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MORE PHLOROGLUCINOLS FROM *HYPERICUM BRASILIENSE**

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Key Word Index—*Hypericum brasiliense*; Guttiferae; phloroglucinols; antibacterial activity; *Bacillus subtilis*; hyperbrasilols B and C; isohyperbrasilol B.

Abstract—Three new phloroglucinols (hyperbrasilols B and C, and isohyperbrasilol B) have been isolated from a petrol extract of the leaves and flowers of *Hypericum brasiliense*. Their structures were established by spectroscopic methods including two-dimensional-NMR heteronuclear correlations. EI and D/Cl-mass spectral, NMR, IR and UV data are reported. All three phloroglucinols were antibacterial against *Bacillus subtilis* in a TLC bioautographic assay.

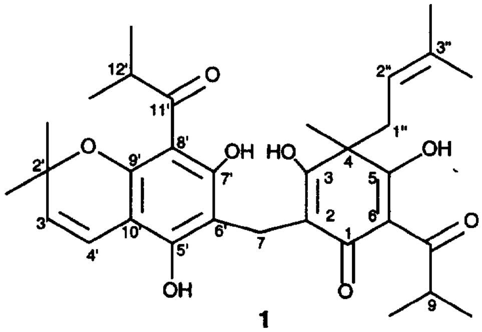
INTRODUCTION

As part of our phytochemical and biological investigations on plants of the genus *Hypericum*, we recently reported on the bioactive constituents of *H. brasiliense*, a species growing in south and south-east Brazil. Xanthones and a new γ-pyrone derivative isolated from the stems and roots showed antifungal properties [2]. In addition, they were found to be inhibitors of monoamine oxidases (MAO), enzymes which are involved in the regulation of some physiological amines and are thought to contribute to the management of depression. Phloroglucinols, including the new compound, hyperbrasilol A, have been isolated from the petrol extract of the leaves and flowers [3]. These compounds exhibited strong antibacterial activity against *Bacillus subtilis* in a TLC bioautographic assay. Further studies on the petrol extract of the leaves and flowers of *H. brasiliense* have now led to the isolation of three additional antibiotic compounds, hyperbrasilol B (**1**), isohyperbrasilol B (**2**) and hyperbrasilol C (**3**).

RESULTS AND DISCUSSION

Leaves and flowers of *H. brasiliense* were extracted with petrol. The resulting extract showed several inhibition zones against *B. subtilis* in a TLC bioautographic assay [4]. After evaporation to dryness, treatment of the petrol extract with acetone provided an acetone-soluble fraction and an insoluble fatty residue. Fractionation of the bioactive acetone-soluble fraction by a combination of silica gel column chromatography, gel filtration on Sephadex LH-20 and centrifugal partition chromatography (CPC) provided compounds **1**–**3**, which appeared as red spots on TLC plates when sprayed with Godin reagent [5]. Their IR spectra showed broad absorption in the 3500–3000 cm^{-1} region, together with intense peaks from 1600 to 1650 cm^{-1}. This suggested compounds **1**–**3** to be phloroglucinol derivatives containing an enolic 1,3-diketo system or a 2-hydroxyaryl ketone [6].

The UV spectrum of **1** exhibited maxima at 357, 287 and 226 nm. A $[M]^+$ was detected in the EI-mass spectrum at m/z 552. The M_r was further confirmed by a $[M+H]^+$ pseudomolecular ion at m/z 553 in the D/Cl mass spectrum. The molecular formula was deduced to be $C_{32}H_{40}O_8$. The ^{1}H and ^{13}C NMR data of **1** (Tables 1 and 2) indicated a close structural similarity to isouliginosin B (**4**), a compound that has been previously isolated from *H. brasiliense* [3]. Compared with that of **4**, however, the NMR spectra of **1** exhibited additional signals corresponding to a prenyl side-chain, while only one methyl group (δ_H 1.51; δ_C 22.8) was found to be present at C-4. In the ^{13}C NMR spectrum of **1**, the resonance of C-4 (δ 49.8) was shifted *ca* 5 ppm downfield from that in isouliginosin B. Hence, the prenyl side-chain was placed at C-4, together with one methyl group. Such a deshielding effect on C-4 when a methyl group is replaced by a prenyl chain has been observed in drummondins [7]. In the ^{1}H NMR spectrum of **1**, a pair of doublets (J = 10 Hz) at δ 5.59 and δ 6.66, respectively, revealed the presence of a 2,2-dimethyl chromene moiety. The fusion of the pyran ring in **1** was inferred by direct comparison of the hydroxyl resonances, in particular HO-7′ (δ 16.35) and HO-5′ (δ 11.40), with those



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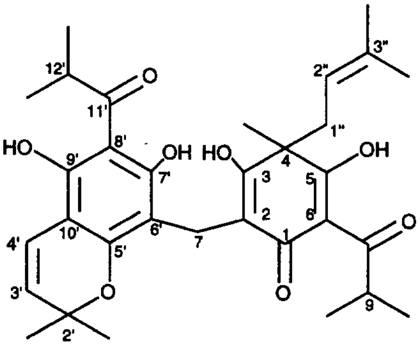

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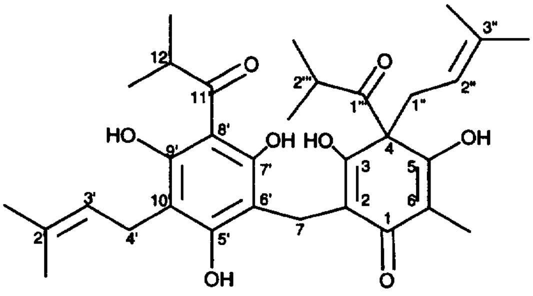

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Table 1. ^{1}H NMR spectral data of compounds **1–3** (*J* values in Hz)*

H	**1**	**2**†	**3**
4-Me	1.51 (*s*)	1.26 (*s*)‡	–
6-Me	–	–	1.17 (*s*)
7	3.55 (*s*)	3.55 (*br s*)	3.41 (*s*)
9	4.19 (*sep J* = 7)	4.20 (*sep J* = 7)	–
9-Me	1.16 (*d J* = 7)	1.17 (*d J* = 7)^a	–
	1.17 (*d J* = 7)	–	
2′-Me	1.53 (*s*)	1.52 (unres.)	1.57 (*s*)
	1.53 (*s*)	1.52 (unres.)	1.72 (*s*)
3′	5.59 (*d J* = 10)	5.48 (*d J* = 10)	5.22 (*br t J* = 7)
4′	6.66 (*d J* = 10)	6.74 (*d J* = 10)	3.20 (*br d J* = 7)
12′	4.00 (*sep J* = 7)	4.11 (*sep J* = 7)	4.19 (*sep J* = 7)
12′-Me	1.20 (*d J* = 7)	1.18 (*d J* = 7)^a	1.11 (*d J* = 7)
	1.21 (*d J* = 7)		
1″	2.8–2.5 (*m*)	2.8–2.5 (*m*)	2.41 (*br d J* = 7)
2″	4.60 (*br t*)	4.8–4.4 (*m*)	4.81 (*br t J* = 7)
3″-Me	1.31 (*s*)	1.49 (unres.)	1.39 (*br s*)
	1.34 (*s*)	1.59 (unres.)	1.44 (*br s*)
2‴	–	–	3.98 (*sep J* = 7)
2‴-Me	–	–	1.05 (*d J* = 7)
			1.07 (*d J* = 7)
3-OH	9.90 (*s*)	9.00 (*s*)	n.o.
5-OH	18.80 (*s*)	18.64 (*s*)	19.40 (*s*)
5′-OH	11.40 (*s*)	–	n.o.
7′-OH	16.35 (*s*)	11.64 (*s*)	14.00 (*s*)
9′-OH	–	14.13 (*s*)	n.o.

*In acetone-d_6 (**1** and **3**) or $CDCl_3$ (**2**): attributions in **1** and **3** are supported by COSY and HETCOR data.

†Quaternary methyl groups were better resolved in acetone-d_6: δ 1.55 (*s*, 4-Me), 1.46 (*s*, 2′-Me_2), 1.23 and 1.31 (2*s*, 3″-Me_2); hydroxyl resonances in acetone-d_6: δ 18.66, 11.71, 14.02 and 9.15 (*br*).

‡Tentative assignment.

n.o., Not observed.

hydroxyl ^{1}H NMR signals for determination of the orientation of the cyclization [8]. Compound **1** has been named hyperbrasilol B.

The UV spectrum of **2** resembled that of **1** with absorption maxima at 357, 279 and 216 nm. The molecular formula was deduced to be $C_{32}H_{40}O_8$ from EI (*m*/*z* 552), D/CI (*m*/*z* 553, $[M+H]^+$) mass spectrometry and NMR data. Hence, compound **2** is an isomer of **1**. NMR data of **1** and **2** were very similar. The only significant difference concerned the signals corresponding to hydroxyl groups. These were detected in the ^{1}H NMR spectrum of **2** at δ 9.00 (HO-3), 11.64 (HO-7′), 14.13 (HO-9′) and 18.64 (HO-5). Thus, the cyclization pattern must involve O-5′ instead of O-9′. and ^{13}C NMR data of **2** are listed in Tables 1 and 2, respectively.

Compound **3** exhibited UV absorption maxima at 348, 301 and 224 nm. The EI-mass spectrum exhibited a $[M]^+$ at *m*/*z* 554. In the D/CI-mass spectrum, a pseudomolecular ion was observed at *m*/*z* 555 ($[M+H]^+$). The difference of 2 amu compared with the M_r of **1** and **2** suggested that **3** contained two uncyclized prenyl chains. NMR data (Tables 1 and 2) confirmed the molecular formula to be $C_{32}H_{42}O_8$. The phloroglucinol moiety was found to be identical to that found in uliginosin A, bearing a prenyl group at C-10′ and an *iso*butyryl substituent at C-8′ [3]. The location of both remaining carbon chains at C-4 was established from the chemical shift of C-4 (δ 57.5) which appeared 8 ppm more downfield than in **1** and **2** and *ca* 13 ppm more deshielded than in compounds bearing a *gem*-dimethyl group. The chemical shift of C-4 was in full agreement with the value (δ 57.4) observed for the corresponding carbon in kolanone (**5**), a polyisoprenylated benzophenone which has been isolated from fruits of *Garcinia kola* [9]. Thus, an isobutyryl sub-

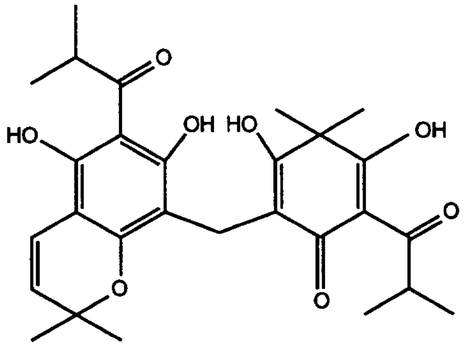
Table 2. ^{13}C NMR spectral data of compounds **1–3** (multiplicities in brackets)*

C	**1**	**2**	**3**
1	199.2 (*s*)	199.1 (*s*)	195.5 (*s*)
2	109.8 (*s*)^{a}	109.8 (*s*)^{a}	109.0 (*s*)^{a}
3	170.7 (*s*)	170.2 (*s*)	183.8 (*s*)
4	49.8 (*s*)	49.7 (*s*)	57.5 (*s*)
4-Me	22.8 (*q*)	22.9 (*q*)	
5	188.5 (*s*)	188.7 (*s*)	199.6 (*s*)
6	114.2 (*s*)^{a}	114.1 (*s*)	105.0 (*s*)^{a}
6-Me	–	–	23.8 (*q*)
7	16.9 (*t*)	17.1 (*t*)	18.9 (*t*)
8	211.9 (*s*)^{b}	212.3 (*s*)^{b}	–
9	37.2 (*d*)	37.4 (*d*)	–
9-Me	19.4 (*q*)	19.5 (*q*)	–
	19.5 (*q*)	19.5 (*q*)	–
2′	79.2 (*s*)	81.5 (*s*)	129.6 (*s*)
2′-Me	27.8 (*q*)	27.6 (*q*)	17.8 (*q*)
	27.9 (*q*)	28.0 (*q*)	25.9 (*q*)
3′	126.1 (*d*)	125.8 (*d*)	125.2 (*d*)
4′	117.5 (*d*)	117.2 (*d*)	22.6 (*t*)
5′	160.0 (*s*)^{c}	160.9 (*s*)^{c}	159.0 (*s*)^{b}
6′	107.2 (*s*)^{a}	106.3 (*s*)^{a}	107.5 (*s*)^{a}
7′	162.7 (*s*)^{c}	161.0 (*s*)^{c}	162.5 (*s*)^{b}
8′	104.3 (*s*)^{a}	106.2 (*s*)^{a}	104.9 (*s*)^{a}
9′	156.1 (*s*)^{c}	155.9 (*s*)^{c}	163.1 (*s*)^{b}
10′	104.2 (*s*)^{a}	102.8 (*s*)^{a}	107.7 (*s*)^{a}
11′	210.8 (*s*)^{b}	211.0 (*s*)^{b}	211.1 (*s*)
12′	39.7 (*d*)	40.1 (*d*)	39.2 (*d*)
12′-Me	18.9 (*q*)	18.7 (*q*)	19.8 (*q*)†
	19.8 (*q*)	19.6 (*q*)	19.8 (*q*)†
1″	39.3 (*t*)	39.1 (*t*)	39.5 (*t*)
2″	118.2 (*d*)	118.1 (*d*)	121.2 (*d*)
3″	136.5 (*s*)	136.7 (*s*)	133.0 (*s*)
3″-Me	17.5 (*q*)	17.6 (*q*)	17.8 (*q*)
	25.6 (*q*)	25.6 (*q*)	25.9 (*q*)
1‴	–	–	197.3 (*s*)
2‴	–	–	33.5 (*d*)
2‴-Me	–	–	19.8 (*q*)†
			19.8 (*q*)†

*In acetone-d_6; assignments are supported by HETCOR data in **1** and **3** and further by COLOC and pulsed-field-gradient HMBC experiments in **3**.

†Broad signal, unresolved.

$^{a-c}$Values with the same superscripts in each column are interchangeable.



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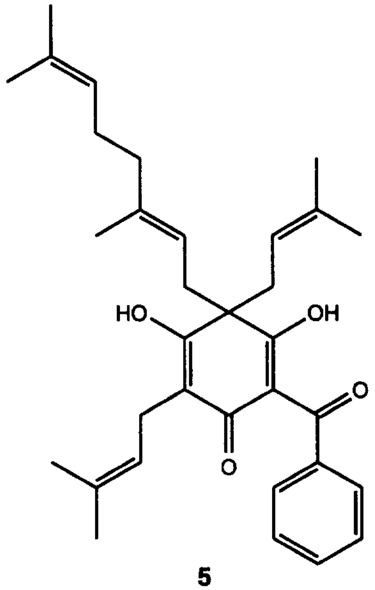

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firmed by a long-range coupling between H-1″ and C-4 in the COLOC spectrum. Conversely, no correlations could be detected from the methine protons of the isobutyryl residue to any C-atom of the filicinic acid ring, neither in COLOC nor in pulsed-field-gradient HMBC [10] experiments. Compound **3** has been named hyperbrasilol C.

All three isolated compounds were inhibitory to *B. subtilis* in a bioautography assay [4]. The minimum quantities of **1–3** required to inhibit the growth of the bacterium on the TLC plate were 0.6, 0.2 and 0.16 μg, respectively. In the same test, the crude petrol extract of **1–3** were comparable to those of japonicine A, uliginosin A, isouliginosin B and hyperbrasilol A, the antibacterial phloroglucinol derivatives previously isolated from *H. brasiliense* [2].

EXPERIMENTAL

General. Mps uncorr. ^{1}H and ^{13}C NMR: 200 and 50 MHz, respectively. HETCOR and COLOC: 400 and 100 MHz, respectively. The pulsed-field-gradient HMBC spectrum of **3** was recorded at 500 MHz. Distinction of carbon multiplicities by the use of DEPT sequences. ^{1}H chemical shifts relative to TMS; ^{13}C chemical shifts referenced to solvent peak. TLC: silica gel precoated Al sheets and RP-18 HPTLC glass plates (Merck). CPC: Pharma-Tech CCC-1000, 2.6 mm i.d. Teflon coils, capacity 660 ml (Pharma-Tech, U.S.A.). MS: triple-stage quadrupole spectrometer; EI: 70 eV; D/CI: positive ion mode, NH the Botany Department, Institute of Biology, Federal University of Rio de Janeiro, Brazil.

Extraction and isolation. Ground, air-dried leaves and flowers (1.31 kg) were extracted at room temp. with petrol. After evapn to dryness, the petrol extract was treated with Me_2CO to afford an Me_2CO-sol. fr. (32 g) and an insol. fatty residue (11.8 g). A portion (17.5 g) of the Me_2CO-sol. fr. was sepd by gel filtration over Sephadex LH-20 with $CHCl_3$–MeOH (1:1) to give 3 frs (A-C). Fr. B, which exhibited antibacterial properties, was further fractionated by CC on silica gel with $CHCl_3$–MeOH mixts of increasing polarity ($CHCl_3$–MeOH 1:0→4:1) to yield seven frs (I–VII). Sepn of fr. II (80 mg) by CPC with *n*-hexane–EtOAc–MeOH–H_2O (10:5:5:1, upper phase as mobile phase) yielded **1** (31 mg). Compound **2** (80 mg) was purified from fr. III (792 mg) by CPC with *n*-hexane–MeCN–MeOH (8:5:2, upper phase as mobile phase) followed by CC on silica gel with *n*-hexane–EtOAc–MeOH–H_2O (10:5:5:1, upper phase). Fr. V was further fractionated using CPC with *n*-hexane–EtOAc–MeOH–H_2O (10:5:5:1, upper phase as mobile phase). Subsequent sepn by CC on silica gel with *n*-hexane–EtOAc–MeOH–H_2O mixtures (10:5:5:1 and 3:7:6:5, respectively; both upper phase), followed by final purification over Sephadex LH-20 with $CHCl_3$–MeOH (1:1) gave **3** (128 mg).

Hyperbrasilol B (**1**). Pale yellow needles from MeCN, mp 114–116°. $[\alpha]_D$ $-73°$ (MeOH; *c* 0.2). TLC (silica gel, $CHCl_3$): R_f 0.67. HPTLC (RP-18, MeCN): R_f 0.15. UV λ^{MeOH} nm (log ε): 357 (4.09), 287 (4.36), 226 (4.46). IR ν^{KBr} cm^{-1}: 3428 (*br*), 3214 (*br*), 2974, 2930, 2872, 1643, 1600, 1465, 1426, 1382, 1361, 1279, 1236, 1198, 1131, 1037, 913, 842, 807, 772, 731. ^1H and ^{13}C NMR: Tables 1 and 2. EIMS *m/z* (rel. int.): 552 ($[M]^+$, 64), 465 (32), 275 (100), 259 (47), 247 (32), 167 (15), 69 (28). D/CIMS *m/z*: 553 ($[M+H]^+$).

Isohyperbrasilol B (**2**). Pale yellow needles from MeOH, mp 68–70°. $[\alpha]_D$ $-56°$ (MeOH; *c* 0.2). TLC (silica gel, $CHCl_3$): R_f 0.66. HPTLC (RP-18, MeCN): R_f 0.18. UV λ^{MeOH} nm (log ε): 357 (4.18), 279 (4.38), 216 (4.46). IR ν^{KBr} cm^{-1}: 3258 (*br*), 2873, 2942, 1645, 1606, 1467, 1428, 1382, 1289, 1235, 1197, 1135, 903, 880, 803, 780, 734. ^1H and ^{13}C NMR: Tables 1 and 2. EIMS *m/z* (rel. int.): 552 ($[M]^+$, 100), 484 (12), 465 (13), 275 (93), 259 (39), 247 (34), 167 (28), 69 (74). D/CIMS *m/z*: 553 ($[M+H]^+$), 263.

Hyperbrasilol C (**3**). Pale yellow needles from MeOH, mp 152–153°. $[\alpha]_D$ $-5°$ (MeOH; *c* 0.25). TLC (silica gel, $CHCl_3$): R_f 0.10. HPTLC (RP-18, MeCN): R_f 0.32. UV λ^{MeOH} nm (log ε): 348 (4.01), 301 (4.17), 224 (4.27). IR ν^{KBr} cm^{-1}: 3474 (*br*), 2974, 2929, 2874, 1619, 1501, 1426, 1383, 1286, 1235, 1207, 1137, 1101, 987, 906, 802. ^1H and ^{13}C NMR: Tables 1 and 2. EIMS *m/z* (rel. int.): 554 ($[M]^+$, 3), 467 (3), 277 (20), 264 (56), 221 (87), 209 (24), 165 (100), 69 (36). D/CIMS *m/z*: 555 ($[M+H]^+$), 335, 279, 265, 148.

Antibacterial testing. Tests were carried out against *B. subtilis* ATCC 6633 using the bioautographic methodology [4] on silica gel glass-backed plates.

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