Biochemical Systematics and Ecology, Vol. 21, No. 5, pp. 621–623, 1993.
Printed in Great Britain.

0305-1978/93 $6.00+0.00

Iridoid Glycosides from *Escallonia* Species

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Key Word Index—*Escallonia*; Saxifragaceae; Escalloniaceae; Cornanae-Hydrangeales; Iridoid glycosides; 6′-*O*-β-D-glucosylasperuloside.

Abstract—Phytochemical study of *Escallonia myrtoidea, E. illinita, E. alpina* and *E. pulverulenta* led to the isolation of asperuloside and other C_{10}-iridoid glycosides. From *E. myrtoidea* a new iridoid glycoside was isolated and identified as 6′-*O*-β-D-glucosylasperuloside by spectroscopic means. The findings are in accordance with the inclusion of *Escallonia* in the Saxifragaceae.

Introduction

The genus *Escallonia* is included in the family Saxifragaceae and belongs to the subfamily Escallonioideae, though some authors consider the latter group as a distinct family (Kausel, 1953; Hegnauer, 1990). The genus contains about 60 species, generally shrubs or evergreen trees, widely spread in the flora of Southern America, especially in the mountains of the Andes (Melchior, 1964; Hoffmann, 1982)

We have studied the Chilean species *E. myrtoidea* Bert., *E. illinita* Presl, *E. alpina* Poepp. et Endl. and *E. pulverulenta* Pers in order to establish the presence of chemical markers of systematic value.

Materials and Methods

CC: silica gel 70–230 mesh (Merck). PC: Schleicher & Schull 2043. TLC: silica gel 60 F_{254} (Merck). Spray reagents: vanillin–HCl (vanillin 2 g, concentrated HCl 4 ml, MeOH 100 ml), 2 N H_2SO_4. ^1H and ^{13}C NMR: Bruker AM 500; TMS as internal reference.

Plant material. E. myrtoidea Bert., *E. illinita* Presl var. *illinita, E. alpina* Poepp. et Endl. var. *carmelitana* and *E. pulverulenta* Pers. var. *glabra* Engler were all collected in Cajòn del Maipo (provincia Cordillera), Region Metropolitana de Santiago de Chile, and identified by Prof. Sebastiàn Teillier at Universidad de Chile, Santiago, Chile, where voucher specimens are deposited.

Extraction and isolation. The aerial parts of each *Escallonia* species were exhaustively extracted with EtOH at room temperature. After evaporation of the solvent, the residue was dissolved in H_2O, charcoal added until a negative vanillin test occurred and the mixture stratified on a Gooch funnel. Elution with H_2O and 5 and 10% aqueous EtOH removed salts and sugars, whereas 30, 50 and 70% aqueous EtOH eluted iridoid containing fractions. The 30 and 50% fractions were mixed after TLC monitoring and chromatographed on silica gel in *n*-BuOH saturated with H_2O, affording iridoid mixtures. Pure iridoids **1**–**4** were obtained by further separation on CC in $CHCl_3$: MeOH 7:3.

The identification of compounds **1**–**3** was carried out by comparison of their physical and spectroscopic data with that previously reported in the literature (El Naggar and Beal, 1980).

Compound **4**. Amorphous powder, $[\alpha]_D^{20} = -148$ (c = 1.0 MeOH), UV (MeOH): λ_{max} 234 nm (log ε = 3.87); IR (KBr): ν_{max} 1760, 1745, 1665 cm^{-1}.

^1H NMR (CD_3OD), δ: 7.30 (1H, *d*, $J_{3,5} = 2.0$ Hz, H-3); 5.95 (1H, *d*, $J_{1,9} = 1.6$ Hz, H-1); 5.72 (1H, *bs*, H-7); 5.57 (1H, *bd*, $J_{5,6} = 6.6$ Hz, H-6); 4.78 (1H, *dd*, $J_{7,10a} = 1.4$ Hz and $J_{10a,10b} = 14.8$ Hz, H-10a); 4.68 (1H, *d*, $J_{1',2'} = 7.8$ Hz, H-1′); 4.66 (1H, *dd*, $J_{7,10b} = 1.4$ Hz, H-10b); 4.26 (1H, *d*, $J_{1'',2''} = 7.8$ Hz, H-1″); 3.94 (1H, *dd*, $J_{5',6'a} = 2.2$ Hz and $J_{6'a,6'b} = 12.2$ Hz, H-6′a); 3.86 (1H, *dd*, $J_{5',6'a} = 2.2$ Hz and $J_{6'a,6'b} = 12.2$ Hz, H-6″a); 3.62–3.70 (2H, m, H-5 and H-6b); 3.30–3.40 (2H, *m*, H-3′ and H-5′); 3.27–3.30 (2H, *m*, H-9 and H-4′); 3.19 (1H, *dd*, $J_{2',3'} = 9.2$ Hz, H-2′); 2.06 (3H, *s*, CH_3—CO).

^{13}C NMR (CD_3OD), ppm: 172.6*, C-11; 172.3*, CH_3–CO; 150.3, C-3; 144.2, C-8; 128.9, C-7; 106.1, C-4; 104.0,

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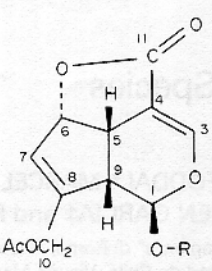

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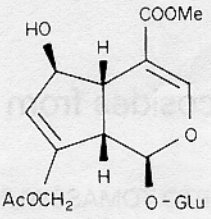
4 R = Gen



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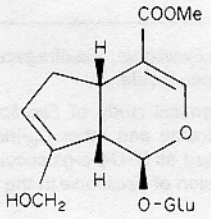

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C-1″; 100.0, C-1′; 93.3, C-1; 86.3, C-6; 78.3†, C-3′; 77.8†, C-5′; 74.6, C-2′; 71.5, C-4′; 64.4, C-6″; 62.7, C-10; 61.9, C-6′; 45.2, C-9; 37.4, C-5; 20.6, CH_3—CO. [*†Values with the same superscript may be interchanged.]

Total hydrolysis of **4**. Compound **4** (30 mg) was dissolved in 1 N H_2SO_4 (5 ml) and refluxed for 6 hr. After removing of degradation products by filtration and after neutralization with $Ba(OH)_2$, the solution was evaporated and the residue chromatographed on silica gel in $CHCl_3$–MeOH 7:3 to give D-glucose (12 mg).

Partial hydrolysis of **4**. Compound **4** (40 mg) was dissolved in 1 N H_2SO_4 (5 ml) and refluxed for 10 min until the solution gave a negative vanillin test. The solution was rapidly frozen and worked up as above. The neutral solution was treated with charcoal (15 g) and the resulting suspension stratified on a Gooch funnel and eluted with a continuous gradient of EtOH (0→30%). Pure gentiobiose (13 mg) was obtained and identified by direct comparison with an authentic sample.

Results and Discussion

The study of the *Escallonia* species led first to the isolation of several common substances. Pinocembrin, kaempferol, rutin and chlorogenic acid were isolated from *E. illinita*; acacetin-7-methyl ether, kaempferol, rutin and chlorogenic acid from *E. pulverulenta*; rutin and chlorogenic acid from *E. alpina*; while only chlorogenic acid was found in *E. myrtoidea*.

Owing to their importance in the chemosystematics of the Sympetalae, iridoid constituents were specifically investigated in the polar extracts of the four *Escallonia* species. After preliminary purification of the ethanolic extract by the charcoal method, repeated column chromatography on SiO_2 in *n*-BuOH led to the isolation of four iridoid glycosides; asperuloside (**1**) was present in all four species. Two more iridoid glucosides, daphylloside (**2**) and geniposide (**3**) were isolated from *E. illinita* and *E. myrtoidea*, respectively.

An unknown compound, **4**, was isolated from *E. myrtoidea*. Substance **4**, $C_{24}H_{32}O_{16}$, was the most polar among the isolated iridoids. It is an amorphous powder with UV and IR spectra very similar to those of **1**. The ^1H NMR spectrum was practically identical to **1**, apart from several additional peaks in the region of the oxymethine protons, suggesting the presence of another sugar unit. This was also confirmed by two signals resonating at δ4.26 and 4.68, as doublets with $J = 7.8$ Hz, which are anomeric protons.

Total hydrolysis of **4** afforded, besides the decomposition products of the aglycone, D-glucose, while partial hydrolysis gave an oligosaccharide, identified as D-gentiobiose.

Both ^{1}H and ^{13}C NMR spectra were in accordance with these identifications, containing signals which correspond to a β-gentiobiosyl moiety. Consequently, all data indicate that **4** is 6′-*O*-β-D-glucosylasperuloside.

Our results agree with a previous chromatographic study, claiming the presence of asperuloside, **1**, in 10 different species of *Escallonia* (Plouvier, 1956). From a chemotaxonomic point of view, this evidence gives support to the classification of the genus *Escallonia* in the Saxifragaceae *s.l.* or, perhaps more aptly, in Cornanae-Hydrangeales *sensu* Thorne (1980). The iridoids of the asperuloside-type that occur in *Escallonia*, as well as iridoids isolated from other plants of the same family (namely deutzioside-type compounds from *Deutzia* spp., montinioside from *Montinia caryophyllacea* Thunb. and hydrangenosides from *Hydrangea* spp. (Hegnauer, 1990), and the great majority of iridoids isolated from Cornanae, appear to have a common biosynthetic origin in iridoidial. This assumes that the hypothesis of two distinct biosynthetic pathways from iridoidial and 8-epi-iridoidial is correct (Inouye and Uesato, 1986; Jensen, 1991).

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