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Antibacterial Activity of Acylated Flavonol Glycoside from *Waltheria indica*

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A rare acylated flavonol glycoside, kaempferol 3-*O*-β-D-(6″-*E*-*p*-coumaryl)-glucopyranoside (**1**), together with five known flavonoids, quercetin 3-*O*-glucopyaranoside (**2**), kaempferol-3-*O*-α-L-rhamnoside (**3**), kaempferol-3-*O*-β-D-glucopyranoside (**4**), quercetin (**5**) and kaempferol (**6**) were isolated from the whole plant of *Waltheria indica*. The structures of these compounds were elucidated by FABMS, UV, ^{1}D and ^{2}D NMR spectroscopy including COSY, HSQC and HMBC experiments and acid hydrolytic and saponification studies. Compound **1** was isolated for the first time from the family Sterculiaceae. These compounds were screened for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Waltheria indica*, Sterculiaceae, Flavonoids, Antibacterial activity.

INTRODUCTION

Waltheria indica (Sterculiaceae) is an erect herb widely distributed throughout the Western Ghats of South India[1]. It was used as an antiinflammatory agent in traditional Indian medicine[2]. Earlier phytochemical studies on *W. indica* revealed the presence of several known anthocyanin and flavonol glycosides[3, 4]. In this paper we report the isolation and structure elucidation of an acylated flavonol glycoside, kaempferol 3-*O*-β-D-(6″-*E*-*p*-coumaryl)-glucopyranoside (**1**) besides quercetin 3-*O*-glucopyranoside (**2**), kaempferol-3-*O*-α-L-rhamnoside (**3**), kaempferol-3-*O*-β-D-glucopyranoside (**4**), quercetin (**5**) and kaempferol (**6**) and their antibacterial activity studies.

EXPERIMENTAL

Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded in KBr disc on Perkin-Elmer 283 double beam spectrophotometer and UV spectra on a Shimadzu UV-240 spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined on a Bruker Avance 400 MHz

spectrometer with IMS as internal standard. ^1H-^1H COSY, HSQC and HMBC spectra were recorded using standard pulse sequences. The FABMS spectrum was obtained on a 700 Jeol mass spectrometer in thioglycerol matrix. Column chromatography was performed on Acme Si gel finer than 200 mesh. All the compounds were tested for their antibacterial activity against *S. aureus* and *E. coli.*

Plant material, extraction and isolation

The whole plant of *W. indica* was collected in September 2001 at Dharmagiri Hills, Tirumala, Andhra Pradesh, India. A voucher specimen (CVR-017) has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati. The air-dried and powdered whole plant (3 kg) of *W. indica* was successively extracted with *n*-hexane, Me_2CO and MeOH. The Me_2CO extract was defatted with *n*-hexane and the residue obtained was purified over a Si gel column using *n*-hexane and EtOAc and their step gradient mixtures as elutes. The *n*-hexane-EtOAc, 1 : 1 and 4 : 6 elutes yielded compounds **5** (20 mg) and **6** (20 mg) respectively. The MeOH extract was solvent fractionated with *n*-hexane and EtOAc. The EtOAc fraction was purified over a silica gel column using EtOAc/MeOH step gradient to yield compounds **4** (15 mg), **3** (12 mg), **2** (20 mg) and **1** (15 mg).

Antibacterial activity

These compounds were screened for antibacterial activity, using paper disc method at 500 ppm concentration using 5 mm disc of filter paper against gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli.* The activity was compared with tetracycline and gentamycine. Generally the compounds were found to have moderate activity against both the bacteria.

Acidic hydrolysis of 1

Compound **1** (4 mg) on acidic hydrolysis with 2 N HCl in MeOH for 2 h after usual work-up gave kaempferol, *trans-p*-coumaric acid and glucose identified by co-PC in BAW.

Alkaline hydrolysis of 1

Compound **1** (5 mg) in 1% KOH was refluxed for 2 h and the reaction mixture on usual work-up gave kaempferol-3-*O*-β-D-glucopyranoside (**4**) and *trans-p*-coumaric acid.

Kaempferol-3-*O*-β-D-(6″-*E*-*p*-coumaryl)-glucopyranoside (1): Pale-yellow amorphous solid, m.p. 252–254°C, $[\alpha]_D^{25}$ –68.6°(c 0.15, MeOH); UV (MeOH): λ_{max} (log ε): 267 (4.35), 315 (4.45), 355 sh (4.29) nm; (MeOH + NaOMe) 280, 315, 378 nm; (MeOH + NaOAc) 279, 315, 360 sh nm; (MeOH + NaOAc + H_3BO_3) 271, 315, 360 sh nm; (MeOH + $AlCl_3$) 278, 305, 320 sh, 399 nm; (MeOH + $AlCl_3$ + HCl) 280, 304, 329, 398 nm; IR (KBr, cm^{-1}) ν_{max}: 3463 ν(OH), 1685 ν(>C=O ester), 1655 ν(>C=O), 1543, 1501; ^1H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH-5), 7.98 (2H, *d*, *J* = 9 Hz, H-2′,6′), 7.36 (2H, *d*,

J = 8.6 Hz, H-2‴,6‴), 7.33 (1H, *d*, J = 16 Hz, H-7‴), 6.85 (2H, *d*, J = 9 Hz, H-3′,5′), 6.78 (2H, *d*, J = 8.6 Hz, H-3‴,5‴), 6.38 (1H, *d*, J = 2.0 Hz, H-8), 6.15 (1H, *d*, J = 2.0 Hz, H-6), 6.11 (1H, *d*, J = 16 Hz, H-8‴), 5.45 (1H, *d*, J = 7.8 Hz, H-1″), 4.16 (2H, m, H-6″), 3.13–3.52 (4H, m); ^{13}C NMR: (Table-1); FABMS m/z: 617 $[M + Na]^+$, 595 $[M + H]^+$, 449 [M + H-*p*-coumaryl]$^+$, 287 [M + H-*p*-coumarylglucosyl]$^+$.

TABLE-1
^{13}C NMR DATA (75 MHz, δ IN ppm, DMSO-d_6)
FOR COMPOUNDS 1 AND 4

C	1	4
2	156.6	156.4
3	133.1	133.3
4	177.4	177.4
5	161.2	161.0
6	98.8	98.4
7	164.2	163.9
8	93.7	93.4
9	156.5	156.4
10	103.9	104.0
1′	120.8	120.9
2′, 6′	130.9	130.9
3′, 5′	115.1	117.5
4′	160.0	159.8
1″	101.0	101.2
2″	74.2	74.0
3″	76.2	76.1
4″	70.0	69.6
5″	74.3	77.0
6″	63.0	60.6
1‴	124.9	—
2‴,6‴	130.2	—
3‴,5‴	115.8	—
4‴	159.8	—
7‴	144.6	—
8‴	113.7	—
9‴	166.2	—

TABLE-2
ANTIBACTERIAL ACTIVITY OF THE COMPOUNDS

Compound	*Staphylococcus aureus*	*Escherichia coli*
1	+++	+++
2	++	+++
3	++	+++
4	+++	++
5	+++	+++
6	++	+
Tetracycline	++++	–
Gentamycine	–	++++

+ = 5–7 mm, ++ = 8–10 mm, +++ = 11–13 mm, ++++ = 14–18 mm.

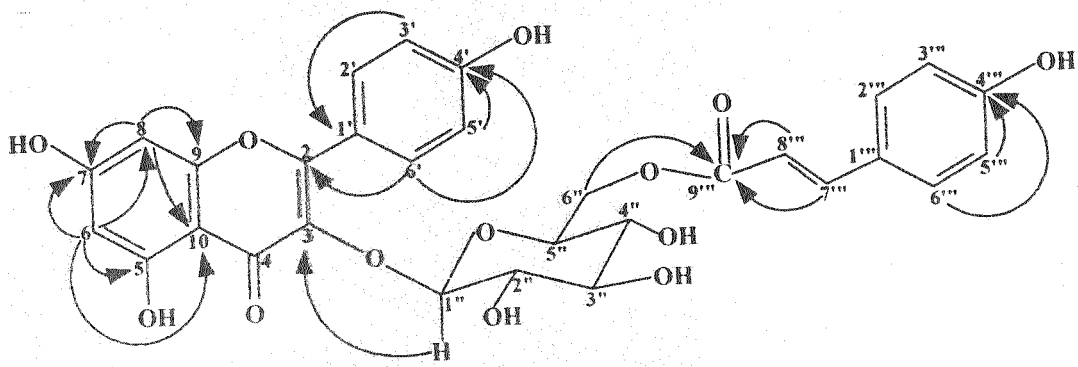
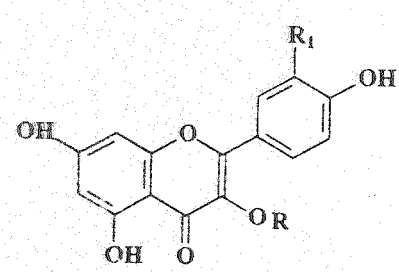


Fig. 1. Selective HMBC (→) corellations for compound 1



2 R = -β-D-glucose; R_1 = OH
3 R = -α-L-rhamnose; R_1 = H
4 R = -β-D-glucose; R_1 = H
5 R = H; R_1 = OH
6 R = R_1 = H

RESULTS AND DISCUSSION

Compound **1** was obtained as pale-yellow amorphous solid and showed a positive reaction with $FeCl_3$. The positive-ion FABMS of compound **1** showed pseudo molecular ion peaks at m/z 617 $[M + Na]^+$ and 595 $[M + H]^+$ corresponding to the molecular formula $C_{30}H_{26}O_{13}$ which is consistent with the presence of 30 carbon signals in its decoupled ^{13}C NMR spectrum. Positive Molisch test, UV absorption maxima of compound **1** in MeOH (267, 315 and 355 sh nm) and with diagnostic reagents suggested compound **1** to be a flavonol glycoside with 5,7,4′-trioxygenation[5]. The presence of *para*-substituted B-ring in compound **1** was evident from the presence of two A_2B_2 doublets at δ 6.85 and 7.98 attributed to 3′,4′

and 2′,6′ protons respectively in its ^{1}H NMR spectrum. Two *meta*-coupled signals at δ 6.15 and 6.38 were assigned to H-6 and H-8 protons respectively of A-ring. These data together indicate that the aglycone moiety is kaempferol and the ^{13}C NMR spectrum of compound **1** was comparable to that of kaempferol itself[6]. The IR spectrum of compound **1** apart from hydroxyl (3463 cm^{-1}) and carbonyl (1655 cm^{-1}) absorption bands, showed an additional carbonyl absorption band at 1685 indicating the presence of an ester group conjugated with a double bond[7,8].

An anomeric proton signal at δ 5.45 (*d*, J = 7.8 Hz) in the ^{1}H NMR spectrum of **1** suggested the presence of a sugar residue with β-configuration. The ^{1}H NMR signals at δ 6.78 (2H, *d*, J = 8.6 Hz) and 7.36 (2H, *d*, J = 8.6 Hz) together with two olefinic doublets at δ 6.11 and 7.33 with large coupling constant (J = 16 Hz) revealed the presence of a *trans-p*-coumaryl moiety in compound **1**, whose presence was evidenced by the formation of *trans-p*-coumaric acid, D-glucose and kaempferol when compound **1** was subjected to total acid hydrolysis. The presence of a fragment at m/z 449 [aglycone + hexose + H$^+$] in its FABMS further supported the presence of a *trans-p*-coumaric acid and kaempferol-3-*O*-β-D-glucopyranoside (**4**), indicating that the glucose residue in compound **1** was linked to C-3 position and the *p*-coumaryl moiety was attached to glucose residue. Comparison of ^{13}C NMR spectral data of compound **1** with **4** (Table-1)[1] showed that the *p*-coumaryl residue in compound **1** was found to be linked to C-6″ hydroxyl of the glucose residue as this carbon signal (63.0 ppm) was shifted to downfield by 2.4 ppm, while the C-5″ signal at 74.3 ppm was shifted upfield by 2.7 ppm[10]. The site of esterification in compound **1** was also revealed by a downfield shift of 0.72 ppm observed for H-6″ (δ 4.16) in its ^{1}H NMR spectrum compared with that of compound **4** (δ 3.44)[10]. The attachment of a *p*-coumaryl moiety at C-6″ of glucose residue in compound **1** was further supported by the presence of a cross peak between H-6″ (δ 4.16) of the glucose residue and the carbonyl carbon (166.2 ppm) of the *p*-coumaryl residue in its HMBC spectrum (Fig. 1). Thus from the foregoing spectral and hydrolytic studies compound **1** was characterized as kaempferol 3-*O*-β-D-(6″-*E*-*p*-coumaryl)-glucopyranoside.

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