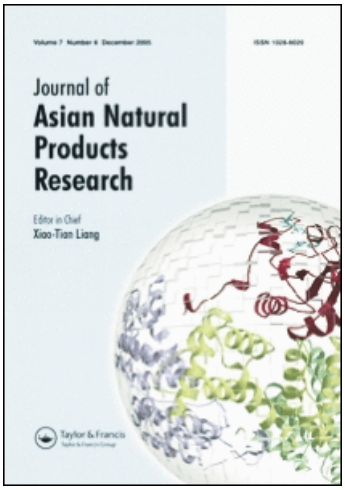

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A new acylated flavone glycoside from *Colebrookea oppositifolia*

Ramireddy Venkata Narahari Reddy[a]; Bandi Anil Kumar Reddy[a]; Duvvuru Gunasekar[a]
[a] Natural Products Division, Department of Chemistry, Sri Venkateswara University, Tirupati, India

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A new acylated flavone glycoside from *Colebrookea oppositifolia*

Ramireddy Venkata Narahari Reddy, Bandi Anil Kumar Reddy and Duvvuru Gunasekar*

Natural Products Division, Department of Chemistry, Sri Venkateswara University, Tirupati, India

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A new acylated flavone glycoside, echioidinin 2′-*O*-β-D-(2″-*O*-acetyl)glucopyranoside (**1**) along with the three known flavonoids, 5,6,7,8,5′-pentamethoxy-3′,4′-methylenedioxyflavone (**2**), 5,2′,6′-trihydroxy-7-methoxyflavone (**3**), and kaempferol 7,4′-dimethyl ether 3-*O*-β-D-glucopyranoside (**4**), were isolated from the roots of *Colebrookea oppositifolia*. The structure of compound **1** was elucidated by extensive spectral and chemical studies.

Keywords: *Colebrookea oppositifolia*; Labiatae; roots; acylated flavone glycoside; echioidinin 2′-*O*-β-D-(2″-*O*-acetyl)glucopyranoside

1. Introduction

Colebrookea oppositifolia Smith (Labiatae) is a densely woolly shrub mostly distributed in the hilly parts of India [1]. In folk medicine, the roots are used for epilepsy and the leaves are applied for wounds and bruises [2]. Previous chemical examination of the bark, stems, leaves, and flowers of *C. oppositifolia* afforded several flavone and flavone glycosides [3–5]. In our systematic search for polyphenolic constituents from Indian medicinal plants, we have investigated the roots of *C. oppositifolia* and report herein the isolation and characterization of a new acylated flavone glycoside, echioidinin 2′-*O*-β-D-(2″-*O*-acetyl)glucopyranoside (**1**) together with the three known flavonoids, 5,6,7,8,5′-pentamethoxy-3′,4′-methylenedioxyflavone (**2**), 5,2′,6′-trihydroxy-7-methoxyflavone (**3**), and kaempferol 7,4′-dimethyl ether 3-*O*-β-D-glucopyranoside (**4**).

2. Results and discussion

Compound **1**, isolated as yellow amorphous powder, showed $[M+H]^+$ peak at *m/z* 489.1317 in its positive ESI-TOF mass spectrum, consistent with the molecular formula $C_{24}H_{24}O_{11}$. This was corroborated by a decoupled ^{13}C NMR spectrum that showed signals for all 24 carbons present in the molecule. Positive Molish's test and the UV absorption maxima (268 and 324 nm) suggested that **1** was a flavone glycoside [6]. A bathochromic shift of 44 nm in band I absorption maximum with $AlCl_3$ and $AlCl_3$/HCl revealed the presence of a chelated hydroxyl in **1** [7]. Addition of NaOAc caused no shift in band II absorption maximum indicating the absence of a free hydroxyl group at C-7 position. The IR spectrum of **1**, apart from hydroxyl (3318 cm^{-1}) and flavone carbonyl (1648 cm^{-1}) absorption bands, showed an additional carbonyl absorption band at 1719 cm^{-1}, indicating the presence of an ester group in **1**.

The ^1H NMR spectrum of **1** showed a D_2O exchangeable downfield signal at δ 12.88 assigned to a chelated hydroxyl group at C-5. A sharp one-proton singlet at δ 7.04 correlated with the carbon at δ 110.5 (C-3) in its HSQC spectrum was ascribed to H-3

*Corresponding author. Email: duvvurusekarg@rediffmail.com

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of a 2′-oxygenated flavones [8]. A pair of *meta*-coupled doublets ($J = 2.2$ Hz) at δ 6.39 and 6.75, each integrating for one proton, were attributed to H-6 and H-8, respectively. It also displayed the characteristic signal pattern of a 2′-oxygenated B-ring [9] with signals at δ 7.92 (1H, dd, $J = 7.8$, 1.6 Hz), 7.57 (1H, ddd, $J = 7.8$, 7.8, 1.6 Hz), 7.32 (1H, dd, $J = 7.8$, 1.6 Hz), and 7.23 (1H, ddd, $J = 7.8$, 7.8, 1.6 Hz), and were assigned to the 6′, 4′, 3′, and 5′ protons, respectively. A sharp three-proton singlet at δ 3.85 was ascribed to a methoxyl group at C-7 as it showed ^3J correlation with this carbon at δ 165.3 in its HMBC spectrum and two strong NOE correlations with H-6 (δ 6.39) and H-8 (δ 6.75) in its NOESY spectrum (Figure 1).

An anomeric proton signal at δ 5.16 (1H, d, $J = 7.4$ Hz) suggested the presence of a sugar residue with β-configuration. The signal at δ 1.98 (3H, s), which, in conjunction with two carbon resonances at δ 170.2 and 20.6 in its ^{13}C NMR spectrum indicated the presence of an acetyl moiety in **1**. Acid hydrolysis of **1** afforded D-glucose and an aglycone, echioidinin [10]. The glucose residue in **1** was found to be linked to C-2′ as the anomeric proton at δ 5.16 was correlated to C-2′ (δ 155.3) in the HMBC spectrum, further supported by a strong NOE correlation between H-1″ (δ 5.16) and H-3′ (δ 7.32) in its NOESY spectrum.

Alkaline hydrolysis of **1** gave a flavone glycoside, echioidinin [11] indicating that the acetyl moiety was attached to the glucosyl residue. The acetyl moiety in **1** was found to be linked to the C-2″ hydroxyl of glucose [12] as this carbon signal was deshielded by 0.3 ppm, while the C-1″ and C-3″ signals were shifted upfield by 2.1 and 2.8 ppm, respectively. Also, in the ^{1}H NMR spectrum, the chemical shift for H-2″ (δ 4.64, dd, $J = 9.0$, 7.4 Hz) was indicative of acylation at C-2″ of the glucose moiety [13]. The site of acylation was further supported by the presence of a crosspeak between H-2″ (δ 4.64) and the carbonyl carbon (δ 170.2) of the acetyl moiety in its HMBC spectrum (Figure 1). On the basis of the foregoing spectral and chemical studies, the structure of compound **1** was established as echioidinin 2′-*O*-β-D-(2″-*O*-acetyl)glucopyranoside.

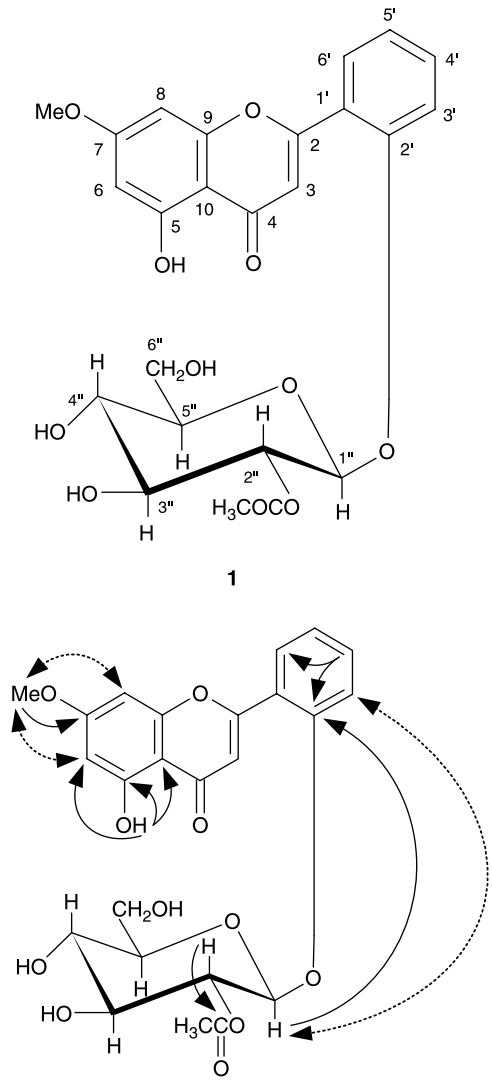

Figure 1. Selected HMBC (⟶) and NOESY (⟷) correlations for **1**.

Compounds **2**–**4** were identified by comparison of their spectral data with the literature values as 5,6,7,8,5′-pentamethoxy-3′,4′-methylenedioxyflavone (**2**) [14], 5,2′,6′-trihydroxy-7-methoxyflavone (**3**) [15], and kaempferol 7,4′-dimethyl ether 3-*O*-β-D-glucopyranoside (**4**) [16].

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured in MeOH at 25°C on a Perkin-Elmer 241 polarimeter. UV absorptions were measured in MeOH on a Shimadzu UV-2450 spectrophotometer. The IR spectra were recorded in KBr discs on a Thermo Nicolet 200 double beam spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 and Bruker AC 300 spectrometers using DMSO-d_6 with TMS as internal standard. $^{1}H-^{1}H$ COSY, HSQC, HMBC, and NOESY (500 ms mixing time) spectra were obtained using the standard pulse sequences. ESI-TOF-MS and ESI-MS/MS were recorded in positive mode on an API Q-STAR PULSA of Applied Biosystems. CC was performed on silica gel (Acme) finer than 200 mesh (0.08 mm). PC was carried out on Whatman No. 1.

3.2 Plant material

The roots of *C. oppositifolia* were collected in December 2004 from Tirumala Hills, Andhra Pradesh, South India. A voucher specimen (DG-046) has been deposited in the herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

3.3 Extraction and isolation

The shade dried and powdered roots of *C. oppositifolia* (2.8 kg) were successively extracted with *n*-hexane (3×9 l), Me_2CO (3×9 l), and MeOH (3×9 l). The Me_2CO extract was defatted with *n*-hexane and the residue obtained, on purification over a silica gel column using *n*-hexane–EtOAc (8:2 and 3:7) step gradient afforded **2** (22 mg) and **1** (34 mg). The MeOH extract was triturated with *n*-BuOH and the *n*-BuOH soluble part, on further purification over a silica gel column using *n*-hexane–EtOAc (7:3 and 4:6) step gradient yielded **3** (26 mg) and **4** (38 mg).

3.3.1 Echioidinin 2′-*O*-β-D-(2″-*O*-acetyl)glucopyranoside (**1**)

Yellow amorphous powder (MeOH); mp 262–264°C; $[\alpha]_D^{25}$ −46.8 ($c = 0.1$, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 268 (4.38) 324 (4.12); (MeOH + NaOAc): 269 324; (MeOH + $AlCl_3$): 274 368; (MeOH + $AlCl_3$/HCl): 272 368; IR (KBr) ν_{max} (cm^{-1}): 3318 (—OH), 2926 (—OMe), 1719 (ester═C═O), 1648 (═C═O), 1612, 1592, 1436, 1309, and 1226; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.88 (1H, s, OH-5), 7.92 (1H, dd, $J = 7.8$, 1.6 Hz, H-6′), 7.57 (1H, ddd, $J = 7.8$, 7.8, 1.6 Hz, H-4′), 7.32 (1H, dd, $J = 7.8$, 1.6 Hz, H-3′), 7.23 (1H, ddd, $J = 7.8$, 7.8, 1.6 Hz, H-5′), 7.04 (1H, s, H-3), 6.75 (1H, d, $J = 2.2$ Hz, H-8), 6.39 (1H, d, $J = 2.2$ Hz, H-6), 5.16 (1H, d, $J = 7.4$ Hz, H-1″), 4.64 (1H, dd, $J = 9.0$, 7.4 Hz, H-2″), 3.85 (3H, s, OMe-7), 3.73 (1H, m, H-6″a), 3.46 (3H, m, H-3″, 5″, 6″b), 3.24 (1H, m, H-4″), and 1.98 (3H, s, OAc-2″); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 182.1 (C-4), 170.2 (O*C*OCH_3-2″), 165.3 (C-7), 161.1 (C-5), 161.0 (C-2), 157.6 (C-9), 155.3 (C-2′), 132.9 (C-4′), 129.2 (C-6′), 122.2 (C-5′), 121.2 (C-1′), 115.8 (C-3′), 110.5 (C-3), 104.8 (C-10), 98.1 (C-1″), 97.9 (C-6), 92.7 (C-8), 77.2 (C-5″), 73.9 (C-3″), 73.4 (C-2″), 69.7 (C-4″), 60.5 (C-6″), 56.1 (OMe-7), and 20.6 (OCO*C*H_3-2″); ESI-MS/MS (positive mode) *m/z*: 489.2 $[M+H]^+$(3), 285.1 $[M+H\text{-acetyl glucosyl}]^+$ (100), 167.0 ($^{1,3}A^+$) (6), and 119.0 ($^{1,3}B^+$) (4); ESI-TOF-MS *m/z*: 489.1317 $[M+H]^+$ (calcd for $C_{24}H_{25}O_{11}$, 489.1396).

3.3.2 Acid hydrolysis of **1**

Compound **1** (10 mg) was dissolved in MeOH solution of 2 N HCl (5 ml) and refluxed at 100°C for 2 h. The reaction mixture was diluted with water (10 ml) and extracted with EtOAc (2×10 ml). The extract was concentrated and the residue on crystallization from MeOH gave yellow needles of echioidinin (4 mg), mp 263–265°C, identified by direct comparison of its spectral data with the literature values [10]. The sugar in the aqueous layer was identified as D-glucose by co-PC (BAW, 4:1:5).

*3.3.3 Alkaline hydrolysis of **1***

A solution of compound **1** (5 mg) in 1% aqueous KOH (5 ml) was refluxed for 2 h. The reaction mixture was acidified with 1 N HCl and extracted with Et_2O (2×5 ml) followed by *n*-BuOH (2×5 ml). The residue obtained from *n*-BuOH extract was crystallized from MeOH to afford echioidinin (3 mg), mp 276–278°C, identified by direct comparison of its spectral data with the literature values [11].

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