Swartziarboreol Diterpenes from *Swartzia langsdorffii* Raddi

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Two new swartziarboreol diterpenes (**1** and **2**) and swartziarboreol C were isolated from the petroleum ether extract of the roots of *Swartzia langsdorffii*. Their structures were elucidated by spectroscopic evidence. The biological activity of the root extracts and **2** was evaluated through bioautography and brine shrimp lethality assays.

The genus *Swartzia* was revised by Cowan,[1] but studies conducted after this review revealed the need for more investigations on this leguminous genus.[2a] Some of the *Swartzia* species were included in two distinct subsections or series, and varieties of the same species have been placed in different series. The genus *Swartzia* Schreb. belongs to tribe Swartzieae, subfamily Papilionoideae (Faboideae) of the Leguminosae (Fabaceae), and consists of about 135 species distributed in tropical America. *Swartzia langsdorffii* Raddi is a native Brazilian tree, which was included in the *Swartzia* ser. *Recurvae* of the section *Swartzia* (see Table 1). [2a−c]

Chemical reports related to *Swartzia* species showed the presence of isoflavonoids,[3a−d] triterpenoidal saponins,[4a−d] and aromatic diterpenes (Table 1).[5] Extracts of some *Swartzia* species[3a] and some of their isoflavonoids[3b,d] also displayed antimicrobial activities.

This work reports the isolation of two new swartziarboreol diterpenes (**1** and **2**) and swartziarboreol C[5] from the petroleum ether extract of *S. langsdorffii* roots, while acetylation of the dichloromethane root extract furnished the diacetate derivative **3**.

NMR data (Tables 2 and 3) of compounds **1**−**3** are very similar to those found for the swartziarboreols (A−E) previously isolated from *S. arboreol* and showed that they possess the same *trans*-decalin system of swartziarboreol C.[5]

Compound **1** has the same skeleton as swartziarboreol C, but the NMR spectra showed the signals of two aromatic methoxy groups [^{1}H NMR (δ 3.79; s; 3H and δ 3.97; s; 3H), ^{13}C NMR (δ 60.7 and 60.5)]. In the HREIMS the molecular ion at *m/z* 356.1982 was compatible with $C_{22}H_{28}O_4$ (calcd for $C_{22}H_{28}O_4$, 356.1988). The fragmentation pattern was similar to that observed for swartziarboreol C; the *m/z* values corresponding to the fragments a [$M^{+\bullet}$ − 15], b [$M^{+\bullet}$ − 85], c [$M^{+\bullet}$ − 97], and d [$M^{+\bullet}$ − 111] are 14 amu higher. This fragmentation pathway is typical for this kind of diterpenoid. [6] The long-range ^{1}H−^{13}C correlations (Figure 2) observed in the HMBC confirmed the fusion of the pyrone ring on the aromatic ring.

Compound **2** showed the signal of one methoxy group [^{1}H NMR (δ 3.78; s; 3H), ^{13}C NMR (δ 61.5)], as in swartziarboreol C, but the signals at δ 23.3, 65.8, and 164.5 in the ^{13}C NMR are compatible with an Ar−CH_2−CH_2−O−CO−Ar system. The long-range ^{1}H−^{13}C correlations observed in the 2D-NMR HMBC spectrum revealed the location of the phenolic and methoxy groups. In the EIMS, fragment ions a, b, c, and d were observed respectively at *m/z* 329, 259, 247, and 233. In HREIMS, the molecular ion at *m/z* 344.1998 was compatible with $C_{21}H_{28}O_4$ (calcd for $C_{21}H_{28}O_4$, 344.1988). The MS/MS experiment selecting the ion *m/z* 329 [287 (17%), 273 (43%), 259 (57%), 247 (73%), 233 (67%)] established that, similarly to the ferruginol fragmentation, [6] ions b, c, and d come from ion a. Compound **2** showed an $[\alpha]^{25}_D$ +117° (*c* 0.8, MeOH), while swartziarboreol C, which was isolated from *S. langsdorffii*, showed an $[\alpha]^{25}_D$ +103° (*c* 0.36, MeOH), very close to that reported.[5] These data, together with the biogenesis established for ferruginol,[7] suggest that compound **2** has the same configuration as swartziarboreols A−E.[5]

Compound **3** was obtained by acetylation of dichloromethane extract and has the same skeleton as swartziarboreol C. However, the NMR spectra showed the signals of two acetyl groups [^{1}H NMR (δ 2.36; s; 6H), ^{13}C NMR (δ 167.7 and 167.9)]. These findings suggested the natural occurrence of an ortho-diphenolic compound, **4**,

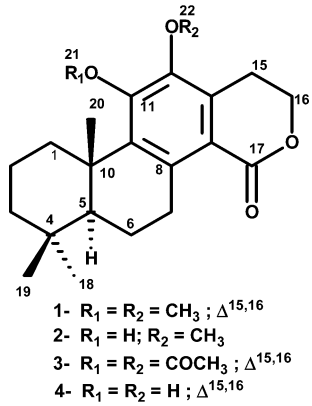

Figure 1. Structures of the swartziarboreol diterpenes **1**, **2**, and **4** and of the derivative **3**.

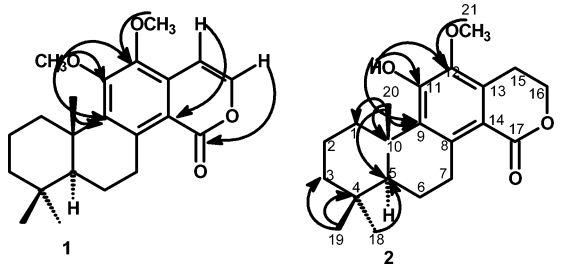

Figure 2. Long-range correlations (^{13}C−^{1}H) in the HMBC experiments for compounds **1** and **2**.

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Table 1. Occurrence of Flavonoids and Terpenoids (++) Isolated from *Swartzia* Species[3–5] as Classified into Section, Subsection, and Series, by Cowan (1967)[a]

	pterocarpan	pterocarpen	isoflavanonol	isoflavone	isoflavanone	diterpenoid	saponin
Swartzia							
Swartzia							
Benthamianae							
S. ulei		++[3a]					
S. laevicarpa	++[3a]						
Recurvae							
*S. leiocalycina**	++[3a]	++[3a]					
S. langsdorffii						++	++[4d]
Terminales							
*S. polyphylla**			++[3c]	++[3c]	++[3b]		
*S. schomburgkii**							++[4c]
Possira							
Possira							
S. simplex var. *grandiflora*							++[4a] ?[b]
S. arborescens						++[5]	
Unifoliolatae							
S. simplex var. *simplex*							++[4b] ?[b]
S. simplex var. *ochnacea*							++[4b] ?[b]

[a] Data from this work and from [3a]Braz Filho et al., 1980; [3b]Osawa et al., 1992; [3c]Dubois et al., 1995, 1996; [4a]Borel et al., 1987, [4b]1987a; [4c]Abdel-Kader et al, 2000; [4d]Magalhães et al. 2003; and [5]Orphelin et al., 1996. [b] ? without information on infraspecific level of the species.

Table 2. ^{13}C NMR Data for **1**, **2**, and the Derivative **3**[a]

position	**1**	**2**	**3**	position	**1**	**2**	**3**
1	37.6	32.0	33.4	13	132.2	131.0	130.7
2	19.8	19.0	21.0	14	115.6	116.0	118.3
3	41.2	41.2	41.0	15	101.2	23.3	100.5
4	33.9	33.0	33.4	16	145.0	65.8	146.4
5	52.0	52.0	50.9	17	161.3	164.5	160.1
6	19.0	18.2	20.4	18	33.8	33.4	33.4
7	33.5	31.5	33.5	19	22.4	19.0	19.4
8	140.5	141.0	143.8	20	21.0	22.1	18.7
9	144.8	136.0	144.0	21	60.7	61.5	167.7
10	41.0	41.0	37.0	22	60.5		167.9
11	157.9	152.0	145.3	23			20.0
12	140.5	141.0	135.3	24			22.0

[a] Chemical shifts in ppm from internal TMS. Obtained in $CDCl_3$. All assignments were performed by a combination of DEPT, ^{13}C NMR, and 2D-NMR experiments: HSQC and HMBC ^{13}C NMR at 125 MHz.

which was reinforced by the isolation of compound **1** when the same extract was submitted to methylation with diazomethane.

The extracts were also submitted to the brine shrimp lethality test[8] and displayed the following toxicity, LC_{50}: MeOH extract, 86.74 μg/mL; petroleum ether extract, 66.06 μg/mL; and CH_2Cl_2 extract, 53.17 μg/mL.

These extracts and compound **2** were then submitted to the bioautography assay[9] against eight fungi and six bacteria. The only positive result was observed for compound **2** against *Staphylococcus aureus*.

Experimental Section

General Experimental Procedures. Thin-layer chromatography (TLC) and preparative TLC were performed using silica gel 60 F_{254} Al sheets (Merck, Germany). IR: KBr. UV/vis spectrum in MeOH: λ_{max} [nm] (log ϵ). NMR spectra were recorded in $CDCl_3$ at 499.88 MHz for ^1H and 125.69 MHz for ^{13}C NMR, with TMS as internal standard. HREIMS and MS/MS experiments: VG Auto Spec 10000 Micromass (Manchester, UK) instrument with an ionizing potential of 70 eV (linked scan at 8 keV collisions with helium). EIMS: *m/z* (rel intensity in %), 70 eV, direct probe. Optical rotations were measured with a Carl Zeiss Jena (Turinger, Germany) Polamat pola-

Table 3. ^1H NMR[a] Data for Swartziarboreol C,[b] **1**, **2**, and the Derivative **3**

position	swartziarboreol C δ_H (mult., Hz)	**1** δ_H (mult., Hz)	**2** δ_H (mult., Hz)	**3** δ_H (mult., Hz)
1 α	1.15 (m)	1.19 (ddd, 13; 6; 4)	1.19 (ddd, 13; 6; 3)	1.20 (m)
1 β	3.16 (m)	2.97 (dt, 13; 4)	3.16 (dt, 13; 3)	3.40 (m)
2 α	1.76 (qt[c], 13; 5; 3.5)	1.75 (m)	1.75 (qt, 13; 4)	1.60 (m)
2 β	1.58 (m)	1.50 (m)	1.55 (dquint[d], 13; 4)	1.75 (m)
3 α	1.29 (m)	1.22 (m)	1.22 (m)	1.30 (m)
3 β	1.44 (m)	1.54 (m)	1.6 (m)	1.42 (m)
5	1.25 (m)	1.25 (m)	1.25 (m)	1.25 (m)
6 α	1.48 (m)	1.96 (ddt, 13; 7; 1.5)	1.90 (dt 13; 4)	1.45 (m)
6 β	1.96 (m)	1.47 (m)	1.49 (m)	1.96 (m)
7 α	3.29 (ddd, 19; 12.5; 7)	3.34 (ddd, 19; 11; 7)	3.23 (m)	3.34 (ddd, 19; 11; 7)
7 β	3.48 (ddd, 19; 5; 1.5)	3.42 (ddd, 19; 7; 3)	3.25 (m)	3.43 (ddd, 19; 11; 7)
11 (OH)	6.65 (s)		6.55 (s)	
15 α, β	6.55 (d, 5.5)	6.71 (d, 6)	2.99 (m)	6.28 (d, 6)
			2.99 (m)	
16 α	7.22 (d, 5.5)	7.20 (d, 6)	4.42 (dt, 10; 4)	7.21 (d, 6)
16 β			4.29 (ddd, 10; 4)	
18	0.97 (s)	0.98 (s)	0.97 (s)	0.98 (s)
19	0.95 (s)	0.95 (s)	0.94 (s)	0.94 (s)
20	1.40 (s)	1.37 (s)	1.36 (s)	1.3 (s)
21	3.84 (s)	3.79 (s)	3.78 (s)	
22		3.97 (s)		
O−CO−CH_3 (2×)				2.36 (s, 6H)

[a] Chemical shifts in ppm from internal TMS. The assignments were based on DEPT, ^{13}C NMR, and 2D-NMR experiments: ^1H–^1HCOSY, HMQC, and HMBC experiments. [b]Isolated from *S. langsdorffi*. [c] qt: quartet of triplets. [d] dquin: doublet of quintets.

rimeter or with an ORD-306 connected to a J-720 JASCO (Tokyo, Japan) instrument.

Plant Collection and Extraction. Samples of the roots (180 g) were obtained by first loosening the soil to avoid any damage to the whole plant. Voucher specimens of Leguminosae Papilionoideae, *S. langsdorffii* Raddi. Brazil, São Paulo, Campinas, Fazenda Santa Elisa, Monjolinho, cultivated, 22/03/97, A. M. G. A. Tozzi, C. C. Santos & J. C. Galvão 97-54 (UEC), were deposited at the herbarium of Botany Department of Campinas State University (UEC), Campinas-SP, Brazil. The plant was identified by the botanist Ana Maria Goulart de Azevedo Tozzi. Dried powdered root (172 g) of *S. langsdorffii* was successively extracted with petroleum ether, CH_2Cl_2, and MeOH for 60 h in a Soxhlet apparatus.

Swartziarboreol Diterpenes Isolation. The petroleum ether extract (2.5 g) was fractionated by rapid CC, using silica gel with CH_2Cl_2 as eluent. The eluent polarity was gradually increased by adding EtOAc, until 100% of this solvent. The 179 fractions collected were monitored through TLC developed in *n*-hexane/EtOAc (80:20 v/v), twice, resulting in 13 groups. Most of the compounds were found in eight groups ranging from fractions 1 to 77. Swartziarboreol C (1.1 mg) and **2** (8.6 mg) were isolated by preparative TLC developed twice with CH_2Cl_2. Compound **1** (2.4 mg) was isolated by preparative TLC developed with *n*-hexane/EtOAc (4:1). The compounds were recovered from TLC plates by extraction with mixtures of CH_2Cl_2 and MeOH.

11-*O*-Methylswartziarboreol C (1): amorphous solid; $[\alpha]^{25}_D$ +51.5° (*c* 0.05, MeOH); ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; EIMS 356 $[M]^{+\bullet}$ (100), 341 $[M^{+\bullet} - Me]$ (40), 313 (8), 298 (10), 285 (21), 271 $[M^{+\bullet} - 85]$ (58), 259 $[M^{+\bullet} - 97]$ (47), 257 (20), 245 $[M^{+\bullet} - 111]$ (78); HREIMS, *m/z* 356.1982 (calcd for $C_{22}H_{28}O_4$, 356.1988).

15,16-Dihydroswartziarboreol C (2): amorphous solid; $[\alpha]^{25}_D$ +117° (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (3.3), 276 (2.0) nm; IR (KBr) ν_{max} 3420, 2926, 1703 (>C=O), 1656, 1577, 1174 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; EIMS 344 $[M]^{+\bullet}$ (100), 329 $[M^{+\bullet} - Me]$ (24), 287 (6), 273 (22), 259 $[M^{+\bullet} - 85]$ (68), 247 $[M^{+\bullet} - 97]$ (83), 233 $[M^{+\bullet} - 111]$ (80); HREIMS, *m/z* 344.1998 (calcd for $C_{21}H_{28}O_4$, 344.1988).

Acetylation. An aliquot of the brine shrimp test bioactive CH_2Cl_2 extract (190 mg) was acetylated at room temperature for 24 h using Ac_2O and pyridine. Compound **3** (5 mg) was isolated after the usual workup, followed by preparative TLC developed three times with $CHCl_3$, and recovered from TLC plates by extraction with mixtures of CH_2Cl_2 and MeOH.

Another aliquot of this CH_2Cl_2 extract (170 mg) was methylated with diazomethane and was further submitted to successive preparative TLC developed twice with *n*-hexane/EtOAc (95:5) and recovered from TLC plates by extraction with mixtures of CH_2Cl_2 and MeOH, affording compound **1** (6 mg).

11,12-Di-*O*-acetylswartziarboreol C (3): amorphous solid; UV (MeOH) λ_{max} (log ϵ) 240 (3.4), 272 (2.0), 330 (1.8) nm; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; EIMS 412 $[M]^{+\bullet}$ (12), 370 $[M - CH_2CO]^{+\bullet}$ (40), 328 $[M - 2 \times CH_2CO]^{+\bullet}$ (100), 313 $[M - 2 \times CH_2CO - Me]^{+\bullet}$ (12), 243 $[M - 2 \times CH_2CO - 85]^{+\bullet}$ (25), 231 $[M - 2 \times CH_2CO - 97]^{+\bullet}$ (20), 217 $[M - 2 \times CH_2CO - 111]^{+\bullet}$ (20); HREIMS, *m/z* 412.1990 (calcd for $C_{24}H_{28}O_6$, 412.1886).

Bioautography Test. The fungi and agar overlays were commercially obtained from the tropical culture collection of the Fundação Tropical de Pesquisa e Tecnologia "André Tosello" Campinas, SP, Brazil. The fungi tested were *Candida albicans* (CCT 0776), *Cladosporium cladosporioides* (CCT 5039), *Aspergillus niger* (CCT 1435), *Penicillium funiculosum* (CCT 0490), *Fusarium oxysporium* (CCT 3244), *Rhizopus orizae* (CCT 4964), *Alternaria alternata* (CCT 1250), and *Aspergillus fumigatus* (CCT 01277). The bacteria tested were *Staphylococcus aureus* (CCT 4295), *Escherichia coli* (CCT 5050), *Bacillus subtilis* (CCT 0089), *Micrococcus luteus* (CCT 2720), *Salmonella typhimurium* (CCT 0528), and *Streptococcus mutans* (CCT 3440). Solutions corresponding to 2 mg/mL of the brine shrimp test bioactive extracts and of the pure compound **2** were made, and 10 μL of each extract solution or 5 μL of the pure compound solution was applied. The inhibition growth halo was compared with those shown by 1 μL of the commercial antibiotics ciclopyrox olamine and chloramphenicol, respectively, against fungi and bacteria. Compounds **1** and **3** were not tested because of insufficient amounts of **1** and because **3** was not a natural compound.

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