Flavonols from Cryptocarya alba

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Ten flavonoids and chlorogenic acid were isolated from the leaves and stems of Cryptocarya alba (Lauraceae). The flavonoids were identified as isorhamnetin, kaempferol, quercetin and their glycosides isorhamnetin-3-O-rhamnoside, isorhamnetin-3-O-galactoside, isorhamnetin-3-O-glucoside, kaempferol-3-O-galactoside, quercetin-3-O-rhamnoside (quercitrin), quercetin-3-O-galactoside (hyperoside), and quercetin-3-O-glucoside (isoquercitrin).

Introduction

Cryptocarya alba (Mol.) Looser, a species of the family Lauraceae, is native to Chile and is distributed from Coquimbo to Valdivia, where it grows as high as 20 m in the coastal mountain range as well as in the Andes, up to 1,500 m above sea level (Hoffmann, 1982). The evergreen trees, also known as “peumo”, “pegu” or “peugo”, are widely used in popular medicine in Chile. Infusions of the bark are used for treating liver diseases and for stopping hemorrhages (Hoffmann, 1982; Wilhelm de Mösbach, 1992), whereas infusions of the leaves, as well as bark, are recommended for the treatment of rheumatism and for wound healing (Hoffmann, 1982). An ointment is prepared from ground seeds to treat vaginal infections. Although C. alba is very much used in popular medicine, its chemical composition is virtually unknown. Previous investigations have reported tanins and resins from its leaves, bark, and fruits (Gautier, 1956), and the composition of essential oils was described from its aromatic leaves (Montes et al., 1988).

Identification

All compounds were identified by 1H NMR. Quercitrin, hyperoside, kaempferol-3-O-galactoside, isorhamnetin-3-O-rhamnoside, isorhamnetin-3-O-galactoside, and isorhamnetin-3-O-glucoside as well as chlorogenic acid were further elucidated by 13C NMR. In addition the structures of kaempferol-3-O-galactoside, isorhamnetin-3-O-rhamnoside and chlorogenic acid were confirmed by EIMS. Isorhamnetin-3-O-rhamnoside, isorham-

Materials and Methods

Plant material

The plant material was collected on October 11, 1991 in Chile, Cuesta La Dormida (33°02’15” S; 71°00’07” W) at 1,250 m above sea level. A voucher specimen (No. 91-0023) has been deposited in the Herbarium at the Pontificia Universidad Católica de Chile, Santiago, Chile.

Extraction and isolation

2.5 kg of air-dried leaves and stems from C. alba were ground with a mill (Laboratory Construction Co., Kansas City, Missouri) to 5 mm particle and macerated with 95% ethanol (ethanol 95% [190 proof], Quantum Chemical Corporation, Cincinnati, Ohio). The crude alcoholic extract was dissolved in water and the aqueous phase was exhaustively extracted with CH2Cl2 followed by EtOAc. CH2Cl2 and EtOAc, both quality AR (ACS), were purchased from Mallinckrodt Chemicals, Paris, Kentucky. The EtOAc extract (20 g) was chromatographed on a 6×60 cm column packed with 200 g of Polyclar AT (polyvinylpyrrolidone, GAF Corporation, Linden, New Jersey) using MeOH–H2O-gradient as eluent (Liu et al., 1989).

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netin-3-O-galactoside, and isorhamnetin-3-O-glucoside were also determined by FABMS. All compounds were purified through a small column of Sephadex LH 20 (Pharmacia LKB, Uppsala, Sweden) before spectral analysis (Liu et al., 1989). The NMR were run on a Varian-Unity 300 spectrometer (1H NMR and 13C NMR frequencies were 300 MHz and 75 MHz, respectively). The solvent for the flavonoids and their glycosides was DMSO- d6, the solvent for chlorogenic acid was CD3OD. The internal reference for all compounds was tetramethylsilane (TMS). EIMS were recorded on a Finnigan MAT 90 (Bremen, Germany) with electron ionization at 70 eV. FABMS were performed using MS-1 of a custom-built four-sector instrument of BEBE Geometry (AMD Intectra, Harpstedt, Germany) equipped with two KWS MC 68000 computer systems for instrument control and data acquisition. The matrix was glycerol. Comparison with authentic samples were done by thin layer chromatography on polyamide and on silica gel as reported previously (Wollenweber et al., 1993; Timmermann et al., 1994). All markers were available in B.T.’s lab. The TLC plates (Polygram, Polyamid-6 UV254 and SIL G/UV254) were purchased from Alltech Associates, Inc., Deerfield, Illinois, manufactures: Macherey-Nagel, Düren, Germany.

Results

The ethanolic extract of leaves and stems from C. alba was dissolved in water and further extracted with CH2Cl2 followed by EtOAc. The EtOAc fraction was subjected to Polyclar AT column chromatography using water-methanol as eluent. Eleven compounds were isolated and characterized as isorhamnetin (22 mg); kaempferol (23 mg); quercetin (32 mg); isorhamnetin-3-O-rhamnoside (36 mg); isorhamnetin-3-O-galactoside (26 mg); isorhamnetin-3-O-glucoside (30 mg); kaempferol-3-O-galactoside (72 mg); quercetin-3-O-rhamnoside (quercitrin) 204 mg; quercetin-3-O-galactoside (hyperoside) 121 mg; quercetin-3-O-glucoside (isodequercitrin) 173 mg and chlorogenic acid (128 mg). Although none of these phenolics is a rare natural product, this is the first time, however, that their occurrence is reported in leaves and stems of Cryptocarya alba.

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