

Triterpenoids from *Acaena pinnatifida* R. et P.

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Eight urs–12–ene triterpenoids, β -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, niga-ichigoside F1, and niga-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 (Muñoz *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*.

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Materials and Methods

General

Mps: uncorr., solvents used for NMR: CDCl₃, CD₃OD, C₅D₅N. The measurements of the NMR spectra were carried out on a Bruker AM 250 NMR spectrometer [¹H NMR (250 MHz), ¹³C NMR (63 MHz)] and on a Varian Unity 300 [¹H NMR (300 MHz), ¹³C 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 CH₂Cl₂–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 μ m) 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) (CH₂Cl₂–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 (CH₂Cl₂–MeOH, 1:1) and silica gel 60 (CH₂Cl₂–EtOAc, 96:4) yielding **2** (48 mg). The CH₂Cl₂ insoluble–part of fraction 7 was dissolved in CH₂Cl₂–MeOH (1:1), chromatographed on silica gel 60 (CH₂Cl₂–EtOAc, 9:1), and applied to preparative TLC (CH₂Cl₂–acetone, 9:1) and purified on Sephadex LH–20 (CH₂Cl₂–MeOH, 1:1) yielding **1** (18 mg) after recrystallization from MeOH–H₂O. 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 μ m, 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_2Cl_2 -MeOH, 5:4:1) yielding **10** (20 mg) after purification on Sephadex LH-20 (CH_2Cl_2 -MeOH, 1:4). The remainder of fraction 9 was applied to VLC on RP-18 (EM, 40–63 μm) with a MeOH- H_2O gradient yielding additional **3** (68 mg) after recrystallization from MeOH- H_2O . Fraction 10 was chromatographed on Sephadex LH-20 (CH_2Cl_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_2Cl_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_2O , 6:4) followed by preparative TLC (EtOAc-MeOH, 85:15) yielding **8** (37 mg) and preparative TLC (hexane-acetone-MeOH, 35:55:10) yielding **6** (17 mg) after purification on Sephadex LH-20 (CH_2Cl_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).

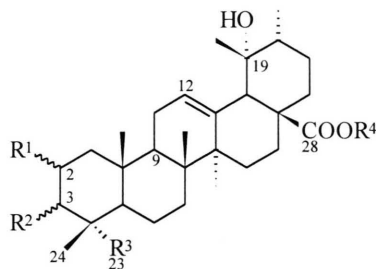
Saponification of **2**

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_2O 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 ^{13}C 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 ^{13}C chemical



- 1:** $\text{R}^1 = \text{R}^4 = \text{H}$, $\text{R}^2 = \beta\text{-OH}$, $\text{R}^3 = \text{CH}_3$
2: $\text{R}^1 = \text{R}^4 = \text{H}$, $\text{R}^2 = \beta\text{-OAc}$, $\text{R}^3 = \text{CH}_3$
3: $\text{R}^1 = \alpha\text{-OH}$, $\text{R}^2 = \beta\text{-OH}$, $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{H}$
4: $\text{R}^1 = \text{R}^2 = \beta\text{-OH}$, $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{H}$
5: $\text{R}^1 = \text{R}^2 = \alpha\text{-OH}$, $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{H}$
6: $\text{R}^1 = \alpha\text{-OH}$, $\text{R}^2 = \beta\text{-OH}$, $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{gluc}$
7: $\text{R}^1 = \alpha\text{-OH}$, $\text{R}^2 = \beta\text{-OH}$, $\text{R}^3 = \text{CH}_2\text{OH}$, $\text{R}^4 = \text{gluc}$
8: $\text{R}^1 = \text{R}^2 = \alpha\text{-OH}$, $\text{R}^3 = \text{CH}_2\text{OH}$, $\text{R}^4 = \text{gluc}$

Fig. 1. Chemical structures of urs-12-ene triterpenoids **1**–**8**.

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 ^{13}C 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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- Brieskorn C. H. und Wunderer H. (1967), Pomol- und Pomonsäure. *Chem. Ber.* **100**, 1252–1265.
- Cheng D.-L. and Cao X.-P. (1992), Pomolic acid derivatives from the root of *Sanguisorba officinalis*. *Phytochemistry* **31**, 1317–1320.
- Doddrell D. M., Khong P. W. and Lewis K. G. (1974), The stereochemical dependence of ^{13}C chemical shifts in olean-12-enes and urs-12-enes as an aid to structural assignment. *Tetrahedron Lett.*, 2381–2384.
- Durham D. G., Liu I. X. and Richards R. M. E. (1996), Unsaturated E-ring triterpenes from *Rubus pinfaensis*. *Phytochemistry* **42**, 505–508.
- Hattori M., Kuo K.-P., Shu Y.-Z., Tezuka Y., Kikuchi T. and Namba T. (1988), A triterpene from the fruits of *Rubus chingij*. *Phytochemistry* **27**, 3975–3976.
- Houghton P. O. and Manby J. (1985), Medicinal plants of the Mapuche. *J. Ethnopharmacol.* **13**, 39–103.
- Jia Z.-J., Liu X.-Q. and Liu Z.-M. (1993), Triterpenoids from *Sanguisorba alpina*. *Phytochemistry* **32**, 155–159.
- Kakuno T., Yoshikawa K. and Arihara S. (1992), Triterpene saponins from fruit of *Ilex crenata*. *Phytochemistry* **31**, 2809–2812.
- Liang G.-Y., Gray A. I. and Waterman P. G. (1989), Pentacyclic triterpenes from the fruits of *Rosa sterilis*. *J. Nat. Prod.* **52**, 162–166.
- Liu Y.-L., Neuman P., Timmermann B. N. and Mabry T. J. (1989), Techniques for flavonoid analysis. *Rev. Latinoamer. Quim. Suppl.* **1**, 90–130.
- Montenegro G., Aljaro M. E., Walkowiak A. and Saenger R. (1981), Seasonality, growth and net-productivity of herbs and shrubs of Chilean matorral. In: *Proc. of the Symposium on Dynamics and Management of Mediterranean-Type Ecosystems*, Conrad C. E. and Oechel W. (ed.), USDA Forest Service General Technical Report PSW-58, San Diego, CA, pp. 129–135.
- Muñoz M., Barrera E. and Meza I. (1981), El Uso Medicinal y Alimenticio de Plantas Nativas y Naturalizadas en Chile. Publicación Ocasional N° 33, edited by Museo Nacional de Historia Natural, Santiago de Chile, p. 62.
- Seo S., Tomita Y. and Tori K. (1975), Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isodon japonicus* Hara tissue cultures. *Tetrahedron Lett.*, pp. 7–10.
- Seto T., Tanaka T., Tanaka O. and Naruhashi N. (1984), β -Glucosyl esters of 19 α -hydroxyursolic acid derivatives in leaves of *Rubus* species. *Phytochemistry* **23**, 2829–2834.
- Shigenaga S., Kouno I. and Kawano N. (1985), Triterpenoids and glycosides from *Geum japonicum*. *Phytochemistry* **24**, 115–118.
- Squeo F. A., Osorio R. and Arancio G. (1994), Flora de Los Andes de Coquimbo: Cordillera de Doña Ana, ed. by Universidad de la Serena, Chile, p. 117.
- Villar A., Payá M., Hortigüela M. D. and Cortes D. (1986), Tormentonic acid, a new hypoglycemic agent from *Poterium ancistroides*. *Planta Med.*, 43–45.
- Wang S., Yi S., Wu L., Li X., Zhao X. and Yang J. (1995), A study on the chemical constituents of *Ligustrum lucidum* Ait. *Shenyang Yaoke Daxue Xuebao* **12**, 25–28.
- Yamagishi T., Zhang D.-C., Chang J.-J., McPhail D. R., McPhail A. T. and Lee K.-H. (1988), The Cytotoxic principles of *Hyptis capitata* and the structures of the new triterpenes Hyptatic acid-A and -B. *Phytochemistry* **27**, 3213–3216.
- Zhou X.-H., Kasai R., Ohtani K., Tanaka O., Nie R.-L., Yang C.-R., Zhou J. and Yamasaki K. (1992), Oleanane and Ursane glucosides from *Rubus* species. *Phytochemistry* **31**, 3642–3644.