One New Royleanumoate from *Teucrium royleanum* Wall. ex Benth

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Received 22 March 2014; Accepted 26 May 2014; Published 12 June 2014

Academic Editor: Valdir Cechinel Filho

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One new royleanumoate, a benzene ester (1), has been isolated from *T. royleanum* Wall. ex Benth along with two known compounds, namely, 3,4-dihydroxymethyl benzoate (2) and oleanolic acid (3). The structure elucidation of the isolated compounds was established on two-dimensional (2D) NMR techniques including heteronuclear multiple bond correlation (HMBC), heteronuclear multiple quantum Coherence (HMQC), and correlation spectroscopy (COSY) experiment.

1. Introduction

The family Lamiaceae is a large family of order Lamiales [1]. It contains about 170 genera and 300 species of worldwide distribution, growing under great variety of soils and climates but more abundant in Mediterranean and mountainous region [2]. Several genera of the family Lamiaceae contain biologically active compounds [3]. *Teucrium* is one of the important genera of this family [4]. The genus *Teucrium* comprises mainly herbaceous plants. It contains about 7,000 species in temperate regions, only four species of which are reported in Pakistan, namely, *Teucrium stocksianum*, *Teucrium scordium*, *Teucrium royleanum*, and *Teucrium quadrijariatum* [5]. Many biological activities have been attributed to the genus *Teucrium*. Some of the species have been used as medicinal plants since time immemorial and are still being used in folk medicine as antispasmodics, tonics, antipyretics, and antiseptics [6]. The literature survey reveals that the terpenoids in these plants have also shown insect antifeedant activity [6–8]. These medicinal properties prompted us to carry out phytochemical investigation on *T. royleanum* in continuation to our ongoing research on this species [9–11]. Our current study has led to the isolation of one new royleanumoate, a benzene ester 1. In addition to the new compound 1, some known compounds 3,4-dihydroxymethyl benzoate 2 and oleanolic acid 3 have been isolated for the first time from this species (see Figures 2, 3, and 4).

2. Material and Methods

2.1. Plant Materials. The aerial parts of *T. royleanum* were collected from Swat (Pakistan) in June 2003 and identified by Professor Dr. Abdul Rashid, Plant Taxonomist, Department of Botany, University of Peshawar, Peshawar, Pakistan, where a voucher specimen (number Shabir 2651979 (PUP)) is deposited.

2.2. Methods for Purification. The powdered air-dried aerial parts of *T. royleanum* (10 kg) were soaked in MeOH (3 × 45 L) at room temperature for about 24 hours. The combined methanolic extract was then concentrated via rotavapour to
**Table 1:** $^1$H-NMR and $^{13}$C-NMR (C$_5$D$_5$N, 400 MHz, C$_5$D$_5$N, 100 MHz), chemical shifts, and multiplicities of (I).

| C. number | Multiplicity (DEPT) | $^{13}$C-NMR ($\delta$) | $^1$H-NMR ($\delta$) | $^{1}$J$_{HH}$ (Hz) |
|-----------|---------------------|------------------------|---------------------|---------------------|
| C-1       | C                   | 173.80                 | —                   | —                   |
| C-2-C-11  | CH$_3$              | 29.25                  | 1.23                | brs                 |
| C-12      | CH$_3$              | 14.11                  | 0.88                | t, $J = 6.4$        |
| C-1'      | C                   | 130.15                 | —                   | —                   |
| C-3'-5'   | CH                  | 115.38                 | 6.75                | d, $J = 10.0$       |
| C-2'-6'   | CH                  | 130.04                 | 7.05                | d, $J = 10.0$       |
| C-4'      | C                   | 153.84                 | —                   | —                   |
| C-1''     | CH$_2$              | 34.38                  | 2.81                | t, $J = 2.8$        |
| C-2''     | CH$_2$              | 64.87                  | 2.24                | t, $J = 2.3$        |

get a thick gummy extract (850 g). The resultant concentrated extract was then dissolved in water and was subjected to solvent-solvent extraction process using n-hexane, chloroform, and n-butanol.

The fraction Tb-SA1 was eluted on a silica gel column loaded with initial chloroform-hexane (1:1) which on further column chromatography in chloroform-hexane (6.5 : 3.5) provided compound 1 as amorphous solid (7 mg).

### 2.3. Physical and Spectral Data of Royleanumoate (I)

- **IR**$_{\text{max}}$ (KBr) cm$^{-1}$ 3440, 1735, 1617
- **EIMS** $m/z$: 121 (100), 107 (6), 71 (11), and 57 (40)
- **FAB+MS $m/z$: 333.3713 (caled. for C$_{21}$H$_{34}$O$_3$)
- **$^1$H-NMR** $^{13}$C-NMR (C$_5$D$_5$N, 400 MHz and 100 MHz): Table 1.

### 3. Results and Discussion

Compound 1 was isolated from the VLC fraction of the chloroform soluble part obtained from the methanol extract of *T. royleanum* Wall. ex Benth as amorphous solid. The Fab $^{+}ve$ of 1 showed the [M + 1]$^+$ at $m/z$ 333.3713 in agreement with the molecular formula C$_{21}$H$_{34}$O$_3$ indicating five degrees of unsaturation. Other prominent mass fragments at $m/z$ 121 (100), 107 (6), 71 (11), and 57 (40) were also observed in the mass spectrum as shown in Scheme 1. The IR spectrum of compound 1 exhibited absorption bands at 1735 (ester C=O), 3430 for (OH), and 1617 for (aryl).

The $^1$H-NMR spectrum corroborated the presence of one methyl, thirteen methylene, and aromatic groups in the high-field region. In the downfield region of the spectrum two doublets at $\delta$ 6.75 and 7.05 each of two protons integration were assigned to C-2', C-6' and C-3', C-5' aromatic protons. The methyl group attached at the terminal position of the aliphatic chain appeared as a triplet at $\delta$ 0.88 with a $J = 6.36$. Similarly, methylene protons at C-1'' and C-2'' at $\delta$ 2.81 and 2.24 show two triplets each of 2 H integration with a $J$ value of 2.81 Hz and 2.25 Hz.

The $^{13}$C-NMR spectrum (BB, DEPT) (Table 1) showed twenty-one signals, including one methyl, thirteen methylene, four methine, and three quaternary carbons. In the downfield region signals appeared at $\delta$ 130.2, 115.4, 130.04, and 153.8 which were assigned to the C-1', C-2', C-6', C-3', C-5', and C-4' of aromatic carbons, while a signal at $\delta$ 173.7 indicated the presence of a carbonyl carbon in the form of ester in the molecule.

Similarly, two signals at $\delta$ 34.4 and 64.9 were assigned to the methylene carbons present in between ether oxygen and aromatic ring, while in the upfield region a signal at $\delta$ 14.1 was assigned to the methyl carbon attached at terminal position of the aliphatic chain. The long-range $^1$H-13C connectivities were established through HMBC technique.

In the HMBC spectrum (Figure 1), the C-1'' methylene protons ($\delta$ 2.81, t) showed correlations with C-2'' ($\delta$ 34.4) and another correlations of C-2'' ($\delta$ 130.04) and C-1' ($\delta$ 153.8), thus supporting the attachment of –CH$_2$–CH$_2$– to the phenol ring at para position. Similarly the two orthoprotons (C-2', C-6') also showed correlations with C-3' and C-5', respectively.

On the basis of all the above spectral data and comparison with the analogous structures in the literature [12] the compound 1 was named as royleanumoate. 3,4-Dihydroxymethyl benzoate 2 and oleanolic acid 3 were also isolated for the

![Scheme 1: The mass spectral fragmentation pattern for royleanumoate (I).](image)
first time from the chloroform soluble fraction of the crude extract of *T. royleanum* and identified by comparison with the literature data [13].

### 4. Conclusion

One new compound (benzene ester 1) and two known compounds (3,4-dihydroxymethyl benzoate 2 and oleanolic acid 3) have been isolated from *T. royleanum* Wall. ex Benth. The isolated compounds were confirmed by two-dimensional NMR technique, IR, and mass spectra.

### Conflict of Interests

Authors have declared that there is no conflict of interests.

### Acknowledgment

The authors are thankful to the Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia, for funding the work through the research Group project no. RGP-210.

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