Triterpenoids from *Acaena pinnatifida* R. et P.

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*Acaena pinnatifida* R. et P., Rosaceae. Isolation, Structure Elucidation, Urs-12-ene Triterpenoids

Eight urs-12-ene triterpenoids, ß-sitosterol, (+)-catechin, and apigenin 7-O-glucoside were isolated from the leaves of *Acaena pinnatifida* R. et P. The triterpenoids were characterized as pomolic acid, pomolic acid-3-acetate, tormentic acid, 2-epi-tormentic acid, euscaphic acid, tormentic acid glucoside, nigai-chigoside F1, and nigai-ichigoside F2.

Introduction

The genus *Acaena* (Rosaceae) comprises 125 species of which 19 occur in Chile (Squeo et al., 1994). *A. pinnatifida* is a pubescent herbaceous cushion plant growing up to 15 cm with a short rhizome and numerous divided leaves. It is one of the dominant species in the subalpine community in the Andes Mountain above 2,800 m.a.s.l. (Montenegro et al., 1981) and is distributed from the Chilean province of Coquimbo in the north to the Magellan Islands in the south. Commonly known as “pimpinela cimarrona”, “cadillo”, or “amor seco”, this plant is used in Chilean folk medicine as a mild astringent and diuretic. Infusions from its leaves and rhizomes are used for the treatment of injuries and diseases of the liver and the urinary tract (Munoz et al., 1981). Leaf infusions are also used by the Mapuche women during menopause (Houghton and Manby, 1985). In spite of wide medicinal uses, the chemistry of this species has not yet been investigated. We report here the isolation of eight known urs-12-ene triterpenoids from *A. pinnatifida*.

Materials and Methods

General

**Mps:** uncorr., solvents used for NMR: CDCl\textsubscript{3}, CD\textsubscript{3}OD, C\textsubscript{5}D\textsubscript{5}N. The measurements of the NMR spectra were carried out on a Bruker AM 250 NMR spectrometer [\textsuperscript{1}H NMR (250 MHz), \textsuperscript{13}C NMR (63 MHz)] and on a Varian Unity 300 [\textsuperscript{1}H NMR (300 MHz), \textsuperscript{13}C NMR (75 MHz)]. CI-MS, EI-MS (70 eV): Finnigan MAT 90.

Plant material

*A. pinnatifida* was collected in January 1994 in Farellones, Chile and identified by Gloria Montenegro. A voucher specimen (5403) is deposited in the herbarium of the Pontificia Universidad Católica de Chile, Santiago, Chile.

Extraction and isolation

Air dried and ground leaves (680 g) of *A. pinnatifida* were exhaustively extracted with CH\textsubscript{2}Cl\textsubscript{2}-MeOH (1:1) yielding 125 g of crude extract. 120 g of this extract were chromatographed in 2 portions on 3 kg silica gel 60 (MN Kieselgel 60, 50-200 μm) with a hexane-EtOAc gradient (0–100% EtOAc) followed by an EtOAc-MeOH gradient (0–100% MeOH) resulting in 12 fractions of increasing polarity. Fraction 4 was chromatographed on Sephadex LH-20 (Pharmacia Biotech AB) (CH\textsubscript{2}Cl\textsubscript{2}-MeOH, 1:1) and silica gel 60 (hexane-EtOAc, 8:2) yielding 9 (200 mg). Fraction 6 was successively applied to column chromatography (CC) on Sephadex LH-20 (CH\textsubscript{2}Cl\textsubscript{2}-MeOH, 1:1) and silica gel 60 (hexane-EtOAc, 9:1) yielding 2 (48 mg). The CH\textsubscript{2}Cl\textsubscript{2} insoluble part of fraction 7 was dissolved in CH\textsubscript{2}Cl\textsubscript{2}-MeOH (1:1), chromatographed on silica gel 60 (CH\textsubscript{2}Cl\textsubscript{2}-EtOAc, 9:1) and applied to preparative TLC (CH\textsubscript{2}Cl\textsubscript{2}-acetone, 9:1) and purified on Sephadex LH-20 (CH\textsubscript{2}Cl\textsubscript{2}-MeOH, 1:1) yielding 1 (18 mg) after recrystallization from MeOH-H\textsubscript{2}O. Fraction 8 was applied to CC on silica gel 60 (hexane-EtOAc-MeOH, 70:25:5) followed by HPLC on silica gel (Alltech Adsorbosil 5 μm, 4.6 x 250 mm, hexane-EtOAc-MeOH, 67:25:8) yielding 4 (9 mg), 5 (18 mg), and 3 (20 mg). Fraction 9 was chromatographed on silica gel 60 (hexane-
CH$_2$Cl$_2$–MeOH, 5:4:1) yielding 10 (20 mg) after purification on Sephadex LH–20 (CH$_2$Cl$_2$–MeOH, 1:4). The remainder of fraction 9 was applied to VLC on RP–18 (EM, 40–63 μm) with a MeOH–H$_2$O gradient yielding additional 3 (68 mg) after recrystallization from MeOH–H$_2$O. Fraction 10 was chromato-}

graphed on Sephadex LH–20 (CH$_2$Cl$_2$–MeOH, 1:1) yielding 3 subfractions 10–1, 10–2, and 10–3. Fraction 10–1 was applied to CC on silica gel (CH$_2$Cl$_2$–MeOH, 8:2) yielding 7 (2.2 g). The remainder of fraction 10–1 was purified with HPLC (RP–18, Econosil 10 μm, 10 x 250 mm, MeOH–H$_2$O, 6:4) followed by preparative TLC (EtOAc–MeOH, 35:55:10) yielding 8 (37 mg) and preparative TLC (hexane–acetone–MeOH, 85:5:5) yielding 6 (17 mg) after purification on Sephadex LH–20 (CH$_2$Cl$_2$–MeOH, 1:1). The MeOH–insoluble, yellow part (120 mg) of fraction 10–3 was identified as 11. That the glucose moiety was present at C–7 and the OH-group at C–4' in 11, was determined by using diphenylboric acid–2-aminoethyl ester (Neu’s natural product Reagent A) (Liu et al., 1989).

**Saponification of 2**

12.5 mg of 2 were saponified in 5% methanolic NaOH at 50 ° for 2 h. After neutralization with HOAc and evaporation in vacuo, H$_2$O was added and the product was extracted with EtOAc yielding 10.9 mg (95%) of 1.

**Results and Discussion**

The air-dried and ground leaves of *A. pinnatifida* were extracted at room temperature with a one-to-one mixture of dichloromethane and methanol. Separation of the crude extract by repeated column chromatography on silica gel and Sephadex LH–20, preparative TLC, and HPLC led to the isolation of triterpenoids 1–8, β-sitosterol (9), (+)-catechin (10), and apigenin 7-O-glucoside (11). The 13C NMR and DEPT spectroscopical data of 1–8 allowed their identification as urs–12-enes. The distinction between urs–12-enes and olean–12-enes, both types of compounds found in the Rosaceae (Shigenaga et al., 1985; Zhou et al., 1992), was made by observing the 13C chemical shifts for the olefinic carbons C–12 and C–13 (Doddrell et al., 1974; Seo et al., 1975).

The known urs–12–enes 1–8 were identified as pomolic acid (1) (Brieskorn and Wunderer, 1967; Cheng and Cao, 1992; Kakuno et al., 1992), pomolic acid acetate (2) (Wang et al., 1995), tormentic acid (3) (Villar et al., 1986; Yamagishi et al., 1988), 2-epi–tormentic acid (4) (Hattori et al., 1988), euscaphic acid (5) (Liang et al., 1989), tormentic acid glucoside (6) (Zhou et al., 1992; Jia et al., 1993), niga-ichigoside F1 (7) (Seto et al., 1984; Durham et al., 1996), and niga-ichigoside F2 (8) (Seto et al., 1984) by comparison of their 1H and 13C NMR data with those reported in the literature. The identity of 2 was confirmed by comparison of its saponification product by TLC and NMR with an authentic sample of pomolic acid. β-Sitosterol (9), (+)-catechin (10), and apigenin 7-O-glucoside (11) were identified by comparison with authentic samples by TLC and NMR.

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