New Butenolides in Plantlets of *Virola surinamensis* (Myristicaceae)

Norberto Peporine LOPES,[a] Pierre Alexandre dos SANTOS,[a] Massuo Jorge KATO,*[,b] and Massayoshi YOSHIDA[b]

[a] *Faculdade de Ciências Farmacêuticas, Universidade de São Paulo; Via do Café S/N, CEP 14.040-903, Ribeirão Preto-SP, Brazil: and* [b] *Instituto de Química-Universidade de São Paulo; C.P. 26077, CEP 05513–970, São Paulo-SP, Brazil.*
Received May 20, 2004; accepted July 15, 2004

A phytochemical investigation in plantlets of the Brazilian medicinal tree *Virola surinamensis* resulted in the isolation and structural determination of four new compounds: 3-hydroxy-4-methyl-2-(11′-piperonyl-*n*-undecyl)-butenolide; 3-hydroxy-4-methyl-2-(7′-piperonyl-*n*-heptyl)-butanolide; 9′-(3,4-methylenedioxy-phenyl)-nonanoic acid and 13′-(3,4-methylene-dioxyphenyl)-tridecanoic acid. Thirteen compounds previously isolated from seeds and adult plants were also reported.

Key words *Virola surinamensis*; butenolide; Myristicaceae

Virola surinamensis (ROL.) WARB., popularly known as "ucuúba", is a myristicaceous tree commonly found in the Amazon River banks.[1)] This species is known by several ethnopharmacological uses.[2)] Its bark resin has been used in the treatment of erysipelas, whereas the tea of leaves is utilized in the treatment of colic and dyspepsia.[3)] Amazon Indians inhale the vapor from leaves to treat malaria, and recently the major constituent nerolidol was demonstrated to inhibit glycoprotein biosynthesis in the trophozoite of *Plasmodium falciparum*.[4)] Flavonoids isolated from roots exhibited antifungal activity against *Cladosporium cladosporioides* as much as 10-fold more potent than the control nystatin.[5)]

The tetrahydrofuran lignans isolated from adult leaves and woods showed high trypanocidal activity against the tripomastigote forms of *Trypanosoma cruzi*, the Chagas disease etiologic agent.[6,7)] Such disease affects more than 18 million people in Latin America, leading to approximately 400000 deaths per year.[8)] Recently, the process of obtaintion and use of the *V. surinamensis* lignans against Chagas's disease was patented (Br, INPI 9.903.472-7).

Farming of medicinal native plants having a potential pharmaceutical application is an important activity for sustainable conservation programs. Previous work showed the presence of antifungal lactone juruenolide C as the major compound in germinated young seedling leaves of *V. surinamensis*.[9)] Nevertheless, since its plantlets has never been fully investigated, we decided to cultivate and to investigate its chemical constituents.

Extracts were fractionated by cromatotron[@] followed by prep. HPLC. Leaves yielded sitosterol, stigmasterol, juruenolide C,[9)] juruenolide D,[10)] juruenolide E (**1**), juruenolide F,[10)] *epi*-juruenolide C[5)] and isoelemicin[11)]; stems yielded sitosterol, galbelgin,[12)] veraguensin,[13)] virolin,[14)] biochanin, A[15)] and a mixture of juruenolide C and *epi*-juruenolide C; roots, yielded sitosterol, iryelliptin B (**2**), **3a** and **3b**, virolin, biochanin A, α,2′-dihydroxy-4,4′-dimethoxydihydrochalcone,[16)] and 7-hydroxyflavanone.[17)] The known compounds were identified by comparison with previously reported data.

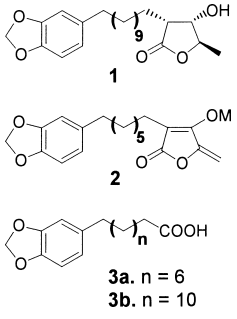
Compound **1** was characterized as a γ-lactone due to IR carbonyl absorption at ν_{max} 1757 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **1** closely resembled those of juruenolide, the major butenolide of wood from *Iryanthera juruensis* and *I. ulei*,[18)] and of juruenolides C, D, F, G previously isolated from *V. surinamensis* seedling leaves and pericarps.[9,11)] The differences in the ^{1}H- and ^{13}C-NMR spectra of juruenolides could be associated to the length of the methylene chain.

In case of novel juruenolide E, n=9 was proposed based on the mass spectral data ([M+H]$^+$, m/z 391 Da) and the elemental analysis. The ^{1}H-NMR chemical shifts and coupling constants referring to the three vicinal chiral positions of the butenolide moieties were superimposable and, thus, **1** and the previously published juruenolides[5,9,10,18)] must have identical relative configurations. Since the positive sign of optical rotation of **1**, was similar to that of other juruenolides, its absolute configuration was likely to be the same.

The ^{1}H-NMR spectrum of **2** revealed the presence of a piperonyl group and a methylene chain, as found in juruenolides. The signals relative to the juruenolide ring were, however, replaced by two hydrogens of an exocyclic methylene (δ 4.98, 4.92, 2d, J=3.0 Hz), as reported for iryelliptin isolated from *I. elliptica*.[19)] IR spectrum analysis showed carbonyl absorption at ν_{max} 1771 cm^{-1}, but no absorption of enolic hydroxyl group was observed. The presence of a methoxyl group in the C-3 position was confirmed by the absence of the enolic hydroxyl group in the IR spectrum and by the signal at δ 4.08 in the ^{1}H-NMR spectrum.

The ^{13}C-NMR spectrum was very similar to that of iryelliptin; the small differences observed could be attributed to the methylene chain length (n=5) and to the absorption at δ 58.8 relative to the MeO-4. Finally, the [M+H]$^+$ was in agreement with the structure of **2** named as iryelliptin B .

Compounds **3a** and **3b** exhibited absorptions characteristic of piperonyl group and methylene chain [$(CH_2)_6$ and $(CH_2)_{10}$, respectively] in their ^{1}H-NMR spectra. In this case, the signals relative to the lactone rings were replaced by a signal at



* To whom correspondence should be addressed. e-mail: majokato@iq.usp.br

δ 2.53 (t, J=7.2), which is associated with the α-carbonyl hydrogens. The IR spectrum shows absorption peaks at ν_{max} 3400—2500 cm^{-1} and at 1722±1 cm^{-1}, assignable to carboxylic acid function. The signals in the ^{13}C-NMR spectrum were in agreement with the proposed structure. The MS data (negative mode) and elemental analysis of **3a** (*m*/*z* 277) and **3b** (*m*/*z* 333) confirmed their structures as 9′-piperonyl-nanoic acid and 13′-piperonyl-tridecanoic acid, respectively.

Previous studies reported the occurrence of lignans in leaves, flavonoids in wood, as well as lignans and flavonoids in roots of adult plants of *V. surinamensis*.[7] The presence of juruenolides (as minor components) was observed only in roots of adult plants. In the present study, roots and stems from plantlet showed similar chemical profiles of adult plants, whereas leaves exhibited higher contents of antifungal juruenolides but no lignans. From the biosynthetic viewpoint, the precursors (C_6C_3 units) were probably uptaken to an alternative route. In adult plants, C_6C_3 acids were shown to derive from the general phenylpropanoid metabolism followed by dimerization of phenyl- or allyl-phenols by oxidative coupling to lignoids.[2] In plantlets, the cinnamoyl-CoA derivatives had it aliphatic chain extended by condensation with acetate units (*via* malonyl-CoA) in which the lactone ring is produced after condensation with pyruvic acid. The occurrence of this biogenetic pathway is supported by the isolation of compounds **3a** and **3b**.

Experimental

General Prep. TLC was carried out on Si gel PF-254 (Merck) and CC on Si gel 60 (40—63 μm; Merck). HPLC separations were carried out on a Perkin Elmer Series 4 liquid chromatography. Optical rotations were measured on a Jasco Digital Polarimeter mod. DIP-370. The ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on a Bruker AC-200 instrument in $CDCl_3$, using TMS as internal standard. ESI-MS and elemental analysis were recorded on Quattro-LC-Micromass and Perkin Elmer CNH-2400 instruments, respectively.

Plant Material Seeds and plantlets (1 to 2 meters height) of *V. surinamensis* were collected in Combú Island (01°30′10″S; 048°27′42″W) underneath the parent specimens Lopes-037, Lopes-038, Lopes-039, and Lopes-040. Dry voucher samples have been deposited in the "S.P.F.-Herbário do Instituto de Biociências da Universidade de São Paulo". Seeds were planted in moistened sand; 60% of the seeds were germinated eight weeks post-plantation. Seedlings were transferred to soil substrates and maintained under greenhouse facilities. Two years later, the plantlets having 1—2 meters height were collected for the phytochemical analysis.

Isolation of Constituents Air-dried, powdered roots (2 g), leaves (2 g), and stems (2 g) were separately extracted with CH_2Cl_2 at room temperature and yielded respectively 250, 230, and 260 mg of crude extracts. The extracts were subjected to circular chromatography (chromatotrom) and eluted with hexane : EtOAc (3 : 2) to give five polled fractions from roots (R1—R5), three fractions from leaves (L1—L3), and five fractions from stems (S1—S5). Sitosterol was the major constituent of R1 and S1. L1 was a mixture of stosterol and stigmasterol. The fractions R2 and R4 were fractionated by prep. TLC (silica gel, hexane : acetone, 4 : 1) yielding the compounds iryelliptin B (**2**, 6 mg), virolin (1.7 mg) and biochanin A (3 mg). The fractions R3 and R5 were subjected to prep. TLC ($CHCl_3$, 9 : 1; hexane : EtOAc, 1 : 1, respectively) yielding 7-hydroxyflavone (4.2 mg), **3a** (6.2 mg), **3b** (5.0 mg) and α,2′-dihydroxy-4,4′-dimethoxydihydrochalcone (3.8 mg), respectively. The fraction L2 submitted to prep. HPLC (RP-8 column, MeOH : H_2O, 4 : 1) gave juruenolide C (8 mg), juruenolide D (4.5 mg), juruenolide E (**1**, 4.2 mg) and juruenolide F (1.5 mg). Fraction L3 was also purified by prep. HPLC (Si-60 column, hexane : EtOAc, 7 : 3) yielding juruenolide C (62 mg), juruenolide D (10 mg), *epi*-juruenolide C (15 mg) and isoelemicin (4.2 mg). Finally the stem fractions were submitted to prep. TLC yielding the following compounds: Fr S2 (hexane : acetone, 4 : 1): juruenolide C+*epi*-juruenolide C (30 mg); Fr S3 ($CHCl_3$: acetone, 95 : 5): veraguensin (2.4 mg) and galbelgin (2.9 mg); Fr S4 (CH_2Cl_2 : acetone, 4 : 1): isoelemicin (3 mg) and Fr S5 (hexane : EtOAc, 1 : 1): virolin (3 mg) and biochanin A (7 mg).

1: (2*S*,3*R*,4*S*)-3-Hydroxy-4-methyl-2-(11′-piperonyl-*n*-undecyl)-butenolide (juruenolide E). Viscous oil. $[\alpha]_D$=+7° (*c*=0.10, MeOH). IR ν_{max} (film) cm^{-1}: 3430, 3020, 1757 (C=O), 1504, 1442. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.58 (1H, m, H-2), 4.17 (1-H, br d, J=5.1 Hz, H-3), 4.49 (1H, br q, J=6.6 Hz, H-4), 1.37 (3H, d, J=6.6 Hz, Me-4), 1.80 (1H, m, H-1′), 1.34 (18H, br s, H-2′—H-10′), 2.50 (2H, t, J=7.7 Hz, H-11′), 6.65 (1H, br s, H-2″), 6.71 (1H, d, J=7.7 Hz, H-5″), 6.60 (1H, br d, J=7.7 Hz, H-6″), 5.90 (2H, s, OCH_2O). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 17.9 (C-5), 23.1 (C-1′), 27.6 (C-2′), 29.0 (C-4′), 29.2 (C-3′), 29.4 (C-5′—C-7′), 31.6 (C-8′), 35.5 (C-9′), 43.6 (C-2), 73.7 (C-3), 82.3 (C-4), 108.0 (C-2″), 108.8 (C-5″), 121.0 (C-6″), 136.7 (C-1″), 145.3 (C-4″), 177.4 (C-1), 100.6 (OCH_2O). ESI-MS $[M+H]^+$ 391. Elemental analysis Found: C, 70.86; H, 8.94. $C_{23}H_{34}O_5$ requires: C, 70.74; H, 8.78%.

2: 3-Hydroxy-4-methyl-2-(7′-piperonyl-*n*-heptyl)-butenolide (iryelliptin B). Viscous oil. IR ν_{max} (film) cm^{-1}: 3020, 1771, 1635, 1504, 1489, 1245. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 4.98, 4.92 (2H, d, J=5.4 Hz, H-5), 2.50 (2H, m, H-7′), 1.50 (2H, m, H-1′), 1.2—1.6 (12H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 6.66 (1H, d, J=1.3 Hz, H-2″), 6.72 (1H, d, J=7.8 Hz, H-5″), 6.61 (1H, dd, J=1.3, 7.8 Hz, H-6″), 5.84 (2H, s, O–CH_2–O), 4.08 (3H, s, OMe). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 23.2 (C-1′), 28.9 (C-2′), 29.1 (C-3′), 29.3, 29.6 (C-4′—C-7′), 31.6 (C-8′), 35.6 (C-9′), 91.0 (C-5), 105.8 (C-2), 136.9 (C-1″), 108.0 (C-2″), 149.8 (C-3″), 147.4 (C-4″), 108.8 (C-5″), 121.0 (C-6″), 161.3 (C-1 or C-4), 162.0 (C-1 or C-4), 169.0 (C-3), 100.6 (OCH_2O). ESI-MS $[M+H]^+$ 345. Elemental analysis Found: C, 70.01; H, 7.04. $C_{20}H_{24}O_5$ requires: C, 69.75; H, 7.02%.

3a: 9′-(3,4-Methylenedioxy-phenyl)-nonanoic acid. Viscous oil. IR ν_{max} cm^{-1}: 3400—2500, 1721, 1600, 1504, 1456, 1258. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.53 (2H, t, J=7.2 Hz, H-1′), 2.33 (2H, t, J=7.5 Hz, H-7′), 1.28 (10H, m, H-2′—H-6′), 6.66 (1H, d, J=1.3 Hz, H-2), 6.72 (1H, d, J=7.8 Hz, H-5), 6.61 (1H, dd, J=1.3, 7.8 Hz, H-6), 5.84 (2H, s, OCH_2O). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 33.5 (C-1′), 24.6 (C-2′), 29.7 (C-3′), 29.2 (C-4′), 29.1 (C-5′), 31.6 (C-6′), 35.6 (C-7′), 108.0 (C-2), 108.8 (C-5), 121.0 (C-6), 100.6 (OCH_2O). ESI-MS $[M-H]^-$ 277. Elemental analysis Found: C, 69.09; H, 7.72. $C_{16}H_{22}O_4$ requires: C, 69.04; H, 7.97%.

3b: 13′-(3,4-Methylenedioxyphenyl)-tridecanoic acid. Viscous oil. IR ν_{max} cm^{-1}: 3380—2500, 1723, 1600, 1504, 1458, 1256. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.43 (2H, t, J=7.2 Hz, H-1′), 2.22 (2H, t, J=7.5 Hz, H-7′), 1.18 (14H, m, H-2′—H-8′), 6.59 (1H, d, J=1.3 Hz, H-2), 6.65 (1H, d, J=7.8 Hz, H-5), 6.52 (1H, dd, J=1.3, 7.8 Hz, H-6), 5.84 (2H, s, OCH_2O). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 33.6 (C-1′), 24.4 (C-2′), 29.6 (C-3′), 29.2 (C-4′), 29.2 (C-5′—C-11′), 32.4 (C-12′), 35.6 (C-13′), 136.8 (C-1), 107.9 (C-2), 147.4 (C-3), 145.3 (C-4), 108.8 (C-5), 120.9 (C-6), 100.6 (OCH_2O). ESI-MS $[M-H]^-$ 333. Elemental analysis Found: C, 71.03; H, 9.02. $C_{20}H_{30}O_4$ requires: C, 71.82; H, 9.04%.

Acknowledgements We thank Dr. Alexandre A. Steiner (St. Joseph's Hospital and Medical Center, Phoenix, AZ, U.S.A.) for the language revisions. This work was supported by CNPq, CAPES, and FAPESP.

References

1) Rodrigues W. A., *Acta Amazonica*, **10**, 39—40 (1980).
2) Kato M. J., "Chemistry of the Amazon," American Chemical Society, Washington, DC, 1995, pp. 168—179.
3) Schultes R. E., Holmstedt B., *Lloydia*, **34**, 61—78 (1971).
4) Lopes N. P., Kato M. J., Andrade E. H. A, Maia J. G. S., Yoshida M., Planchart A. R., Katzin A., *J. Ethnopharm.*, **67**, 313—319 (1999).
5) Lopes N. P., Kato M. J., Yoshida M., *Phytochemistry*, **51**, 29—33 (1999).
6) Lopes N. P., Chicaro P., Kato M. J., Albuquerque S., Yoshida M., *Planta Medica*, **64**, 667—669 (1998).
7) Lopes N. P., Blumenthal E. E. A., Cavalheiro A. J., Kato M. J., Yoshida M., *Phytochemistry*, **43**, 1089—1092 (1996).
8) WHO Tropical Disease Research 1999: Progress 1997—98: W.H.O.: Geneva.
9) Lopes N. P., Franca S. C., Pereira A. M. S., Maia J. G. S., Kato M. J., Cavalheiro A. J., Gottlieb O. R., Yoshida M., *Phytochemistry*, **35**, 1469—1470 (1994).
10) Lopes N. P., Silva D. H. S., Kato M. J., Yoshida M., *Phytochemistry*, **49**, 1405—1410 (1998).
11) Barbosa-Filho J. M., Silva M. S., Yoshida M., Gottlieb O. R., *Phytochemistry*, **28**, 2209—2211 (1989).
12) Sumathykutty M. A., Rao J. M., *Phytochemistry*, **30**, 2075—2076 (1991).

13) Talapatra B., Chaudhuri P. K., Talapatra S. K., *Phytochemistry*, **21**, 747—750 (1982).
14) Barata L. E. S., Baker P. M., Gottlieb O. R., Rúveda E. A., *Phytochemistry*, **17**, 783—786 (1978).
15) Santos S. A., Carvalho M. G., Braz-Filho R., *J. Braz. Chem. Soc.*, **6**, 349—352 (1995).
16) Martinez J. C., Cuca L. E., *J. Nat. Prod.*, **50**, 1045—1047 (1987).
17) Bohlmann F., Jakupovic J., *Phytochemistry*, **18**, 1189—1194 (1979).
18) Vieira P. C., Yoshida M., Gottlieb O. R., Paulino-Filho H. P., Nagem T. J., Braz-Filho R., *Phytochemistry*, **22**, 711—713 (1983).
19) Braz-Filho R., Dias D. P. P., Gottlieb O. R., *Phytochemistry*, **19**, 455—457 (1980).