (*1a*)

Colourless crystals; m.p. 112°C ($CHCl_3$); $[\alpha]_D^{20}$ 0° ($CHCl_3$; *c* 1.53). IR ν_{max} cm^{-1}: 3428, 2943, 1734, 1593, 1515, 1250, 1030. UV λ_{max} nm ($\log\varepsilon$): 233 (4.27), 285 (3.74). $\lambda_{max.}^{MeOH+MeONa}$ nm ($\log\varepsilon$): 239 (4.40), 294 (3.75). ^{1}H NMR (90 MHz): δ 1.90–2.15 (2H, m, H-8 and H-8′), 2.06 (6H, s, OAc-9 and OAc-9′), 2.61 (4H, d, J=7.2 Hz, H-7 and H-7′), 3.75 (6H, s, OMe-3 and OMe-3′), 3.95 (2H, dd, J=11.4 and 5.6 Hz, H-9a and H-9′a), 4.23 (2H, dd, J=11.4 and 5.6 Hz, H-9b and H-9′b), 5.40 (2H, br s, OH-4 and OH-4′), 6.40 (2H, d, J=2.0 Hz, H-2 and H-2′), 6.50 (2H, dd, J=2.0, 8.0 Hz, H-6 and H-6′), 6.72 (2H, d, J=8.0 Hz, H-5 and H-5′). ^{13}C NMR (22.4 MHz, $CDCl_3$): Table 1. EIMS *m*/*z* (rel. int.): 446 $[M]^+$ (30), 447 $[M+1]^+$ (8.2), 448 $[M+2]^+$ (1.61), 137 (100), 43 (12).

3.5. rac-(8α,8′β)-4-Hydroxy-3-methoxy-3′,4′-methylenedioxylignan-9,9′-diyl diacetate (*1b*)

Viscous oil. $[\alpha]_D^{20}$ 0° ($CHCl_3$; *c* 0.18). IR ν_{max} cm^{-1}: 3452, 2928, 1736, 1590, 1506, 1243, 1037. UV λ_{max} nm ($\log\varepsilon$): 232 (4.20), 286 (3.63). $\lambda_{max}^{MeOH+MeONa}$ nm ($\log\varepsilon$): 236 (4.30), 290 (3.63). ^{1}H NMR (90 MHz): δ 2.00–2.20 (2H, m, H-8 and H-8′), 2.06 (6H, s, OAc-9 and OAc-9′), 2.62 (4H, d, J=6.6 Hz, H-7 and H-7′), 3.82 (3H, s, OMe-3), 4.00 (2H, dd, J=11.2 and 5.3 Hz, H-9a and H-9′a), 4.17 (2H, dd, J=11.2 and 5.3 Hz, H-9b and H-9′b), 5.50 (1H, br s, OH-4), 5.92 (2H, s, OCH_2O-3′,4′), 6.40–6.62 (4H, m, H-2, H-2′, H-6 and H-6′), 6.70 (1H, d, J=8.4 Hz, H-5′) and 6.81 (1H, d, J=8.8 Hz, H-5). ^{13}C NMR (22.4 MHz, $CDCl_3$): Table 1. EIMS *m*/*z* (rel. int.): 444 $[M]^+$ (38), 445 $[M+1]^+$ (10.1), 446 $[M+2]^+$ (2.8), 137 (94), 135 (100), 43 (18).

3.6. (8R,8′R)-4′-Hydroxy-3′-methoxy-3,4-methylenedioxylignan-9,9′-olide (*2a*)

Viscous oil; $[\alpha]_D^{20}$ −28.9 ($CHCl_3$; *c* 0.150) (lit. (Evcim et al., 1986), $[\alpha]_D^{25}$ −31.6 ($CHCl_3$; *c* 0.11)). IR, UV, ^{1}H NMR and EIMS in Evcim et al. (1986). ^{13}C NMR (22.4 MHz, $CDCl_3$) δ (DEPT): 34.8 (CH_2, C-7), 38.3 (CH_2, C-7′), 41.3 (CH, C-8′), 46.4 (CH, C-8), 55.5 (CH_3, CH_3O-3′), 71.2 (CH_2, C-9′), 100.0 (CH_2, CH_2O_2-3,4), 108.2 (CH, C-5), 109.5 (CH, C-2), 111.0 (CH, C-2′), 114.5 (CH, C-5′), 121.3 (CH, C-6), 122.3 (CH, C-6′), 129.7 (C, C-1′), 131.4 (C, C-1), 144.4 (C, C-4′), 146.6 (C, C-4 and C-3′), 147.8 (C, C-3), 178.5 (C, C-9).

Acknowledgements

This work was supported by COLCIENCIAS (Colombia) (grant RC No. 008-94 cod. 1101-05-002-92) and by the Departamento de Química de la Universidad Nacional de Colombia.

References

Agrawal, P. K., & Rastogi, R. P. (1982). *Phytochemistry*, *21*, 1459.
Corothie, E., & Nakano, T. (1969). *Planta Medica*, *17*, 184.
Corrie, J. E., Green, G. H., Ritchie, E., & Taylor, W. C.(1970). *Australian Journal Chemistry*, *23*, 133.
Evcim, U., Gözler, B., Freyer, A. J., & Shamma, M. (1986). *Phytochemistry*, *25*, 1949.
Fonseca, S. F., Campello, J. de P., Barata, L. E., & Ruveda, E. (1978). *Phytochemistry*, *17*, 499.
Hernandez, J. D., Roman, L. U., Espiñeira, J., & Nathan, P. J. (1983). *Planta Medica*, *47*, 215.
Kato, M. J., Lopes, L. M. X., Paulino-Filho, H. F., Yoshida, M., & Gottlieb, O. R. (1985). *Phytochemistry*, *24*, 533.
Kawanishi, K., & Hashimoto, Y. (1987). *Phytochemistry*, *26*, 749.
Kawanishi, K., Uhara, Y., & Hashimoto, Y. (1985). *Phytochemistry*, *24*, 1773.
Lopes, L. M. X., Yoshida, M., & Gottlieb, O. R. (1982). *Phytochemistry*, *21*, 751.
Lopes, L. M. X., Yoshida, M., & Gottlieb, O. R. (1983). *Phytochemistry*, *22*, 1516.
Lopes, L. M. X., Yoshida, M., & Gottlieb, O. R. (1984). *Phytochemistry*, *23*, 2021.
Lopes, L. M. X., Yoshida, M., & Gottlieb, O. R. (1984). *Phytochemistry*, *23*, 2647.
Martínez V., J. C., & Torres, R. (1997). *Phytochemistry*, *44*, 1179.
Martínez V., J. C., Cuca, L. E., & Martínez, P. (1985). *Revista Colombiana de Quí*mica, *14*(1,2), 117.
Rodrigues-Fo, E., Fernandes, J. B., Vieira, P. C., & Da Silva das G.F., M. F. (1992). *Phytochemistry*, *31*, 2115.
Shultes, R. E., & Holmstedt, D. B. (1971). . *Lloydia*, *34*, 61.
Von Rothz, R., Cuca, L. E., & Martínez, V. J. C. (1990). *Revista Colombiana de Quí*mica, *19*(1), 97.
Von Rothz, R., Cuca, L. E., & Martínez, V. J. C. (1987). *Revista Colombiana de Quí*mica, *16*(1,2), 51.