New Prenylated Quinones from *Peperomia galioides*

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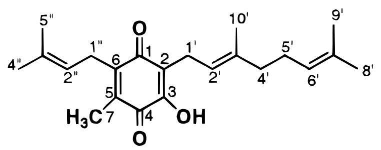
Two new prenylated quinones, piperogalone (**1**) and galopiperone (**2**), and a new prenylated dihydroquinone, hydropiperone (**3**), were isolated from *Peperomia galioides* H.B.K (Piperaceae). Hydropiperone exhibited potent antiparasitic activity against three species of *Leishmania*.

Previous phytochemical studies of a petroleum ether extract of *Peperomia galioides* H.B.K. (Piperaceae) afforded three interesting prenylphenols: grifolin, grifolic acid, and piperogalin.[1] In a continuation of our investigations on this extract, we have isolated three new minor constituents, piperogalone, galopiperone, and hydropiperone, to which structures **1**, **2**, and **3** have been assigned.



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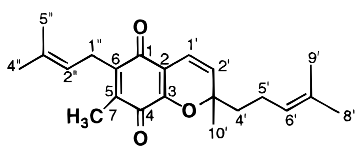

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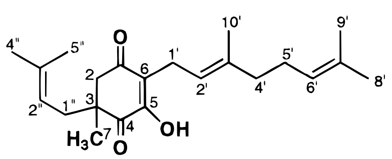

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We have previously shown[1] that the petroleum ether extract has a significant in vitro activity on three species of *Leishmania*, responsible for leishmaniasis, and on three strains of *Trypanosoma cruzi*, the causative factor for Chagas' disease. The activities of the major isolated compounds have been reported already.[1] One of the new compounds, hydropiperone (**3**), displays a promising activity against promastigote forms of three species of *Leishmania*.

The petroleum ether extract (7% of dried material) was purified by chromatographic methods on Si gel (column chromatography and TLC) and resulted in the isolation of two new prenylated quinones **1** and **2** (piperogalone and galopiperone) and the 2,3-dihydroquinone **3** (hydropiperone).

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The HREIMS spectrum of **1** shows a molecular ion peak at m/z 342.2183, corresponding to the molecular formula $C_{22}H_{30}O_3$. The presence of conjugated carbonyl groups is deduced from the IR spectrum (broad absorption at 1641 cm^{-1}) and is substantiated by two ^{13}C-NMR signals at δ 187.3 and 184.9 indicative of a quinone moiety.[2] A bathochromic shift, obtained in the UV spectrum after addition of base, indicates the presence of a phenolic or enolic function, also deduced from the IR absorption at 3407 cm^{-1}. The ^{1}H-NMR spectrum shows the singlet of an aromatic methyl group at δ 2.01 and two methylene groups resonating at δ 3.12 and 3.22 as doublets. The quinone nucleus is thus substituted by two alkenyl moieties further identified by a COSY experiment as a geranyl chain and a prenyl group.

The homonuclear ^{1}H−^{1}H and heteronuclear ^{1}H−^{13}C correlations clearly indicated the presence of the prenyl group, corresponding with the methylene at δ 3.22, the ethylenic proton at δ 4.97, and two methyl groups at δ 1.65 and 1.74 (Table 1). Other signals in the ^{1}H-NMR spectrum were attributed to the geranyl moiety, in particular the ethylenic protons at δ 5.15 and 5.05 and the isolated methylene doublet at δ 3.12 bonded with the quinone nucleus. The ^{13}C-NMR chemical shift for the C-10′ methyl group indicated the *E*-geometry about the asymmetrically substituted double bond.[3]

The respective positions of the four substituents of the quinone were then deduced from the long-range ^{1}H−^{13}C experiment (HMBC) and NOE (Table 1), which further confirmed the attributions of ^{1}H and ^{13}C signals of the geranyl and prenyl chains.

The two alkenyl methylene groups at δ 3.12 and 3.22 were both ^3J correlated with the C-1 carbonyl group at δ 187.3, thus indicating the position of the geranyl and prenyl chains at C-2 and C-6, respectively. The proton signal at δ 3.12 was also ^3J correlated with the OH-bearing carbon at δ 152.4, indicating a C-3 position for the hydroxy group, and subsequently the position of the methyl group at C-5. Further confirmation of this position was brought by the observation of ^3J correlations of the methyl protons with C-6 (δ 137.2) and with the second carbonyl group (δ 184.9) located at C-4. The structure of the new prenylated quinone, named piperogalone, is thus established as structure **1**. The spatial proximity between the methyl group and the prenyl methylene was further supported by a NOE experiment.

Table 1. NMR Data for Quinones **1** and **2** (in CD_3COCD_3)

	1			2		
position	δ ¹H[a]	δ ¹³C[b]	HMBC (H to C)[a]	δ ¹H[a]	δ ¹³C[b]	HMBC (H to C)[a]
1		187.3			184.5	
2		120.4			115.6	
3		152.4			151.3	
4		184.9			182.7	
5		145.2			142.9	
6		137.2			139.7	
7[d]	2.01, s	11.4	C-4, C-5, C-6	1.98 s	11.9	C-4, C-5, C-6
1′	3.12 d (7)	22.6	C-1, C-2, C-3, C-2′, C-3′	6.45 d (10)	116.4	C-3, C-3′
2′	5.15 tq (7, 1)	121.6	C-1′, C-4′, C-10′	5.71 d (10)	129.7	C-1′, C-3′
3′		136.5			83.4	
4′	1.96 t (7)	40.4		1.95−2.05 m	42.2	
5′	≈2.00 m	27.3		2,08−2,13 m	23.4	
6′	5.05 m	125.0	C-8′, C-9′	5.09 br t (7)	124.9	C-5′
7′		131.4			132.4	
8′	1.61 br s	25.8		1.60 br s	25.9[c]	C-6′, C-7′, C-9′
9′	1.55 br s	17.7		1.53 br s	17.8	C-6′, C-7′, C-8′
10′	1.74 br s	16.2		1.42 s	27.5	C-2′, C-3′, C-4′
1″[d]	3.22 d (6)	26.3	C-1, C-5, C-6, C-2″, C-3″	3.20 d (7)	26.1	C-1, C-5, C-6, C-2″, C-3″
2″	≈4.97 m	120.6	C-4″, C-5″	4.96 br t (7)	120.8	
3″		133.7			133.9	
4″	1.65 d (1.3)	25.8		1.64 br s	25.8[c]	C-2″, C-3″, C-4″
5″	1.74 br s	18.0		1.72 br s	18.1	C-2″, C-3″, C-5″

[a] Spectra were recorded at 400 MHz (*J* values are given in Hz). [b] Spectra were recorded at 50 MHz. [c] The assignments may be interchanged. [d] NOE enhancements were observed between CH_3-7 and CH_2-1″ for **1**.

The second compound isolated from *P. galioides*, named galopiperone (**2**), is another new quinone as shown by the IR absorption at 1668 and 1647 cm^{-1} and the two carbonyl signals at δ 184.5 and 182.7 in the ^{13}C-NMR spectrum. The ^1H- and ^{13}C-NMR data of **1** and **2** are very similar (Table 1) and prove the existence of a prenyl group at C-6 and a methyl group at C-5, but the substituents at C-2 and C-3 are modified.

The molecular formula ($C_{22}H_{28}O_3$) of **2**, deduced from the HREIMS, suggests an oxidative cyclization between the geranyl chain and the C-3 OH group. This assertion is confirmed by the disappearance of the conjugated OH group in the UV and IR spectra, and the presence of an oxygen-bearing quaternary carbon at δ 83.4 in the ^{13}C-NMR spectrum. The dihydropyrane ring is further characterized by the typical coupling constant ($J = 10$ Hz) between C-1′ and C-2′, and ^3J correlations in HMBC between H-1′ and both C-3 and C-3′. The cyclization between the C-3 OH group and the C-3′ position of the geranyl chain is also proved by the significant downfield shift (+9.3 ppm) of the C-10′ methyl group in the ^{13}C-NMR spectrum.

The absence of specific rotation for **2** indicates that the oxidative cyclization of **1** into **2** is not stereoselective and suggests that galopiperone (**2**) could be an artifact. This hypothesis is confirmed by the spontaneous conversion of piperogalone into galopiperone after a few weeks, even at +4 °C.

Hydropiperone (**3**) was obtained as a yellow oil, whose elemental composition was determined as $C_{22}H_{32}O_3$ by HREIMS, two mass units more than **1**. The IR spectrum shows characteristic absorption bands for H-bonded OH (ν_{max} 3389 cm^{-1}) and quinonoid CO (1669 cm^{-1}), confirmed by two ^{13}C-NMR resonances at δ 195.6 and 198.7.[4] In comparison with piperogalone (**1**), the ^1H- and ^{13}C-NMR spectra of **3** (Table 2) exhibited similar signals indicative of a *E*-geranyl chain. The resonances of a prenyl moiety are also observed, but the methylene group appears as part of an AMX system at δ 2.37 and 2.24, indicating the absence of free rotation between the quinonoid nucleus and the prenyl chain. On the EIMS, the base peak at m/z 275 is presumably due to the loss of the prenyl side chain and confirms the presence of this group.

The reduction of one double bond in the quinone nucleus, suggested by the IR spectrum, the molecular formula, and the downfield shift of the two carbonyl groups in the ^{13}C-NMR spectrum, is confirmed by the observation of two high-field resonances at δ 47.0 and 48.0, corresponding to a quaternary carbon and an inequivalent methylene group (δ ^1H 2.65 and 2.75), respectively.

The structure for hydropiperone, therefore, can be proposed as **3**. The respective positions of the substituents were determined on the basis of HMBC correlations, which indicated the position of the geranyl chain at C-6, of the OH group at C-5, and of both the methyl group and the prenyl chain at C-3.

The geminal position of the two latter groups was substantiated by NOE enhancements between the methyl group, the prenyl methylene, and the methylene at C-2 (Table 2). The NOE experiments also provided some information about the molecular conformation. Irradiation of the C-7 methyl group resulted in the enhancement of the methylene groups at C-2 (δ 2.75 and 2.65) and C-1″ (δ 2.37 and 2.24), suggesting the equatorial position of the methyl group. Significant enhancements were also observed between the C-5″ methyl group (δ 1.56) and the methylene at C-1″ (δ 2.24 and 2.37), indicating a *cis* position of this methyl.

Hydropiperone was isolated as a racemic mixture, as shown by the absence of specific rotation.

Piperogalone (**1**), galopiperone (**2**), and hydropiperone (**3**) belong to the rare class of prenylated quinones or dihydroquinones.[5−7] They are probably derived biogenetically from the prenylated diphenols reported earlier in the same plant,[1] by oxidative procedures accompanied by the introduction of a prenyl chain at C-5 or C-6 of the diphenol precursor.

Despite the very small amount of compounds available, we investigated the antiparasitic activity for two of them, according to Mahiou *et al.*[1], Hocquemiller *et*

Table 2. NMR Data for 2,3-Dihydroquinone **3** (in CD_3COCD_3)

position	δ ^{1}H[a]	δ ^{13}C[b]	HMBC (H to C)[a]	NOESY[a]
1		195.6		
2	2.65 d (16)	48.0	C-1, C-3, C-4, C-7, C-1″	H-7
	2.75 d (16)			
3		47.0		
4		198.7		
5	OH 2.84 s	153.9		
6		125.3		
7	1.22 s	23.2	C-2, C-4, C-1″	H-2, H-1″
1′	3.09 d (7)	21.9	C-1, C-5, C-6, C-2′, C-3′	H-2′, H-10′
2′	5.14 br t (7)	119.9		H-1′, H-4′
3′		135.8		
4′	1.95 t (7)	39.3	C-2′, C-3′, C-5′, C-6′, C-10′	H-2′, H-6′
5′	≈2.00 m	26.2	C-4′, C-6′, C-7′	H-6′, H-10′
6′	5.07 m	123.9		H-4′, H-5′
7′		129.6		
8′	1.62 d (≈1)	24.6[c]		
9′	1.58 br s	16.5[d]		
10′	1.72 br s	15.1		H-1′, H-5′
1″	2.37 dd (16, 8)	37.9	C-2, C-4, C-7, C-2″, C-3″	H-7, H-2″
	2.24 dd (16, 8)			
2″	5.03 m	118.5		H-1″, H-4″
3″		135.2		
4″	1.65 br s	24.8[c]		H-2″
5″	1.56 br s	16.7[d]		H-1″

[a] Spectra were recorded at 400 MHz (*J* values are given in Hz). [b] Spectrum was recorded at 75 MHz. [c,d] The assignments may be interchanged.

al.,[8] and Fournet.[9] Galopiperone (**2**) and hydropiperone (**3**) were inactive against the trypomastigote form of *Trypanosoma cruzi*, responsible for Chagas' disease (gentian violet displayed a total lysis of the parasites at 250 μg/mL).[9] Concerning the activity against leishmaniasis, hydropiperone (**3**) displayed a promising toxicity against *Leishmania braziliensis*, *Leishmania donovani*, and *Leishmania amazonensis* at 25 μg/mL, with a total lysis of the parasites at 100 μg/mL. (Pentamidine displayed a total lysis of the parasites at 5 μg/mL and glucantime 40–50% lysis at 100 μg/mL.) These results, however, are somewhat lower than those obtained with the prenylated diphenols precedently reported.[1]

Experimental Section

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 AC 200 P spectrometer operating at 200 and 50 MHz, respectively, and a Bruker ARX 400 spectrometer operating at 400 and 100 MHz, respectively. EIMS and CIMS spectra were obtained on a Kratos MS-80 spectrometer.

Plant Material. *P. galioides*, described by A. Bonpland *et al*. in 1815,[10] was collected by A. Fournet in April 1986 along the old road of Chulumani, near the village of Unduavi, Yungas, department of La Paz, Bolivia (altitude, 3000 m). The botanical identification of the species was made by Dr. H. A. Valdebenito, Department of Botany, Ohio State University, Columbus, OH. A voucher specimen (no. AF 615) is deposited in the National Herbarium of Bolivia, La Paz, and in the Ohio State University Herbarium, Columbus, OH.

Extraction and Isolation. The air-dried *P. galioides* (whole plant, 206 g) was extracted in a Soxhlet apparatus with petroleum ether, CH_2Cl_2, and MeOH successively, which gave 14 g, 9 g, and 14 g of crude extracts, respectively. Si gel GF_{254} was used for TLC. The petroleum extract (7% of dried material) was first purified by a liquid/liquid partition between 1 M NaOH and CH_2Cl_2. The aqueous layer was acidified with 1 M HCl and further extracted with Et_2O. The CH_2Cl_2 extract was submitted to several purifications using flash chromatography on Si gel (0.032–0.063 mm) affording piperogalone (24 mg), galopiperone (12 mg), and hydropiperone (30 mg).

Piperogalone (1): $C_{22}H_{30}O_3$; yellow oil; UV (EtOH) λ max (log ϵ) 204 (4.43), 277 (3.98); (OH^-) 232 (3.96), 280 (3.85) nm; IR (dry film) ν max 3407 (br), 1641, 1620, 1450, 1370, 1309 cm^{-1}; EIMS *m/z* 342.2183 ($M^{\bullet+}$, 8) [342.2194 calcd], 259 (30), 257 (26), 217 (18), 203 (29), 91 (22), 77 (18), 69 (100); CIMS *m/z* 343 (MH^+, 100); for ^{1}H and ^{13}C NMR (CD_3COCD_3) data, see Table 1.

Galopiperone (2): $C_{22}H_{28}O_3$; red oil; $[\alpha]^{20}D$ 0 (*c* 0.07, EtOH); UV (EtOH) λ max (log ϵ) 204 (4.08), 276 (3.52) nm; IR (dry film) ν max 1668, 1647, 1622, 1450, 1370, 1267 cm^{-1}; EIMS *m/z* 340.2022 ($M^{\bullet+}$, 9) [340.2038 calcd], 257 (75), 215 (11), 165 (68), 164 (31), 83 (40), 69 (100); CIMS *m/z* 341 (MH^+, 100); for ^{1}H and ^{13}C NMR (CD_3COCD_3) data, see Table 1.

Hydropiperone (3): $C_{22}H_{32}O_3$; yellow oil, $[\alpha]^{20}D$ 0 (*c* 0.15, EtOH); UV (EtOH) λ max (log ϵ) 201 (4.17), 242 (3.61), 350 (3.50) nm; (OH^-) 205 (3.56), 242 (3.71), 352 (3.62); IR (dry film) ν max 3389 (br), 1669, 1377, 1055 cm^{-1}; EIMS *m/z* 344.2365 ($M^{\bullet+}$, 4) [344.2351 calcd], 276 (20), 275 (100), 207 (76), 153 (54), 137 (20), 123 (20), 69 (99); CIMS *m/z* 345 (MH^+, 100); for ^{1}H and ^{13}C NMR (CD_3COCD_3) data, see Table 2.

Biological Assays. *In Vitro* Study on the Promastigote Form of *Leishmania*.[8] Hydropiperone (**3**) was dissolved in DMSO and evaluated against the promastigote forms of *L. amazonensis* (strain LV-79 = IFLA/BR/67/PH8), *L. donovani* (strain PP-75 = MHOM/IN/83/HS70), and *L. braziliensis* (strain 2903 = MHOM/RR/M2903). Microorganisms are deposited in the Instituto de Investigaciones en Ciencias de la Salud (IICS), Asunciòn, Paraguay. Each assay was performed three times. The viability of the parasites was estimated by direct observation with an inverted microscope after a 24-h incubation at 28 °C. The positive controls were glucantime (Rhône-Poulenc, France) and pentamidine (May and Baker, UK).

***In Vitro* Study on the Trypomatigote Form of *T. cruzi*.**[9] Galopiperone (**2**) and hydropiperone (**3**) were dissolved in DMSO and evaluated against the trypomatigote forms of *T. cruzi* (strain Y). The microorganism is deposited in the Instituto de Investigaciones en Ciencias de la Salud (IICS), Asunciòn, Paraguay. Each assay was performed three times. The parasites were counted after 24 h incubation at 4 °C. The positive control was gentian violet.

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References and Notes

(1) Mahiou, V.; Roblot, F.; Hocquemiller, R.; Cavé, A.; Rojas de Arias, A.; Inchausti, A.; Yaluff, G.; Angelo Barrios, A.; Fournet, A. *J. Nat. Prod.* **1995**, *58*, 324−328.
(2) Bonny, M. L.; Capon, R. J. *J. Nat. Prod.* **1994**, *57*, 539−540.
(3) Ampofo, S. A.; Roussis, V.; Wiemer, D. F. *Phytochemistry* **1987**, *26*, 2367−2370.
(4) Tanaka, H.; Koyama, Y.; Nagai, T.; Marumo, H.; Omura, S. *J. Antibiotics* **1975**, *28*, 868−875.
(5) Thomson, R. H. Naturally Occurring Quinones; Academic Press: London, 1971; pp 93−197.
(6) Thomson, R. H. *Naturally Occurring Quinones III, Recent Advances*; Chapman and Hall: London, 1987; pp 31−71.
(7) Howard, B. M.; Clarkson, K. *Tetrahedron Lett.* **1979**, *46*, 4449−4452.
(8) Hocquemiller, R.; Cortes, D.; Arango, G. J.; Myint, S. H.; Cavé, A.; Angelo, A.; Muños, V.; Fournet, A. *J. Nat. Prod.* **1991**, *54*, 445−452.
(9) Fournet, A. Plantes médicinales boliviennes antiparasitaires (leishmaniose et maladie de Chagas): *Galipea longiflora* Krause (Rutaceae), *Pera benensis* Rusby (Euphorbiaceae) et *Ampelocera edentula* Kuhlm (Ulmaceae). Ph.D. Thesis, Université Paris-Sud, Châtenay-Malabry, France, 1991, pp 83−84.
(10) Bonpland, A.; Kunth, C.; de Humboldt, A. *Nov. Gen. Sp.* **1815**, *71i*, t17.

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