120.68 (*d*, C-6'), 124.24 (*d*, C-3), 127.60 (*d*, C-5'), 140.76 (*s*, C-9), 144.59 (*s*, C-5), 161.09 (*s*, C-7), 161.32 (*s*, C-3'), 163.54 (*s*, C-7'). Diacetate, crystals, mp 198–202° (MeOH). EIMS (probe, 70 eV) *m/z* 394 $[M]^+$. $^1$H NMR (500 MHz, $CDCl_3$); $\delta$1.26 (3H, *s*, 2-Me), 1.51 (3H, *s*, 2-Me), 2.46 (6H, s, -OCOMe × 2), 5.71 (1H, *d*, $J$ = 10.03, H-3), 6.48 (1H, *d*, $J$ = 0.85 Hz, H-8), 6.69 (1H, *d*, $J$ = 10.03, 0.85 Hz, H-4), 7.32 (1H, *dd*, $J$ = 7.9 Hz, H-5'), 7.44 (1H, *dd*, $J$ = 1.50, 7.90 Hz, H-4'), 8.11 (1H, *dd*, $J$ = 1.50, 7.90 Hz, H-6').


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*Phytochemistry*, Vol. 29, No. 3, pp. 1011–1012, 1990.
Printed in Great Britain.

0031-9422/90 $3.00 + 0.00
Pergamon Press plc

# 6-HYDROXYKAEMPFEROL 6,4'-DIMETHYL ETHER 3-GALACTOSIDE FROM *EUPATORIUM GLANDULOSUM*

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(*Received in revised form* 9 *August* 1989)

**Key Word Index**—*Eupatorium glandulosum*; Asteraceae; flowers; flavonoids; 3,5,7-trihydroxy-6,4'-dimethoxyflavone 3-galactoside.

**Abstract**—3,5,7-Trihydroxy-6,4'-dimethoxyflavone (betuletol) and its 3-galactoside have been characterized from the flowers of *Eupatorium glandulosum*.

## INTRODUCTION

*Eupatorium glandulosum* H.B & K [1] growing in and around Ooty hills in South India has not been examined previously for its chemical constituents. We here report the identification of an uncommon methoxylated flavonol and its 3-galactoside from the flowers.

## RESULTS AND DISCUSSION

The structure of the aglycone betuletol(6-hydroxykaempferol 6,4'-dimethyl ether) was established by mp, IR, UV, $^1$H NMR, $^{13}$C NMR, mass spectral data, demethylation and finally by co-chromatography with authentic sample [2]. The glycoside, purified through CC (silica gel; $CHCl_3$–$Me_2CO$, 1:9), $C_{23}H_{24}O_{12}$, gave à light red colour with magnesium/hydrochloric acid, green with ferric chloride and a positive Molisch's test. It was purple under UV, yellow under UV/$NH_3$ and had $\lambda_{max}^{MeOH}$ 273, 341 nm, indicating a flavonol glycoside [3, 4]. The FDMS showed the psuedomolecular ion peak at *m/z* 493 $[M+H]^+$ suggesting that the compound was a hexoside of betuletol. On acid hydrolysis it gave betuletol and D-galactose in a 1:1 ratio. The purple UV fluorescence of the glycoside in comparison with yellow of the aglycone suggested involvement of the 3-OH in glycosylation [5]. This was supported by a hypsochromic shift of 23 nm (band I) in the UV spectrum of the glycoside compared to aglycone. Its $^1$H NMR spectrum gave further evidence for the aglycone as well as sugar components. The appearance of the anomeric proton as a doublet at $\delta$5.44 ($J$ = 7.6 Hz) showed the $\beta$-linkage of galactose [5]. The $^{13}$C NMR spectrum also revealed the nature of the aglycone, sugar, position of glycosylation and orientation and ring size of the sugar (see Experimental). The absorption frequency of C-3 (ipsocarbon) and C-2 and C-4 (orthocarbons) of the glycoside showed characteristic up- and downfield shifts with respect to the aglycone carbons, in agreement with 3-*O*-glycosylation. The occurrence of the anomeric carbon signal at $\delta$101.69 is in agreement with the anomeric carbon of the 3-*O*-linked $\beta$-D-galactopyranoside [6]. The compound gave a hexaacetate whose UV and $^1$H NMR spectral data were consistent

Table 1. $^1$H NMR chemical shifts of compounds **1**–**4** [270 MHz, DMSO-$d_6$, $\delta$ppm, TMS internal, multiplicity and coupling constant (Hz) shown in parentheses]

| H | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 8 | 6.90 (*s*) | 6.88 (*s*) | 6.90 (*s*) | 6.87 (*s*) |
| 2′ | 8.10 (*d*, 8.8) | 7.83 (*d*, 8.7) | 8.10 (*d*, 8.8) | 8.07 (*d*, 8.8) |
| 3′ | 6.94 (*d*, 8.8) | 7.27 (*d*, 8.7) | 6.88 (*d*, 8.9) | 7.21 (*d*, 8.8) |
| 5′ | 6.94 (*d*, 8.8) | 7.27 (*d*, 8.7) | 6.88 (*d*, 8.9) | 7.21 (*d*, 8.8) |
| 6′ | 8.10 (*d*, 8.8) | 7.83 (*d*, 8.7) | 8.10 (*d*, 8.8) | 8.07 (*d*, 8.8) |
| 1″ | — | — | 5.44 (*d*, 7.6) | 5.49 (*d*, 7.8) |
| 2″–6″ | — | — | 5.21–3.5 (*m*) | 5.4–5.08 (*m*) |
| OMe | 3.92 (*s*) | 3.99 (*s*) | 3.92 (*s*) | 3.99 (*s*) |
| | 3.74 (*s*) | 3.85 (*s*) | 3.74 (*s*) | 3.86 (*s*) |
| OH | 12.45 (*br s*) | — | 12.59 (*br s*) | — |
| | 10.17 (*br s*) | — | 10.24 (*br s*) | — |
| | 9.57 (*br s*) | — | — | — |
| OAc | — | 2.48 (*s*) | — | 2.50 (*s*), 2.34 (*s*) |
| | — | 2.35 (*s*) | — | 2.14 (*s*), 2.13 (*s*) |
| | — | 2.32 (*s*) | — | 1.99 (*s*), 1.93 (*s*) |

**1** betuletol, **2** betuletol acetate, **3** betuletol 3-*O*-galactoside, **4** acetate.

with betuletin 3-*O*-galactoside structure, which is a new naturally occurring glycoside.

## EXPERIMENTAL

*Plant material.* The flowers of *Eupatorium glandulosum* were collected from Lovedale, Nilgiris, Tamil Nadu, India. A voucher specimen (No. 2/89) is deposited in Pondicherry University, Pondicherry, India. Fresh flowers (2 kg) were extracted with 95% EtOH (3 × 6 l) and concd *in vacuo* to afford a residue (2 g). This residue found to be a mixture of flavonoids by PC was dissolved in minimum of MeOH and chromatographed over a column of silica gel using $CHCl_3$ and $CHCl_3$ containing increasing percentage of $Me_2CO$. $CHCl_3$–$Me_2CO$ (3:1) fraction yielded betuletol (50 mg) and $CHCl_3$–$Me_2CO$ (1:9) gave betuletrin (200 mg).

Table 2. $^{13}$C NMR chemical shifts of compounds **1** and **3** (broad band decoupled, 67.89 MHz, $\delta$ppm, DMSO-$d_6$, TMS internal)

| C | 1 | 3 |
|---|---|---|
| 2 | 159.25 | 160.00 |
| 3 | 135.64 | 133.31 |
| 4 | 176.07 | 177.79 |
| 5 | 151.50 | 151.60 |
| 6 | 131.50 | 131.78 |
| 7 | 152.00 | 156.80 |
| 8 | 91.80 | 91.32 |
| 9 | 148.05 | 151.75 |
| 10 | 104.50 | 105.32 |
| 1′ | 122.16 | 120.82 |
| 2′ | 129.54 | 131.00 |
| 3′ | 115.43 | 115.09 |
| 4′ | 158.53 | 158.69 |
| 5′ | 115.43 | 115.09 |
| 6′ | 129.54 | 131.00 |
| 1″ | — | 101.69 |
| 2″ | — | 71.20 |
| 3″ | — | 73.13 |
| 4″ | — | 67.88 |
| 5″ | — | 75.70 |
| 6″ | — | 60.15 |
| OMe (6) | 60.05 | 60.15 |
| (4′) | 57.00 | 56.46 |

*Betuletol* 3-*galactoside*. $C_{23}H_{24}O_{12}$, light yellow needles, mp 142–144°. $[\alpha]_D^{28}$ −50.4° (pyridine; *c* 1.0). UV $\lambda_{max}^{MeOH}$ 271, 341, (MeOH + NaOMe) 279, 386, (MeOH + $AlCl_3$) 279, 298sh, 360, (MeOH + $AlCl_3$ + HCl) 281, 298sh, 360, (MeOH + NaOAc) 274, 357, (MeOH + NaOAc + $H_3BO_3$) 270, 344 nm. IR $\nu_{max}^{KBr}$ 3300 *br*, 1640, 1590, 1480, 1350, 1260, 1210, 990, 850 cm$^{-1}$. $^1$H NMR: see Table 1; $^{13}$C NMR: see Table 2. FDMS, *m/z* (rel. int.) 493 ([M + H]$^+$, 100); PC ($R_f$ × 100, Whatman 1, ascending, 28° + 2) 41 ($H_2O$), 65 (15% HOAc), 72 (BAW), 89 (phenol), 92 (Forestal) and 73 (*t*-BAW). Betuletrin hexaacetate. ($Ac_2O$, pyridine, 30°, 24 hr) mp 197–198°. UV $\lambda_{max}^{MeOH}$ 257, 310 nm. $^1$H NMR: see Table 1.

*Acknowledgements*—We thank Prof. E. Wollenweber, (Institut für Botanik, Technische Hochschule Darmstadt, Schnittspahnstrasse-3, F.R.G.) for providing authentic sample of betuletol, Dr K. P. Madhusudanan (Medicinal Chemistry Division, Central Drug Research Institute, Lucknow, India) for mass spectral data and the sophisticated instrument facility, Indian Institute of Science, Bangalore, India for 270 MHz $^1$H and 67.89 MHz $^{13}$C NMR spectra. Thanks are also due to Institut Francaise de Pondicherry, India for identification of the plant.

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