SHORT COMMUNICATION

Isolation of Linoleic and \( \alpha \)-Linolenic Acids as COX-1 and -2 Inhibitors in Rose Hip

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Rose hip has previously shown clinical efficacy in the treatment of osteoarthritis, and organic solvent extracts of rose hip have showed inhibition of cyclooxygenase-1 and -2. A petroleum ether extract of rose hip was fractioned by VLC on silica; on a C-18 column and by HPLC. Each step was COX-1/2 activity-guided. The bioassay-guided fractionation led to the isolation of linoleic acid (the IC\(_{50}\) for COX-1 was 85 \( \mu \)M and 0.6 \( \mu \)M for COX-2) and \( \alpha \)-linolenic acid (the IC\(_{50}\) for COX-1 was 52 \( \mu \)M and 12 \( \mu \)M for COX-2). The COX-2/COX-1 ratio was 0.007 for linoleic acid and 0.2 for \( \alpha \)-linolenic acid. Linoleic acid and \( \alpha \)-linolenic acid contribute to the COX-1 and -2 inhibitory activity of rose hip. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: cyclooxygenase; linoleic acid; \( \alpha \)-linolenic acid; Rosa canina; rose hip.

INTRODUCTION

The dry powder of Rosa canina L. fruit (rose hip) is a popular herbal remedy for arthritis. A recent systematic review on the clinical evidence of rose hip and seed indicated that it is effective against osteoarthritis (Chrubasik et al., 2006). An attempt to isolate antichemotactic constituents led to the isolation of galactolipid (1, Fig. 1) from a dichloromethane extract (Larsen et al., 2003). An ethanol extract of R. canina possessed significant inhibitory activity against in vivo inflammatory models, from which it was concluded that the inhibitory activity is possibly obtained through cyclooxygenase inhibition (Orhan et al., 2007). Organic solvent extracts of R. canina showed good inhibition of both COX-1 and 2 (Jäger et al., 2007). Consequently, the observed clinical effect of rose hip powder is not restricted to the presence of 1. In the present study compounds exhibiting COX inhibitory activity were isolated by bioassay-guided fractionation.

MATERIALS AND METHODS

Ground rose hip (Rosa canina L., Rosaceae) powder in the form of the standardized product Langelands-Hyben\(^*\) was obtained from Hyben Vital International ApS, Denmark.

For determination of the best extraction method, rose hip powder (1 g) was extracted with petroleum ether, dichloromethane, methanol and water (10 mL) individually or serially by sonication for 30 min. The extracts were filtered through Whatman No. 1 filter paper and taken to dryness under vacuum.

For isolation, 500 g rose hip powder was extracted with 5 L of petroleum ether. The mixture was sonicated for 30 min, left to macerate overnight and then sonicated for a further 30 min, before filtration through Whatman No. 1 filter paper. The filtrate was taken to dryness under vacuum.

A part of the residue (4 g) was subjected to vacuum liquid chromatography (VLC) on a 200 g silica gel 60 (0.040–0.063 mm) column. The column was eluted with 500 mL petroleum ether followed by 200 mL each of the following ratios of petroleum ether: ethyl acetate (95:5; 90:10; 70:30; 60:40; 50:50; 25:75) and 200 mL ethyl acetate and 200 mL methanol. The fractions were taken to dryness, evaluated by TLC (Merck Silica gel 60 F\(_{254}\) 0.25 mm TLC plate eluted with petroleum ether: ethyl acetate 80:20 and the spots visualized with anisaldehyde–sulfuric acid R and heating) and tested for COX-1 and -2 inhibitory activity.

The active fraction from VLC was dissolved in acetonitrile: 0.1% TFA 90:10 and passed through a...
Bakerbond C18 column. The column was eluted with acetonitrile: 0.1% TFA 90:10. Fractions were evaluated by TLC (Merek RP-18 F_{254}, 0.25 mm TLC plate eluted with acetonitrile: 0.1% TFA 90:10 and derivatized with anisaldehyde–sulphuric acid R and heating) and tested for COX-1 and -2 inhibitory activity.

The active fraction from the C18 column was subjected to preparative HPLC on a Gilson HPLC (Gilson 215 Liquid Handler; Gilson 805 Manometric module; Gilson 306 Pump; Gilson 819 Injection Valve Activation) fitted with a Gilson 170 diode array detector. The column was a XTerra RP18 10 μm (150 mm × 19 mm i.d.). The eluent was acetonitrile: 0.1% TFA 90:10 at a flow rate of 15 mL/min. The fractions were evaluated by TLC on RP-18 plates and tested for COX-1 and -2 inhibitory activity.

Structure elucidation was done by 1H-NMR at 300 MHz on a Varian Mercury instrument. The spectra of deuterated methanol solutions were recorded.

The isolated compounds were converted to their methyl esters by derivatization. The compound (2 mg) was reacted with 20 μg EDAC (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride) and DMAP (4-((dimethylamino)pyridine) (13 mg) in 500 μL methanol. After 30 min reaction time, 2 mL 0.1 M HCl and 1 mL heptane were added and the mixture partitioned. The heptane phase was analysed by GC-MS on an Agilent 6890N Network GC system coupled to a 5973 Network Mass Selective Detector. GC conditions: injector temperature: 150 °C; temperature programme: start 100 °C, 20 °C/min to 300 °C and 300 °C for 5 min; column: HP5MS.

COX-1 and -2 activity were assayed as outlined by Jäger et al. (1996) and Zschocke and Van Staden (2000) for COX-1 and -2, respectively, with COX-1 from sheep seminal vesicles (Sigma) and human recombinant COX-2 (Sigma). Both assays were performed in duplicate with double determinations for each sample per assay. Data were fitted into Grafit® software for estimation of IC_{50} values. The IC_{50} value for indomethacin was 4.3 μM for COX-1 and 144 μM for nimesulide for COX-2.

**RESULTS AND DISCUSSION**

The methanol, dichloromethane and petroleum ether extracts all showed dose-dependent inhibition of both COX-1 and COX-2, whereas the water extract did not show any activity in the assays (results not shown). These results were in accordance with previously obtained results (Jäger et al., 2007). The petroleum ether extract had the lowest IC_{50} values, which were 18 μg/mL for COX-1 and 90 μg/mL for COX-2.

Petroleum ether was therefore used as the extraction solvent in the bioassay-guided isolation. This extract was fractionated by VLC where the active compounds were eluted with petroleum ether: ethyl acetate 25:75 and ethyl acetate. These two fractions were combined and passed through a C-18 column to remove very lipophilic, non-active compounds before fractionation on HPLC. Two peaks, eluting at 17 min and 17.7 min, were active in the COX assays. The two compounds were identified as linoleic acid (2, Fig. 1) and α-linolenic acid (3, Fig. 1) by H-NMR and comparison with published data (Gunstone et al., 1994; Faulh et al., 2000). The structures were confirmed by GC-MS after derivatization to methyl esters. The methyl esters co-chromatographed with authentic standards and the EIMS spectra were superimposable.

The compounds were tested in the COX assays. The IC_{50} values for linoleic acid were 85 μM for COX-1 and 0.6 μM for COX-2, and for α-linolenic acid the values were 52 μM for COX-1 and 12 μM for COX-2. The COX-2/COX-1 ratio was 0.007 for linoleic acid and 0.2 for α-linolenic acid, meaning that both compounds were selective inhibitors of COX-2. Previously, both fatty acids have been shown to be COX inhibitors, with the IC_{50} values for linoleic acid at 170 μM for COX-1 and 94 μM for COX-2, and for α-linolenic acid the values were 93 μM for COX-1 and 12 μM for COX-2, resulting in a COX-2/COX-1 ratio of 0.6 for linoleic acid and 0.1 for α-linolenic acid (Ringbom et al., 2001). The results are in broadly in accordance with the study by Ringbom et al. (2001), except for the IC_{50} value for linoleic acid for COX-2, where our value is two orders of magnitude lower. Therefore authentic standard linoleic acid was tested, and an IC_{50} value obtained that was in accordance with the results.

The crude petroleum ether extract showed predominantly COX-1 activity, whereas the two isolated fatty acids were COX-2 selective. Some of the early-eluting fractions from the VLC possessed moderate COX-1 inhibition, but no COX-2 inhibition. Compounds in these fractions could change the balance causing the crude extract to be predominantly COX-1 active, even though the two isolated fatty acids are COX-2 selective.

TLC analysis showed that the two fatty acids also were present in the dichloromethane and methanol extracts, which could, at least in part, account for the COX-1 and -2 activities of these extracts.

The galactolipid with effect on chemotaxis previously isolated from rose hip (Larsen et al., 2003) did not exhibit COX inhibition in the present study. However, the galactolipid contains two α-linolenoyl residues. If the galactolipid is metabolized in the body in the same way as triglycerides, it is possible that α-linolenic acid formed from the galactolipid could contribute to the COX-inhibitory activity of the extracts.

This study indicates that part of the clinically observed effect on osteoarthritis by rose hip might be due to inhibition of cyclooxygenase 1 and -2 by the fatty acids linoleic acid and α-linolenic acid.

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REFERENCES


