Granulosin, a New Chromone from *Galipea granulosa*

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A novel chromone, granulosin (**1**), has been isolated from the bark of *Galipea granulosa*. The extract of the bark, as well as granulosin (**1**), exhibited lethality in the brine shrimp test. The structure of granulosin (**1**) as 2-propyl-7,8-(methylenedioxy)chromone was established via spectroscopic analysis.

The genus *Galipea* Aubl. (Rutaceae) is composed of approximately 20 species, with these plants being principally found in northern South America.[1] Numerous 2-substituted quinoline alkaloids have been isolated from extracts of *G. bracteata*[2] and *G. longiflora*,[1,3] while extracts of *G. trifoliata* have yielded coumarins[4] and glycosylated flavones.[5,6] Four quinoline alkaloids (2-*n*-propylquinoline, 2-*n*-pentylquinoline, 2-(1′-pentenyl)-quinoline, and 2-[3′,4′-(methylenedioxy)phenethyl]quinoline have been isolated from extracts of the stems of the Brazilian *G. bracteata*.[2] Each of these alkaloids was observed to inhibit the growth of shoots and roots of lettuce, while three of the compounds (the latter three) exhibited molluscicidal activity against the aquatic snail, *Biomphalaria glabrata*.[2] Five novel 2-alkyl- and 2-arylquinoline alkaloids were isolated from extracts of the stembark of the Bolivian medicinal plant, *G. longiflora*.[1] This plant has been traditionally employed in the therapy of parasitic infections, particularly cutaneous leishmaniasis, by the Indians of Bolivia.[1,3] In addition, five other alkaloids were isolated from the same extract, including two 2-alkylquinolines, one 2-arylquinoline, and two furo[2,3-*b*]quinolines.[1] Petroleum ether and $CHCl_3$ extracts of the stem, rootbark, and leaves of *G. longiflora* were noted to possess in vitro inhibitory activity against the growth of *Leishmania braziliensis* and *Trypanosoma cruzi*, organisms responsible for the human protozoan diseases leishmaniasis and South American trypanosomiasis (Chagas' disease), respectively.[3] Bioassay-guided fractionation of these extracts afforded 13 active 2-substituted quinoline alkaloids, including four new 2-alkylquinoline compounds (chimanines A, B, C, and D), eight known 2-substituted quinolines, and one known furo[2,3-*b*]-quinoline.[3,7] 2-Phenylquinoline and 2-*n*-pentylquinoline, the major alkaloids of this extract, were evaluated for in vivo antiprototozal activity in mice infected with *Leishmania amazonensis*. 2-Phenylquinoline was observed to be as potent as the reference antiprotozoan drug, meglumine antimonate, against one strain of the organism, but less active than the reference drug against a second strain, while 2-*n*-pentylquinoline was found to be inactive.[7] Three coumarins were isolated from petroleum ether extracts of the rootbark and stembark of the French Guyanan *G. trifoliata*, including the novel coumarin, galipein.[4] In a subsequent study, six flavone *C*-glycosides were isolated from extracts of the stembark of *G. trifoliata*, including two new compounds, 6,8-di-*C*-glucosylgenkwanin and 2″-*O*-xylosyl-6,8-di-*C*-glucosylgenkwanin.[5] Some 5 years later, four glycoflavones were also isolated from the stembark, including the novel 2″-*O*-*β*-xylosyl-8-*C*-*β*-D-galactosylapigenin.[6]

In our continuing investigation of the medicinal plants of Costa Rica, we have isolated and identified a new chromone, granulosin (**1**), from an EtOH extract of the bark of *Galipea granulosa* Kallunki. The extract, displaying lethality in the brine shrimp lethality test (BST 30 ppm),[8] was originally worked up in a manner consistent for isolation of alkaloids via treatment with dilute HCl, filtration, alkalinization with NH_4OH, and extraction with $CHCl_3$. Chromatography of the $CHCl_3$ extract (BST 22 ppm) over Si gel, and elution with $CHCl_3$ followed by $CHCl_3$−MeOH mixtures afforded granulosin (BST 6 ppm), mp 102−103 °C. The UV spectrum was characteristic of a chromone (benzo-*γ*-pyrone) and displayed absorption maxima at 317 nm (sh) (log ϵ 3.59), 257 (4.33), 251 (4.31), 237 (sh) (4.19), and 225 (4.27).[9−11] In addition, strong FTIR bands observed at 1654 (C═O), 1636 (C═C), 1610 (Ar), and 1499 cm^{-1} (Ar) were also consistent for a chromone.[11,12] The HREIMS displayed a parent ion and base peak at *m*/*z* 232 (obsd 232.0730; calcd 232.0736 for $C_{13}H_{12}O_4$), with other significant fragment ions at *m*/*z* 204 (69%)-(obsd 204.0417; calcd 204.0423 for $C_{11}H_8O_4$) and 164 (84)(obsd 164.0109; calcd 164.0110 for $C_8H_4O_4$). The prominent EIMS fragment ions at *m*/*z* 204 ($M^+ - [CH_2{=}CH_2]$)(M − 28)and 164 ($M^+ - [CH_3CH_2CH_2C{\equiv}H]$)-

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Table 1. Proton and Carbon Chemical Shift Assignments[a] and Long-range Connectivities Observed in the HMBC Spectrum of Granulosin (**1**)

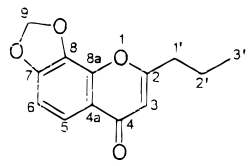
	NMR chemical shift (ppm)[b]		
position	^{1}H (mult, J = Hz)	^{13}C	long-range couplings[c]
2		168.7	H-3, H-1′, H-2′
3	6.07 (s)	108.7	H-1′
4		176.5	H-5
4a		119.12*	H-3, H-6
5	7.74 (d, 8.5)	119.28*	H-6
6	6.90 (d, 8.5)	106.1	
7		151.5	H-5, H-6, H-9
8		133.8	H-6, H-9
8a		140.7	H-5
9	6.16 (s)	102.8	
1′	2.58 (t, 7.3)	35.4	H-3, H-2′, H-3′
2′	1.77 (m)	19.6	H-1′, H-3′
3′	1.01 (t, 7.3)	13.0	H-1′, H-2′

[a] The ^{1}H−^{13}C correlations were based on a HMQC spectrum (DMSO-d_6). [b] An asterisk indicates that chemical shifts are interchangeable. [c] Long-range couplings were based on a HMBC spectrum (DMSO-d_6) and are specified from the proton indicated to the specified carbon.

(M-68), arising via facile retro-Diels-Alder fragmentations of **1**, indicated that the presence of a C-2 propyl group (M-28) in a methylenedioxy-containing chromone ring.[13,14] The ^{1}H-NMR spectrum of **1** was relatively simple and indicated the presence of one propyl group attached to a γ-pyrone ring, one aromatic methylenedioxy group, two ortho-coupled aromatic protons, and one proton as part of a γ-pyrone ring (ene−one system). The presence of a lowfield aromatic doublet at δ 7.74 was characteristic of an aromatic proton in a peri-relationship with a carbonyl group, and suggested the placement of this proton at C-5.[15] Furthermore, because this proton was ortho-coupled to its H-6 neighbor at δ 6.90, this directed the placement of the methylenedioxy function to the C-7 and C-8 position. Finally, the one-proton singlet at δ 6.07 was characteristic of the H-3 proton in benzo-γ-pyrones.[11] The ^{13}C-NMR spectrum revealed the presence of 11 distinct carbon resonances, with two additional partially overlapping resonances (δ 119.12 and 119.28). A close inspection of the spectrum revealed the presence of one methyl (δ 13.0) and two methylenes (δ 19.6 and 35.4) that form the propyl group. In addition, the aromatic region contained a total of seven resonances, including three protonated carbons (δ 106.1, 108.7, and 119.28), and four quaternary carbons (δ 119.12, 133.8, 140.7, and 151.5). The methylene carbon of the methylenedioxy group was observed at δ 103.8, and the benzo-γ-pyrone carbonyl carbon at δ 176.5. Finally, the presence of the downfield C-2 resonance at δ 168.7 was characteristic of this carbon atom in numerous benzo-γ-pyrones.[11,16,17] Confirmation of the ^{1}H- and ^{13}C-NMR chemical shift assignments was accomplished via the HMQC spectrum, in which one-bond proton−carbon chemical shift correlations were established, and via the HMBC spectrum in which long-range heteronuclear correlations were ascertained (Table 1). The structure of granulosin (**1**) was thus assigned as 2-propyl-7,8-(methylenedioxy)chromone. To our knowledge, this is both the first reported isolation of this chromone from nature and the first report of constituents from *Galipea granulosa*.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns hot-stage apparatus



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and are uncorrected. The UV spectrum was recorded on a Hewlett-Packard HP-845 UV-vis spectrophotometer, while the FTIR spectrum was determined on a Nicolet, Impact 410 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker Model WH-300 (300 MHz) spectrometer, with $CDCl_3$ as a solvent. The HMQC and HMBC experiments were performed in DMSO-d_6 on a Varian Unity 400 spectrometer operating at a proton observation frequency of 399.952 MHz and equipped with a Z·SPEC MD-400-3 microdual or MID-400-3 micro inverse probe obtained from Nalorac Cryogenics Corp. (Martinez, CA). The EIMS were recorded on a Fison VG Autospec Spectrometer (high resolution) or a Fison VG Analytical 70-G Spectrometer. GCMS was performed on a Hewlett-Packard 5890 GC connected to a Hewlett-Packard 5971a mass spectrometer. Column chromatography was carried out on Si gel 60 (70−230 mesh; Merck, Darmstadt, Germany), and column fractions were monitored via TLC over precoated sheets of Si gel 60 F_{254} (0.2-mm layer thickness) (E. Merck) under UV (254 and 365 nm) and by Dragendorff reagent.

Plant Material. *G. granulosa* Kallunki was collected in March 1993, at San Ramón, Alajuela, Costa Rica, and identified by Dr. Jorge Gómez-Laurito, Herbarium of the National Museum (CR), San Jose, and Herbarium of the University of Costa Rica (USJ). A voucher specimen (no. 50088-USJ) is on deposit at the Herbarium of the University of Costa Rica (USJ).

Extraction and Isolation. The air-dried, ground bark (400 g) was exhaustively extracted by percolation with EtOH, and the solvent was evaporated to afford a dark brown residue (20 g) that displayed lethality in the brine shrimp test (BST 30 ppm). The residue was suspended in 5% HCl (400 mL), filtered, alkalinized with NH_4OH, and extracted with $CHCl_3$ (3 × 300 mL). The $CHCl_3$ extracts were pooled, dried (anhydrous Na_2SO_4), and filtered, and the filtrate was evaporated to afford a residue (2.5 g)(BST 22 ppm). This residue was chromatographed over Si gel (75 g) in $CHCl_3$, and elution was achieved via $CHCl_3$ and $CHCl_3$−MeOH mixtures of increasing polarity. Elution with $CHCl_3$−MeOH (95:5) afforded several pooled fractions that deposited crystalline material upon evaporation. Repeated recrystallization of this crystalline material from MeOH afforded granulosin (**1**) (about 500 mg) (BST 6 ppm).

Granulosin (1): translucent white plates, mp 102−103 °C; IR ν max (film, NaCl) 1654, 1636, 1610, 1499, 1458, 1394, 1334, 1294, 1257, 1213, 1175, 1117, 1068, 1025, 980, 929, 826, and 747 cm^{-1}; UV λ max (MeOH) (log ϵ) 317 (sh) (3.59), 257 (4.33), 251 (4.31), 237 (sh) (4.19), and 225 (4.27) nm; ^{1}H- and ^{13}C-NMR data, see Table 1; EIMS m/z 232 (M^+, 100), 217 (M^+ − Me, 3), 204 (M^+ −[CH_2=CH_2], 69), 175 (15), 164 (M^+ −[CH_3-CH_2CH_2C≡H], (84), 136 (6), 106 (15), 94 (6), 78 (9); HREIMS m/z [M^+] 232.0730 ($C_{13}H_{12}O_4$ requires

232.0736), 204.0417 ($C_{11}H_8O_4$ requires 204.0423), 164.0109 ($C_8H_4O_4$ requires 164.0110).

Brine Shrimp Assay. The extract, fractions, and compound were evaluated for lethality in the brine shrimp lethality test using previously described methods.[8]

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