Pergamon

0031-9422(95)00464-5

Phytochemistry, Vol. 40, No. 4, pp. 1259–1261, 1995

0031 9422/95 $9.50 + 0.00

LIGNANS FROM KERNELS OF *VIROLA MICHELII* HECKEL

MARIA CRISTINA SILVA VIDIGAL, ALBERTO JOSÉ CAVALHEIRO,* MASSUO JORGE KATO*† and MASSAYOSHI YOSHIDA*

Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, 30161-970 Belo Horizonte, M.G., Brazil; *Instituto de Química, Universidade de São Paulo, 05599-970 São Paulo, S.P., Brazil

(*Received in revised form* 15 *May* 1995)

Key Word Index — *Virola michelii*; Myristicaceae; kernels; seedlings; furofuran; dibenzylbutyrolactone; dibenzylbutyrolactol; dibenzylbutanediol; tetrahydrofuran lignans.

Abstract— A new tetrahydrofuran lignan, the (+)-(7*S*,8*R*,8′*R*)-3′,4′-dimethoxy-3,4-methylenedioxy-7,9′-epoxylignan-9-ol, was isolated from kernels of germinated seeds of *Virola michelii*, besides 10 previously described lignans: (+)-sesamin, (+)-fargesin, (+)-phillygenin, (−)-hinokinin, (−)-kusunokinin, (−)-dimethylmatairesinol, (+)-lariciresinol dimethyl ether, (−)-dihydrocubebin, (−)-2,3-desmethoxy-*seco*-isolintetralin and (−)-3′,4′,-dimethoxy-3′,4′-desmethylenedioxycubebin.

INTRODUCTION

The genus *Virola* (Myristicaceae) includes 59 species distributed in one South American rain forest (Venezuela, Guianas and Brazil). *Virola michelii* Heckel is widely spread in the Brazilian Amazon, and it is popularly called 'ucuúba' [1, 2]. Phytochemical analysis carried out on a morphologically related species *V. venosa* revealed the accumulation of flavones in flowers and pericarps at an early phase of fruit development [3]. During maturation such flavones are substituted by lignans, which also accumulate in arils and seeds. Apparently, no detectable changes occur in the lignan content during germination [3]. In one case of *V. surinamensis*, a butenolide polyketide and neolignans have also been isolated as major components in leaves from seedlings and adult plants [4]. The aim of this work addressed phytochemical investigations on germinated seeds taken from seedlings collected beneath the parent tree of *V. michelii*. Besides 10 previously reported lignans, one new tetrahydrofuran lignan (**1a**) is described.

RESULTS AND DISCUSSION

Chromatographic fractionation of the dichloromethane extracts from kernels of the germinated seeds of *V. michelii* afforded lignans belonging to five different structural types: (+)-sesamin [5–7], (+)-fargesin [8–10], (+)-phillygenin [7, 11] (furofuran lignans), (−)-hinokinin [12], (−)-kusunokinin [12], (−)-dimethylmatairesinol [12] (dibenzylbutyrolactone lignans), (+)-(7*S*,8*R*,8′*R*)-3′,4′-dimethoxy-3,4-methylenedioxy-7,9′-epoxylignan-9-ol, (+)-lariciresinol dimethyl ether [13, 14] (tetrahydrofuran lignans), (−)-dihydrocubebin [15, 16], (−)-2,3-desmethoxy-seco-isolintetralin [16] (dibenzylbutanediol lignans) and (−)-3′,4′-dimethoxy-3′,4′-desmethylenedioxycubebin [17] (dibenzylbutyrolactol lignan). The structural elucidation of these lignans was attained by spectroscopic techniques and by direct comparison with authentic samples.

The new tetrahydrofuran lignan **1a** possesses the molecular formula $C_{21}H_{24}O_6$ as determined from low resolution mass spectroscopy (M^+ 372) and from ^1H and ^{13}C counting in NMR spectra. Its IR spectrum exhibited a broad band at 3430 cm^{-1} assignable to a hydroxyl group. The ^1H NMR spectrum showed one methylenedioxyphenyl group at δ5.95, two methoxyl groups at δ3.87 and six aromatic protons (δ6.71–6.85), indicating two trisubstituted benzene rings. Additionally, its ^1H NMR spectrum revealed for the aliphatic protons the same profile as observed for lignans **1b** and **1c** [18], with a doublet at δ4.79 (6.5 Hz, H-7), two nonequivalent oxymethylene protons (*dd*, 6.4 and 8.4 Hz, H-9′a or H-9′b) at δ4.05 and a multiplet at δ3.72–3.96 (2 H-9, H-9′a or H-9′b); one doublet–doublet at δ2.93 (4.6 and 12.8 Hz, H-7′a or H-7′b) and a multiplet at δ2.35–2.80 (H-8, H-8′, and H-7′a or H-7′b). Its mass spectrum gave a base peak at *m/z* 151 (100%), assignable to 3,4-dimethoxytropylium ion and ArCH = $^+$OH (Ar = 3,4-methylenedioxyphenyl) besides another at *m/z* 149 (methylenedioxybenzoyl ion). Both ions arise from a typical cleavage of the tetrahydrofuran lignan. The definition of the substituents in each aromatic ring as depicted for the structure **1a** was also supported by the ^{13}C NMR data obtained for **1a**–**1c** and dihydrocubebin (Table 1). The chemical shifts for aliphatic and aromatic carbons of **1a** were assigned by comparison with those of lariciresinol dimethyl ether (**1b**) [14], dihydrosesamin (**1c**) [18] and dihydrocubebin [15,

†Author to whom correspondence should be addressed.

16]. The assignments of chemical shifts for tetrahydrofuran carbons were assigned by direct comparison to that of lariciresinol [14] or using dihydrocubebin as a model compound (Table 1). An important effect observed on chemical shifts of aromatic carbons is due to a β-effect caused by the oxygen of the tetrahydrofuran ring on C-1 of compounds **1a** (137.0) and **1c** (137.2) as compared to dihydrocubebin (134.3), and a γ-effect on carbons C-2/C-6 of **1a** (106.3/119.1) and **1c** (106.2/119.0) as compared to dihydrocubebin (108.1/121.9).

The accumulation of furofuran, tetrahydrofuran, dibenzylbutanediol and dibenzylbutyrolactone lignans in seeds has been previously demonstrated in *V. venosa* [3]. Nevertheless, remarkable differences were observed in *V. elongata* in which these lignans accumulate only in its pericarps, arils and seed coat, while in kernels the presence of aryltetralins and aryltetralone neolignans are ubiquitous [19].

The absolute configurations for all isolated lignans were established as $8R/8'R$ from optical rotation signals and/or circular dichroism curves. Indeed, the biosynthetic sequence (+)-furofuran, (+)-tetrahydrofuran, (−)-butanediol and (−)-butyrolactone, involving the series $8R/8'R$, has recently been proved to occur enzymically in *Forsythia intermedia* plants [20] and might be the case in *Virola* species, considering solely their absolute configurations.

Table 1. ^{13}C NMR data for **1a**, **1b**, **1c** and dihydrocubebin

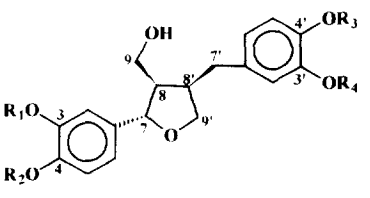
Carbons	1a	1b	1c [18]	Dihydro cubebin
C-1	137.0	135.9	137.1	134.3
C-2	106.3	108.9	106.2	108.1
C-3	147.8*	149.0	147.8	147.6
C-4	147.4*	148.9	147.8	145.8
C-5	108.0	110.9*	108.2*	109.3
C-6	119.1	117.9	119.0	121.9
C-7	82.8	82.7	82.8	35.9
C-8	52.7	52.5	52.6	44.3
C-9	60.9	60.8	60.8	60.4
C-1′	132.9	132.9	134.2	134.3
C-2′	111.3*	111.3*	108.0*	108.1
C-3′	148.9	148.4	145.9	147.6
C-4′	146.9	147.4	145.3	145.8
C-5′	111.9*	111.9*	108.9	109.3
C-6′	120.5	120.5	121.3	121.9
C-7′	33.1	33.2	33.3	35.9
C-8′	42.3	42.3	42.3	44.3
C-9′	72.9	72.9	72.9	60.4
$-OCH_3$	55.9	55.8 55.9	—	—
$-OCH_2O-$	100.9	—	100.9 100.8	100.8

*Values may be reversed.

EXPERIMENTAL

General. Prep. TLC was carried out on Silica gel PF-254 (Merck) and Alumina GF-254 (Merck) and CC on Silica gel 60H (0.005–0.045 mm) (Merck) and Alumina-90 (Merck). Mps were obtained on Electrothermal equipment and are uncorrected. Optical rotations were measured on a Polamat A-Carl Zeiss and CD with a dichrograph Jobin Yvon CD6. The ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Bruker-AC 200 in $CDCl_3$ with TMS as int. standard. EIMS were obtained at 70 eV on HP 5988-A.

Plant material. Seedlings of *V. michelii* Heckel were collected in April of 1989, at Gavião Reserve (INPA-WWF), Manaus–Caracaraí Road, Amazonas State, Brazil. The specimen was identified by William Rodrigues, Departamento de Botânica, Instituto Nacional de Pesquisas da Amazônia (Manaus).



	R_1	R_2	R_3	R_4
1a		$-CH_2-$	$-CH_3$	$-CH_3$
1b	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$
1c		$-CH_2-$	$-CH_2-$	

Extraction and isolation of the constituents. The dried kernels (24.98 g) of the seedling of *V. michelii* were ground and exhaustively extracted with CH_2Cl_2 at room temp. The CH_2Cl_2 extracts were concd *in vacuo* to give a brown mass (15.63 g, 62.6%). A portion of this extract (10 g), submitted to CC using solvents of increasing gradient of polarity (*n*-hexane, EtOAc and EtOH), gave different groups of frs (1–14). Frs 1–5 (2395 mg) contained a mixt. of non-polar materials that was not further investigated. Frs 6 and 7 (135 mg) were submitted to prep. TLC (CH_2Cl_2–Me_2CO, 0.25%) to give (+)-sesamin (5.2 mg), (+)-fargesin (8.3 mg) and (−)-hinokinin (37.2 mg). Frs 8–12 (1321 mg) were subjected to CC on silica gel and eluted with CH_2Cl_2–Me_2CO, MeOH to give frs A–N. Frs B–D yielded (−)-kusunokinin (688.6 mg) and frs E–G afforded (−)-dimethylmatairesinol (175.1 mg). Frs H and I were pooled and recrystallized with MeOH to give (+)-phillygenin (11.2 mg). Fr K was purified by prep. TLC (CH_2Cl_2–Me_2CO, 5%) and yielded **1a** (4.7 mg) and (−)-3′,4′-dimethoxy-3′,4′-demethylenedioxycubebin (11.5 mg). Fr. L was purified by prep. HPLC (Lichrosorb RP-8 column; 250 × 22 mm; flow rate 12 ml min^{-1}; detection at 254 nm) with H_2O–MeOH [3:17 → 3:22 (30 min)] to give (−)-dihydrocubebin (2.0 mg). Frs 13 and 14 (562 mg) were subjected to CC on alumina *in vacuo* using solvents of increasing polarity (CH_2Cl_2, Me_2CO and MeOH) affording 8 frs (a–h). Fr. b and frs d and f afforded (−)-dimethylmatairesinol (27.6 mg) and (+)-lariciresinol dimethyl ether (**1b**, 21.8 mg), respectively. Frs g and h were purified by HPLC (Perkin-Elmer C-18 column; 250 × 4.5 mm; flow

rate 2 ml min^{-1}; detection at 254 nm) with H_2O–MeOH (3:7) to give (−)-2,3-desmethoxy-*seco*-isolintetralin (15.1 mg). All spectral data for the known compounds were similar to those for authentic samples or lit. values. The $[\alpha]_D^{25}$ values for (+)-sesamin, (+)-fargesin and (+)-phillygenin were taken from the ORD curves.

(+)-*Sesamin*. Solid, mp 120–121° (MeOH), lit. [6], mp 120–121°. $[\alpha]_D^{25}$ +33.2° (MeOH; *c* 0.01), lit. [7] $[\alpha]_D^{25}$ +69.2° ($CHCl_3$; *c* 0.68). CD (MeOH; *c* 0.01): $[\Delta\varepsilon]_{232}^{max}$ +2.61, $[\Delta\varepsilon]_{288}^{max}$ +0.88.

(+)-*Fargesin*. Solid, mp. 115–117° (hexane), lit. [8] mp 139°. $[\alpha]_D^{25}$ +125.8° (MeOH; *c* 0.01), lit. [21] $[\alpha]_D^{25}$ +112° ($CHCl_3$; *c* 0.17). CD (MeOH; *c* 0.01): $[\Delta\varepsilon]_{235}^{max}$ +2.94, $[\Delta\varepsilon]_{290}^{max}$ −0.60.

(+)-*Phillygenin*. Solid, mp 130–132° (hexane), lit. [7] mp 135–136°. $[\alpha]_D^{25}$ +62.7° (MeOH; *c* 0.01), lit. [7] $[\alpha]_D^{25}$ +91.6° ($CHCl_3$; *c* 0.5). CD (MeOH; *c* 0.01): $[\Delta\varepsilon]_{234}^{max}$ +1.89, $[\Delta\varepsilon]_{275}^{max}$ +0.12.

(−)-*Hinokinin*. Pale solid, mp 92–94°, lit. [12] mp 92–95° (MeOH). $[\alpha]_D^{25}$ −22.9° ($CHCl_3$; *c* 1.4), lit. [12] $[\alpha]_D^{25}$ −26.3° ($CHCl_3$; *c* 0.123).

(−)-*Kusunokinin*. Viscous oil. $[\alpha]_D^{25}$ −87° ($CHCl_3$; *c* 1.4), lit. [22] $[\alpha]_D^{23}$ −31.4° ($CHCl_3$; *c* 1.0).

(−)-*Dimethylmatairesinol*. Crystals, mp 125–126° (MeOH), lit. [12] mp 127–128° (MeOH). $[\alpha]_D^{25}$ −32.5° ($CHCl_3$; *c* 0.4), lit. [12] $[\alpha]_D^{25}$ −39° ($CHCl_3$; *c* 0.18).

(7*S*,8*R*,8′*R*)-3′,4′-*Dimethoxy*-3,4-*methylenedioxy*-7,9′-*epoxylignan*-9-*ol* (**1a**). Viscous oil, $[\alpha]_D^{25}$ +4.5° (Me_2CO; *c* 0.2). IR ν_{max}^{film} cm^{-1}: 3430, 3009, 2928, 1608, 1515, 1489, 1444, 1249, 1039, 934, 863, 810. MS *m/z* (rel. int.): 372 ([M]$^+$, 87, calc. for $C_{21}H_{24}O_6$: 372.417); 358 (1), 354 (4), 233 (5), 208 (2), 203 (13), 194 (18), 178 (16), 176 (10), 151 (100), 149 (43), 121 (13), 91 (13). ^{1}H NMR: *d* 2.35–2.80 (3H, *m*, H-8, H-8′, H-7′a or H-7′b), 2.93 (1H, *dd*, *J* = 4.6, 12.8 Hz, H-7′a or H-7′b), 3.72–3.96 (3H, *m*, 2 H-9, H-9′a or H-9′b), 3.87 (6H, *s*, $OCH_3 \times 2$), 4.05 (1H, *dd*, *J* = 6.4, 8.4 Hz, H-9′a or H-9′b), 4.79 (1H, *d*, *J* = 6.5 Hz, H-7), 5.95 (2H, *s*, OCH_2O-), 6.71–6.85 (6H, *m*, Ar-H). ^{13}C NMR: Table 1.

(+)-*Lariciresinol dimethyl ether* (**1b**). Viscous oil, lit. viscous oil [14]. $[\alpha]_D^{25}$ +58° (Me_2CO; *c* 0.9), lit. [13] $[\alpha]_D^{25}$ +11° (Me_2CO; *c* 0.9).

(−)-*Dihydrocubebin*. Crystals, mp 99–100° (hexane), lit. [23] mp 101–102° (petrol). $[\alpha]_D^{25}$ −21° ($CHCl_3$; *c* 0.4), lit. [23] $[\alpha]_D^{25}$ −36.8° ($CHCl_3$; *c* 2.2).

(−)-2,3-*Desmethoxy*-seco-*isolintetralin*. Viscous oil. $[\alpha]_D^{25}$ −15° ($CHCl_3$; *c* 2.4), lit. [16] $[\alpha]_D^{28}$ −1.6° ($CHCl_3$; *c* 1.3).

(−)-3′,4′-*Dimethoxy*-3′,4′-*desmethylenedioxycubebin*. Viscous oil $[\alpha]_D^{25}$ −110° ($CHCl_3$; *c* 0.2), lit. [17] $[\alpha]_D^{25}$ −15.88° ($CHCl_3$; *c* 0.17).

Acknowledgements—This work was supported by Conselho Nacional de Desenvolvimento Cientifico e Technológico (CNPq) and Fundação de Amparo Pesquisa do Estado de São Paulo (FAPESP, grant 88/4316-9).

REFERENCES

1. Rodrigues, W. A. (1972) *Acta Amazonica* **2**, 29.
2. Rodrigues, W. A. (1980) *Acta Amazonica* **10**, 1.
3. Kato, M. J., Yoshida, M. and Gottlieb, O. R. (1992) *Phytochemistry* **31**, 283.
4. Lopes, N. P., França, S. C., Pereira, A. M. S., Maia, J. G. S., Kato, M. J., Cavalheiro, A. J., Gottlieb, O. R. and Yoshida, M. (1994) *Phytochemistry* **35**, 1469.
5. Agrawal, P. K. and Thakur, R. S. (1985) *Magn. Reson. Chem.* **23**, 389.
6. Greger, H. and Hofer, O. (1980) *Tetrahedron* **36**, 3551.
7. Iida, T., Nakano, M. and Ito, K. (1982) *Phytochemistry* **21**, 673.
8. Kakisawa, H., Kusumi, T., Hsu, H. Y. and Chen, Y. P. (1970) *Bull. Chem. Soc. Jpn* **43**, 3631.
9. Pelter, A., Ward, R. S., Rao, E. V. and Sastry, K. V. (1976) *Tetrahedron* **32**, 2783.
10. Corrie, J. E. T., Green, G. H., Ritchie, E. and Taylor, W. C. (1970) *Aust. J. Chem.* **23**, 133.
11. Rahman, M. M. A., Dewick, P. M., Jackson, D. E. and Lucas, J. A. (1990) *Phytochemistry* **29**, 1971.
12. Lopes, L. M. X., Yoshida, M. and Gottlieb, O. R. (1983) *Phytochemistry* **22**, 1516.
13. Ayoub, S. M. H., Maat, L., Peters, J. A. and Linders, J. T. M. (1985) *Magn. Reson. Chem.* **23**, 385.
14. Ayoub, S. M. H. and Kingston, D. G. I. (1984) *J. Nat. Prod.* **47**, 875.
15. Anjaneyulu, A. S. R., Ramaiah, P. A., Row, L. R., Venkateswarlu, R., Pelter, A. and Ward, R. S. (1981) *Tetrahedron* **37**, 3641.
16. Satyanarayana, P. and Venkateswarlu, S. (1991) *Tetrahedron* **47**, 8931.
17. Sumathykutty, M. A. and Rao, J. M. (1988) *Indian J. Chem.* **27B**, 388.
18. Lin-Gen, Z., Seligmann, O., Lotter, H. and Wagner, H. (1983) *Phytochemistry* **22**, 265.
19. Kato, M. J., Yoshida, M. and Gottlieb, O. R. (1990) *Phytochemistry* **29**, 1799.
20. Ozawa, S., Davin, L. B. and Lewis, N. G. (1993) *Phytochemistry* **32**, 643.
21. Takahashi, K., Hayashi, Y. and Takani, M. (1970) *Chem. Pharm. Bull.* **18**, 421.
22. Takaoka, D., Takamatsu, N., Saheki, Y., Kôno, K., Nakaoka, C. and Hiroi, M. (1975) *Nippon Kagaku Kaishi* 2192.
23. Tillekeratne, L. M. V., Jayamanne, D. T., Weerasuria, K. D. V. and Gunatilaka, A. A. L. (1982) *Phytochemistry* **21**, 476.