

Supporting Information

General Experimental Procedures. NMR spectra were recorded on a Varian 300 spectrometer operating at 300 MHz for ^1H and at 75 MHz for ^{13}C NMR spectra, respectively. COSY and NOESY spectra were recorded with standard pulse set up in the Varian program. Chloroform- d_1 and methanol- d_4 were used as solvent and TMS as internal standard. The optical rotation was determined on a Perkin-Elmer 241 polarimeter. *n*-Hexane, MeOH, MeCN, CH_2Cl_2 , CHCl_3 and DMSO were all of HPLC grade from Merck (Darmstadt, Germany). D-Galactose and glycerol were of highest quality from Fluka (Buchs, Switzerland) and aqueous NaOH (50%) was obtained from J.T. Baker (Netherlands). Open-column chromatography was performed with silica gel 60 (63–200 μm , Merck). TLC: silica gel 60 F₂₅₄ Al sheets (0.1 mm) (Merck), developed using 10% H_2SO_4 in MeOH followed by heating. Preparative HPLC was carried out using a Merck L-6200 intelligent pump equipped with a Merck L-4200 UV detector. Separations were performed on a RP- C_{18} column (particle size 5 μm ; 250 × 20 mm i.d., Develosil ODS-HG-5, Nomura Chemical Co., Japan) protected with a guard cartridge (50 × 20 mm i.d.) packed with the same material as the column. Analytical HPLC was carried out on a SUMMIT/Dionex HPLC system (Dionex Denmark A/S, Denmark) equipped with a diode array detector (DAD) operating between 200–650 nm. Separations were performed on a LiChrospher 100 RP- C_{18} (particle size 5 μm ; 244 × 4 mm i.d., Merck) column. High performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) was carried out on a Dionex series 300DX ion chromatograph system using a CarboPac PA10 column (250 × 4 mm i.d., Dionex Denmark A/S, Denmark). Eluant 52 mM NaOH and flow rate 1.5 mL/min.

Extraction and Isolation: Dried and milled fruits of dog rose (1 kg) were sequentially extracted with *n*-hexane, CH₂Cl₂, MeOH and water. The dog rose powder was first submerged in *n*-hexane (2 L) overnight at room temperature, filtered and the powder washed with *n*-hexane (2 × 500 mL). The combined *n*-hexane solutions were evaporated to dryness under reduced pressure at below 40 °C. The powder was then submerged in CH₂Cl₂, MeOH and water, subsequently, following the same procedure as described above for extraction with *n*-hexane. The resulting *n*-hexane (30 g), CH₂Cl₂ (10 g), MeOH (35 g) and water extracts (125 g) were tested for inhibition of chemotaxis of human peripheral blood neutrophils *in vitro*. The activity was confined to the CH₂Cl₂ extract which was subjected to silica gel (400 g) open-column chromatography (column dimensions, 5 × 50 cm), eluting with a stepwise gradient of CH₂Cl₂-MeOH mixtures (100 mL 100:0, 100 mL 99:1, 100 mL 98:2, 150 mL 95:5, 150 mL 90:10, 450 mL 80:20, 950 mL 0:100) to give 20 fractions (fr. 1 to fr. 20, each fraction 100 mL). The individual fractions were concentrated *in vacuo* (below 40 °C) and tested for inhibition of chemotaxis of human peripheral blood neutrophils *in vitro*. The activity appeared to be confined to one major constituent in fr. 10-12 as shown by TLC (CH₂Cl₂-MeOH-H₂O, 70:30:3, *R_f* 0.46) and HPLC-DAD (see below). Fr. 10-12 (850 mg) was further separated by preparative HPLC on a RP-C₁₈ column eluting with a stepwise MeCN-H₂O gradient (150 mL 25:75; 150 mL 50:50; 200 mL 60:40; 200 mL 70:30; 200 mL 80:20; 350 mL 90:10 and 400 mL 100:0, column temperature 35 °C, flow rate = 7 mL/min, UV detection at 203 nm, injection volume = 5 mL) to give 14 fractions (fr. 10-12.1 to fr. 10-12.14) of which only fr. 10-12.11 (*t_R* ~ 163-179 min, 1145-1255 mL, fraction volume = 110 mL) showed high activity. The active principle in fr. 10-12.11 was found to be confined to one compound that was obtained as a colorless oil (250 mg) and identified as (2*S*)-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-

trienoyl]-3-*O*- β -D-galactopyranosyl glycerol (**1**). The purity of the galactolipid **1** (> 98%) was determined by analytical HPLC-DAD using a LiChrospher RP-C₁₈ column and eluting with a MeCN–20% MeCN (aq) gradient (0–10 min (0:100), 10–25 min (from 0:100 to 50:50), 25–55 min (from 50:50 to 100:0), 55–64 min (100:0), gradient linear programmed, column temperature 35 °C, flow rate = 1 mL/min, injection volume = 20 μ l, UV detection at 203 nm, t_R (compound **1**) = 54 min). The structure of **1** was identified from the ¹H- and ¹³C-NMR data (see Table I and Table II) and $[\alpha]_D^{26}$ ($[\alpha]_D^{26}$ -3.0° (c 0.4 CHCl₃)) and by comparison with literature data.^{1–3} The structure of **1** was further confirmed by basic methanolysis and acidic hydrolysis. Basic methanolysis² yielded methyl linolenate as the only methyl ester as shown by GC-MS, whereas acid hydrolysis in 4 N HCl afforded D-galactose and glycerol as shown by HPAEC-PAD.

Table I. $^1\text{H-NMR}$ spectral data (300 MHz, CDCl_3 and CD_3OD , δ -values in ppm) for compound **1**.

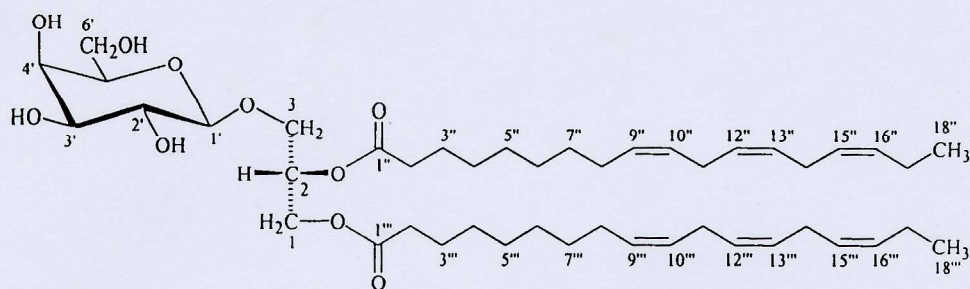
H	δ_{H} (multiplicity, J in Hz) in CDCl_3	δ_{H} (multiplicity, J in Hz) in CD_3OD
1'	4.24 (<i>d</i> , 6.8)	4.23 (<i>d</i> , 6.6)
2'	3.63 (<i>m</i>)	3.51 (<i>dd</i> , 6.6, 9.7)
3'	3.54 (<i>m</i>)	3.45 (<i>dd</i> , 2.1, 9.7)
4'	3.91 (<i>m</i>)	3.84 (<i>dd</i> , 0.5, 2.1)
5'	3.58 (<i>m</i>)	3.48 (<i>m</i>)
6'a	3.88 (<i>m</i>)	3.73 (<i>dd</i> , 6.6, 12.0)
6'b		3.75 (<i>dd</i> , 4.4, 12.0)
1a	4.22 (<i>dd</i> , 6.8, 12.0)	4.22 (<i>dd</i> , 6.9, 12.0)
1b	4.40 (<i>dd</i> , 2.0, 12.0)	4.43 (<i>br d</i> , 12.0)
2	5.28 (<i>m</i>)	5.27 (<i>m</i>)
3a	3.72 (<i>dd</i> , 6.0, 11.0)	3.71 (<i>dd</i> , 5.4, 11.0)
3b	3.99 (<i>m</i>)	3.98 (<i>dd</i> , 5.4, 10.8)
2'',2'''	2.32 (<i>br t</i> , 7.0)	2.32 (<i>br t</i> , 7.0)
3'',3'''	1.60 (<i>m</i>)	1.60 (<i>m</i>)
4''-7'', 4'''-7'''	1.32 (<i>m</i>)	1.35 (<i>m</i>)
8'',17'',8''',17'''	2.08 (<i>m</i>)	2.08 (<i>m</i>)
9'',10'',12'',13'',15'',16''	5.36 (<i>m</i>)	5.35 (<i>m</i>)
9''',10''',12''',13''',15''',16'''		
11'',14'', 11''', 14'''	2.81 (<i>br t</i> , 6.8)	2.82 (<i>br t</i> , 6.8)
18'', 18'''	0.97 (<i>t</i> , 7.5)	0.98 (<i>t</i> , 7.5)

Abbreviations for multiplicity: *d* = doublet, *dd* = double doublet, *m* = multiplet, *t* = triplet.
br = broad. Assignments based on $^1\text{H-}^1\text{H-COSY}$ and $^1\text{H-}^1\text{H-NOESY}$ NMR experiments.

Table II. ^{13}C -NMR spectral data (75 MHz, CDCl_3 and CD_3OD , δ -values in ppm) for compound 1.

Assignments	Multiplicity*	δ_{C} (CDCl_3)	δ_{C} (CD_3OD)
C-1'	<i>d</i>	104.3	106.3
C-2'	<i>d</i>	71.6	73.3
C-3'	<i>d</i>	73.7	75.7
C-4'	<i>d</i>	69.5	71.1
C-5'	<i>d</i>	74.8	77.7
C-6'	<i>t</i>	62.4	63.3
C-1	<i>t</i>	63.1	64.9
C-2	<i>d</i>	70.4	72.7
C-3	<i>t</i>	68.4	69.6
C-1'', C-1'''	<i>s</i>	174.1, 173.7	176.1, 175.8
C-2'', C-2'''	<i>t</i>	34.5, 34.3	36.0, 35.8
C-3'', C-3'''	<i>t</i>	25.1 ^a	26.8 ^a
C-4'', C-4'''	<i>t</i>	29.8 ^b	31.6 ^b
C-5'', C-5'''	<i>t</i>	29.5 ^b	31.2 ^b
C-6'', C-6'''	<i>t</i>	29.4 ^b	31.1 ^b
C-7'', C-7'''	<i>t</i>	29.3 ^b	31.0 ^b
C-8'', C-8'''	<i>t</i>	27.4	29.0
C-9'', C-9'''	<i>d</i>	132.2 ^c	133.8 ^c
C-10'', C-10'''	<i>d</i>	130.4 ^c	132.1 ^c
C-11'', C-11'''	<i>t</i>	25.9 ^a	27.4 ^a
C-12'', C-12'''	<i>d</i>	128.6 ^c	130.2 ^c
C-13'', C-13'''	<i>d</i>	128.5 ^c	130.2 ^c
C-14'', C-14'''	<i>t</i>	25.8 ^a	27.3 ^a
C-15'', C-15'''	<i>d</i>	128.0 ^c	129.9 ^c
C-16'', C-16'''	<i>d</i>	127.3 ^c	129.2 ^c
C-17'', C-17'''	<i>t</i>	20.8	22.3
C-18'', C-18'''	<i>q</i>	14.5	15.5

*Multiplicity determined by DEPT and HETCOR-NMR experiments. Abbreviations for multiplicity: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet. ^{a, b, c} In the same column: These assignments may be interchanged.



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References

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2. Murakami, A.; Nakamura, Y.; Koshimizu, K.; Ohigashi, H. *J. Agric. Food Chem.* **1995**, *43*, 2779–2783.
3. Olugbade, T. A.; Adesanya, S. A. *Phytochemistry* **2000**, *54*, 867–870.