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A FLAVONE GLYCOSIDE FROM *ANDROGRAPHIS ALATA*

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Key Word Index—*Andrographis alata*; Acanthaceae; whole plant; flavone glycoside; echioidinin 5-*O*-*β*-D-glucopyranoside.

Abstract—A new flavone glycoside, echioidinin 5-glucoside along with its known aglycone, echioidinin have been isolated from the whole plant of *Andrographis alata*. The structure of the new compound was established as 5,2′-dihydroxy-7-methoxyflavone 5-*O*-*β*-D-glucopyranoside on the basis of spectral and chemical studies. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Andrographis alata Nees (Acanthaceae) is an erect herb which occurs widely in South India [1]. A number of unusual flavones and flavone glycosides have been reported from *Andrographis species* [2–10] but this plant has not been investigated so far. Our phytochemical investigation of the whole plant of *Andrographis alata* has resulted in the isolation of a new flavone glycoside, echioidinin 5-glucoside (**2**) together with its known aglycone, echioidinin (**1**).

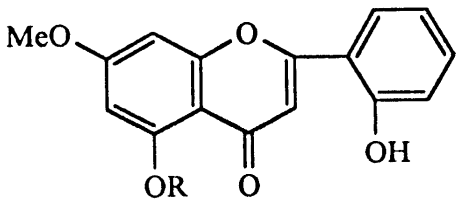
RESULTS AND DISCUSSION

The defatted methanolic extract of the whole plant of *A. alata* on purification over a silica gel column, afforded **1** and **2**. Compound **1** was identified as echioidinin by comparison of its mp and spectral data with published values [2, 11].

The positive FAB mass spectrum of **2** showed $[M + H]^+$ peak at *m*/*z* 447 corresponding to the molecular formula $C_{22}H_{22}O_{10}$ and a prominent fragment at *m*/*z* 285 $[M + H\text{-}162]^+$ indicating the loss of an hexose moiety. The UV absorption maxima of **2** at 257, 305 and 330 nm is typical of a flavone derivative [12]. It responded positively to the Molisch test indicating it to be a flavone glycoside. The addition of $AlCl_3$ and NaOAc caused no shift in its UV spectrum indicating the absence of free hydroxyl groups at C-5 and C-7, respectively. The IR spectrum of **2** showed an hydroxyl absorption band at 3200 and a carbonyl absorption band at 1652 cm^{-1}.

The ^1H NMR spectrum of **2** exhibited a methoxyl singlet at δ 3.89 and a one-proton singlet at δ 7.06 characteristic of C-3 proton of a 2′-oxygenated flavone [13]. Two *meta* coupled doublets at δ 6.91 and 6.98 were assigned to C-6 and C-8 protons, respectively. A broad singlet at δ 10.70 exchangeable with D_2O was assigned to a nonchelated hydroxyl group at C-2′ since the signal due to H-3 in the penta-acetate of **2** appeared at a significantly higher field (δ 6.14) than in **2** (δ 7.06) [13]. It also displayed the characteristic signal pattern of a 2′-oxygenated B-ring [9] at δ 7.94 (*dd*, 1H, *J* = 2, 8 Hz), 7.38 (*dt*, 1H, *J* = 2, 8 Hz) and 7.02–7.04 (m, 2H), which were assigned to the C-6′, C-4′ and C-3′, 5′ protons, respectively. The spectrum also showed the presence of a glucose moiety [δ 4.78 (*d*, *J* = 7 Hz, H-1″) and 3.35-3.85 (*m*, 5H)].

Acid hydrolysis of **2** with 2N HCl afforded glucose and an aglycone identified as echioidinin (**1**) [2, 11]. The presence of a chelated hydroxyl signal at δ 12.87 in the aglycone (**1**) and not in the gly-



1: R = H
2: R = Glc

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Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (75.4 MHz, ppm, DMSO-d_6)

C	1	2
2	161.4	158.5
3	109.1	112.0
4	182.0	177.1
5	161.0	158.0
6	97.8	103.3
7	165.1	163.5
8	92.4	96.4
9	157.3	158.7
10	104.6	109.1
1′	117.0	117.0
2′	156.7	156.6
3′	116.9	116.9
4′	132.8	132.4
5′	119.3	119.2
6′	128.4	128.2
1″	–	104.1
2″	–	73.5
3″	–	77.5
4″	–	69.8
5″	–	75.6
6″	–	60.8
7-OMe	55.9	55.9

coside (**2**) indicated that the glucose moiety in **2** must be attached to the C-5 hydroxyl group. The 5-*O*-glycosylation in **2** was further confirmed by a comparison of ^{13}C NMR data of **2** with **1** (Table 1) which showed upfield shifts of 3.0 and 4.9 ppm for the C-5 and C-4 resonances, and downfield shifts of 5.5 and 4.5 ppm for the C-6 and C-10 resonances, respectively [14]. The coupling constant (J = 7 Hz) of the anomeric proton signal at δ 4.78 indicated the β-configuration of the glucopyranoside. Thus, **2** was characterized as echioidinin 5-*O*-β-D-glucopyranoside.

EXPERIMENTAL

General

Mps: uncorr. UV and IR: MeOH and KBr discs, respectively. ^1H and ^{13}C NMR: 300.13 and 75.43 MHz, respectively in DMSO-d_6 and $CDCl_3$ with TMS as int. standard. EIMS at 70 eV (direct probe). FABMS was registered in positive ion mode using a glycerol matrix. CC: Silica gel finer than 200 mesh (0.08 mm).

Plant material

The whole plant of *Andrographis alata* Nees was collected from the Talakona hills, Andhra Pradesh, India, in January 1995. A voucher specimen has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

Extraction and isolation

Dried and powdered whole plant (2.5 kg) were successively extracted with n-hexane, Me_2CO and MeOH. The MeOH extract was defatted with n-hexane and the residue obtained on CC over silica gel using $CHCl_3$–EtOAc step gradient afforded **1** (150 mg) and **2** (260 mg).

Echiodinin (**1**)

Green–yellow needles (MeOH); mp 264–265°; UV λ_{max}^{MeOH} nm (log ϵ): 265 (4.40), 335 (4.19); +NaOMe: 265, 390; +NaOAc: 265, 335; +$AlCl_3$: 250, 274, 285 sh, 315 sh, 355; +$AlCl_3$/HCl: 235, 265, 315, 355; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2980, 1654 (C=O), 1615, 1520, 1456, 1360, ^1H NMR (DMSO-d_6): δ 12.87 (OH-5), 10.86 (OH-2′), 7.90 (1H, *dd*, J = 2,8 Hz, H-6′), 7.40 (1H, *dt*, J = 2,8 Hz, H-4′), 7.11 (1H, *s*, H-3), 6.99-7.06 (2H, *m*, H-3′, 5′), 6.73) (1H, *d*, J = 2.5 Hz, H-8). 6.35 (1H, *d*, J = 2.5 Hz, H-6), 3.90 (3H, *s*, OMe-7), ^{13}C NMR: Table 1; ELMS m/z (rel. int): 284 $[M]^+$ (100), 267 $[M–OH]^+$ (1) 255 $[M–CHO]^+$ (5), 254 $[M–CO]^+$ (2), 166 $[A_1]^+$ (4), 118 $[B_1]^+$ (1).

Echioidinin 5-*O*-β-D-*glucopyranoside* (**2**)

Pale yellow needles (MeOH), mp 245–246°; FAB-MS (positive mode) m/z (rel. int): 447 $[M + H]^+$ (5), 285 $[M + H\text{-glucosyl}]^+$ (23); UV λ_{max}^{MeOH} nm (log ϵ) 257 (4.38), 305 (4.33) and 330 (4.25); +NaOMe: 257 sh, 300 sh, 400 + NaOAc: 257, 305, 342 sh; +$AlCl_3$: 257, 315, 348 sh; +$AlCl_3$/HCl: 257, 288 sh, 335; IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 2950, 1652 (C=O), 1618, 1560; ^1H NMR (DMSO-d_6): δ 10.70 (OH-2′), 7.94 (1H, *dd*, J = 2, 8 Hz, H-6′), 7.38 (1H, *dt*, J = 2, 8 Hz, H-4′), 7.02–7.04 (2H, *m*, H-3′, 5′), 7.06 (1H, *s*, H-3), 6.98 (1H, *d*, J = 2.5 Hz, H-8), 6.92 (1H, *d*, J = 2.5 Hz, H-6), 4.78 (1H, *d*, J = 7 Hz, H-1″), 3.91 (3H, *s*, OMe-7), 3.35–3.85 (5H, *m*, sugar protons), ^{13}C NMR: Table 1.

Acid hydrolysis of **2**

A MeOH soln. of **2** (20 mg) in 2N HCl (5 ml) heated at 100° for 1 h gave glucose and (**1**) (12 mg) identified by mp, UV, IR, ^1H and ^{13}C NMR analysis.

Acetylation of **2**

A mixture of **2** (10 mg), Ac_2O (2 ml) and C_5H_5N (1 ml) was kept at room temp. for 48 h and poured into crushed ice to yield the penta-acetate of **2** as colourless crystals (12 mg) from MeOH, mp 120° IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O of OAc), 1654 (C=O), 1619, 1465, 1433, 1369, 1345, 1196; ^1H NMR ($CDCl_3$) δ: 7.47 (2H, *m*, H-4′, 6′), 7.12 (1H, *m*, H-3′), 6.96 (1H, *m*, H-5′), 6.67 (1H, *d*, J = 2.5 Hz, H-8), 6.63 (1H, *d*, J = 2.5 Hz, H-6), 6.14 (1H, *s*, H-3), 5.08 (1H, *d*, J = 7 Hz, H-1″), 5.10-5.19 (4H, *m*, H-2″, 3″, 4″, 5″), 4.19–4.27 (2H, *m*, CH_2-6″), 3.89 (3H, *s*, OMe), 2.42 (3H, *s*, OAc-2′), 1.91–2.08 (12H, *m*, 4 × OAc).

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