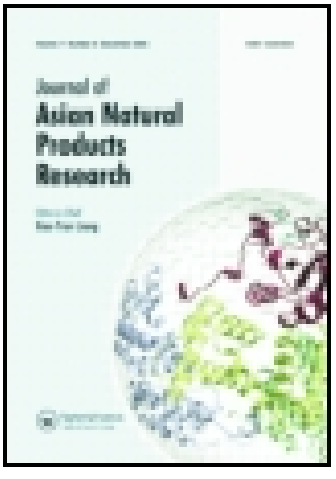

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Royleanumioside – a new phytotoxic triterpenoid from *Teucrium royleanum*

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Royleanumioside (**1**), a new lupane type triterpenoid, was secluded from the $CHCl_3$ soluble fraction of *Teucrium royleanum*. The structure of royleanumioside (**1**) was ascertained by spectral studies including 1D and 2D NMR spectroscopy and by the assessment with the reported data in literature. This new identified compound **1** was further tested for its phytotoxic activity against lettuce seedlings. Interestingly royleanumioside (**1**) had shown moderate phytotoxic activity.

Keywords: *Teucrium royleanum*; Lamiaceae; lupane type triterpenoid; phytotoxicity

1. Introduction

The genus *Teucrium* belongs to the family Lamiaceae and is consisted of 300 species [1]. Several species of the genus *Teucrium* are known for their biological activities such as antiseptics, antipyretics, and antispasmodics. Some species of this genus were also used as tonics [2,3]. Aerial parts of *Teucrium* spp. showed hepatotoxic effect when used as decoction in herbal medicine and this may be due to the presence of diterpenoids in this genus [4–7]. Other biological activities reported from this species are insect antifeedant activity and antioxidant activity of essential oil [2,8–11]. Previously some interesting natural products have further been reported from the aerial parts of *Teucrium royleanum* [12]. This background of *T. royleanum* incited us for phytochemical and biological evaluation of this plant. During the course of our ongoing project, we have isolated and identified several compounds. In this study, we account for the isolation, characterization, and phytotoxic activities of one new compound named royleanumioside (**1**), a lupane type triterpenoid.

2. Results and discussion

The chloroform-soluble fraction of the aerial parts of *T. royleanum* was subjected to column chromatography (CC) over silica gel eluting with different mobile phases.

Compound **1** was isolated as an amorphous powder. The HR-FAB-MS (positive ion mode) provided the molecular ion peak at *m/z* 853.6929 $[M + H]^+$, corresponding to the molecular formula $C_{54}H_{92}O_7$, with nine degrees of unsaturation. The infrared (IR) spectrum showed strong absorptions at 3360 (O—H), 2900 (C—H), 1740 (C═O), 1630 (C═C), and

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1250 (C—O) cm^{-1}. The ^1H NMR spectrum exhibited seven tertiary methyl singlets at δ 0.67, 0.77, 0.85, 0.89, 0.97, 0.99, and 1.54 (all, 3H, s), a trisubstituted double bond at δ 5.33 (1H, t, $J = 3.4$ Hz). The ^1H NMR spectrum also showed two broad doublets for exocyclic double bond at δ 4.70 and 4.62 (1H each, $J = 2.0$ Hz) [13]. The signals for sugar moiety appeared at δ 4.35 (1H, d, $J = 7.6$ Hz, H-1′), 3.32–3.34 (1H, m, H-2′), 3.54 (1H, m, H-3′), 3.44–3.46 (1H, m, H-4′), 3.41–3.43 (1H, m, H-5′), 4.43 (1H, dd, $J = 12.0$, 4.6 Hz), and 4.25 (1H, dd, $J = 12.0$, 1.9 Hz). The presence of acyl group was evident from ^1H NMR signals at δ 0.65 (3H, t, $J = 7.4$ Hz), 1.23 (26H, br s), and 2.32 (2H, t, $J = 7.4$ Hz). The ^{13}C NMR spectrum (BB and DEPT) of **1** revealed the presence of 8 methyl, 27 methylene, 11 methine, and 8 quaternary carbon atoms. It included signals for four olefinic carbons (δ 140.2, 121.4 and 147.4, 111.1), a hydroxyl-bearing carbon (δ 79.4), sugar moiety (δ 101.1, 73.3, 76.2, 70.2, 73.6, and 63.5), and acyl moiety (δ 174.2, 34.1, 24.8, 29.5, and 13.8). A triplet at δ 5.33 (1H, $J = 3.4$ Hz) in the ^1H NMR spectrum and characteristic EI-MS fragments at *m/z* 216 and 201 resulted from *retro*-Diels-Alder cleavage in the ring C indicated the position of a second double bond at C-12 [14]. The fragments at *m/z* 208 and 189 indicated a hydroxyl group at ring A or B. The hydroxyl group was assigned to C-3 on the basis of HMBC correlations of H-3 to C-1 and C-2 and was further confirmed through the characteristic shifts of C-2 and C-4 in the ^{13}C NMR spectrum. The β-orientation of the C-3 hydroxyl group was decided by measuring the *J* values of the double doublet at δ 3.57 (1H, dd, $J = 9.5$, 4.8 Hz) [15]. The acid hydrolysis of **1** provided various products among which the glycone was separated and identified as D-glucose through its optical rotation sign, retention time in GC, and by co-TLC with an authentic sample. The position of the sugar moiety was confirmed by long range HMBC experiments in which H-3 (δ 3.57) showed ^3J correlation with the anomeric carbon (δ 101.1) and the anomeric proton (δ 4.35) showed ^3J correlation with C-3 (δ 79.4). The position of aliphatic ester was deduced at C-6′ of the sugar moiety which is due to the downfield shifts of H-6′ (δ 4.43 and 4.25), C-6 (δ 63.5) and upfield shift of C-5′ (δ 73.6) and finally confirmed by HMBC correlations of H-6′ (δ 4.43 and 4.25) with C-1″ (δ 174.2) (Figure 1). On the basis of these evidences, the structure of **1** was elucidated as lupa-12,

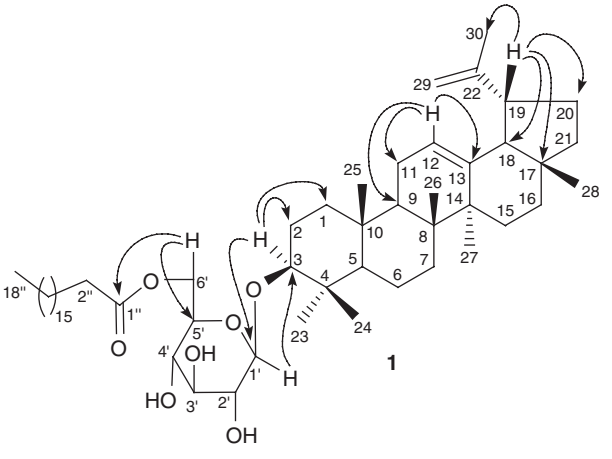

Figure 1. Structure and key HMBC correlations of royleanumioside (**1**).

20(29)-dien-6′-stearoyl 3-*O*-β-D-glucopyranoside.

The results of the phytotoxic bioassay against lettuce seedlings (*Lactuca sativa* cv. Great Lakes 366) are reported in Figure 2. Compound **1** exhibits moderate phytotoxicity. The effects produced by our tested compound **1** on the roots inhibition are presented in Figure 2. The overall effect on *L. sativa* inhibition was 22–28.6%, while interestingly low inhibition had been shown at higher concentration and none of the concentration has an EC_{50} value. The available data on the lupanic triterpenes showed stimulation of the germination of *L. sativa* [16]. The saponins are another class of closely related secondary metabolites exhibiting slight inhibitory activities [17].

3. Experimental

3.1 General experimental procedures

JASCO DIP-360 Polarimeter (JASCO, Inc., Easton, MD, USA) was used to determine optical rotations. IR spectrum was recorded on 460 Shimadzu Spectrometer (Shimadzu, Tokyo, Japan). The ^{1}H, ^{13}C NMR, and HMBC spectra were taken on Bruker NMR-spectrophotometer (Bruker Instruments, Inc., Karlsruhe, Germany) operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Mass spectra were recorded on JMS-DA 500 mass spectrometers (JEOL Company, Tokyo, Japan). Ceric sulfate spray was used for visualization of the TLC plates under UV at 254 and 366 nm. CC was carried out using silica gel (70–230 and 230–400 mesh; E-Merck, Darmstadt, Germany). Aluminum sheets precoated with silica gel 60 F 254 (0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as the spray reagent.

3.2 Plant material

The aerial parts of the plant *T. royleanum* were collected in June 2003 from Swat (Pakistan) and deposited in the Department of Botany with voucher specimen [No. Shabir 2651979(PUP)]. It was identified by Plant Taxonomist Professor Dr A. Rashid, Botany Department, University of Peshawar, Pakistan.

3.3 Extraction and isolation

The dried and powdered whole plant (10 kg) of *T. royleanum* was exhaustively extracted in MeOH for 3 weeks and filtration and evaporation of the solvent afforded a crude extract (550 g), which was extracted with *n*-hexane, $CHCl_3$, and *n*-BuOH. The chloroform extract (50 g) was subjected to CC on silica gel using

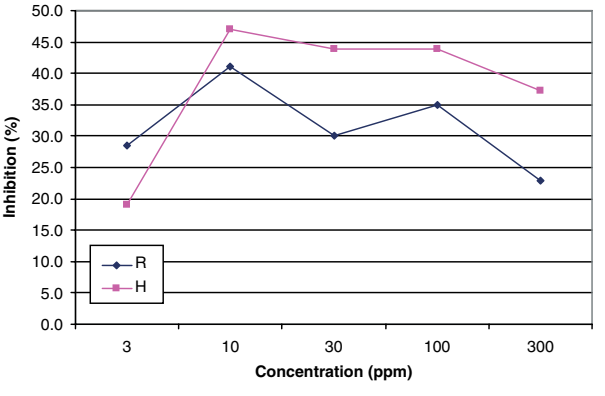

Figure 2. Inhibitory effects of royleanumioside (**1**) (R, root; H, hypocotyl).

n-hexane, *n*-hexane–$CHCl_3$, $CHCl_3$–MeOH and finally, pure MeOH as mobile phases which yielded 11 fractions. Fraction no. 7 of the first column was loaded on silica gel and eluted with MeOH–$CHCl_3$ (1:9) to give compound **1** (11 mg).

3.3.1 *Royleanumioside (**1**)*

Colorless amorphous solid; $[\alpha]_D^{25}$ −34 ($c = 1.2$, CHCl3); IR ν_{max} cm^{-1} (KBr): 3360, 2900, 1740, 1630, 1250. ^{1}H NMR (CD_3OD): 5.33 (1H, t, $J = 3.4$ Hz, H-12), 4.70 (1H, d, $J = 2.0$ Hz, H-29a), 4.62 (1H, d, $J = 2.0$ Hz, H-29b), 4.43 (1H, dd, $J = 12.0$, 4.6 Hz, H-6′), 4.35 (1H, d, $J = 7.6$ Hz, H-1′), 4.25 (1H, dd, $J = 12.0$, 1.9 Hz, H-6′), 3.57 (1H, dd, $J = 9.5$, 4.8 Hz, H-3), 3.53–3.55 (1H, m, H-3′), 3.44–3.46 (1H, m, H-4′), 3.41–3.43 (1H, m, H-5′), 3.32–3.34 (1H, m, H-2′), 2.75–2.77 (1H, m, H-19), 2.32 (2H, t, $J = 7.4$ Hz, H-2″), 1.59–1.61 (2H, m, H-3″), 1.54 (3H, s, CH_3-30), 1.23 (26H, br s, H-4″-H-16″), 0.99 (3H, s, CH_3-27), 0.97 (3H, s, CH_3-24), 0.89 (3H, s, CH_3-23), 0.85 (3H, s, CH_3-28), 0.77 (3H, s, CH_3-25), 0.67 (3H, s, CH_3-26), 0.65 (3H, t, $J = 7.4$ Hz, H-18″). ^{13}C NMR (CD_3OD): 174.2 (C-1″), 147.4 (C-22), 140.2 (C-13), 121.4 (C-12), 111.1 (C-29), 101.1 (C-1′), 79.4 (C-3), 76.2 (C-3′), 73.6 (C-5′), 73.3 (C-2′), 70.2 (C-4′), 63.5 (C-6′), 56.6 (C-18), 55.9 (C-5), 50.0 (C-9), 49.3 (C-19), 42.1 (C-4), 42.0 (C-14), 39.6 (C-21), 38.6 (C-1), 36.5 (C-10), 36.4 (C-17), 34.1 (C-2″), 31.8 (C-11), 31.7 (C-7), 29.5 (C-4″–C-16″), 29.0 (C-16), 27.9 (C-15), 24.8 (C-3″), 24.1 (C-2), 22.5 (C-6), 19.1 (C-27), 18.5 (C-30), 18.4 (C-24), 17.5 (C-23), 13.9 (C-28), 13.8 (C-18″), 11.8 (C-25), 11.6 (C-26). El-MS: *m/z* 423 (15), 407 (10), 216 (20), 208 (25), 201 (17), 189 (34), 69 (24), 175 (19). HR-FAB-MS: *m/z* 853.6929 $[M + H]^+$ (calcd for $C_{54}H_{93}O_7$, 853.6921).

3.4 *Acid hydrolysis of 1*

Compound **1** was refluxed with 2 M HCl in MeOH (5 ml) at 80°C for 2 h in a water bath. The reaction mixture was evaporated, and the hydrolysate after dilution with H_2O (10 ml) was extracted with $CHCl_3$ (10 ml × 3). The $CHCl_3$ extracts were evaporated to afford glycone which was identified as D-glucose by the sign of its optical rotation ($[\alpha]_D^{25}$ + 51.8 ($c = 0.02$, MeOH)), retention time in GC ($R_t = 11.481$), and by co-TLC with an authentic sample.

3.5 *Phytotoxicity bioassay*

A filter paper (27 mm Ø, type 1, Toyo Roshi Kaisha, Ltd, Tokyo, Japan) was placed in a glass petri dish (27 mm Ø), followed by the addition of a test solution to the filter paper in the petri dish and drying completely *in vacuo* at 40°C. After addition of distilled water (0.7 ml), six pre-germinated (16 h at 20°C in the dark) seedlings of lettuce (*L. sativa* cv. Great Lakes 366) were placed on the filter paper, and incubated for 48 h at 20°C in the dark. The inhibitory activity on root elongation was detected by measuring the length of the root and comparing the data obtained with that of untreated controls.

Disclosure statement

No potential conflict of interest was reported by the authors.

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