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Note

Pentadecanoic acid β-D-glucoside from *Clerodendrum inerme*

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A new aliphatic glucoside, isolated from the hexane extract of aerial parts of *Clerodendrum inerme*, has been characterized as pentadecanoic acid-β-D-glucoside, on the basis of spectral data analyses and chemical studies. Stigmasterol glucoside was also isolated from the same extract for the first time from this plant species. The butanol extract afforded acacetin and apigenin.

Keywords: *Clerodendrum inerme*, Verbenaceae, pentadecanoic acid-β-D-glucoside, stigmasterol glucoside, acacetin, apigenin.

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Extensive chemical studies of the constituents of *Clerodendrum inerme* G. (Verbenaceae) have been reported and led to isolation of sterols, diterpenes, flavones and iridoids[1]. Recently, we have reported the isolation and structure elucidation of two new sterols and an aliphatic ketone[2] as well as three other unknown neo-clerodane type diterpenes[3] from the hexane extract of aerial parts of *C. inerme*. In continuation, a new aliphatic glucoside namely, pentadecanoic acid-β-D-glucoside **1**, (**Figure 1**) was encountered together with stigmasterol glucoside **2** from the polar fractions of the same extract, the structural determination of which is described herein. From the butanol extract two flavonoids, namely, acacetin **3** (5,7-dihydroxy-4'-methoxyflavone) and apigenin **4** (5,7,4'-trihydroxyflavone) were also isolated.

Results and Discussion

Compound **1** was isolated from the fractions eluted with hexane-ethyl acetate (25:75), m.p. 138-40°C, $[\alpha]_D$ -39.2°. Its FAB mass showed the molecular ion peak at m/z 427 $[M+Na, C_{21}H_{40}O_7]^+$. The IR spectrum revealed absorptions at 3424 cm^{-1} (*br*) for a polyhydroxy unit and ester carbonyl at 1720 cm^{-1}. IR bands at 2923, 2855, 1466, and 1352 cm^{-1} (stretching and bending vibrations of CH_2 and CH_3 groups) and at 765, 720 cm^{-1} indicated straight chain nature of the compound **1**. From the proton nuclear magnetic resonance (^{1}H NMR) spectrum of compound **1**, which has complex signals corresponding to protons on oxygenated carbon atoms between δ 4.2-4.6 and an anomeric hydrogen at 5.0, it can be inferred that the compound has a sugar moiety. This fact was supported by the appearance of signals between δ 62.9-97.1 in the ^{13}C NMR spectrum. The triplet at δ 2.2 (2H, J = 7.5Hz) in the ^{1}H NMR indicated that a $-CH_2-CH_2-$ group is attached to a carbonyl group.

The ^{13}C NMR spectrum showed signals attributable to a β-D-glucopyranosyl moiety and signals, ($-CH_3$ × 1, $-CH_2$ × 12, $-CH_2CO$ × 1) due to the aglycone moiety. The appearance of anomeric carbon at δ 97.1 was indicative of the fact that the glycosidic linkage was with a carboxylic group instead of with an alcoholic group, as in the case of glycosidic linkage at OH group, the anomeric carbon appears at δ 105.4. Other carbons appeared at δ 62.9, 72.1, 73.2, 78.3 and 79.0 for a β-D-glucosidic linkage[4].

Acid hydrolysis of **1** yielded glucose (compared with authentic glucose on HPLC; mobile phase, acetonitrile-water (80:20), carbohydrate column 250 × 4.5mm I.D., 4 μm, Waters; flow rate 1 mL/min, detector-R.I.). Since only D-type is known for naturally occurring glucose, the component sugar in **1** was tentatively assigned to be D-type. The aglycone moiety was identified as pentadecanoic acid, m.p. 54-56°C (lit. m.p. 53°C)[5].

Thus, the compound **1** was characterized as pentadecanoic acid-β-D-glucoside.

Stigmasterol glucoside **2** was isolated from later fractions eluted with the same ratio of hexane-ethyl acetate (25:75) and confirmed by co-comparison with authentic standard[6]. On acidic hydrolysis **2** yielded stigmasterol as the genin part and glucose as the sugar moiety.

Elution of butanol extract with chloroform-methanol resulted in isolation of acacetin **3** and apigenin **4**. Their identities were confirmed by comparison with literature data (m.p., UV, NMR)[7].

Fatty acid esters serve as an essential source of energy for plant growth[8]. Also, odd number carbon

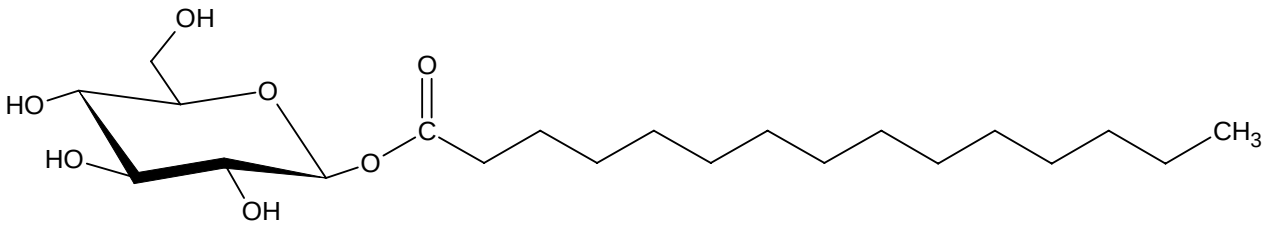

Figure 1

fatty acid esters with glycosides are effective as hair growth stimulants[9]. Apigenin has been shown to possess anti-inflammatory, anti-carcinogen and free radical scavenging properties in many *in vitro* systems[10,11]. Acacetin has been reported to possess anti-peroxidative, anti-inflammatory, and anti-plasmodial effects as well as to be anti-mutagenic[10,12].

Experimental Section

Melting points reported are uncorrected. The 300 MHz NMR spectra were recorded in pyridine or $CDCl_3$ with tetramethyl silane (TMS) as internal standard. The ^{13}C NMR (broad band and DEPT) spectra were recorded at 75 MHz. TLC was performed on 60 F_{254} precoated silica gel plates (Merck) and the spots were visualized by exposure to I_2 vapours or by spraying with vanillin : sulphuric acid : ethanol (1 g:5m:95mL) reagent followed by heating the plate at 110ºC for 15 min.

Plant material. Aerial parts of *Clerodendrum inerme* were collected from Lucknow in October 2000 and a voucher specimen (No. CIMAP–8199) has been deposited in the herbarium of this institute.

Extraction and Isolation. Air-dried and finely powdered aerial parts of the plant (8.2 kg) were extracted as reported earlier[2]. The hexane extract (80.46 g) was subjected to column chromatography on silica gel, 1.5 kg (60–120 mesh). Elution was carried out in varying percentage of EtOAc in hexane. Fractions eluted with hexane–EtOAc (25:75) afforded compounds **1** and **2**. Compound **1** (0.0177 g) was obtained in crystalline form after crystallization from CH_3COCH_3. Compound **2** (0.1775 g) was obtained as colourless crystals from $CHCl_3$–CH_3COCH_3. The *n*-butanol extract (30.25 g) was subjected to column chromatography on silica gel, 0.9 kg (60–120 mesh). Elution was carried out in varying percentage of $CHCl_3$ in MeOH. On crystallization from MeOH, fractions eluted with $CHCl_3$–MeOH (98 : 2) afforded compound **3** (0.0100 g) while those eluted with $CHCl_3$–MeOH (95 : 5) afforded compound **4** (0.0150 g).

Pentadecanoic acid β-D-glucoside 1: Solid; m.p.138-40º; IR (cm^{-1}) 3424, 2923, 2855, 1720, 1466, 1352, 765, 720; ^1H NMR (300 MHz, pyridine): δ 0.83 (3H, *t*, *J* = 7.5Hz, CH_3), 1.25-1.95 (*m*,-$(CH_2)_n$-), 2.20 (2H, *t*, *J* = 7.5Hz, -CH_2CO-), 4.25-4.61 (*m*, CH and CH_2 of glucose), 5.01 (H-1', anomeric proton of glucose);^{13}C NMR (pyridine): δ 176.0 (C-1), 36.1 (C-2), 26.3-34.5 (C-3 to C-13), 24.5 (C-14), 14.0 (C-15), glucose moiety; 97.1 (C-1') , 73.2 (C-2'), 79.0 (C-3'), 72.1 (C-4'), 78.3 (C-5'), 62.9 (C-6'); FAB MS m/z (rel. int.): 427 $[M + Na]^+$ ($C_{21}H_{40}O_7$, 23.3).

Hydrolysis of 1: 0.0080 g of **1** was refluxed in 7% methanolic sulphuric acid (10 mL) on a water-bath for 6 hr. An equal volume of water was added and again heated for 1 hr. Methanol was removed, under reduced pressure, water being added from time to time. The precipitated aglycone was extracted with $CHCl_3$, dried over anhydrous Na_2SO_4 and $CHCl_3$ was removed under vacuum. The residue afforded 0.0043 g of genin, identified as pentadecanoic acid. The aqueous layer from the above experiment was neutralised with $BaCO_3$. Precipitated $BaSO_4$ was filtered, concentrated and the concentrate was analysed through HPLC using RI detector and identified as glucose.

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