SPECIALIA

A new chalcone glucoside and cernuoside from the flowers of *Acacia dealbata*

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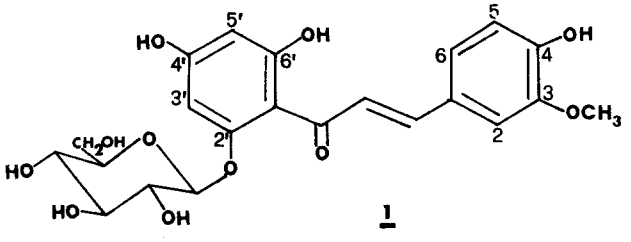
Summary. A new chalcone glucoside has been isolated from the flowers of *Acacia dealbata* and shown to be 4,2′,4′,6′-tetrahydroxy-3-methoxychalcone 2′-O-β-D-glucoside (**1**) by chemical degradations and spectroscopic methods. Cernuoside (4,6,3′,4′-tetrahydroxyaurone 4-O-β-D-glucoside), (**2**) has also been found in this plant material.

Earlier work on the polyphenolic constituents of *Acacia dealbata* (Leguminosae) has led to the identification of 2 flavonol glycosides (myricetin 3-glucoside[2] and rutin[3]), a 5-deoxyflavonol (fisetin[4]), a leucoanthocyanidin (mollisacacidin[5]) and a flavanone glycoside (naringenin 5-O-diglucoside[6]). Moreover the presence of anthochlor pigments in the flowers of this plant has recently been shown by the isolation of 2 chalcone glycosides[7] (isosalipurposide and chalcononaringenin 2′-xyloside). The present study deals with the isolation of 2 further anthochlors (**1** and **2**) from the yellow flowers of *Acacia dealbata.*

Material and methods. For paper chromatography and TLC the solvent mixtures used, with their abbreviations, are as follows: A, 1-butanol-acetic acid-water (4:1:5, upper phase); B, acetic acid-water (5:95); C, 1-butanol-ethanol-water (4:1:2.2); D, ethyl acetate-butanone-formic acid-water (5:3:1:1); E, 1-butanol-pyridine-water (6:4:3); F, 1-butanol-acetic acid-ethyl ether-water (9:6:3:1); G, water; H, acetic acid-water (3:7); I, benzene-pyridine-formic acid (36:9:5); L, chloroform-methanol-butanone (70:10:6); M, benzene-methanol-butanone (3:1:1); N, chloroform-ethyl acetate (1:1); O, benzene-acetone (9:1.5); P, phenol saturated with water.



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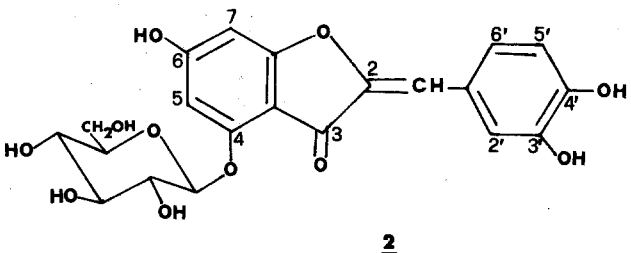

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Fresh flowers (50 g) of *Acacia dealbata* Link (collected in Catania) were extracted 3 times with boiling 95% ethanol; the combined extracts were filtered, concentrated to a small volume in vacuo, re-filtered and evaporated to dryness in vacuo giving an orange-coloured residue (ca. 12 g). Anthochlor pigments **1** (circa 10 mg) and **2** (circa 25 mg) were isolated by preparative chromatography on Whatman 3MM paper in solvent A. Bands were cut off, eluted with 70% ethanol, concentrated in vacuo and rechromatographed in solvents B and C; further purification was obtained by preparative SiO_2 TLC in solvent D.

The UV-spectrum of pigment **1** (R_f values on Whatman No.1 paper: 0.69 in solvent A; 0.77 in solvent C; 0.30 in solvent G; 0.70 in solvent P) showed λ_{max}^{MeOH} (nm) 310 (sh), 368; +NaOMe 245 (sh), 340 (sh), 433 (increase in intensity); +$AlCl_3$ 260, 327, 420; +$AlCl_3$/HCl 260, 320, 418; +NaOAc 260 (sh), 340 (sh), 395. These spectral properties are consistent[8] with pigment **1** (color reactions: brown to orange in UV+NH_3) being a polyhydroxychalcone with free hydroxyl groups at positions 4 and 2′. Partial acid hydrolysis with 10% acetic acid (3.5 h under reflux), total acid hydrolysis with 2N HCl (2 h at 100 °C) and treatment with β-glucosidase gave D-glucose and homoeridodictyol (5,7,4′-trihydroxy-3′-methoxyflavanone). The sugar was identified by paper chromatography (solvents A and E), SiO_2 TLC (solvent F) and by the use of glucose oxidase; homoeriodictyol was identified by paper co-chromatography with authentic sample (solvents A, G and H), SiO_2 TLC (solvent I), polyamide TLC (solvents L and M) and UV-spectral analysis with shift reagents[8]. On methylation (methyl iodide in dimethylformamide in the presence of silver oxide), this chalcone glucoside gave a methyl ether, λ^{MeOH} 350 nm, which on acid hydrolysis with 0.3 N HCl (4 h under reflux) isomerized to a flavanone since it gave eriodictyol tetramethyl ether (5,7,3′,4′-tetramethoxyflavanone) and 2,3,4,6-tetra-O-methyl-D-glucose. The methylated sugar was identified by SiO_2 TLC (solvent N) and paper co-chromatography according to Petek[9]; eriodictyol tetramethyl ether was identified by UV-spectroscopy and alkaline degradation[10] to give di-O-methylphloroglucinol and 3,4-dimethoxycinnamic acid which were identified by SiO_2 TLC (solvent O). Thus pigment **1** must be 4,2′,4′,6′-tetrahydroxy-3-methoxychalcone 2′-O-β-D-glucoside, a name which derives from its isomeric flavanone. Pigment **2** was identified as cernuoside (4,6,3′,4′-tetrahydroxyaurone 4-O-β-D-glucoside) by UV-spectral analysis with usual shift reagents[8], paper co-chromatography with authentic material (solvents A, H and P) and SiO_2 TLC (solvent D).

This identification was confirmed by total acid hydrolysis, partial acid hydrolysis and treatment with β-glucosidase to give D-glucose and aureusidin (4,6,3′,4′-tetrahydroxyaurone). D-glucose was identified as above; aureusidin was identified by paper co-chromatography with authentic sample (solvents A, H and P) and UV-spectral analysis with shift reagents[8].

Results and discussion. Although several natural products based upon 3,4,2′,4′,6′-pentahydroxychalcone are known[11], pigment **1** is the first chalcone whose substitution pattern is related to that of the flavanone homoeriodictyol. Cernuoside (**2**) is reported for the first time in the Leguminosae; this anthochlor pigment was first isolated from *Oxalis cernua*[12] (Oxalidaceae) and later found in 4 species of Gesneriaceae (*Chirita micromusa*[13], *Cyrtandra oblongifolia*[14], *Didymocarpus malayanus*[14] and *Petrocosmea kerrii*[15]) and in the Plumbaginaceae (*Limonium bonduelli*[13] and *Limonium* cv. Gold Coast[16]). It is interesting to note that anthochlor pigments **1** and **2** as well as anthochlors (isosalipurposide and chalcononaringenin 2′-xyloside) previously isolated[7] from *Acacia dealbata* have phloroglucinol A-ring structures. The occurrence of 4 such pigments in this plant is quite exceptional since resorcinol based-A-ring anthochlors are considered a biochemical characteristic of the family Leguminosae[11].

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Starfish saponins VII. Structure of luzonicoside, a further steroidal cyclic glycoside from the pacific starfish *Echinaster luzonicus*[1,2]

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Summary. On the basis of comparative chemical and spectral data, the structure of the major saponin, luzonicoside, from the starfish *Echinaster luzonicus* has been elucidated as **2.** This is a further example of a novel class of steroidal cyclic glycoside from starfish of the genus *Echinaster.* Its structure includes a Δ^7, 3β,6β-dioxygenated-23-oxosteroidal moiety, already found in the saponins of *Echinaster sepositus,* and a trisaccharide moiety, β-D-galactopyranosyl-(1 → 2) α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranosyl, bridging C-3 and C-6 of the steroid.

Recently we have elucidated[6] the structure of the major saponin, sepositoside A, from *Echinaster sepositus* as **1.** On very mild acid treatment (1 *N*-HCl, r.t.) it gives the corresponding opened glycoside **3**, while it is hydrolyzed to its sugars and the 3β-hydroxy-5α-cholesta-8,14-dien-23-one[7], by prolonged acid treatment. The structures of the minor saponins from the same sources possess the same cyclic trisaccharide moiety bridging C-3 and C-6 of the steroid, and the differences reside in the steroidal side-chains, characterized by epoxide functionalities at C-22 and C-23 (part structures **5**–**7**)[8].

In this paper we describe the discovery of a further example of this novel class of steroidal cyclic glycoside from a starfish of the same genus, *E. luzonicus,* collected near Nouméa, Nouvelle Calédonie. The extraction and isolation of the saponins has followed the same procedure used before[6]. Fresh animals (4 kg), collected in october 1979, were extracted with water and the extracts were lyophilized (290 g). After removal of fat materials by washing with $CHCl_3$, the saponins were recovered from the aqueous solution by Amberlite XAD-2 and purified by silica gel column chromatography followed by reverse phase HPLC[9] (C-18 μ-Bondapak; CH_3OH:H_2O, 55:45) to give 0.75 g of luzonicoside.

Luzonicoside (**2**) analyzed for $C_{44}H_{67}O_{17}Na$, was levorotatory, $[\alpha]_D - 66°$ (H_2O), and was indistinguishable from sepositoside A (**1**) in TLC and HPLC. On acid hydrolysis it yielded the 3β-hydroxy-5α-cholesta-8,14-dien-23-one, but, unlike **1**, D-galactose, L-arabinose and D-glucuronic acid. In the 270-MHz NMR-spectrum (DMSO) it showed a broad signal at δ5.47 ($W^{1}/_{2} = 12$ Hz) corresponding to the olefinic proton at C-7, 2 methyl singlets at δ0.556 and 0.825 corresponding to CH_3-18 and CH_3-19, respectively and methyl doublets at δ0.85 and 0.83 for CH_3-21, -26 and -27. The same signals were also observed in the spectrum of **1**; the spectrum of **2** also contained 3 doublets assigned to the anomeric protons at δ4.32 (J = 7.5 Hz), 4.65 (J = 7.5 Hz) and 5.05 (J = 7.0 Hz). Similarly the comparison of the ^{13}C-NMR spectrum of **2** with that of the known **1** indicated that the steroidal parts of the molecules were identical (all resonances associated with the steroidal carbons had identical