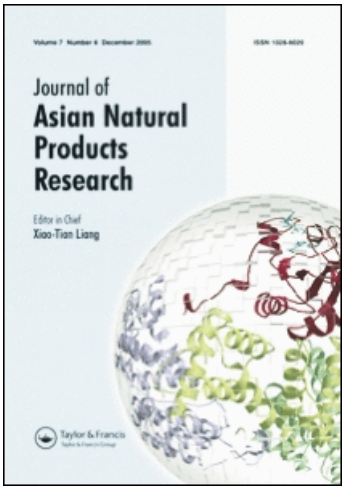

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Note: Novel acylkaempferol glycoside from the endemic species, Vernonia travancorica Hook. f.

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Note

NOVEL ACYLKAEMPFEROL GLYCOSIDE FROM THE ENDEMIC SPECIES, *VERNONIA TRAVANCORICA* HOOK. f.

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Vernonia travancorica Hook. f. is a taxon, endemic to the evergreen forests of Western Ghats (above 1000 m), India. From the aqueous EtOH extract of the air-dried corymbose inflorescence a novel secondary metabolite, 3-*O*-β-[β-(6‴-acetyl)-D-glucopyranosyl(1→2)]-D-glucopyranosyl kaempferol (**1**), has been isolated, together with three other compounds, *viz.*, 4′-methoxykaempferol (**2**), its 3-O-β-D-glucopyranoside (**3**) and kaempferol (**4**). The structure of the new natural product has been established based on chemical analysis, including characteristic colour reactions, rigorous hydrolytic procedures involving acid, alkali and enzyme, as well as spectral (UV, ^{1}H and ^{13}C NMR and FABMS) evidences.

Keywords: *Vernonia travancorica*; Asteraceae; Flavonoids; 3-*O*-β-[β-(6‴-Acetyl)-D-glucopyranosyl(1→2)]-D-glucopyranosyl kaempferol

INTRODUCTION

The genus *Vernonia* Schreb (family: Asteraceae; tribe: Vernonieae) consists of more than 1000 species of herbs, shrubs and small trees, found chiefly in the tropics and subtropics of the world, with about 52 of them found in India [1]. Numerous reports are available on the chemistry of *Vernonia* and a vast majority of these concern the characterisation of sesquiterpene lactones, as well as triterpenes, sterols and steroidal saponins, acetylenes and other compounds. Some of these compounds have been demonstrated to exhibit antimicrobial and cytotoxic effects [2,3]. A survey of the flavonoid constitution of this genus prompts one to deduce that they largely biosynthesise flavones, flavonols and their methyl ethers, with flavanones existing to a limited extent [4,5]. *Vernonia travancorica* Hook. f. is a small (6–7 m tall) tree, endemic to the evergreen forests of the Western Ghats of Karnataka, Kerala and Tamil Nadu [6,7]. As there is no recorded chemical examination of

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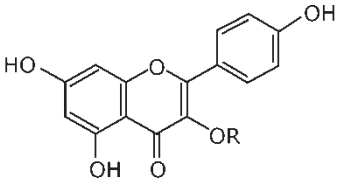
this taxon, a systematic analysis of the air-dried corymbose inflorescence was taken up, which resulted in the isolation and characterisation of a novel secondary metabolite, 3-*O*-β-[β-(6‴-acetyl)-D-glucopyranosyl(1→2)]-D-glucopyranosyl kaempferol (**1**), about which the present article is concerned, together with the rare 4′-methoxykaempferol (**2**) and its 3-*O*-β-D-glucopyranoside (**3**) as well as the ubiquitous kaempferol (**4**). All four compounds are reported from this species for the first time.

RESULTS AND DISCUSSION

The aqueous alcoholic extract of the corymbose inflorescence of the endemic taxon *V. travancorica*, upon solvent exraction, followed by extensive chromatographic separations yielded a novel acylated glycoside (**1**). The compound ($C_{29}H_{32}O_{17}$) was isolated as a yellow powder that gave yellow colour with alkalis, pink with Mg–HCl, olive green with Fe^{III} and a positive Molisch test. It appeared purple under UV, changing to yellow in UV/NH_3 and had λ_{max} (MeOH) 254sh, 267, 298sh, 348 nm, characteristic of a 3-*O*-glycosylated flavonol. Hydrolysis with 2 M HCl yielded a flavonol, identical in all respects to authentic kaempferol [8] and a sugar (identified by co-chromatography as D-glucose) in the molar ratio 1:2. Cold alkaline hydrolysis (2M NaOH) afforded a glycoside (**1a**), whose paper chromatographic mobility suggested it to be a diglycoside. This diglycoside yielded D-sophorose upon H_2O_2 oxidation. Further, the glycoside **1a** produced kaempferol and D-glucose (1:2) as 2 M HCl hydrolytic products, and a kaempferol glycoside **1b** and D-glucose (1:1) as the partial hydrolytic products. Compound **1b** also yielded kaempferol and D-glucose when subjected to both 2 M HCl and β-glucosidase enzyme hydrolyses. The UV spectral characteristics of the methanolic solution of **1** in the presence of diagnostic shift reagents fairly resembled those of kaempferol 3-*O*-β-D-sophoroside and, hence, based on the electronic spectral and chemical evidences one could assign, without much hesitation, the structures of kaempferol 3-*O*-β-D-sophoroside and kaempferol 3-*O*-β-D-glucopyranoside [9,10] respectively to compounds **1a** and **1b**.



1. R= β-[β-(6'''-acetyl)D-glucopyranosyl (1 ⟶ 2)]D-glucopyranosyl

1a. R = β-[β-D-glucopyranosyl(1 ⟶ 2)]D-glucopyranosyl

1b. R =β-D-glucopyranosyl

The ^{1}H NMR spectrum of the compound exhibited resonances characteristic of a kaempferol skeleton together with two distinct anomeric proton resonances at δ 5.51 and 4.16 ppm, each consisting of a doublet with $J = 7.0$ Hz. The magnitude of the coupling constants of the anomeric protons was indicative of β-linked glucose moieties [11]. The ^{13}C NMR spectrum of the compound contained carbon resonances that reasonably agreed with the expected kaempferol 3-*O*-β-D-sophoroside [12] and in addition contained peaks at δ 169.09 and at δ 20.22 ppm. These two resonances correlated well with the carbonyl carbon and methyl carbon resonances of an acetyl moiety, whose presence in compound **1** could also

be recognised from the sharp singlet at δ 2.18 ppm of its ^{1}H NMR spectrum, integrating to 3 protons. That the acetylation was only at the terminal sugar in the compound could be inferred from the fact that cold alkaline hydrolysis of **1** yielded **1a**. Further, the characteristic downfield shift of the C-6‴ (δ 63.70 ppm) of **1** compared with the resonance frequency of the corresponding carbon (δ 61.4 ppm) of **1a** and the concomitant upfield shift of C-5‴ (δ 73.83 ppm) of **1** in relation to C-5‴ (δ 76.7 ppm) of **1a** [12] strongly favoured the site of acetylation as C-6‴. The interglycosidic linkage could also be deduced from the appreciable downfield shift of the resonance position of the ipso carbon, C-2″ (δ 82.34 ppm), which accords with the C-2″ of **1a** (δ 82.0 ppm), accompanied by the characteristic upfield shift of C-1″ ($\Delta\delta$ 1.88 ppm, compared to **1b**). Based on the fully assigned ^{1}H and ^{13}C resonances and the products isolated from hydrolysis, compound **1** was characterised as 3-*O*-β-[β-(6‴-acetyl)-D-glucopyranosyl(1→2)]-D-glucopyranosyl kaempferol (kaempferol 3-*O*-[6‴-acetyl] sophoroside). The same was also substantiated by the FABMS of **1**, which displayed a quasi-molecular ion peak at *m*/*z* 653 [$(M + H)^+$] and characteristic fragment ion peaks at *m*/*z* 611 [653 − acetyl] (**1a**), 449 [653 − acetylglucose] (**1b**) and 286 [aglycone + H^+].

EXPERIMENTAL

General Experimental Procedures

Column chromatography was carried out on silica gel 60–120 mesh obtained from Qualigens Fine Chemicals (Glaxo Smith Kline Pharmaceuticals Limited), Mumbai, India. UV spectra were recorded on a Shimadzu UV-160 spectrophotometer. The FAB mass spectrum was recorded on a JEOL SX 102/DA-6000 Mass Spectrometer/Data System using argon/xenon (6 kV, 10 mA) as the FAB gas and *m*-nitrobenzyl alcohol as the matrix at room temperature. The ^{1}H NMR (200 MHz) spectrum was recorded on a Bruker DPX-200 and the ^{13}C NMR (125.7 MHz) spectrum on a Bruker DRX-500 spectrometer, using DMSO-d_6 as solvent.

Plant Material

Fresh corymbose inflorescence were collected near Walayar estate, Tinnevelly District, Tamil Nadu, India and authenticated by Dr V.M. Meher-Homji, Department of Ecology, French Institute, Pondicherry, and a voucher specimen has been deposited in this Centre.

Extraction and Isolation

The air-dried and coarsely powdered inflorescence (890 g), were exhaustively extracted with boiling 90% EtOH (3 × 4 L) and the combined extract was concentrated under reduced pressure. The aqueous extract was then subjected to solvent extraction using C_6H_6, Et_2O and EtOAc, successively, and concentrated *in vacuo* to yield C_6H_6 (1.218 g), Et_2O (0.325 g) and EtOAc (0.780 g) fractions. The EtOAc concentrate was separated by column chromatography (Sephadex LH 20, 90% aq. MeOH) to yield two fractions, F_1 and F_2. F_1 was chromatographed on silica gel ($CHCl_3$–MeOH 9:1 → 0:10) to yield compounds **1** and **3**, each of which was further purified using a Sephadex column (MeOH), followed by recrystallisation from MeOH–Me_2CO (1:1). F_2 was separated by preparative paper chromatography (Whatman No. 3, 50% aq. HOAc, descending, 28°C, 10 h) and processed to yield compounds **2**–**4**, whose structures were identified by chemical and spectral methods and further confirmed by comparison with authentic samples [8,13].

Kaempferol 3-*O*-β-[β-(6‴-Acetyl)-D-glucopyranosyl(1→2)]-D-glucopyranoside (1)

$C_{29}H_{32}O_{17}$, yellow powder (53 mg), from MeOH–Me_2CO (1:1). UV λ_{max} (MeOH) (nm): 254 sh, 267, 298 sh, 348; (+NaOAc) 273, 305, 368; (+NaOAc + H_3BO_3) 267, 300, 350; (+NaOMe) 274, 320, 390; (+$AlCl_3$) 274, 300, 326, 390 sh; (+$AlCl_3$ + HCl) 273, 300 sh, 326, 390 sh; ^{1}H NMR (200 MHz, DMSO-d_6, TMS internal standard, δ, ppm): 8.18 (2H, d, $J = 8.5$ Hz, H-2′,6′), 7.02 (2H, d, $J = 8.5$ Hz, H-3′,5′), 6.51 (1H, d, $J = 2.0$ Hz, H-8). 6.20 (1H, d, $J = 2.0$ Hz, H-6), 5.51 (1H, d, $J = 7.0$ Hz, H-1″), 4.16 (1H, d, $J = 7.0$ Hz, H-1‴), 2.18 (3H, s, acetyl CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6, TMS int., δ, ppm) (the superscripts [a] and [b] denote exchangeable sets of ^{13}C-resonances): 178.20 (C-4), 169.09 (acetyl-CO), 164.99 (C-7), 160.68 (C-5), 160.47 (C-4′), 157.44 (C-2), 157.20 (C-9), 133.65 (C-3), 130. 95 (C-2′,6′), 121.62 (C-1′), 115. 95 (C-3′,5′), 104. 38 (C-10), 103. 65 (C-1‴), 99.52 (C-1″), 97. 56 (C-6), 95.00 (C-8), 82.34 (C-2″), 77.62[a] (C-5″) 77.34[a] (C-3″), 76.58[a] (C-3‴), 74.96 (C-2‴), 73.83 (C-5‴), 71. 02[b] (C-4‴), 70.85[b] (C-4″) 63.70 (C-6‴), 61.94 (C-6″), 20.22 (acetyl CH_3). MS (FAB, *m/z*, relative intensity as %): 653 [$(M + H)^+$, 62], 611[653 − acetyl, 9], 449 [653 − acetylglucose, 56], 286 [aglycone + H^+, 65].

Hydrolysis Procedure

(i) Cold alkaline hydrolysis: 2 M NaOH, room temperature, 2 h. (ii) Acid hydrolysis: 2 M HCl, 100°C, 3 h. (iii) Partial hydrolysis: 1% HCl, 100°C, 10 min. (iv) Enzyme hydrolysis: β-glucosidase, 0.1 M HOAc–NaOAc (pH 5.0), 38°C, 24 h.

Hydrogen Peroxide Oxidation

30% H_2O_2, 0.1 M NH_3, 28°C, 24 h. Products were analysed according to the procedures described in [14].

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