

# Iridoids from *Plantago carinata* Schrad.

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Iridoids, *Plantago*

The new iridoid 10-acetylaucubin and the well known iridoids aucubin, melittoside and monomelittoside as well as the rare 6-epiaucubin and 3,4-dihydroaucubin were isolated from aerial parts of *Plantago carinata* Schrad. This is the first isolation of 6-epiaucubin from Plantaginaceae plants and of 3,4-dihydroaucubin and monomelittoside from *P. carinata* Schrad.

## Introduction

*Plantago* plants have been used in folk medicine. Part of the biological activity is due to the iridoid glucosides. In *P. carinata* Schrad. (*P. subulata*) the presence of aucubin (**1**) and melittoside (**2**) was shown by TLC only [1]. In this work we report the isolation and identification of the iridoids found in aerial parts of *Plantago carinata* Schrad. [2].

## Results and Discussion

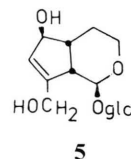
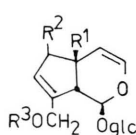
Six iridoid glucosides were isolated from the BuOH fraction of the MeOH extract by vacuum liquid chromatography (VLC) [3] and HPLC separations.

The main components were identified as aucubin (**1**) and monomelittoside (**3**) from <sup>1</sup>H NMR data [4, 5] and comparison with authentic samples.

The other iridoids were identified with the known melittoside (**2**) [6] and the rare iridoid glucosides 6-epiaucubin (**4**) and 3,4-dihydroaucubin (**5**). The identification of the latter two compounds was achieved on the basis of <sup>1</sup>H NMR [7, 8] and DCI MS (NH<sub>3</sub>) data [9]. This is the first isolation of 6-epiaucubin from Plantaginaceae plants and of monomelittoside and 3,4-dihydroaucubin from *P. carinata*.

The remaining one is a novel iridoid named 10-acetylaucubin (**6**). A molecular adduct ion (M + NH<sub>4</sub>)<sup>+</sup> at *m/z* 406 consistent with the empirical formula C<sub>17</sub>H<sub>24</sub>O<sub>10</sub> of **6** could be observed in the DCI MS spectrum. The elimination of acetic acid and ketene from the molecular adduct ion and from some other fragment ions indicated the pres-

ence of an acetoxy group in the molecule. The peaks at *m/z* 198 and 180 due to the sugar part showed that the acetoxy group is not attached to the latter. The <sup>1</sup>H NMR spectrum of **6** resembles that of **1** with the exception of the appearance of a signal for an acetoxy group (three proton singlet at 2.15 ppm) and deshielding of the 2H-10 from 4.19 and 4.26 ppm [3] to 4.86 and 4.92 ppm, which showed the presence of a 10-CH<sub>2</sub>OAc group. The <sup>13</sup>C NMR data proved this suggestion (see Experimental). After acid hydrolysis glucose was identified. Acetylation of **6** afforded aucubin hexaacetate identified by spectral data and comparison with an authentic sample.



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
<b>1</b>	H	β-OH	H
<b>2</b>	Oglc	β-OH	H
<b>3</b>	OH	β-OH	H
<b>4</b>	H	α-OH	H
<b>6</b>	H	β-OH	Ac

## Materials and Methods

The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were measured on a Bruker 250 MHz spectrometer. TMS was used as internal standard. DCI MS were recorded with MAT-44 using NH<sub>3</sub> as reagent gas by temperature of emitter 86 °C and source temperature 152 °C. The HPLC separations were achieved on a Perkin Elmer chromatograph with a RP-18 reversed phase column Whatman ODS-3 (250 × 4.6, 10 μm) and mobile phase MeOH–H<sub>2</sub>O mixtures.

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*Plant material*

Aerial parts of *Plantago carinata* Schrad. were collected in August 1988 in flowering in the south Pirin mountain. Voucher specimen No. 148258 was deposited in the Herbarium of the Institute of Botany with Botanical Garden, Bulg. Acad. Sci.

*Isolation*

350 g dried above-ground parts of *P. carinata* were three fold extracted with EtOH. The alcoholic concentrate was dissolved in H<sub>2</sub>O and consistently extracted with Et<sub>2</sub>O and BuOH. 10 g from the BuOH fraction (12 g) were treated with active charcoal. Elution was carried out with H<sub>2</sub>O, H<sub>2</sub>O-EtOH (10:1), EtOH (2 fractions – 320 mg and 470 mg, respectively) and EtOH-acetone (1:1) (840 mg). An aliquote part (290 mg) of the second EtOH fraction was separated by HPLC with 10% MeOH as mobile phase and flow rate 3 ml/min. Pure **1** (135 mg), **2** (20 mg), **4** (9 mg) and **5** (12 mg) were isolated.

The EtOH-acetone fraction was separated by VLC on silica gel and eluted with CHCl<sub>3</sub>-EtOH: (5.5:1) – 65 mg fraction, (5:1–4:1) – 110 mg, (4:1–2:1) – 440 mg. 27 mg of the last fraction were separated by prep. TLC on silica gel with CHCl<sub>3</sub>-EtOH (2:1) to give **1** (12 mg) and **3** (10 mg). The 65 mg fraction was separated by HPLC (20% MeOH, 3 ml/min) to give pure **6** (7 mg).

*10-acetylaucubin (6)*

Amorphous solid. UV:  $\lambda_{\max}$  (MeOH) nm: 211; DCI MS:  $m/z$  (rel. int.): 406 [M + NH<sub>4</sub>]<sup>+</sup> (100), 388 [M + NH<sub>4</sub> – 18]<sup>+</sup> (16), 364 [M + NH<sub>4</sub> – 42]<sup>+</sup> (22), 346 [M + NH<sub>4</sub> – 60]<sup>+</sup> (18), 244 [M + NH<sub>4</sub> – 162]<sup>+</sup> (6), 226 [M + NH<sub>4</sub> – 162 – 18]<sup>+</sup> (16), 209 [M + NH<sub>4</sub> – 180 – 17]<sup>+</sup> (30), 166 [M + NH<sub>4</sub> – 180 – 60]<sup>+</sup> (21), 198 [glc + NH<sub>4</sub>]<sup>+</sup> (6), 180 [glc + NH<sub>4</sub> – 18]<sup>+</sup> (28); <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  6.31 dd (1H,  $J = 1.7$ ,  $J = 6.5$ , H-3), 5.94 m (1H, H-7), 5.28 d (1H,  $J = 5.1$ , H-1), 5.09 m (1H, H-4), 4.92 d (1H,  $J = 15.2$ , H-10), 4.86 d (1H,  $J = 15.2$ , H-10), 4.55 bs (1H, H-6), 3.93 dd (1H,  $J = 12.1$ ,  $J = 1.7$ , H-6'), 3.76 dd (1H,  $J = 12.1$ , H-6'), 3.18 t (1H, H-9), 2.81 m (1H, H-5), 2.15 s (3H, CH<sub>3</sub>CO); <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  168.96 (CH<sub>3</sub>CO); 145.95 (C-8), 140.96 (C-3), 128.83 (C-7), 105.48 (C-4), 99.99 (C-1'), 97.85 (C-1), 82.09 (C-6), 78.55 (C-3'), 77.09 (C-5'), 74.04 (C-2'), 70.68 (C-4'), 62.45 (C-6'), 62.26 (C-10), 47.2 (C-9), 45.49 (C-5), 20.02 (CH<sub>3</sub>CO).

*Acetylation of 6*

Compound **6** was treated with pyridine-Ac<sub>2</sub>O in the usual manner to give aucubin hexaacetate.

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