Journal of Natural Products
Vol. 58, No. 2, pp. 324-328, February 1995

PIPEROGALIN, A NEW PRENYLATED DIPHENOL FROM *PEPEROMIA GALIOIDES*

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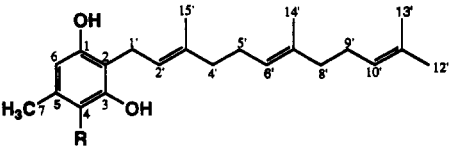
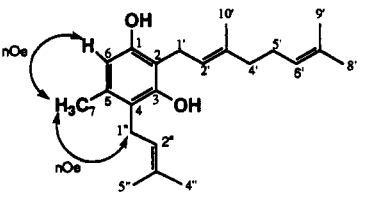
ABSTRACT.—A petroleum ether extract of *Peperomia galioides* showed significant in vitro activity against three *Leishmania* species and *Trypanosoma cruzi*. Three major prenylated diphenols, including two known compounds, grifolic acid [**1**], and grifolin [**2**], and the new substance piperogalin [**3**], have been isolated. Structures were established on the basis of spectral analysis including 2D nmr spectroscopy.

As part of a search for naturally occurring antiparasitic agents, in collaboration with ORSTOM (Institut Français de Recherche Scientifique pour le Développement en Coopération) and IBBA (Instituto Boliviano de Biologia de Altura), we have investigated the chemical composition of a petroleum ether extract of *Peperomia galioides* H.B.K. (Piperaceae). This species, collected in Bolivia in the Department of La Paz, has not been investigated for its chemical composition so far. It was tested in preliminary studies by IBBA and ORSTOM, with the activity of different extracts of *P. galioides* having been measured in vitro against three species of *Leishmania* responsible for leishmaniasis and against three stains of *Trypanosoma cruzi*, the factor responsible for Chagas disease (1). The interesting activity of the petroleum ether extract that was observed prompted us to investigate its chemical constituents. Three major terpenoids were isolated from this extract. Two of these, grifolin [**2**] (2–4) and grifolic acid [**1**] (4) have already been isolated from organisms in the Basidiomycetes. The third compound, piperogalin [**3**], is novel.

The powdered whole plant was successively extracted in a Soxhlet apparatus with petroleum ether, CH_2Cl_2, and then with MeOH. The petroleum ether extract (7% of dried material) was purified by the usual chromatographic methods on Si gel (cc and tlc) and liquid/liquid partition between 1 N NH_4OH and CH_2Cl_2. From the aqueous layer, acidified with 1 N HCl and further extracted with Et_2O, was isolated a *para*-hydroxysalicyclic acid derivative containing a farnesyl moiety, namely, grifolic acid [**1**], a compound encountered previously only from the basidiomycete *Albatrellus cristatus* (4). From the CH_2Cl_2 layer were further separated two neutral phenolic compounds, grifolin [**2**] and



1 R=COOH
2 R=H



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piperogalin [**3**]. The structures of both products were determined using ^{1}H- and ^{13}C-nmr and long-range heteronuclear correlations (COLOC). Grifolin [**2**] is the decarboxylated derivative of grifolic acid [**1**], and was isolated earlier from *Grifola confluens, Albatrellus ovinus,* and *A. cristatus* (2–4).

Piperogalin [**3**] is a new prenylated derivative isolated as a brown oil. The cims showed a $[M+H]^+$ ion peak at *m/z* 329 corresponding to the same molecular formula $C_{22}H_{32}O_2$ as grifolin [**2**], as confirmed by hrms. The uv spectrum also showed a bathochromic shift upon addition of a base, indicating the phenolic nature of this compound. The ^{1}H-nmr spectrum exhibited in the aromatic region two D_2O-exchangeable singlets at 7.77 and 6.53 ppm assigned to two phenolic functions, one singlet of an aromatic proton at 6.25 ppm, and the singlet of an aromatic methyl group at 2.11 ppm. The aromatic ring is thus substituted by two alkenyl moieties, as shown by two benzylic methylene groups resonating at 3.26 and 3.36 ppm as doublets.

Homonuclear ^{1}H-^{1}H and heteronuclear ^{1}H-^{13}C nmr correlations clearly indicated the presence of a prenyl group, corresponding with the benzylic methylene at 3.26 ppm and ethylenic proton at about 5.07 ppm, and a geranyl moiety bearing the methylene doublet at 3.36 ppm and ethylenic protons at 5.23 and 5.03 ppm. The respective positions of the five aromatic substituents were deduced from a long-range ^{1}H-^{13}C nmr experiment (COLOC) and by nOe observations (see Table 1).

The methylene group at δ 3.36 of the geranyl moiety was clearly three-bond coupled with both OH-bearing carbons at δ 153.1 and 153.3, indicating the position of this substituent at C-2. The other methylene at δ 3.26 was also correlated with C-3 (δ 153.1) and with the CH_3-bearing carbon at δ 131.3, thus

TABLE 1. Nmr Data for **3** (CD_3COCD_3, 400 MHz).

Position	δ ^{1}H[a]	δ ^{13}C	COSY	H-C long range correlations
1	7.77 s (OH)	153.3[c]		C-2
2		112.4		
3	6.53 s (OH)	153.1[c]		C-2, C-4
4		117.9		
5		131.3[d]		
6[b]	6.25 s	109.0		C-2, C-4, C-7
7[b]	2.11 s	18.9		C-4, C-5, C-6
1′	3.36 br d (7)	22.3	H-2′, H-10′	C-1, C-2, C-3, C-3′
2′	5.23 tq (6 and 1.3)	123.3[e]	H-1′, H-10′	
3′		131.6[d]		
4′	1.95 t (8)	39.6	H-5′	
5′	2.01–2.03 m	26.1	H-4′, H-6′	
6′	ca. 5.03 m	124.2	H-5′, H-8′, H-9′	
7′		130.8		
8′	1.62 d (ca. 1)	21.9	H-6′	
9′	1.55 br s	16.8	H-6′	
10′	1.76 d (ca. 1)	15.4	H-1′, H-2′	C-2′, C-3′, C-4′
1″[b]	3.26 br d (7)	24.8	H-2″, H-4″, H-5″	C-3, C-4, C-5, C-3″
2″	ca. 5.07 m	123.7[e]	H-1″, H-4″, H-5″	
3″		130.0		
4″	1.63 d (1.4)	21.9	H-2″, H-1″	
5″	1.73 d (ca. 1)	17.0	H-2″, H-1″	

[a]*J* values are given in Hz.
[b]NOe enhancements were observed between CH_3-7 and both H-6 and CH_2-1″.
[c,d,e]The assignments may be interchanged.

locating the prenyl moiety at C-4 and the methyl group at C-5. Further confirmation of the structure was given by the correlation of the CH_3 protons (δ 2.11) with C-4 (δ 117.9) and the three-bond coupling of H-6 with C-2 and C-4. Long-range correlations also indicated the respective positions of the hydroxy groups at C-1 (δ 7.77) and C-3 (δ 6.53).

NOe experiments were in good agreement with the proposed structure **3** for piperogalin. Significant enhancements were observed between the methylene group of the prenyl moiety (δ 3.26) and the aromatic methyl group (δ 2.11), and between the latter and the phenyl proton (δ 6.25).

During preliminary studies, carried out by IBBA, three extracts were prepared from *Peperomia galioides*, namely, petroleum ether, EtOAc, and EtOH extracts. Biological activity of the crude extracts was measured in vitro (Table 2) on the promastigote form of three species of *Leishmania* (*L. braziliensis, L. amazonensis, L. chagasi*), and on the epimastigote form of three strains of *Trypanosoma cruzi* (*T.c. tulahuen, c8cl*1, and *tehuentepec*). The best activity was obtained with the petroleum ether extract showing a total lysis of *Leishmania chagasi* at 10 μg/ml.

The three extracts obtained after the successive extraction of *P. galioides* with petroleum ether, CH_2Cl_2, and MeOH were tested on the bloodstream form of *Trypanosoma cruzi* encountered during the acute stage of the disease. The trypomastigotes were isolated from blood samples of *T. cruzi*-infected mice, as described in Ref. (5). From these results (Table 3), it appears that the petroleum ether extract exhibits a significant activity (% lysis ≥ 60%) against *T. cruzi* and contains the most active products. Grifolic acid [**1**], grifolin [**2**], and piperogalin [**3**] were tested against *T. cruzi* by the same method (Table 3). From these results, grifolin [**2**] exhibited a significant activity (% lysis ≥ 60%) against *T. cruzi*.

The crude extracts and the three isolated compounds were also tested on four strains of *Leishmania* (Table 4), under the same conditions as described by Hocquemiller *et al.* (1). Piperogalin [**3**] showed the best activity of the com-

TABLE 3. In Vitro Study of *Peperomia galioides* Extracts and Compounds **1**, **2**, and **3** on the Trypomastigote Form of *Trypanosoma cruzi*, strain Y.

Extract/Compound[a]	Activity (% lysis)
Petroleum ether extract	72
CH_2Cl_2 extract	31
MeOH extract	0
Grifolic acid [**1**]	0
Grifolin [**2**]	68
Piperogalin [**3**]	42

[a]Concentration=250 μg/ml.

TABLE 2. In Vitro Inhibitory Effects of *Peperomia galioides* Extracts on *Leishmania* sp. (Promastigote Form) and *Trypanosoma cruzi* (Epimastigote Form).

Extract	Concentration μg ml^{-1}	Organism/activity[a]					
		Leishmania braziliensis	*Leishmania amazonensis*	*Leishmania chagasi*	*T.c.*[b] *tulahuen*	*T.c.*[b] *c8cl*1	*T.c.*[b] *tehuentepec*
Petroleum ether	100	+++	+++	+++	+++	+++	+++
	50	+++	+++	+++	+++	+++	+++
	25	+++	+++	+++	+++	+++	+++
	10	++	++	+++	0	0	0
	5	0	0	0	0	0	0
EtOAc	100	+++	+++	+++	+++	+++	+++
	50	+++	+++	+++	+++	+++	+++
	25	+++	+++	+++	+++	+++	+++
	10	0	0	0	0	0	0
EtOH	100	+++	+++	+++	+++	+++	+++
	50	0	0	0	0	0	0

[a]0: no lysis; ++: modified or immobilized parasites; +++: total lysis of parasites.
[b]*T.c.*=*Trypanosoma cruzi*.

TABLE 4. In Vitro Study of *Peperomia galioides* Extracts and Compounds **1**, **2**, and **3** on the Promastigote Form of *Leishmania* species.

Exract/Compound	Concentration µg/ml	*L. braziliensis* (2903)[a]	*L. donovani* (PP 75)[a]	*L. amazonensis*[a]
Petroleum ether extract	100	+++	+++	+++[b]
	50	+++	+++	+++
	25	++	++	++
CH_2Cl_2 extract	100	+++	+++	+++[b]
	50	+++	+++	+++
	25	+++	++	++
MeOH extract	100	+	+	++[c]
	50	+	+	+
Grifolic acid [**1**]	100	++	+++	+++[c]
Grifolin [**2**]	100	+++	+++	+++[c]
	50	+++	+++	+++
	25	+++	+++	+++
	10	++	++	++
Piperogalin [**3**]	100	+++	+++	+++[c]
	50	+++	+++	+++
	25	+++	+++	+++
	10	++*	++*	++*

[a]0: no lysis; +: parasites less mobile than controls; ++: modified or immobilized parasites; +++: total lysis of parasites; *: more than 90% lysis.
[b]*L. amazonensis* (LV 79).
[c]*L. amazonensis* (H 142).

pounds tested against the three species of *Leishmania*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Philips PU 8700 spectrophotometer and ir spectra on a Perkin-Elmer 841 spectrometer. All ^{1}H- and ^{13}C-nmr spectra were recorded in CD_3COCD_3 (δ ppm) on a Bruker AM 400 or a AC 200 nmr spectrometer operating at 400 and 100 MHz or 200 and 50 MHz, respectively. Eims and cims spectra were obtained on Kratos MS-80 and Kratos MS-9 mass spectrometers. Si gel GF_{254} was used for tlc.

PLANT MATERIAL.—*Peperomia galioides* described by A. Bonpland, C. Kunth, and A. de Humboldt in 1815 (6) was collected by A. Fournet in April 1986, along the old road of Chulumani, near the village of Unduavi, Yungas, Department of La Paz (altitude 3000 m), Bolivia. The botanical identification was carried out by Dr. H.A. Valdebenito, Department of Botany, Ohio State University, Columbus, OH. Voucher specimens are deposited (No. AF 615) in the National Herbarium of Bolivia (La Paz) and at Ohio State University in Columbus.

EXTRACTION AND ISOLATION.—Dried and powdered plant samples (10 g) were macerated for two days with petroleum ether, EtOAc, and EtOH (50 ml for each extract). The extracts were filtered and evaporated to dryness, affording the three residues, which were used for preliminary biological testing.

For the phytochemical work described in this paper, the air-dried whole plant of *Peperomia galioides* (206 g) was successively extracted in a Soxhlet apparatus with petroleum ether (bp 40–65°), CH_2Cl_2, and MeOH, which gave 14 g, 9 g, and 14 g of each crude extract, respectively.

The petroleum ether extract (7% of dried material) was first purified by a liquid/liquid partition between 1 N NH_4OH and CH_2Cl_2. The aqueous layer was acidified with 1 N HCl and further extracted with Et_2O, affording grifolic acid [**1**] (10% of the petroleum ether extract). The CH_2Cl_2 layer was fractionated by flash chromatography on Si gel (0.032–0.063 mm), eluted with CH_2Cl_2, to which increasing percentages (0–100%) of MeOH were added. The first fractions were separated again using flash chromatography, and eluted with cyclohexane-EtOAc (100:7) to afford grifolin [**2**] (22% of the petroleum ether extract) and piperogalin [**3**] (26% of the petroleum ether extract).

Grifolic acid [**1**].—$C_{23}H_{32}O_4$, brown oil; uv λ max (EtOH) (log ε) 204 (4.36), 215 (4.35), 256 (3.76), 281 (3.41) nm; (OH^-) (log ε) 213 (4.16), 223 (4.42), 282 (3.95), 326 (3.31) nm; ir (film) ν max 3400, 1630, 1452, 1380, 1268 cm^{-1}; eims *m/z* 372.2288 ([M]$^+$, 21) (372.2300 calcd), 344 (18), 328 (10), 217 (96), 191 (84), 175 (47), 137 (100), 121 (48); ^{1}H nmr (200 MHz, CD_3COCD_3) δ 1.54 (6H, s, CH_3-13′ and 14′), 1.62 (3H, s,

CH_3-12′), 1.75 (3H, s, CH_3-15′), 1.86–2.03 (8H, m, H-4′, 5′, 8′, and 9′), 2.46 (3H, s, CH_3-7), 3.31 (2H, d, J=7 Hz, H-1′), 4.97–5.18 (2H, m, H-6′ and 10′), 5.25 (1H, br t, J=7 and ca. 1 Hz, H-2′), 6.32 (1H, s, H-6); ^{13}C nmr (100 MHz, CD_3COCD_3) δ 15.3 (C-14′), 15.5 (C-15′), 16.9 (C-13′), 21.7 (C-1′), 23.5 (CH_3-7), 25.0 (C-12′), 26.4 (C-5′), 26.6 (C-9′), 39.6 and 39.7 (C-4′ and C-8′), 103.9 (C-4), 110.7 (C-6), 112.8 (C-2), 122.9 (C-2′), 124.2 (C-6′), 124.4 (C-10′), 130.7 (C-11′), 134.0 (C-7′), 134.5 (C-3′), 140.7 (C-5), 159.9 (C-3), 164.0 (C-1), 174.0 (COOH).

Grifolin [**2**].—$C_{22}H_{32}O_2$, brown oil; uv λ max (EtOH) (log ε) 207 (4.64), 231 sh (4.00), 273 (3.23); (OH^-) (log ε) 223 (4.35), 248 sh (3.84), 291 (3.41) nm; ir (film) ν max 3462, 2971, 2924, 2857, 1634, 1592, 1450, 1042 cm^{-1}; eims *m/z* 328.2428 ($[M]^+$, 28) (328.2402 calcd), 191 (26), 177 (18), 175 (35), 137 (100); ^{1}H nmr (200 MHz, CD_3COCD_3) δ 1.56 (6H, s, CH_3-13′ and 14′), 1.63 (3H, s, CH_3-12′), 1.76 (3H, s, CH_3-15′), 1.87–2.07 (8H, m, H-4′, 5′, 8′, and 9′), 2.10 (3H, s, CH_3-7), 3.33 (2H, d, J=7 Hz, H-1′), 5.06–5.09 (2H, m, H-6′ and 10′), 5.30 (1H, br t, J=7 and ca. 1 Hz, H-2′), 6.19 (2H, s, H-4 and 6), 7.80 (2H, s, OH-1 and 3); ^{13}C nmr (50 MHz, CD_3COCD_3) δ 15.6 (C-14′), 15.8 (C-15′), 17.3 (C-13′), 20.8 (CH_3-7), 22.1 (C-1′), 25.4 (C-12′), 26.8 (C-5′ and C-9′), 39.8 and 40.0 (C-4′ and C-8′), 107.8 (C-4 and C-6), 112.0 (C-2), 123.9 (C-2′), 124.5 and 124.6 (C-6′ and C-10′), 130.8 (C-11′), 133.7 (C-7′), 134.6 (C-3′), 136.0 (C-5), 156.0 (C-1 and C-3).

Piperogalin [**3**].—$C_{22}H_{32}O_2$, brown oil; uv λ max (EtOH) (log ε) 207 (4.66), 277 (3.60) nm; (OH^-) 216 (4.56), 294 (3.64) nm; ir (film) ν max 3433 (br), 2972, 2929, 2855, 1622, 1452, 1375, 1168, 1053 cm^{-1}; eims *m/z* 328.2403 ($[M]^+$, 43) (328.2402 calcd), 243 (27), 205 (72), 203 (90), 189 (54), 149 (100), 69 (83); cims *m/z* 329 (100), 273 (98), 205 (35); for ^{1}H- and ^{13}C-nmr (CD_3COCD_3) data, see Table 1.

BIOLOGICAL ASSAYS.—*In vitro study on the promastigote form of* Leishmania *and on the epimastigote form of* Trypanosoma cruzi.—General method according to Hocquemiller *et al.* (1). Briefly, the extracts and pure compounds were dissolved in DMSO. Each assay was performed three times. The viability of the parasites was estimated by direct observation after 24 h incubation at 28°, with an inverted microscope. The baseline drugs were glucantime (Rhône-Poulenc, France), pentamidine (May and Baker, UK), and gentian violet.

In vitro study on the trypomastigote form of Trypanosoma cruzi.—General method according to Fournet (5). Briefly, the extracts and pure compounds were dissolved in DMSO. Each assay was performed three times. The parasites were counted after 24 h incubation at 4°. The baseline drug was gentian violet.

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Received 21 September 1994