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NEOLIGNANS OF *VIROLA SURINAMENSIS*

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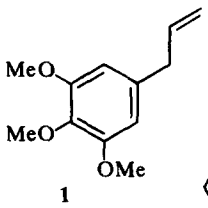
Key Word Index—*Virola surinamensis*; Myristicaceae; elemicin; 8,8′-neolignans; galbacin; veraguensin; 8-*O*-4′-neolignans; surinamensin; virolin.

Abstract—Elemicin, galbacin, veraguensin and two new neolignans, surinamensin and virolin, were isolated and characterized from the leaves of *Virola surinamensis*.

INTRODUCTION

In connection with a project designed to provide naturally occurring compounds active against the penetration of cercaria of *Schistosoma mansoni*, more than 40 plant species were collected at the delta of the Amazonas river and submitted to biological testing [1]. Since the hexane extract of the leaves of *Virola surinamensis* (Rol.) Warb. (Myristicaceae) showed the strongest activity, effort was devoted to the isolation and structural elucidation of the components.

The isolation, which involved solvent fractionation and Si gel column chromatography of the extract, led to elemicin **1**, the known 8,8′-neolignans, galbacin **2** and veraguensin **3**, and the new neolignans of type 8-*O*-4′, surinamensin **4a** and virolin **4b** [2]. The synthesis of **4a** and related compounds, prepared to confirm the proposed structures, was also undertaken. The biological activity of the extract was shown to be due only to compounds **4a** and **4b**.



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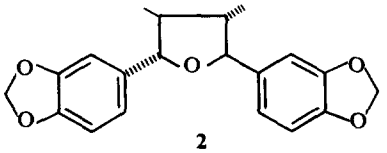

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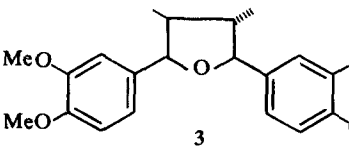
RESULTS AND DISCUSSIONS

The fractions that contain the neolignan surinamensin were resistant to all attempts at separation on a preparative scale, and most of the work on its constitution was carried out on a mixture of two components. By repetitive analytical HPLC a 3 mg pure sample was obtained, which by high resolution MS revealed the composition of the natural product, $C_{22}H_{28}O_6$. The strong IR band at 3500 cm^{-1} revealed the presence of an OH group, that was shown to be alcoholic, since no shift was observed by addition of alkali in the UV spectrum and by comparison of the PMR spectrum of surinamensin and its acetate (Table 1). The 100 MHz PMR spectrum, further to corroborating the existence of an OH group (δ 3.3, *br s*, disappearing on the addition of 1 drop of D_2O), indicated the presence of 4 OMe groups (δ 3.82, 3.86 and 3.90) and



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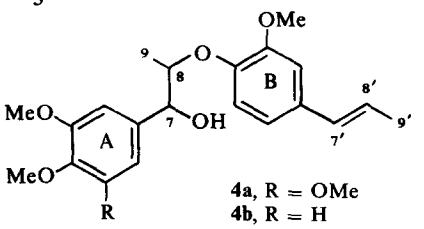


4a, R = OMe
4b, R = H

Table 1. PMR data for surinamensin and surinamensin acetate*

	Me-9	Me-9′	H-7		H-8	H-7′	H-8′	OMe	Ar-2,6	Ar3′,5′,6′	Others
Surinamensin	1.19	1.87	4.62		4.14	6.37	6–6.24	3.82, 3.86, 3.9	6.61	6.9	3.3
	d, J = 6	*d*, J = 5.5	*d*, J = 8		*m*	*d*, J = 16	*m*	(3.8, 3.5, 3.65)†	*s*	*m*	*br s*, —O<u>H</u>
Surinamensin	1.17	1.82	5.85		4.54	6.32	6–6.2	3.81, 3.84	6.58	6.86	2.00
acetate	*d*, J = 7	*d*, J = 6	*d*, J = 7		*m*	*d*, J = 16	*m*		*s*	*m*	OCO<u>CH</u>$_3$
Surinamensin	1.2	1.87	4.85	*erythro*	4.36	6.39	6.1–6.28	3.82, 3.84, 3.88	6.62	6.94	3.62
(synthetic)	*d*, J = 6	*d*, J = 5.5	*d*, J = 3		*m*	*d*, J = 16	*m*	3.92	*m*	*m*	*s*, —O<u>H</u>
			4.62		4.18						
			d, J = 8	*threo*	*m*						

*The spectra were recorded at 100 MHz in $CDCl_3$ soln, using TMS as int. ref.† δ of methoxylgroups in $d_6C_6H_6$ soln.

other features of the structure of surinamensin. The doublets centred at δ 1.19 (3H, J = 6 Hz) and δ 1.87 (3H, J = 5.5 Hz) could be ascribed to C-9 and C-9′ Me groups, while the doublet at δ 4.62 (1H, J = 8 Hz) and the multiplet at δ 4.14 (1H), were assigned to the methines at C-7 and C-8. The aromatic protons of C-2 and C-6 appeared as a singlet at δ 6.61 and the remaining ones as a multiplet at δ 6.9. Two additional signals, a doublet at δ 6.37 and a multiplet (1H, δ 6–6.24), were assigned to the protons of the *trans* double bond moiety at C-7′ and C-8′. Double irradiation experiments at 100 MHz confirmed most of the above assignments. Irradiation at δ 1.87 simplifies the olefinic pattern, showing two doublets with J = 16 Hz, while irradiation at δ 1.19 transforms the multiplet at δ 4.14 into a doublet with J = 8 Hz. As expected, irradiation at the olefinic region or at δ 4.14 collapses the doublets at δ 1.87 and δ 1.19 into two singlets respectively.

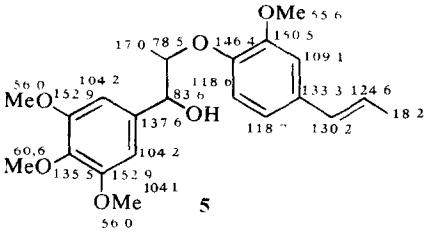
That the A ring of surinamensin **4a** is 3,4,5-trioxygenated, was established on the basis of the known shielding effect observed on the methoxyl groups with an unsubstituted *ortho* position, when $d_6C_6H_6$ is used as solvent for recording the PMR spectrum [3], and by identification of 3,4,5-trimethoxybenzoic acid amongst the products of the $KMnO_4$ (Me_2CO) oxidation of surinamensin. Analysis of its MS confirmed the trimethoxybenzylic alcohol and isoeugenol moieties, on the basis of the ions at *m/e* 195 and 164, respectively. Further support for the proposed structure of surinamensin **4a**, was obtained from the ^{13}C NMR spectrum (cf. **5**) [4].

By phenol oxidation coupling of arylpropenoids, mixtures of *erythro* and *threo*-neolignans related to **4a** were previously obtained, and their configurations assigned by analysis of their PMR spectra [5]. Comparison of the δ and J values of the methines at C-7 and C-8 (Table 1) with the reported values, indicated that **4a** corresponds to the *threo* series.

Indication of the constitution of the contaminant of surinamensin was obtained by oxidation of the mixture with DDQ [6]. Chromatographic analysis of the reaction mixture revealed two products which were isolated by PLC and submitted to MS analysis. One of these products (M^+, *m/e* 386) was shown to be the oxidation product **9a** of surinamensin, and the other one, (M^+, *m/e* 356) was identified as the oxidation product of virolin (**4b**), by comparison with an authentic sample of **9b** prepared as shown below. A careful inspection of the MS of samples of surinamensin and its acetylderivative, showed also the presence of virolin and its corresponding derivative.

A diastereoisomeric mixture of *erythro*- and *threo*-surinamensin was prepared following the method described by Adler *et al.* [7], by treatment of 3,4,5-trimethoxybenzaldehyde with ethylmagnesium bromide and the use of solid CO_2 in H_2O to decompose the magnesium complex, the substituted benzylic alcohol **6** was obtained. Oxidation of **6** with Jones reagent afforded the corresponding ketone **7**, which on bromination gave a mixture of bromoderivatives, including the bromoketone **8a**, isolated by PLC. The treatment of **8a** with the sodium salt of isoeugenol in DMF produced **9a**, purified by PLC. $NaBH_4$ reduction of **9a** gave the diastereoisomeric mixture represented by **4a**, analyzed by PMR spectroscopy (Table 1). Again double irradiation experiments were used to confirm the assignments. Irradiation at δ 1.2 simplified the C-8 methine signals and two doublets at δ 4.18 and 4.36 with J = 8 and 3 Hz, corresponding to the *threo* and *erythro* isomers respectively, could be easily observed.

The ketone **9b**, used for comparison with the DDQ oxidation product of virolin (**4b**), was prepared by the method described above using the bromoketone **8b** and the sodium salt of isoeugenol.



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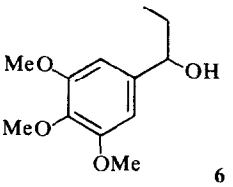

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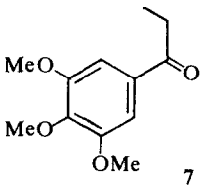

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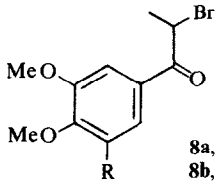
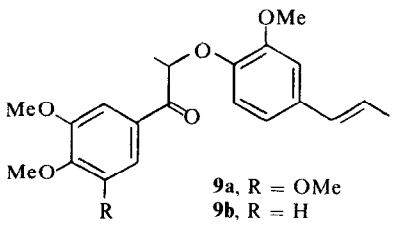


8a, R = OMe
8b, R = H



9a, R = OMe
9b, R = H

EXPERIMENTAL

Extraction. Plant material was collected at Aurá Forest Reserve, Belém (Estado do Pará, Brazil) and identified by Dr. João Murça Pires (Herbarium sample No. 126.206) EMBRAPA, Departmento de Botânica, Ministério de Agricultura, 66000, Belém, PA, Brazil. Dried powdered leaves (2.25 kg) of *V. surinamensis* were Soxhlet extracted with hexane for 72 hr, the extract evapd *in vacuo* afforded a green oily residue (145 g) which was suspended in MeOH–H_2O (7:3) (500 ml), stirred at room temp. for 3 hr, filtered through a celite pad and successively extracted with hexane (3 × 300 ml) and Et_2O (3 × 500 ml). The combined hexane extracts were evapd to dryness yielding a residue (65 g), which was dissolved in MeOH–H_2O (5:1), stirred 2 hr and extracted with $CHCl_3$, evapn of the $CHCl_3$ extracts yielded a yellowish oily residue (29 g). Part of the last residue was chromatographed on a Si gel column and eluted with hexane, C_6H_6, C_6H_6–EtOAc and EtOAc, yielding the following compounds in order of elution: galbacin, **2** (8.2 g), elemicin, **1** (4.6 g), veraguensin, **3** (0.7 g) and surinamensin, **4a** (0.5 g). The combined Et_2O extracts were evapd to dryness yielding a yellowish oily residue, which was chromatographed on a Si gel column and eluted with hexane, C_6H_6, C_6H_6–EtOAc and EtOAc, yielding the following compounds in order of elution: veraguensin, **3** (3.7 g) and surinamensin, **4a** (8.2 g).

Elemicin **1**. Viscous oil, PMR and MS data in agree with lit. [8].

Galbacin **2**. Mp 114–118° (from hexane–EtOAc), MS *m/e* (rel. int.) 340 [M^+] (45), PMR (100 MHz, $CDCl_3$): δ 1.05 (6H, *d*, *J* = 6 Hz, C-9 and C-9'), 1.78 (2H, *m*, C-8 and C-8'), 4.61 (2H, *d*, *J* = 9 Hz, C-7 and C-7'), 5.96 (4H, *s*, 2 × —O—C<u>H</u>$_2$—O—), 6.82–6.93 (6H; *m*, aromatic protons). Identical with an authentic sample (mmp and TLC).

Veraguensin **3**. Mp 127–128° (from Et_2O), $[\alpha]_D$ +40° ($CHCl_3$, *c* = 1.0), MS *m/e* (rel. int.) 372 [M^+] (34). PMR (100 MHz, $CDCl_3$): δ 0.66 (3H, *d*, *J* = 7 Hz, C-9), 1.07 (3H, *d*, *J* = 7 Hz, C-9'), 1.8 (1H, *m*, C-8), 2.25 (1H, *m*, C-8'), 3.84, 3.86, 3.88, 3.9 (4 × OC<u>H</u>$_3$), 4.4 (1H, *d*, *J* = 9 Hz, C-7'), 5.12 (1H, *d*, *J* = 8 Hz, C-7), 6.86–7.08 (6H, *m*, aromatic protons). Identical with authentic sample (mmp and TLC).

Surinamensin **4a**. Yellowish oil, after many attempts to purification by column chromatography and PLC, still showed tailing spots. By HPLC (analytical column, Micropack SI 10, 50 cm, UV detector and using hexane–CH_2Cl_2–*iso*-PrOH (98:1:1) it was shown to be a *ca* 4:1 mixture of two components, $[\alpha]_D$ +52° ($CHCl_3$, *c* = 1.0), MS (high resoultion), found: 388.1913, calc. for $C_{22}H_{28}O_6$, 388.1886, MS *m/e* (rel. int.) 388 [M^+] (7), 358 (2), 253 (5), 224 (25), 208 (16), 195 (40), 180 (17), 169 (20), 164 (100), 154 (12), 151 (12), 137 (9), 91 (12); IR (film) ν 3500, 1601, 1520, 1475, 1440, 1265, 960 cm^{-1}; UV λ_{max}^{EtOH}: 225(*sh*) nm, 262, 285(*sh*), 295; no change was observed by addition of an EtOH soln of NaOH.

Surinamensin acetate. Prepared using Ac_2O and C_5H_5N, viscous oil, MS *m/e* (rel. int.) 430 [M^+] (31).

Oxidation of surinamensin. To a stirred soln of surinamensin (0.25 g) in Me_2CO (10 ml) powdered $KMnO_4$ (0.231 g) was added over 1 hr. After being stirred for an additional 2 hr at room temp., the mixture was reflected for 15 min and the Me_2CO evapd *in vacuo*. To the residue 10% aq. KOH (30 ml) was added and filtered, the filtrate extracted with Et_2O and acidified with conc HCl and extracted again with $CHCl_3$. Evapn of the $CHCl_3$ extracts gave an oil, which by sublimation gave pure 3,4,5-trimethoxybenzoic acid, mp 168–170°. Identical with authentic sample (mmp).

Oxosurinamensin **9a** *and oxovirolin* **9b**. DDQ (0.057 g) was added to a soln of surinamensin (0.07 g) in dioxan (1 ml). After being stirred for 20 hr at room temp., the mixture was filtered and the residue thoroughly washed with Et_2O. The product obtained by evapn of the solvent was submitted to PLC (C_6H_6–EtOAc, 9:1) on Si gel and the isolated products analyzed by MS. Oxosurinamensin, MS *m/e* (rel. int.) 386 [M^+] (43), 195 (100), 164 (30), oxovirolin, MS (high resolution), found: 356.1593, calc. for $C_{21}H_{24}O_5$ 356.1624. Identical with the synthetic compounds **9a** and **9b** respectively prepared as described below (TLC, MS).

1-(3,4,5-Trimethoxyphenyl)propan-1-ol **6**. 3,4,5-Trimethoxybenzaldehyde (0.98 g) in Et_2O (15 ml) was added gradually to a stirred soln of ethylmagnesium bromide, prepared from EtBr (1.6 ml) and Mg (0.5 g) in Et_2O (10 ml). After being stirred for a few min, powdered solid CO_2 (20 g) in H_2O (10 ml) was added and the product extracted with Et_2O. The combined Et_2O extracts were washed, dried and evapd to leave an oily residue (0.76 g), which was used for the next step without further purification. MS *m/e* (rel. int.) 226 [M^+] (50), PMR (60 MHz, $CDCl_3$): δ 0.83 (3H, *t*, *J* = 7 Hz, C-3), 1.56 (2H, *q*, *J* = 7 Hz, C-2), 3.2 (1H, *s*, —OH), 3.62 (9H, *s*, 3 × OMe), 4.3 (1H, *t*, *J* = 7 Hz, C-1), 6.4 (2H, *s*, aromatic protons).

1-(3,4,5-Trimethoxyphenyl)propan-1-one **7**. To a cold soln of **6** (0.55 g) in Me_2CO (10 ml) was added dropwise Jones reagent (1 ml) prepared from CrO_3 (2.67 g), H_2SO_4 (2.3 ml) and H_2O (10 ml). When the soln became slightly reddish, H_2O (10 ml) was added and the soln extracted with Et_2O. The combined Et_2O extracts were washed, dried and evapd leaving a yellowish oily residue, which crystallized spontaneously, mp 51–53° (from hexane–C_6H_6), MS *m/e* (rel. int.) 224 [M^+] (34), 195 (100), PMR (60 MHz, $CDCl_3$): δ 1.2 (3H, *t*, *J* = 7 Hz, C-3), 2.96 (2H, *q*, *J* = 7 Hz, C-2), 3.91 (9H, *s*, 3 × OMe), 7.23 (2H, *s*, aromatic protons).

1-(3,4,5-Trimethoxyphenyl)-2-bromopropan-1-one **8a** A $CHCl_3$ (5 ml) soln of Br_2 (0.87 ml) was added gradually to a stirred soln of **7** (0.38 g) in $CHCl_3$ (10 ml) at 0°. Arter being stirred for 4 hr, the reaction mixture was washed × 3 with aq. 10% $NaHCO_3$, dried and evapd to dryness. The semicrystalline residue (0.28 g) was submitted to PLC for the isolation of **8a** (0.085 g), MS *m/e* (rel. int.) 304 (7), 302 (14), 195 (100); PMR (60 MHz, $CDCl_3$): δ 1.9 (3H, *d*, *J* = 7 Hz, C-3), 3.93 (9H, *s*, 3 × OMe), 5.3 (1H, *q*, *J* = 7 Hz, C-2), 7.33 (2H, *s*, aromatic protons). Some of 1-(3,4,5-trimethoxyphenyl)-2,2-dibromopropan-1-one was also isolated (0.06 g), MS *m/e* (rel. int.) 382 (6), 380 (4); PMR (60 MHz, $CDCl_3$): δ 2.75 (3H, *s*, C-3), 3.96 (9H, *s*, 3 × OMe), 7.65 (2H, *s*, aromatic protons).

1-(3,4,5-Trimethoxyphenyl)-2-[2-methoxy-4-(E)-propenylphenoxy]propan-1-one **9a**. The Na salt of isoeugenol (0.12 g) prepared from Na and freshly dist. isoeugenol in EtOH, was added to a soln of **8a** (0.15 g) in DMF (3 ml). After being stirred 18 hr, H_2O was added and the mixture extracted with Et_2O. The combined Et_2O extracts were washed with 0.2 N aq. NaOH, with H_2O and dried. The residue was purified by PLC (Si gel, C_6H_6–EtOAc, 9:1) giving **9a** (0.132 g), as a crystalline product, mp 100.3–100.6° (EtOH). MS (high resolution), found: 386.1700, calc. for $C_{22}H_{26}O_6$, 386.1729; PMR (100 MHz, $CDCl_3$): δ 1.71 (3H, *d*, *J* = 7 Hz, C-3), 1.83 [3H, *d*, *J* = 6 Hz, (*E*)—CH=CH—C<u>H</u>$_3$], 3.84, 3.88 and 3.9 (12H, 4 × OMe), 5.33 (1H, *q*, *J* = 6 Hz, C-2), 5.98–6.4 [2H, *m*, (*E*)—C<u>H</u>=C<u>H</u>—], 6.7–7.45 (5H, *m*, aromatic protons).

Erythro *and* threo-*1-(3,4,5-trimethoxyphenyl)-2-[2-methoxy-4-(E)-propepylphenoxy]propan-1-ol* **4a**. A soln of **9a** (0.1 g) in EtOH (4 ml) was gradually added to $NaBH_4$ (0.02 g) in EtOH (0.5 ml). After being stirred 18 hr, H_2O and a few drops of HOAc were added and the mixture extracted with Et_2O, the combined Et_2O extracts washed with aq. $NaHCO_3$, dried and evapd giving an oil residue (0.091 g). MS (high resolution), found 388.1896, calc. for $C_{22}H_{28}O_6$: 388.1886.

1-(3,4-Dimethoxyphenyl)-2-[2-methoxy-4-(E)-propenylphenoxy]propan-1-one **9b**. The procedure to prepare **9b** was the same as described above for compound **9a**. From **8b** (0.27 g) [9], **9b** was obtained (0.17 g), mp 123–125° (from hexane–Me_2CO), after PLC purification, MS (high resolution) found: 356.1615, calc. for $C_{21}H_{24}O_5$: 356.1624, PMR (100 MHz, $CDCl_3$): δ 1.72 (3H, *d*, *J* = 6 Hz, C-3), 1.85 [3H, *d*, *J* = 6 Hz, (*E*)—CH=CH—C<u>H</u>$_3$], 3.86, 3.94, 3.96 (9H, 3 × OMe), 5.41 (1H, *q*, *J* = 6 Hz, C-2), 5.94–6.4 [2H, *m*, (*E*)—C<u>H</u>=C<u>H</u>—], 6.72–7.9 (6H, *m*, aromatic protons).

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