An Antiinflammatory Galactolipid from Rose Hip (*Rosa canina*) that Inhibits Chemotaxis of Human Peripheral Blood Neutrophils in Vitro

Erik Larsen,[†] Arsalan Kharazmi,[‡] Lars P. Christensen,*[,†] and S. Brøgger Christensen[§]

Department of Food Science, Danish Institute of Agricultural Sciences, Research Centre Aarslev, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark, Department of Clinical Microbiology, University Hospital (Rigshospitalet), Tagensvej 20, DK-2200, Copenhagen, Denmark, and Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100, Copenhagen Ø, Denmark

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The galactolipid (2*S*)-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-3-*O*-*β*-D-galactopyranosyl glycerol (**1**) isolated from dried and milled fruits of *Rosa canina* by bioassay-guided fractionation is an antiinflammatory agent with inhibitory effects on chemotaxis of human peripheral blood neutrophils in vitro. The inhibition of cell migration is not related to toxicity. The presence of **1** in rose hips may explain the clinically observed antiinflammatory properties of rose hip herbal remedies.

A recent randomized, placebo-controlled, double-blind clinical study has shown that standardized preparations of the fruits of dog rose (*Rosa canina* L., Rosaceae) or just rose hips improve mobility and reduce pain in the knee and hip joint of patients with osteoarthritis.[1] In contrast to the presently used drugs, e.g., aspirin or corticosteroids, no side effects were observed after treatment with rose hips.[1] Polymorphonuclear leukocytes (PMNs) and monocytes are involved in the inflammatory process and tissue damage in inflammatory diseases such as osteoarthritis and arteriosclerosis.[2,3] The damage is caused by the release of proteolytic and hydrolytic enzymes as well as toxic reactive oxygen radicals in the tissue and joints.[4] Since extracts of rose hips have been shown to inhibit chemotaxis of peripheral blood PMNs and monocytes in vitro,[3,5] the ability of rose hip extracts and fractions of the extracts to reduce chemotaxis of PMNs was used as the basis for an assay for isolation of an antiinflammatory agent.

Dried and milled fruits of dog rose were sequentially extracted with *n*-hexane, CH_2Cl_2, MeOH, and water, and the extracts evaporated in vacuo to dryness. Following testing of the resulting residues in the bioassay it was found that the activity was confined to the CH_2Cl_2 extract. Bioassay-guided chromatographic fractionation of the CH_2Cl_2 extract by open-column chromatography (CC) on silica gel followed by preparative HPLC showed that the activity was exclusively confined to a single compound that was 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**) (Figure 1). The identification of the fatty acid residues and the sugar moiety on the active galactolipid **1** was achieved by characterization of the fatty acid obtained by basic methanolysis and D-galactose obtained by acidic hydrolysis, respectively. Consistency between the published 1D and 2D NMR data and the optical rotation reported for **1** and that observed for the isolated compound finally established the structure.[6a−c] The configuration at C-2 in the glycerol moiety of **1** is presumed to be *S* on the basis of a comparison of the specific rotation with literature values.[6b] Compound **1** has previously been isolated from various plant sources[6] and from cyanobacterium (*Phormidium tenue*),[7] but this is the first time that **1** has been described in the Rosaceae family. Compound **1** has been shown to possess antitumor-promoting properties,[7,8] as well as antiinflammatory effects[6b] that are not related to inflammatory conditions, such as osteoarthritis and arteriosclerosis.

Compound **1** showed a strong inhibitory effect on the migration of human peripheral blood PMNs toward the chemotactic factor zymosan-activated human serum (ZAS) with over 60% inhibition from 100 down to 1 μg/mL (Experimental Section). Cell viability tests showed that the PMNs cells were viable even at concentrations between 50 and 100 μg/mL of **1**, which strongly inhibited chemotaxis, indicating that the inhibition of cell migration is not related to toxicity and that compound **1** is not toxic at any of the tested concentrations (Experimental Section). Consequently, compound **1** may explain the antiinflammatory properties of rose hip, and hence is a possible candidate for the development of new drugs to treat symptoms associated with inflammatory diseases, such as osteoarthritis and arteriosclerosis.

Experimental Section

General Experimental Procedures. Silica gel (63−200 μm, Merck) was used for CC. Preparative HPLC was carried out using a Merck L-6200 intelligent pump equipped with a Merck L-4200 UV detector and a Develosil ODS-HG-5 RP-18 column (5 μm; 250 × 20 mm, Nomura Chemical Co.), flow = 7 mL/min, detection at 203 nm. HPLC-DAD was performed with a LiChrospher 100 RP-18 column (5 μm; 244 × 4 mm, Merck) using a MeCN−20% MeCN(aq) gradient (0:100 → 100:0) in 64 min, detection at 203 nm, flow = 1 mL/min.

Plant Material. Dried and milled fruits of dog rose were obtained from Hyben Vital International ApS (Tullebølle, Langeland, Denmark) and are identical with the commercially available product. A voucher is deposited at the Department of Ecology, Botanical Section, Royal Veterinary and Agricultural University, Frederiksberg C, Denmark (No. LPC 001).

Extraction and Isolation. Dried and milled fruits of dog rose (1 kg) were sequentially extracted with 3 L of *n*-hexane, CH_2Cl_2, MeOH, and H_2O. The CH_2Cl_2 extract (10 g) was separated by CC with a CH_2Cl_2−MeOH gradient (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 of 100 mL each. The antiinflammatory activity was confined to fractions 10−12. Fractions 10−12 (850 mg) was further fractionated by preparative HPLC using a MeCN−H_2O gradient (1:3, 1:1, 6:4, 7:3, 4:1, 9:1, 10:0) to give 14 fractions, of which only fraction

* To whom correspondence should be addressed. Tel: +45 63 90 43 43. Fax: +45 63 90 43 95. E-mail: larsp.christensen@agrsci.dk.
[†] Danish Institute of Agricultural Sciences.
[‡] University Hospital (Rigshospitalet).
[§] The Danish University of Pharmaceutical Sciences.

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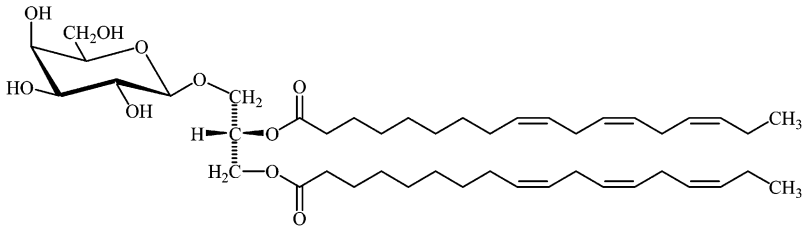

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Figure 1. Chemical structure of the antiinflammatory galactolipid **1** isolated from rose hip.

11 (=10−12.11) showed high activity. Fraction 10−12.11 was found to consist of only compound **1** ($t_R \approx 163-179$ min; 250 mg). The purity of **1** (>98%) was determined by HPLC-DAD (**1**, $t_R = 54$ min).

(2*S*)-1,2-Di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-3-*O*-*β*-D-galactopyranosyl glycerol (1): colorless oil; $[\alpha]^{26}_D$ −3.0° (*c* 0.4 $CHCl_3$), lit.[6b] −4.0° (*c* 0.48 $CHCl_3$). The structure was identified from the 1D and 2D NMR data (^1H, ^{13}C, $^1H-^1H$-COSY, $^1H-^1H$-NOESY, HETCOR) and by comparison with literature data[6a−c] and by basic methanolysis and acidic hydrolysis. Basic methanolysis[6b] yielded methyl linolenate as the only methyl ester, whereas acid hydrolysis afforded D-galactose and glycerol.

Polymorphonuclear Leukocytes (PMNs). PMNs were isolated from the peripheral blood of healthy individuals in citrated glass. The cells were separated by dextran density gradient and lymphoprep separation.[9] The purity of PMNs was greater than 98%. Cell viability of isolated PMNs as well as PMNs in preparations containing compound **1** (50 and 100 μg/mL) and in the control (DMSO) was nearly 100% as determined by the trypan blue exclusion method. All experiments were performed twice in duplicate.

Bioassay for Chemotaxis. Compound **1** was dissolved in DMSO (20 mg/mL) and diluted in minimal essential medium,[5] to final concentrations of 100, 50, 10, 1, and 0.1 μg/mL for use in the cell function assays. The bioassay was performed using a modified Boyden chamber technique as previously described.[9] The purified PMNs were preincubated with the different dilutions of **1** for 30 min at 37 °C. Following preincubation, the chemotaxis of the cells toward the chemotactic factor ZAS was tested. The inhibiting activity of **1** at 100, 50, 10, 1, and 0.1 μg/mL was 82, 77, 62, 64, and 7%, respectively. The control (DMSO) showed no inhibiting activity. The migrated cells were counted by a computer-assisted image analysis system.[9] All experiments were performed twice in duplicate (data presented are mean values).

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Supporting Information Available: Detailed description of the isolation procedure and identification data (tabulated ^1H and ^{13}C NMR data in $CDCl_3$ and CD_3OD) for compound **1**.

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