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# FLAVONOL GLYCOSIDES FROM LEAVES OF *EUPATORIUM GLANDULOSUM*

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**Key Word Index**—*Eupatorium glandulosum*; Asteraceae; leaves; flavonol glycosides; 4′-methylquercetagetin 7-*O*-(6″-*E*-caffeoyl-β-D-glucopyranoside); quercetagetin 7-*O*-(6″-acetyl-β-D-gluco-pyranoside).

**Abstract**—Two new flavonol glycosides: 4′-methylquercetagetin 7-*O*-(6″-*E*-caffeoyl-β-D-glucoside) and quercetagetin 7-*O*-(6-acetyl-β-D-glucoside) along with the 7-*O*-glucosides of 6-hydroxykaempferol, quercetin and quercetagetin have been isolated and characterized from the leaves of *Eupatorium glandulosum*.

## INTRODUCTION

*Eupatorium glandulosum* (Asteraceae), earlier examined in our laboratories, revealed the presence of three rare flavonoids: betuletol (6-hydroxykaempferol 6,4′-dimethyl ether), betuletol 3-galactoside and 6-hydroxykaempferol 7-(6″-caffeoyl glucoside) [1, 2]. In view of the novelty of the compounds, especially the acylglycoside and the reported analgesic activity of this type of compound in mice and rats [3], larger quantities of the leaves of the same plant have been re-investigated for additional flavonoids. Two new flavonol glycosides, along with three known compounds, have been isolated and characterized.

## RESULTS AND DISCUSSION

Adopting multistep chromatographic separation [4] (DCCC, column, Centrifugal TLC, TLC and PC) six flavonol glycosides (**1**–**6**) were isolated from the ether insoluble fraction of the aqueous alcoholic concentrate of the leaves of *E. glandulosum* (see Experimental). Only one of them (6-hydroxykaempferol 7-*O*-caffeoyl glucoside **6**), has been previously known in this plant. While quercetagetin 4′-methyl ether 7-*O*-(6″-*E*-caffeoyl-β-D-glucopyranoside) (**1**) and quercetagetin 7-*O*-(6″-acetyl-β-D-glucopyranoside) (**2**) are new natural products, quercetagetin 7-*O*-β-D-glucoside (**3**), 6-hydroxykaempferol 7-*O*-β-D-glucoside (**4**) and quercetin 7-*O*-β-D-glucoside (**5**) are reported for the first time in this plant.

Compound **1** on acid hydrolysis yielded an aglycone, D-glucose and *E*-caffeic acid in almost equal molar ratio. The $\lambda_{max}$ of the aglycone (in MeOH and in presence of NaOAc, NaOMe, $AlCl_3$ $AlCl_3$/HCl, NaOAc/$H_3BO_3$) along with the development of the characteristic green colour with $NH_3$, indicated it to be a quercetagetin derivative with no free hydroxyl at C-4′ [5, 6]. The $^1$H NMR spectrum indicating the presence of 3,5,6,7,3′,4′-oxygenated flavone skeleton and an OMe group was in further support of 4′-*O*-methylquercetagetin [5]. The presence of 4′-OMe was further confirmed by the increase in peak intensity of 5′-H by selective irradiation of – OMe protons. Thus, the compound was identified as a caffeoyl glucoside of 4′-*O*-methylquercetagetin. That only the 7-OH group of the aglycone was involved in glycosylation was evident from: (a) the identical $\lambda_{max}$ of aglycone and glycoside in MeOH, $AlCl_3$ and $AlCl_3$/HCl [5–7], (b) the absence of any shift in Band II by NaOAc [5–7] and absence of Band III (between 305 and 350 nm) in NaOAc indicating [8] the absence of free7-OH, (c) the 8 nm bathochromic shift in Band II of the glycoside compared to quercetin revealing a free 6-OH [9] which was further confirmed [6] by the presence of the shoulder around 433 nm in the $AlCl_3$/HCl spectrum. A 6-*O*-glycosyl structure was ruled out from the absence of shoulder around 385 nm in $AlCl_3$ and $AlCl_3$/HCl spectra [6, 7]. The site of attachment of glucose as well as stereochemistry of the caffeoyl moiety was determined by analysing the $^1$H NMR signals (and *J*) of the $C\underline{H}_2OH$ of glucose and –C$\underline{H}$ = C$\underline{H}$– of caffeoyl part respectively and comparing them with 7-*O*-(6″-*E*-caffeoyl-β-D-glucoside) [2]. The anomeric proton of glucose appearing as doublet ($J$ = 7.1 Hz) at 5.1 indicated the β-configuration. FABMS of the glycoside showing peaks at 695 $(M + K)^+$, 679 $(M + Na)^+$, 347 (caffeoyl glucose + H)$^+$ and 333 (aglycone + H)$^+$ was in further support of the above structure (the quantity of the glycoside was insufficient to further confirm the structure by $^{13}$C NMR

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analysis [2]). Based on these findings and on the observation that the most favoured site of acylation in glucose is C-6 [10], the glycoside was constituted as 4′-methylquercetagetin-7-*O*-(6″-*E*-caffeoyl-β-D-glucopyranoside).

Compound **2** had $\lambda_{max}$ almost identical with quercetagetin. On acid hydrolysis it yielded quercetagetin and D-glucose. Characteristic shifts with diagnostic reagents revealed the absence of a free 7-OH indicating it to be the 7-*O*-glucoside of quercetagetin. $^1$H NMR spectrum of **2** was very similar to that of **1** except the absence of an OMe signal in the aglycone part and acetyl (2.03) replacing caffeoyl part. FABMS showing peaks at *m/z* 523 $(M + H)^+$ and 319 (aglycone + H) supported the structure as an acetylglucoside of quercetagetin. Analysis of the $^1$H NMR spectrum, as in the case of **1**, favoured the site of acylation at C-6 and the stereochemistry of the glycoside linkage as β. Thus, **2** is quercetagetin 7-*O*-(6″-acetyl-β-D-glucopyranoside). Both **1** and **2** are new natural products. The other three glycosides have been found to be quercetagetin 7-*O*-glucoside, 6-hydroxykaempferol 7-*O*-glucoside and quercetin 7-*O*-glucoside. The detailed $^{13}$C NMR spectral data for quercetagetin 7-*O*-glucoside is reported for the first time. **1** is the second glycoside of the very rare aglycone, 4′-methylquercetagetin, the first being the 3-*O*-arabinoside [11]. Similarly **2** is the fifth glycoside of the rare quercetagetin, others being the 3-*O*-rhamnoside, 7-*O*-glucoside, 3-glucoside and 3,7-diglucoside [12]. Our results show that biosynthesis of 6-acylated glucosides of 6-hydroxyflavonols is an important characteristic of *E. glandulosum* and warrant further examination of other *Eupatorium* species.

## EXPERIMENTAL

*Plant material.* Leaves of *E. glandulosum* were obtained from Lovedale, Nilgiris, Tamil Nadu, India (voucher specimen No. 2/89 [1, 2]).

*Isolation.* Fresh leaves (1.5 kg) were extracted with boiling 90% EtOH (3 × 5 l). The combined extract was concd *in vacuo*. The crude solid obtained from the aqueous alcoholic concentrate, after digestion with petroleum (b.r. 60–80°) and $Et_2O$ was dissolved in MeOH and subjected to ascending DCCC using BAW upper layer as mobile phase and lower layer as stationary phase. The mixt. of flavonoids obtained from different frs was found to be almost same, glycosidic in nature and free from aglycones and non-flavonoids. The total yield of purified flavonoid was 5 g (1.2% on dry basis).

1.0 g of the mixture was column chromatographed over polyamide SC 6 using toluene:ethyl methyl ketone:methanol (25-42-33) as developing solvent. 300 ml frs were collected serially and tested by PC. Frs 7–9 contained six flavonoid compounds. These were further separated by multistage chromatographic techniques. Preparative PC (ascending) of fr. 9 on Whatman 3 using 50% HOAc (16 h) yielded one pure compound (**6**) from the low $R_f$ zone. The upper bands of frs 7 and 8 were separately chromatographed on Sephadex LH20. Sephadex frs 4 and 5 of frs 7, 3 and 4 of frs 8, being similar on PC were mixed and processed by centrifugal chromatography on polyamide DC6 using water–ethyl methyl ketone–acetylacetone–methanol (13:3:3:1) mixture with two runs. Three bands were separated. Two bands on elution with methanol yielded **1** and **2** in pure form. The compound from third band was chromatographed on Sephadex LH20, the initial frs gave **4** and the later frs **5**. Compound **3** was obtained from the lower band in PC (60% HOAc, two runs) of polyamide frs 8 and 9 after passing through Sephadex LH20.

*4′-methylquercetagetin7-O-(6″-O-E-caffeoylglucopyranoside*) (**1**). Positive FAB-MS *m/z*: 695 $(M + K)^+$ (7%), 679 $(M + Na)^+$ (100), 347 (caffeoyl glucose + H)$^+$ (24), 333 (aglycone + H)$^+$ (29) and 163 (caffeic acid + H)$^+$ (15%). UV $\lambda_{max}^{MeOH}$ nm: 245, 253sh, 279, 338, 382sh (MeOH + NaOMe) 265, 379dec (MeOH + $AlCl_3$) 245, 266, 287, 376, 433sh (MeOH + $AlCl_3$ + HCl) 267, 338, 389, 433 (MeOH + NaOAc) 267, 370 (MeOH + NaOAc + $H_3BO_3$) 256sh, 285, 348, $^1$H NMR (200 MHz, $CD_3OD$, TMS as int. standard): 7.68 (1H, *d*, *J* = 2 Hz, H-2′), 7.65 (1H, *dd*, *J* = 8 Hz and 2 Hz, H-6′), 7.45 (1H, *d*, *J* = 15.9 Hz, H-3‴ of caffeoyl), 6.86 (1H, *d*, *J* = 8 Hz, H-5′), 6.83 (1H, *s*, H-8), 6.73 (1H, *d*, *J* = 2 Hz, H-5‴), 6.56 (1H, *dd*, *J* = 8 Hz and 2 Hz, H-9‴), 6.49 ((1H, *d*, *J* = 8 Hz, H-8‴), 6.17 (1H, *d*, *J* = 15.9 Hz, H-2‴) 5.06 (1H, *d*, *J* = 7 Hz, H-1″ of glucose) 4.3 and 4.24 (1H each, *dd* each, $H_2$-6″), 3.86 (3H, *s*, $OCH_3$-4′). TLC: 28 (cellulose, t-BAW, 3:1:1)[a], 32 (microcrystalline cellulose, BAW, 4:1:3)[b], 56 (BEW = 4:1:4)[c], 49 (60% HOAc)[d], 17 (polyamide DC6, $H_2O$, MEK, $(Ac)_2CH_2$, MeOH, 13:3:3:1)[e].

*Quercetagetin 7-O-(6″-O-acetyl-β-D-glucopyranoside*) (**2**). Positive FAB-MS *m/z*: 523 $(M + H)^+$ (30%), 319 (aglycone + H)$^+$ (100%). UV $\lambda_{max}^{MeOH}$ nm: 242sh, 259, 277sh, 373 (MeOH + NaOMe) 266, 285sh, 412dec. (MeOH + $AlCl_3$) 279, 443 (MeOH + $AlCl_3$ + HCl) 253, 270, 390, 425 (MeOH + NaOAc) 262sh, 287sh, 403 (MeOH + NaOAc + $H_3BO_3$) 270, 386. $^1$H NMR, (200 MHz, $CD_3OD$): 7.72 (1H, *d*, *J* = 2 Hz, H-2′), 7.63 (1H, *dd*, *J* = 8.4 Hz and 2 Hz, H-6′), 6.87 (1H, *d*, *J* = 8.4 Hz, H-5′), 6.86 (1H, *s*, H-8), 5.01 (1H, *d*, *J* = 7.2 Hz, H-1″), 4.49 and 4.21 (1H each, *dd* each, $H_2$-6″), 2.03 (3H, *s*, $OCOCH_3$-6″). TLC: 24[a], 28[b], 48[c], 47[d] and 23[e].

*Quercetagetin 7-O-β-glucoside* (**3**). Positive FAB-MS *m/z*: 481 $(M + H)^+$ (44%), 319 (aglycone + H)$^+$ (100%). $^1$H NMR, (400 MHz, DMSO-$d_6$, TMS): 7.69 (1H, *d*, *J* = 2.1 Hz, H-2′), 7.53 (1H, *dd*, *J* = 8.5 Hz and 2.1 Hz, H-6′), 6.91 (1H, *s*, H-8), 6.89 (1H, *d*, *J* = 8.5 Hz, H-5′) 5.0 (1H, *d*, *J* = 7 Hz, H-1″), 3.76–3.2 (unresolved *m*, other sugar protons). $^{13}$C NMR (100 MHz, DMSO-$d_6$, TMS, broad band decoupled): 176.4 (C-4), 151.9 (C-7), 148.5 (C-9), 148.1 (C-4′), 147.9 (C-2), 145.8 (C-5), 145.4 (C-3′), 130.9 (C-3), 130.1 (C-6), 123.4 (C-1′) 120.3 (C-6′), 115.9 (C-5′). 115.8 (C-2′), 105.5 (C-10), 101.5 (C-1″), 94.1 (C-8), 77.6 (C-5″), 76.2 (C-3″), 73.6 (C-2″), 70.2 (C-4″) 61.1 (C-6″). TLC: 9[a], 13[b], 21[c], 27[d] and 27[e].

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