PERGAMON

Phytochemistry 52 (1999) 935–937

PHYTOCHEMISTRY

Dihydroechioidinin, a flavanone from *Andrographis echioides*

B. Jayaprakasam[a], A.G. Damu[a], D. Gunasekar[a,*], A. Blond[b], B. Bodo[b]

[a]*Natural Products Division, Department of Chemistry, Sri Venkateswara University, Tirupati, 517 502, India*
[b]*Laboratoire de Chimie des Substances Naturelles, URA 401 CNRS, Museum National d'Histoire Naturelle, 63 rue Buffon, 75005, Paris, France*

Received 15 March 1999; accepted 12 May 1999

Abstract

A reinvestigation of the whole plant of *Andrographis echioides* has led to the isolation of a new flavanone, dihydroechioidinin together with four known flavones, echioidinin, echioidin, skullcapflavone I 2′-*O*-methyl ether, and skullcapflavone I 2′-*O*-glucoside. The structure of dihydroechioidinin was established as (2*S*)-5,2′-dihydroxy-7-methoxyflavanone on the basis of spectral and chemical evidence.

Keywords: *Andrographis echioides*; Acanthaceae; Flavanone; Dihydroechioidinin

1. Introduction

Andrographis echioides Nees (Acanthaceae) is an erect herb widely distributed in the dry districts of tropical India and Sri Lanka (Gamble, 1956). In traditional medicine, the leaf juice of this plant is used as a remedy for fevers (Kirtikar & Basu, 1975). Previous study on the leaves of this species has resulted in the isolation of two new flavones, echioidinin (**3**) (Govindachari, Parthasarathy, Pai & Subramaniam, 1965) and echioidin (**4**) (Govindachari et al., 1965). As part of our ongoing studies on the species of *Andrographis* (Damu, Jayaprakasam & Gunasekar, 1998; Damu, Jayaprakasam, Rao & Gunasekar, 1998; Damu, Jayaprakasam, Gunasekar, Blond & Bodo, 1999), we reinvestigated the whole plant of *A. echioides* and report here the isolation and characterization of a new flavanone, dihydroechioidinin (**1**) and two known flavones, skullcapflavone I 2′-methyl ether (**2**) and skullcapflavone I 2′-*O*-glucoside (**5**) in addition to previously reported flavones **3** and **4**.

* Corresponding author. Tel.: +91-8574-42471; fax: +91-8574-27499.

2. Results and discussion

Dihydroechioidinin (**1**) isolated as colourless needles, showed $[M]^+$ peak at *m*/*z* 286 in its EIMS, consistent with the molecular formula $C_{16}H_{14}O_5$. This was supported by the appearance of 16 carbon signals in its ^{13}C NMR spectrum. The UV spectrum of **1** in methanol (285 and 332 (*sh*) nm) suggested a flavanone structure (Mabry, Markham & Thomas, 1970). Its UV spectral maxima was unaffected by the addition of sodium acetate indicating the absence of a free 7-hydroxyl. A bathochromic shift of 23 nm in its UV absorption maxima on addition of $AlCl_3$ and $AlCl_3$/HCl suggested the presence of a chelated hydroxyl group at C-5 position (Mabry et al., 1970).

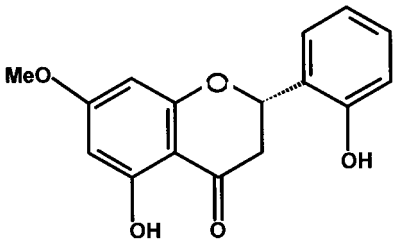
The ^1H NMR spectrum of **1**, showed two D_2O exchangeable downfield signals at δ 12.14 and 8.79 indicating the presence of a chelated hydroxyl at C-5 and a non-chelated hydroxyl group, respectively. The presence of three sets of double doublets at δ 5.81 (1H, J = 13.0, 3.0 Hz), 3.12 (1H, J = 17.2, 13.0 Hz) and 2.85 (1H, J = 17.2, 3.0 Hz) are typical of H-2, H-3_{ax} and H-3_{eq}, respectively of a flavanone moiety (Mabry et al., 1970). Two *meta* coupled doublets at δ

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00295-2

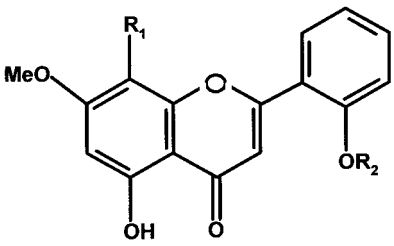
6.04 and 6.09, each integrating for one proton, were assigned to H-6 and H-8, respectively. It also showed the presence of a methoxyl group at δ 3.85 and was placed at C-7 as it showed ^3J coupling with C-7 at 168.8 ppm in its HMBC spectrum. The EI mass spectrum of **1** showed two *retro* Diels-Alder fragments at *m/z* 167 and 120 consistent with the presence of a hydroxyl and a methoxyl group in ring A, and a hydroxyl group in ring B, respectively. The chemical shift of C-2 in 2′-unsubstituted flavanones, usually appears at 79.0 ppm in the ^{13}C NMR spectrum (Agrawal, 1989). However, in dihydroechioidinin (**1**), the C-2 signal appeared at 75.5 ppm, which is unusually upfield, indicating the presence of non-chelated hydroxyl (δ 8.79) at C-2′ position of the B-ring (Agrawal, 1989). The chemical shifts of B ring carbons of **1** are consistent with literature values of 2′-hydroxyflavanones (Agrawal, 1989). Moreover, a typical ABCD spectrum at δ 6.93, 6.95, 7.21 and 7.53, established the presence of four adjacent aromatic protons in ring B. The ^{1}H and ^{13}C NMR assignments for **1** were unambiguously assigned based on ^{1}H-^{1}H COSY and HMBC studies. The laevorotatory nature of **1** indicated the normal flavanone stereochemistry, *S* at C-2. Thus the structure of dihydroechioidinin was established as (2*S*)-5,2′-dihydroxy-7-methoxyflavanone (**1**).

A final proof of the proposed structure for dihydroechioidinin was obtained by dehydrogenation (Goel, Mahesh & Seshadri, 1958) of **1** which resulted in a flavone whose physical and spectral data agreed well with those of echioidinin (**3**) (Damu et al., 1998; Govindachari et al., 1965).

Although dihydroechioidinin (**1**) has been reported synthetically (Viswanathan & Sidhaye, 1976), this is the first report of its isolation from a natural source. Incidentally, the occurrence of **1**, constitutes the first report of a 2′-oxygenated flavanone in *Andrographis*.



1



	R_1	R_2
2	OMe	Me
3	H	H
4	H	Glc
5	OMe	Glc

3. Experimental

3.1. General

Mps: Uncorr. UV: MeOH. IR: KBr. ^{1}H and ^{13}C NMR: 300.13 and 75.43 MHz, respectively in Me_2CO-d_6 and DMSO-d_6 with TMS as int. standard. EIMS: 70 eV. HMBC: optimized for a *J* value of 7 Hz. CC: Acme silica gel finer than 200 mesh (0.08 mm).

3.2. Plant material

The whole plant of *A. echioides* Nees was collected from Tirupati, Andhra Pradesh, India in May 1998. A voucher specimen (DG-199) has been deposited in the herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

3.3. Extraction and isolation

The dried and ground whole plant (3 kg) was successively extracted with *n*-hexane, Me_2CO and MeOH. The hexane extract on purification over a silica gel column using C_6H_6 as eluent yielded 25 mg of **1** and 100 mg of **2**. The Me_2CO extract on CC over silica gel using hexane–EtOAc step gradient afforded 60 mg of **3**, 50 mg of **4** and 60 mg of **5**.

3.3.1. Dihydroechioidinin (*1*)

Colourless needles ($CHCl_3$), mp 200–201°, $[\alpha]_D^{28°}$ −19.7° (MeOH; *c* 0.13). UV λ_{max}^{MeOH} nm (log ϵ): 285 (4.17), 332 *sh* (3.29); +NaOAc: 285, 332 *sh*; +$AlCl_3$: 308, 367; +$AlCl_3$/HCl: 308, 367. IR ν_{max}^{KBr} cm^{-1}: 3421 (—OH), 3239, 2829, 1645 (>C=0), 1601, 1507, 1456, 1345. ^{1}H NMR (300 MHz, Me_2CO-d_6): δ 12.14 (OH-5), 8.79 (OH-2′), 7.53 (1H, *dd, J* = 7.5, 2.0 Hz, H-6′), 7.21 (1H, *ddd, J* = 7.5, 7.5, 2.0 Hz, H-4′), 6.95 (1H, *dd, J* = 7.5, 2.0 Hz, H-3′), 6.93 (1H, *ddd, J* = 7.5, 7.5,

2.0 Hz, H-5′), 6.09 (1H, *d*, J = 2.3 Hz, H-8), 6.04 (1H, *d*, J = 2.3 Hz, H-6), 5.81 (1H, *dd*, J = 13.0, 3.0 Hz, H-2), 3.85 (3H, *s*, OMe-7), 3.12 (1H, *dd*, J = 17.2, 13.0 Hz, H-3_{ax}), 2.85 (1H, *dd*, J = 17.2, 3.0 Hz, H-3_{eq}). ^{13}C NMR (75 MHz, Me_2CO- d_6): δ 197.7 (C-4), 168.8 (C-7), 165.0 (C-5), 164.4 (C-8a), 154.8 (C-2′), 130.2 (C-4′), 127.2 (C-6′), 126.2 (C-1′), 120.7 (C-5′), 116.3 (C-3′), 103.7 (C-4a), 95.5 (C-6), 94.5 (C-8), 75.5 (C-2), 56.2 (OMe-7), 42.5 (C-3). EIMS *m/z* (rel.int.): 286 $[M]^+$ (55), 268 (100), 193 (16), 167 (82), 138 (21), 120 (17).

*3.3.2. Skullcapflavone I 2′-methyl ether (**2**)*

Yellow needles ($CHCl_3$), mp 190–191°. UV, IR, ^{1}H NMR and EIMS data were similar to literature values (Jalal, Overton & Rycroft, 1979).

*3.3.3. Echioidinin (**3**)*

Green-yellow needles (MeOH), mp 264–265°. UV, IR, ^{1}H and ^{13}C NMR, and EIMS data were identical with published data (Damu et al., 1998; Govindachari et al., 1965).

*3.3.4. Echioidin (**4**)*

Yellow needles (MeOH), mp 276–278° (dec). FABMS (positive mode) *m/z* (rel.int.): 447 $[M+H]^+$ (50), 285 $[M+H\text{-glucosyl}]^+$ (100). ^{1}H NMR (300 MHz, Me_2CO-d_6 + DMSO-d_6): δ 12.92 (OH-5), 7.95 (1H, *dd*, J = 7.5, 2.0 Hz, H-6′), 7.57 (1H, *ddd*, J = 7.5, 7.5, 2.0 Hz, H-4′), 7.43 (1H, *dd*, J = 7.5, 2.0 Hz, H-3′), 7.31 (1H, *ddd*, J = 7.5, 7.5, 2.0 Hz, H-5′), 7.17 (1H, *s*, H-3), 6.71 (1H, *d*, J = 2.0 Hz, H-8), 6.34 (1H, *d*, J = 2.0 Hz, H-6), 5.20 (1H, *d*, J = 7.0 Hz, H-1″), 3.90 (3H, *s*, OMe-7), 3.22–3.80 (6H, *m*, sugar protons). EIMS *m/z* (rel.int.): 284 $[M\text{-glucosyl}]^+$ (100), 267 (7), 255 (11), 166 (19), 122 (9), 118 (9). UV and IR data were consistent with literature values (Govindachari et al., 1965).

*3.3.5. Skullcapflavone I 2′-O-β-D-glucopyranoside (**5**)*

Yellow amorphous powder, mp 260–262°. FABMS (positive mode) *m/z* (rel.int.): 477 $[M+H]^+$ (46), 315 $[M+H\text{-glucosyl}]^+$ (100). UV, IR, ^{1}H NMR and EIMS data were in good agreement with reported data (Gupta, Taneja & Dhar, 1996).

*3.4. Dehydrogenation of **1***

A mix. of 10 mg of **1**, 0.13 g of KOAc and 25 mg of I_2 in glacial HOAc (1 ml) was heated under reflux in an oil bath for 2 h. The reaction mix. was cooled and poured into crushed ice and extracted with EtOAc. The solvent was removed in vacuo and a satd soln of $NaHSO_3$ was added to the residue to destroy excess of I_2. It was filtered and the residue obtained was crystallized from MeOH to yield green-yellow needles, mp 264–265°, identical in all respects with echioidinin (**3**) (Damu et al., 1998; Govindachari et al., 1965).

Acknowledgements

The authors thank Mr. J. P. Brouard, Laboratoire de Chimie des Substances Naturelles, MNHN, Paris, France for providing mass spectral data. BJ is grateful to CSIR, New Delhi for financial assistance.

References

Agrawal, P. K. (1989). *The Carbon-13 NMR of flavonoids* (pp.96–97; 100–108). Amsterdam: Elsevier.

Damu, A. G., Jayaprakasam, B., & Gunasekar, D. (1998). *Journal of Asian Natural Products Research*, *1*, 133.

Damu, A. G., Jayaprakasam, B., Rao, K. V., & Gunasekar, D. (1998). *Phytochemistry*, *49*, 1811.

Damu, A. G., Jayaprakasam, B., Gunasekar, D., Blond, A., & Bodo, B. (1999). *Phytochemistry* (in press).

Gamble, J. S. (1956). *Flora of the Presidency of Madras*. Botanical Survey of India, Calcutta, Vol. 2, p. 1051.

Goel, R. N., Mahesh, V. B., & Seshadri, T. R. (1958). *Proceedings of Indian Academy of Sciences*, *47*, 184.

Govindachari, T. R., Parthasarathy, P. C., Pai, B. R., & Subramaniam, P. S. (1965). *Tetrahedron*, *21*, 2633; 3715.

Gupta, K. K., Taneja, S. C., & Dhar, K. L. (1996). *Indian Journal of Chemistry*, *35B*, 512.

Jalal, M. A. F., Overton, K. H., & Rycroft, D. S. (1979). *Phytochemistry*, *18*, 149.

Kirtikar, K. R., & Basu, B. D. (1975). In *Indian medicinal plants*, *3*. New Delhi: Periodical Experts.

Mabry, T. J., Markam, K. R., & Thomas, M. B. (1970). *The systematic identification of flavonoids*. New York: Springer.

Viswanathan, N., & Sidhaye, A. R. (1976). *Indian Journal of Chemistry*, *14B*, 644.