Phytochemistry, Vol. 25, No. 7, pp. 1770–1771, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

AN ACYLATED FLAVONE APIGENIN 7-*O*-β-D-(4″-*CIS*-*p*-COUMAROYL)GLUCOSIDE FROM *ECHINOPS ECHINATUS*

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(*Received* 12 *August* 1985)

Key Word Index—*Echinops echinatus*; Compositae; glycosylflavones; apigenin 7-*O*-glucoside; apigenin 7-*O*-β-D-(4″-*cis*-*p*-coumaroyl)glucopyranoside; FD-MS; ^{13}C NMR.

Abstract—Besides apigenin 7-*O*-glucoside, a new acylated flavone has been identified in *Echinops echinatus* as apigenin 7-*O*-β-D-(4″-*cis*-*p*-coumaroyl)glucoside from spectral and chemical analysis.

Echinops echinatus L. (syn. *M. compositae*, voucher specimen deposited at the CIMAP Herbarium) is a pubescent annual plant which is distributed throughout India, ascending to 1700 m on the hills. The plant was collected from Kurukshetra, India. It is traditionally used as nerve tonic in hoarse cough, hysteria and ophthalmia [1]. Earlier work on *E. echinatus* led to the isolation of triterpenoids [2].

We report here isolation and characterization of two *O*-glycosylflavones from the aerial parts of *E. echinatus* including one new compound. Compound **1**, isolated from the alcoholic extract through fractionation with chloroform, has been found to be apigenin 7-*O*-glucoside by spectral and chemical analysis [3].

Compound **2**, isolated from ethanolic extract through *n*-butanol extraction, appeared as a violet spot on chromatograms under UV light and UV spectral data indicated an apigenin glycosidic structure [4]. Whereas the UV spectrum of **1** showed two bands at λ_{max} 271 (Band II) and 233 (Band I) nm of nearly equal intensity, Band I (316 nm) was much higher than Band II (267 nm) in the UV spectrum of **2**, suggesting the presence of an additional chromophore [5]. Also the IR band at 1702 cm^{-1} showed the presence of an ester moiety. However, the usual diagnostic reagents disclosed the presence of a 7-*O*-substituted apigenin structure with free 5- and 4′-hydroxyl groups [4].

On acid hydrolysis, **2** gave D-glucose and apigenin (UV, ^{1}H NMR and MS data). On mild alkaline hydrolysis, **2** afforded **1** indicating the acylation in the glycosidic part of the molecule. FD-MS showed the molecular ion peak at 578 corresponding to the presence of an ion $[M+Na]^+$ at 601. The peak at *m/z* 414 $[M - HOC_6H_4\text{–}CH{=}CH\text{–}COOH]^+$ confirmed the presence of a hydroxycinnamoyl moiety attached to the 7-*O*-hexosyl residue of apigenin 7-*O*-glucoside.

The ^{1}H NMR spectrum of **2** showed all protons characteristic of the molecule (see Experimental) and the appearance of the olefinic protons (H-α and H-β) at 6.10 and 6.91 ($J = 11$ Hz each) respectively suggested the presence of *cis*-*p*-coumaroyl moiety [6] (for *trans*-*p*-coumaroyl moiety, $J = 16$ Hz [7]).

The ^{13}C NMR signals for the carbon atoms of the flavanoid nuclei of **1** and **2** and the *cis*-*p*-coumaroyl moiety of **2** respectively (see Experimental) are in conformity with the formulations outlined above. The chemical shifts for glucose carbons in **1** and **2** (Table 1) indicated its β-pyranose form by comparison with the ^{13}C NMR of methyl-β-D-glucopyranoside [8].

Also in the ^{1}H NMR spectrum of **2**, the anomeric proton (H-1″) at δ5.2 ($J = 8$ Hz) showed the presence of a β-glucosyl moiety. In **2**, the resonances assigned to the *O*-glucosyl carbons are indicative of the cinnamoylation at position C-4 because of the upfield shift of C-5 and C-3 by δ2.55 and 2.60 and downfield shift of C-4 by δ1.40 respectively [9, 10]. Apigenin 7-*O*-(4″-coumaroyl)glucoside has been reported [7] and the IR spectra of **2** and the authentic sample were found to be nonsuperimposable. Also the UV and ^{1}H NMR data of **2** differed from those of an authentic sample [7]. Thus the structure of **2** was confirmed as apigenin 7-*O*-β-D-(4″-*cis*-*p*-coumaroyl)glucopyranoside. As the *trans* is more stable than the *cis*, it is a unique addition to the known acylated flavone glycoside of biogenetic importance.

EXPERIMENTAL

NMR: Varian FT-80A, δ values; solvent DMSO-d_6; mps uncorr., FD-MS on a JEOL JMS-O1 SG-2; silica gel BDH India.

Extraction. Fresh aerial parts of *E. echinatus* (1.5 kg) were extracted with 90% EtOH (7 × 15 l.). After concn under red. pres. and filtration, the residue was diluted with 200 ml of H_2O and extracted with hexane, $CHCl_3$ and *n*-BuOH successively.

Isolation. A crude yellow solid was separated on addition of MeOH–Me_2CO to the *n*-BuOH extract and was further washed repeatedly by Me_2CO, followed by MeOH. Thus the residue obtained was chromatographed over a column of silica gel using *iso*-PrOH–$CHCl_3$ of increasing polarities. The residue obtained from the 35% *iso*-PrOH–$CHCl_3$ was further purified from hot MeOH and finally by prep. TLC in EtOAc–Me_2CO (4:1) to afford the pure compound **2** (50 mg). The concn of *n*-BuOH washing on chromatography over silica gel afforded from 25% MeOH–$CHCl_3$ a yellow compound which was further purified by prep. TLC (silica gel, MeOH–$CHCl_3$, 1:3) to yield the homogeneous compound **1** (30 mg).

Apigenin 7-O-β-D-glucoside (**1**). Mp 209°; TLC (silica

Table 1. Chemical shift data for sugars in the ^{13}C NMR of 1 and 2

Compound	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
1	99.90	73.20	77.20	69.60	76.40	60.70
2	99.90	73.20	74.80	71.00	73.85	60.60

Chemical shifts in δ (ppm); solvent DMSO-d_6 39.5.

gel): R_f 0.65 (EtOAc–MeOH–H_2O, 63:12:9), 0.70 (EtOAc–Me_2CO–AcOH, 55:50:2); UV λ_{max}^{MeOH} nm: 268, 333; ^{13}C NMR (DMSO-d_6): 182.2 (C-4) 164.6 (C-2), 163.10 (C-7), 162.0 (C-5), 161.4 (C-4'), 157.2 (C-9), 129.01 (C-2', 6'), 120.9 (C-1'), 116.2 (C-3', 5'), 105.3 (C-10), 102.9 (C-3), 99.50 (C-6), 94.9 (C-8); for sugar carbon resonances see Table 1. On hydrolysis it afforded apigenin and D-glucose.

Apigenin 7-O-β-D-(4″-cis-p-coumaroyl)glucopyranoside (2). Found C 62.1; H 5.41% $C_{28}H_{38}O_{14}$ requires C, 62.3; H, 5.48%; mp 248° (dec.); TLC (silica gel): 0.85 (EtOAc–MeOH–H_2O, 63:12:9), 0.70 (EtOAc–Me_2CO–AcOH, 70:30:1), 0.81 (EtOAc–Me_2CO, 4:1); (cellulose), 0.25 (30% AcOH); UV λ_{max}^{MeOH} nm: 226, 268, 316; + $AlCl_3$ 226, 276, 298, 323, 383; + $AlCl_3$ + HCl 276, 275, 297, 323, 385; + NaOMe 242 sh, 298 sh, 273, 375; + NaOAc 268, 376; + NaOAc + H_3BO_3, 268, 375; IR ν_{max}^{KBr} cm^{-1}: 3700–3040 (OH), 1702 (ester C=O) 1656 (>C=O), 1600, 1510, 1495 (aromatic), 1445, 1240, 1170, 830; ^1H NMR (DMSO-d_6): δ7.95 (2H, *d*, *J* = 10 Hz, H-2', 6'), 6.91 (1H, *d*, *J* = 11.0 Hz, H-β), 7.50 (2H, *d*, *J* = 10 Hz, H-2‴ and 6‴), 6.65–7.15 (6H, *m*, H-6, 8, 3', 5', 3‴, 5‴), 6.5 (1H, *s*, H-3), 6.10 (1H, *d*, *J* = 11.0 Hz, H-α), 5.2 (1H, *d*, *J* = 8 Hz, H-1″) 4.6–4.95 (*m*); ^{13}C NMR (DMSO-d_6): δ182.20 (C-4), 166.2 (C=O, *p*-Cou), 164.6 (C-2), 163.15 (C-7), 161.20 (C-5), 161.12 (C-4'), 160.20 (C-4‴), 157.10 (C-9), 145.25 (C-β), 130.4 (C-2‴, 6‴), 128.9 (C-2', 6'), 125.15 (C-1‴), 121.05 (C-1'), 115.80/115.90 (C-3', 5' and C-3‴, 5‴), 114.20 (C-α), 105.25 (C-10), 103.08 (C-3), 99.80 (C-6), 95.08 (C-8), for sugar carbon resonances see Table 1 (carbon numbers bearing the superscript ‴ are of *cis*-p-coumaroyl moiety).

Acid hydrolysis. Compound 2 was dissolved in MeOH–4 N HCl (1:1) and heated at 100° for 2 hr. After repeated evapn of the solvent, the pptd residue was filtered off and purified by prep. TLC on silica gel in MeOH–$CHCl_3$ (1:4) to afford apigenin. The sugar in aq. phase was concd and run on PC giving glucose (co-PC) (*n*-BuOH–pyridine–H_2O, 6:4:3, *n*-BuOH–AcOH–H_2O, 4:1:5).

Alkaline hydrolysis. Compound 2 was dissolved in MeOH–4 N KOH (1:1) and left in a closed flask for 2 hr at room temp. After acidification with 4 M HCl and evapn to dryness under red. pres., the residue was extracted with EtOAc and *n*-BuOH. The *n*-BuOH extract showed the same UV, ^{13}C NMR data and chromatographic behaviour as 1.

Acknowledgements—We thank Dr. K. P. Madhusudanan, CDRI, Lucknow, for FD-MS analysis and are grateful to Dr. Chr. Karl, Walda Ag, B.R.D. for the generous gift of apigenin 7-*O*-(4″-*p*-coumaroyl)glucoside. We are also grateful to our Director for constant encouragement and to the Instrumental Centre for providing necessary spectral facilities.

REFERENCES

1. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *Glossary of Indian Medicinal Plants* p. 104. CSIR, New Delhi.
2. Sen, A. B., Dhar, M. M. and Shukla, Y. N. (1968) *J. Indian Chem. Soc.* **45**, 697.
3. Redaelli, C., Formentini, L. and Santaniell, E. (1980) *Phytochemistry* **19**, 985.
4. Mabry, T. J., Markham, K. P. and Thomas, M. B. (1970) in *The Systematic Identification of Flavonoids*. Springer, Heidelberg.
5. Chopin, J., Dellamonica, G., Markham, K. R., Ramachandran Nair, A. G. and Gunasegaran, R. (1984) *Phytochemistry* **23**, 2106.
6. Romnssi, G., Parodi, B. and Sancassan, F. (1984) *Justus Liebigs Ann. Chem.* **11**, 1867.
7. Karl, C., Muller, G. and Pedersen, P. A. (1976) *Phytochemistry* **15**, 1084.
8. Gorin, P. A. J. and Mazurek, M. (1975) *Can. J. Chem.* **53**, 1212.
9. Bundle, D. R., Jennings, H. J. and Smith, I. C. P. (1973) *Can. J. Chem.* **51**, 3812.
10. Vignon, M. R. and Vottero, Ph. J. A. (1976) *Tetrahedron Letters* 2455.