Constituents of *Hypericum laricifolium* and Their Cyclooxygenase (COX) Enzyme Activities

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Investigation of the aerial parts of the medicinal plant *Hypericum laricifolium* led to the isolation of two new natural products, hentriacontanyl caffeate (1a), nonacosanyl caffeate (1b). In addition, stigmasterol, β-sitosterol, 3-epi-betulinic acid (2), caffeic acid (3), ferulic acid, docosanol, p-hydroxybenzoic acid, 3,4-dimethoxy benzoic acid, quercetin (4), quercetin-3-O-galactoside (5), quercetin-3-O-rutinoside (6), quercetin-3-O-rhamnoside (7), quercetin-3-O-glucuronide (8) and shikimic acid were also isolated. The structures were determined by 1D- and 2D-NMR, mass spectrometry, and chemical transformations. The anti-inflammatory effects of the isolated compounds were discussed briefly.

Key words *Hypericum laricifolium*; Guttiferae; caffeate ester; flavonol; cyclooxygenase-1 (COX-1); cyclooxygenase-2 (COX-2)

The genus *Hypericum* (Guttiferae) encompasses approximately 400 species, of which ten morphologically and chemically distinct species grow in Central Europe. Several species have been used in folk medicine. There is a growing interest in constituents of the genus because a number of species have been found to possess various biological properties. Preparations of *H. perforatum* L. are now commercialized in Europe for the management of various depressive disorders. Antifungal γ-pyrone and xanthones, dianthrones (hypericin), spiroterpenoids, antifungal and antimalarial phloroglucinol, and antibacterial phloroglucinols have been reported in the literature for this genus. The investigated plant, *H. laricifolium* H.B.K., with the common name “Romerillo”, has been used in Ecuadorian traditional medicine as a diuretic and for provoking menstruation. This herb was selected for this study primarily because it has not previously been investigated, chemically or pharmacologically. In addition, hyperforin and extracts of the related species, *H. perforatum*, has shown effects on inhibition of COX-1 and 5-lipoxygenase, which prompted an investigation of COX-1 and -2 enzyme activity for *H. laricifolium* as a part of our continuing search for identification of natural products as inhibitors of prostaglandin biosynthesis. This paper reports methods and results of isolating and characterizing 14 compounds from the EtOAc extract of *H. laricifolium*, and of investigating COX-1 and -2 enzymes activity for the extract and isolated compounds.

Results and Discussion

All the known compounds were identified by their spectral properties and, where appropriate, by their melting points and/or optical rotations. In several cases (all unnumbered compounds), identification was confirmed by direct comparison with authentic samples.

From the less polar fractions of the ethyl acetate extract of *H. laricifolium*, a caffeic acid ester of long-chained aliphatic alcohols (1) was isolated as a semisolid. It has been found to be a mixture by analysis of electron impact mass spectrum (EI-MS), which showed a major peak at *m/z* 614, C_{40}H_{70}O_{12}, corresponding to hentriacontanyl caffeate and a minor peak at *m/z* 586, C_{38}H_{66}O_{13} corresponding to nonacosanyl caffeate with approximately ratio 13:3, which was estimated accordin
the tested compounds, quercetin (4) inhibited COX-1 by 44±
2% at 200 μM concentration, and ester caffeates (1) inhibited
the same enzyme at 1000 μM by 52±2%. Indomethacin (1.7
μM) was used as a positive control yielding 43±3% inhibi-
tion of COX-1. Due to the solubility problems of both com-
pounds no IC₅₀ value was obtainable. On the other hand,
caffeic acid (3) inhibited COX-2 by 32±16% at 100 μM concentra-
tion, it showed no dose-dependent inhibi-
tion as we explained in our previous paper.16) The other iso-
lated compounds and the extract showed less than 30% inhibi-
tion of COX-1 and COX-2, and were considered inactive.

Experimental

General Procedures 1D- and 2D-NMR spectra were recorded on a
400 MHz Varian VXR-400 NMR instrument. The EI-MS, and the EI-MS
(with glycercol as matrix) spectra, with a JEOL JMS SX/SX102A instrument.

MPLC was performed using SEPARO AB MPLC equipment (Baeckstrom
SEPARO AB, Lidingo, Sweden). For this, SEPARO variable-length glass
columns with inner diameter of 1.5 or 2.5 cm were used, packed with silica
gel 60, 40–63 mm (Merck). An FMI Labr pump, model QD (Fluid Metering
Inc., Oyster Bay, NY, U.S.A.) was used at a flow rate of 20–30 ml/min.
Frations of 1 ml were collected with a Gilson 201 fraction collector. The
columns were eluted with continuous gradients running from hexane, over
CH₂Cl₂ to MeOH and H₂O afforded by a SEPARO constant-volume-mixing
chamber combined with an open reservoir. The mixing chamber initially
contained 50 ml non-polar solvent, and the reservoir contained the first of
15–20 premixed binary (less polar/more polar solvent) gradient mixtures,
of 20–40 ml each, which were successively fed to the reservoir during the
separation.

Plant Material Hypericum laricifolium H.B.K. consisting of leaves,
stem, and flowers was collected by Dr. Felipe Ghia in July, 1992 at Ha-
cienda El-Tablon, Sitio Palupugil via Quito-Papallacta, 3100 m altitude,
Provincia de Pichincha, Ecuador. Voucher specimens, F.G. 845, are de-
posited in the Herbario Economico, Escuela Politecnica Nacional (EPN),
Quito, Ecuador.

Extraction and Isolation H. laricifolium was dried in the dark at 40°C
in a ventilated hood. The plant (1.78 kg), after being ground, was extracted
(at room temp.) three times with light petroleum (40–60°C) (6 l) and then
three times with ethanol (5 l) for 48 h each time with occasional stirring.
The extracts were evaporated in vacuo to give 15.1 g and 135 g of semisolid ma-
terial respectively. The ethanol extract was chromatographed on silica gel (42 g)
using CH₂Cl₂–EtOAc and purified by re-

Cyclooxygenase-1 and -2 Catalysed Prostaglandin Biosynthesis Assays

Inhibition on COX-1 and COX-2 catalysed prostaglandin biosynthesis in
vitro was performed according to Noreen et al.17) COX-1 (prostaglandin en-
doperoxide H synthase-1) was prepared from bovine seminal vesicles, and
COX-2 (prostaglandin endoperoxide H synthase-2) was obtained from sheep