Activity guided fractionation and structure elucidation of GOPO

Dried and milled fruits of rose hip (1000 g) were sequentially extracted with *n*-hexane, CH_2Cl_2, MeOH and water. The rose hip 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 CH_2Cl_2, MeOH and water, subsequently, following the same procedure as described above for extraction with *n*-hexane. The resulting *n*-hexane (30 g), CH_2Cl_2 (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 CH_2Cl_2 extract which was subjected to silica gel (400 g) open column chromatography (column dimensions, 5 × 50 cm), eluting with a stepwise gradient of CH_2Cl_2–MeOH mixtures (100:0, 99:1, 98:2, 95:5, 90:10, 80:20, 0:100) to give 20 fractions (F1 to F20, 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 F10–F12 as shown by TLC (CH_2Cl_2/MeOH/H_2O, 70:30:3, R_f 0.46) and analytical HPLC (see below). Fractions 10–12 (850 mg) was further separated by preparative HPLC using a RP-C_{18} column eluting with a stepwise CH_3CN–water gradient (25:75; 50:50; 60:40; 70:30; 80:20; 90:10 and 100:0, column temperature: 35 °C, flow rate: 7 ml/min, UV detection: 203 nm, injection volume: 5 ml) to give 14 fractions (F10–12.1 to F10–12.14) of which only F10–12.12 (t_R ~ 140–150 min) that eluted between 90–100% CH_3CN showed high activity. The active principle in F10–12.12 was found to be confined to one compound that was obtained as a colorless oil (250 mg) and identified as (2*S*)-3-*O*-*β*-D-galactopyranosyl-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-*sn*-glycerol. This compound was designated as GOPO. The purity of GOPO (> 98%) was determined by analytical HPLC-DAD with a 100% CH_3CN–20% CH_3CN (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 µl, UV detection: 203 nm, t_R (GOPO) 54 min), and tested for the inhibition of chemotaxis of human peripheral blood neutrophils *in vitro* at the following concentrations: 100, 50, 10, 1 and 0.1 µg/ml. The characterization of the active principle from *R. canina* was performed by 1D- and 2D-NMR experiments as well as by comparisons of its physical