Benzophenones from *Hypericum carinatum*

Ana Paula M. Bernardi,[†] Alexandre B. F. Ferraz,[†] Daniela V. Albring,[†] Sérgio A. L. Bordignon,[†] Jan Schripsema,[‡] Raquel Bridi,[§] Carlos Severo Dutra-Filho,[§] Amélia T. Henriques,[†] and Gilsane Lino von Poser*[,†]

Programa de Pós Graduação em Ciências Farmacêuticas, UFRGS, Avenida Ipiranga, 2752, 90610-000 Porto Alegre, RS, Brazil, Grupo Metabolômica, LCQUI/CCT, Universidade Estadual do Norte Fluminense, Avenida Alberto Lamego, 2000, 28015-620 Campos dos Goytacazes, RJ, Brazil, and Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600, 90035-003 Porto Alegre, RS, Brazil

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Two new benzophenones were isolated from the leaves of *Hypericum carinatum*. Their structures were established on the basis of 2D NMR spectroscopic analyses and mass spectrometry as cariphenone A (6-benzoyl-5,7-dihydroxy-2,2,8-trimethyl-2*H*-chromene) (**1**) and cariphenone B (8-benzoyl-5,7-dihydroxy-2,2,6-trimethyl-2*H*-chromene) (**2**). Five known compounds, the phloroglucinol derivative uliginosin B (**3**), 1-eicosanol, sitosterol, stigmasterol, and campesterol, were also characterized. Compounds **1**−**3** were evaluated for their total antioxidant capacity through a total radical-trapping parameter assay. Only compound **1** showed moderate antioxidant activity, exhibiting inhibition of chemiluminescence similar to that of quercetin at the same concentration.

The widespread interest in the antidepressive activity of *Hypericum perforatum* (Guttiferae) has encouraged us to further survey the chemical composition of the *Hypericum* species native to southern Brazil. Recently, in our laboratory some species were investigated, affording benzopyrans and phloroglucinol derivatives.[1,2] In this paper, we report the structural elucidation of two new benzophenones isolated from the aerial parts of *Hypericum carinatum* Griseb. (Guttiferae), native to southern Brazil.

Guttiferae is a rich source of benzophenones, a group of substances with cytotoxic and anti-HIV[3,4] activities. This group of compounds has been isolated from genera such as *Allamblackia*, *Clusia*, *Ochrocarpus*, and *Vismia*.[3−6] The benzophenones found in Guttiferae may be divided into two main groups: prenylated trihydroxybenzophenones and polyisoprenylated benzophenones in which the acetate-derived ring has been modified.[7]

It has been reported that some benzophenones (i.e., garcinol) possess free radical scavenging abilities.[8,9] In this way, the antioxidant capacity of cariphenones A and B as well as uliginosin B was assessed by total radical-trapping antioxidant parameter (TRAP) assay, a convenient initial screening method for antioxidative natural products.[10]

The *n*-hexane extract of the aerial parts of *H. carinatum* yielded two main compounds, which were separated by chromatographic techniques. Compound **1** was obtained as a yellow oily material and gave a positive reaction to methanolic ferric chloride reagent. Peaks in the mass spectrum at *m/z* 77 $[Ph]^+$ and *m/z* 105 $[Ph - CO]^+$ indicated a compound with a monosubstituted benzene ring. The NMR spectra of **1** indicated that two aromatic ring systems were present, one fully substituted and the other monosubstituted (Table 1). The wide differences in chemical shifts between the oxygenated and nonoxygenated aromatic carbons, observed in the ^{13}C NMR spectrum of **1**, were indicative of a phloroglucinol oxidation pattern in the fully substituted aromatic ring. The carbonyl group (δ 197.2 ppm) showed an IR absorption band at 1630 cm^{-1}, suggesting hydrogen bonding with a hydroxyl group.[11] The IR spectrum of **1** also showed absorptions related to hydrogen-bonded (3250 cm^{-1}), *gem*-dimethyl (1340 and 1320 cm^{-1}), and aromatic groups (1600 and 1470 cm^{-1}).

Table 1. ^1H NMR (δ, in $CDCl_3$, 400 MHz) and ^{13}C NMR (δ, in $CDCl_3$, 100 MHz) Data for Compounds **1** and **2**[a]

	^1H		^{13}C		
	1	**2**		**1**	**2**
H-3	5.49 (1H, d, J = 10.0)	5.31 (1H, d, J = 10.0)	C-2	77.8	77.3
H-4	6.59 (1H, d, J = 10.0)	6. 49 (1H, d, J = 10.0)	C-3	125.8	125.5
H-11; H-12 (2-Me)	1.46 (6H, s)	0.98 (6H, s)	C-4	116.0	115.8
H-13 (Me)	1.99 (3H, s)	2.10 (3H, s)	C-5	154.5	156.0
5-OH	8.79 (1H, s)	5.38 (1H, s)	C-6	103.9	101.8
7-OH	9.09 (1H, s)	12.70 (1H, s)	C-7	159.6	162.4
2′, 6′	7.64 (1H, d)	7.47 (1H, d)	C-8	104.8	105.4
3′, 5′	7.54 (1H, t)	7.37 (1H, t)	C-9	159.4	154.1
4′	7.60 (1H, t)	7.44 (1H, t)	C-10	102.2	101.2
			C-11	28.4	27.1
			C-12	28.4	27.1
			C-13	7.1	7.0
			CO	197.2	200.5
			C-1′	139.7	142.8
			C-2′	127.7	127.1
			C-3′	129.4	127.5
			C-4′	132.3	129.9
			C-5′	129.4	127.5
			C-6′	127.7	127.1

[a] H, multiplicities, coupling constants in Hz.

In the ^1H NMR spectrum of **1** (Table 1) the appearance of a pair of doublets at δ 6.59 and 5.49 ppm (each 1H, J = 10.0 Hz) together with a six-proton singlet at δ 1.46 indicated the presence of a dimethylpyran ring system in the molecule.

The presence of two hydroxyls was confirmed by the ^1H NMR spectrum, which exhibited sharp singlets at δ 9.09 and 8.79 ppm. The chemical shifts are characteristic of two hydroxyls associated with a single carbonyl. Due to the alternating hydrogen-bonding, the chemical shifts are intermediate between the chemical shift of a non-hydrogen-bonded hydroxyl (about 6 ppm) and a permanently hydrogen-bonded hydroxyl (about 12 ppm). The observed values

* To whom correspondence should be addressed. Tel: 55-51-3316-5456. Fax: 55-51-3316-5456. E-mail: gilsane@farmacia.ufrgs.br.
[†] Programa de Pós Graduação em Ciências Farmacêuticas, UFRGS.
[‡] Universidade Estadual do Norte Fluminense.
[§] Instituto de Ciências Básicas da Saúde, UFRGS.

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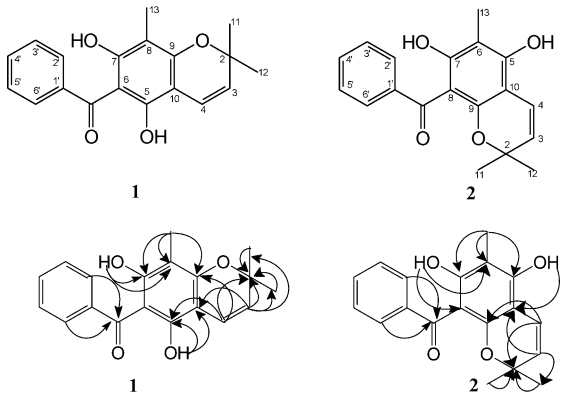

Figure 1. $^{1}H-^{13}C$ long-range correlations by HMBC spectra (optimized for $J = 8$ Hz) of **1** and **2**.

were analogous to those observed for other similar dihydroxybenzophenones.[11−13]

The structure of **1** was confirmed by HMBC data. Besides the expected cross-peaks for the dimethylbenzopyran moiety, H-4 with C-9, C-5, and C-2; H-8 with C-10, C-2, C-11, and C-12 (2-Me_2), H-11, and H-12 (2-Me_2) with C-2, the following correlations were observed: 7-OH with C-7 and C-8; 5-OH with C-10 and C-5; and H-13 with C-7, C-8, and C-9. The HREIMS data (M+, *m/z* 310.1207) established a molecular formula of $C_{19}H_{18}O_4$ (calcd 310.1205) for compound **1**, which was named cariphenone A (6-benzoyl-5,7-dihydroxy-2,2,8-trimethyl-2*H*-chromene).

Compound **2** was isolated as an amorphous yellow powder and shares several ^{1}H and ^{13}C NMR spectral characteristics (Table 1) with other similar benzophenones.[7,13] As for compound **1**, the structural analyses were supported by earlier studies.[14,15] The HREIMS data (M+, *m/z* 310.1209) established a molecular formula of $C_{19}H_{18}O_4$ (calcd 310.1205) for compound **2**. The IR spectrum of **2** displayed vibration bands due to hydrogen-bonded hydroxyl (3250 cm^{-1}) and carbonyl (1630 cm^{-1}) groups, benzene ring moieties, and other functionalities. Its UV spectrum exhibited maxima at 216, 254, and 294 nm and was similar to that of compound **1**. From the above data, it was inferred that compound **2** could also be a benzophenone. As in compound **1**, the second benzene ring (ring B) has a phloroglucinol oxidation pattern and was fully substituted by two hydroxyl groups, one methyl, and one dimethylbenzopyran ring. The HMBC spectrum showed correlations of the hydrogens of the methyl group with C-7, C-6, and C-5. Except for the absence of characteristic signals for a prenyl group at C-6, the spectra are identical to those of isovismiaphenone B.[7,10,11,13,16−20] In compound **2** the pyran ring is formed by the cyclization of a precursor prenyl side chain with a hydroxyl group at C-9. The presence of two signals at δ_H 12.7 and 5.38 ppm indicated that two hydroxyl groups were present, one hydrogen-bonded to the carbonyl group of the benzophenone moiety. As opposed to the 7-OH chemical shift value (δ_H 9.09 ppm) in compound **1**, the 7-OH chemical shift in compound **2** is at δ_H 12.7 ppm.[13,21] Thus, two hydroxyl functionalities were assigned to C-7 and C-9 in ring B, and ring C was fused to ring B at C-10 and C-5. Therefore, the structure **2** was assigned to the new compound, cariphenone B (8-benzoyl-5,7-dihydroxy-2,2,6-trimethyl-2*H*-chromene).

In addition to these two new natural compounds, the fractionation by silica gel column chromatography of the *n*-hexane extracts yielded a mixture of sitosterol (58.9%), stigmasterol (33.4%), and campesterol (3%). Besides this mixture of sterols, 1-eicosanol was also isolated.

The benzophenones and the phloroglucinol derivative were tested for their total antioxidant capacity through the TRAP measurement. This method is based on the fact that free radicals produced from ABAP oxidize luminol, leading to the formation of luminol radicals, which in turn emit light. Antioxidants can inhibit this chemiluminescence, and the time this inhibition lasts, called induction time, is directly proportional to the total antioxidant potential. The induction time of the samples was compared to that from Trolox (a water-soluble α-tocopherol analogue). Figure 2 shows the effect of cariphenone A (**1**) (1.6 and 3.2 mM), Trolox (0.16 mM), and quercetin (3.2 mM) on TRAP measurement. It can be seen in Figure 2 that cariphenone A (**1**) exhibited free radical scavenging activity, as manifested in the reduction of luminescence intensity. This method is most likely due to trapping peroxyl radicals.[10,22] Although Trolox was added at a different concentration than cariphenone A, it can be seen that the curve of Trolox is situated between the curves of both concentrations used of cariphenone A, indicating that this compound may have an antioxidant potential about 10−20 times lower than that of Trolox. Comparing cariphenone A and quercetin, a reference scavenger molecule, at the same concentration

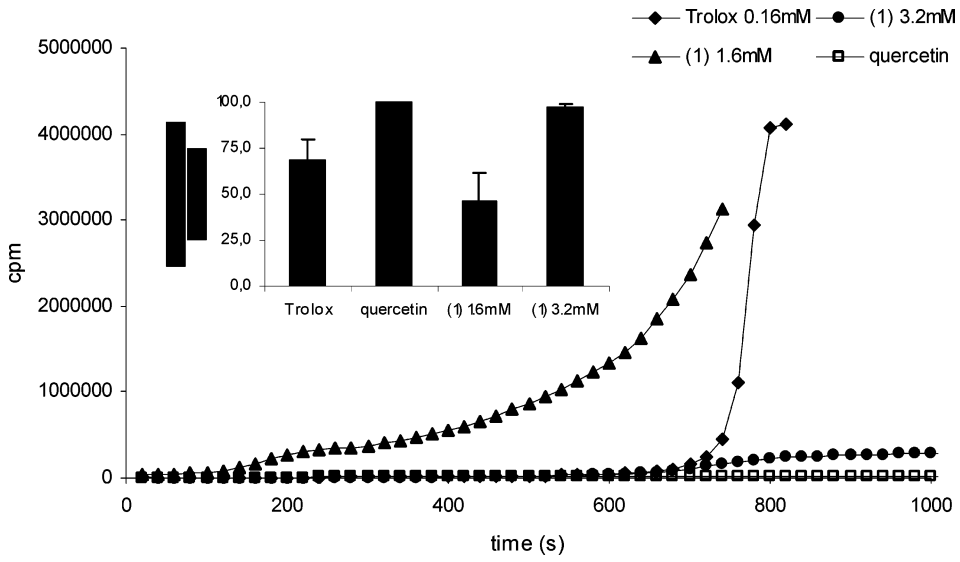

Figure 2. Effect of cariphenone A (**1**) and quercetin on the TRAP measurement. Data are representative for three independent experiments. Data displayed in the bar graphic (inset) were calculated at 700 s and represented by mean ± SE.

(3.2 mM), the profiles observed in the TRAP assay were similar for both substances, which inhibited the chemiluminescence for a period greataer than 900 s. Cariphenone B and uliginosin B showed no antioxidant activity in this assay (results not shown).

Experimental Section

General Experimental Procedures. NMR data (400 MHz for ^{1}H and 100 MHz for ^{13}C) were measured on a JEOL Eclipse 400 spectrometer in $CDCl_3$ using the solvent peak as internal standard; for 2D experiments standard pulse sequences from Delta software (version 3.2) were used. The HMQC experiment was optimized for $J = 140$ Hz. The HMBC experiment was optimized for $J = 8$ Hz. HREIMS: 70 eV. Mass spectra were obtained through GC-MS analysis on a Shimadzu QP5050 system: temp program 100−280 °C (15°/min), injector 280 °C, transferline 280 °C, column DB-1, **1** eluted at 7.09 min and **2** at 7.05 min. Preparative TLC: 20 × 20 cm plates coated with 0.5 mm layer of silica gel GF_{254} (Merck); bands were detected under UV light (254 nm).

Plant Material. Plant material of *H. carinatum* Griseb. (aerial parts) was collected in Glorinha, RS, in December 2002. The species was identified by one of us (S.B.), and a voucher specimen was deposited (Bordignon 1520) in the herbarium of the Universidade Federal do Rio Grande do Sul (ICN).

Extraction and Isolation. Air-dried and powdered plant material (78 g) was extracted in a Soxhlet apparatus with *n*-hexane over 12 h, and then the extract was evaporated to dryness under reduced pressure. The *n*-hexane extract (3.1 g) of *H. carinatum* was submitted to column chromatography (50 × 3 cm) on silica gel using an *n*-hexane/$CHCl_3$ gradient system followed by preparative-TLC on silica gel with $CHCl_3$/*n*-hexane (1:1). Compounds **1** (31 mg) and **2** (17 mg) were isolated.

Cariphenone A (1): viscous yellow oil; R_f 0.79 ($CHCl_3$); UV λ^{MeOH}_{max} nm (log ϵ) 234 (2.81), 282 (2.87), 322 (2.62); IR $\nu^{CHCl_3}_{max}$ cm^{-1} 3250, 2900, 2850, 1630, 1600, 1470, 1340, 1320, 1260, 1190, 1160, 1030, 800; EIMS *m/z* (rel int) [M^+] 310 (23), 295 (100), 257 (19), 255 (10), 217 (49), 179 (12), 105 (7), 84 (25), 77 (11), 49 (9); HREIMS 310.1207 found, calc for $C_{19}H_{18}O_4$ 310.1205; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$), see Table 1.

Cariphenone B (2): amorphous yellow powder; R_f 0.50 ($CHCl_3$); UV λ^{MeOH}_{max} nm (log ϵ) 216 (2.86), 254 (2.50), 294 (2.70); IR ν $^{CHCl_3}_{max}$ cm^{-1} 3250, 2900, 2850, 1630, 1600, 1550, 1470, 1340, 1320, 1280, 1150, 700; EIMS *m/z* (rel int) [M^+] 310 (20), 295 (100), 217 (58), 147 (14), 105 (23), 83 (10), 77 (48), 43 (23); HREIMS 310.1209 found, calc for $C_{19}H_{18}O_4$ 310.1205; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$), see Table 1.

TRAP Assay. TRAP, representing the total antioxidant capacity of the compounds, was determined by measuring the chemiluminescence intensity of luminol induced by 2,2′-azo-bis(2-amidinopropane) (ABAP) according to the method of Lissi et al. (1992).[23] Sample solutions (1.0 and 0.5 mg/mL) of cariphenone A, cariphenone B, and uliginosin B were prepared in 0.1 M glycine buffer, pH 8.6, using Tween 80 (10%). The solvent alone was analyzed in the experimental procedures, and there was no effect on the measured parameter.

The background chemiluminescence was measured by adding 3 mL of 10 mM ABAP dissolved in 0.1 M glycine buffer, pH 8.6, into a glass scintillation vial. A 10 μL sample of luminol (4 mM) was added to each vial, and the chemiluminescence was measured. This was considered to be the initial value. Trolox (10 μL, 0.16 mM), cariphenone A, and cariphenone B (3.2 and 1.6 mM) or uliginosin B (2.0 and 1.0 mM) were separately added, and the chemiluminescence was measured. The time necessary for the chemiluminescence intensity shows a rapid increase [induction time (IT)]. IT is directly proportional to the antioxidant capacity of the compound, and the IT of each sample was compared with the IT of Trolox.

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