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Terpens from aerial parts of *Euphorbia splendida*

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Euphorbia splendida Mobayen (Euphorbiaceae) is one of the *Euphorbia* species that has not been studied so far. The aerial parts of this plant was collected from Arak, Iran and extracted with methanol (by maceration). The dried extract was subjected to exhaustive separation by column chromatography and preparative TLC. Two terpens, a lupane-type triterpenoid and a diterpene with a tricyclic lathyrane skeleton, were isolated and purified and identified as 3 β -28-dihydroxylup-20(29)-ene (betulin) and decipinone respectively for the first time from this plant. The structures of the isolated compounds were elucidated on the basis of spectroscopic methods including 1 and 2 D-NMR, IR, UV and MS.

Key words: Chromatography, diterpene, triterpen, betulin, decipinone.

INTRODUCTION

Euphorbia is the largest genus in the plant family Euphorbiaceae, comprising about 2000 known species and ranging from annuals to trees. Over 82 species of this genus have been found in Iran (Jassbi, 2006). Some species of *Euphorbia* have been used as medicinal plants for the treatment of skin diseases, gonorrhoea, intestinal parasites, inflammation and wart cures (Singla and Pathak, 1990). Plants of this genus are known for their rich content in secondary metabolites. Indeed, numerous studies undertaken on this genus have revealed presence of triterpenes, diterpenes, steroids (Singla and Pathak, 1990) and macrocyclic diterpenes (Duarte et al., 2006). Besides the well known skin irritant and tumor promoting tiglane, ingenane and daphnane diterpenes (Evans and Taylor, 1983), some diterpenes of *Euphorbia* have been found to possess various activities such as cytotoxic, antitumor, antibacterial (Singla and Pathak, 1990), antinociceptive (Ahmad et al., 2005) and inhibitor of multidrug resistant tumor cells (Hohmann et al., 2000).

Euphorbia splendida Mobayen is a plant distributed in west of Iran with 30 - 50 cm height and several stems which have flowers with sytium arrangements (Ghahraman, 1986). According to the best of our knowledge, the chemical constituents of this plant have not been investigated so far. In this study we report the isolation and structure elucidation of a lathyrane diterpene (decipinone) and a lupane-type triterpenoid (betulin) from the aerial parts of *E. splendida*.

MATERIALS AND METHODS

All solvents and chemical reagents were purchased from Merk (Darmstot, Germany). The FT-IR spectra were recorded on a vector 22 instrument. The ¹H-NMR was recorded on a Bruker AM 500 and AMX 600 NMR (Avance) instruments using the UNIX data system at 500 and 600 MHz, respectively. The ¹³C-NMR spectrum was recorded at 125 and 150 MHz, respectively using CDCl₃ as solvent. ¹H-¹³C HMBC and HMQC were recorded as mentioned above. EI-MS spectra were recorded on a Finnigan MAT 312. HR-EIMS were carried out on Jeol JMS 600 mass spectrometer. Column chromatography was carried out on silica gel (M&N), 70-230 and 230-400 meshes. Compounds on the TLC were detected at 254 and 366 nm and by ceric sulfate as spraying reagent.

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Plant material

The aerial parts of *E. splendida* were collected from Arak, Province of Markazi, Iran, in May 2007 and identified by Mrs. Dr. M. Nori (Faculty of science, Arak university, Arak, Iran) and a voucher specimen is deposited at the collection of pharmacy school of Shaheed Beheshti university, Tehran, Iran (Voucher number 15678H).

Extraction and isolation

The fresh aerial parts of *E. splendida* were dried under shadow for three weeks. The powdered materials (4 Kg) were extracted three times with methanol by maceration at room temperature (seven day for each time). The solvent was evaporated under vacuum to give 500 g of extract. The obtained dry extract was suspended in water followed by extraction with hexane, CHCl_3 and n-butanol for three times consecutively. The CHCl_3 fraction (100 g) was chromatographed on silica gel column (70 - 230 mesh) using hexane, gradient of hexane/EtOAc up to 100% EtOAc and then followed by gradient of EtOAc/MeOH up to 100% MeOH to collect 95 fractions of 100 ml volume. Fraction 17 (A-P) was selected from eluted fraction of n-hexane/EtOAc (8:2). Fraction 17F was rechromatographed by column chromatography with n-hexane/EtOAc (9:1 to 6:4), yielding a compound that was crystallized from MeOH, to afford 20 mg of compound 1 (17F₂). Fraction 18 (A-G) was selected from eluted fraction of n-hexane/EtOAc (7:3). Fraction 18 F was chromatographed on a preparative TLC plate (5%Acetone/ CHCl_3) to yield an impure compound that was further purified by preparative HPLC (MeOH) to afford 15 mg of compound 2 (18 F₃).

RESULTS

Fraction 17 F₂ gave 20 mg of a white needle crystal after evaporating the solvent. The compound had melting point of 246 - 248°C and showed the following spectral characteristics:

Compound 1. m.p. 246-248°C; HR-EI MS: $m/z = 442.3788 [M]^+$, (calcd. for $\text{C}_{30}\text{H}_{50}\text{O}_2$); UV max (EtOH): 210 nm; IR (KBr) max: 3400 (OH), 2950, 1650 (C=C), 1460, 1350, 1020 cm^{-1} ; EI-MS: $m/z = 442 [M]^+$, 412 (50), 411 (100), 399 (31), 385 (37), 207 (72), 203 (87), 189 (95), 175 (30), 135 (27), 121 (18), 107 (15), 95 (18); ^1H -NMR (500 MHz, CDCl_3) and ^{13}C -NMR: (Table 1).

Fraction 18 F gave 15 mg of compound 2 (18 F₃) and showed the following spectral characteristics:

Compound 2. m.p. 245-247 °C; HR-EI MS: $m/z = 654.2582 [M]^+$, (calcd. for $\text{C}_{35}\text{H}_{42}\text{O}_{12}$); UV max (MeOH): 198, 227, 272 nm; IR (KBr) max: 3500 (OH), 2950, 1750, 1710 (C=O), 1600 (C=C), 1450, 1000, 700 cm^{-1} ; EI-MS: $m/z = 654 [M]^+$, 594 (65), 550 (20), 534 (40), 490 (42), 474 (15), 460 (35), 432(27), 414 (12), 383 (9), 310 (29), 281 (31), 264 (32), 251 (39), 237 (59), 175 (22), 157 (18), 131 (19), 121 (6), 105 (100), 77 (29).

^1H -NMR (500 MHz, CDCl_3) and ^{13}C -NMR: (Table 2).

DISCUSSION

Compound obtained from fraction 17 F was identified as betulin and Compound obtained from fraction 18 F was identified as Decipinone (figure1) by interpretation of their MS, NMR and IR spectra as well as by comparison of their spectral data with those reported in the literatures (Tinto et al., 1992; Hayek et al., 1989; Ahmad & Jassbi, 1998).

Compound 1, obtained as white crystals, showed $[M]^+$ peak at $m/z = 442$ in its EIMS and molecular formula $\text{C}_{30}\text{H}_{50}\text{O}_2$ on the basis of its HR-EIMS (442.3788 $[M]^+$). It was considered to be a triterpenoid due to a positive Liebermann-Burchard test. Analysis of the IR spectrum suggested that it contained a hydroxyl group (3400 cm^{-1}) and a terminal double bond (2950, 1650, 880 cm^{-1}).

The assignment of the signals of ^1H and ^{13}C signals was performed through analysis of the HMQC, HMBC, COSY and NOESY experiments and the results were consistent with a lupane type triterpene.

The ^1H -NMR spectrum (Table 1) exhibited signals for six singlet methyl groups at δ 0.74, 0.8, 0.95, 0.96, 1, 1.66 and one isopropenyl moiety at δ 1.67, indicating a lupane skeleton (Mahato and Kundu, 1994), two diastereotopic protons for a methylene group (attached to hydroxyl) at δ 3.31 and 3.78 ppm (H-28 and H-28', dd, $J = 10.7, 4.3$) and two exocyclic methylene protons at δ 4.66, 4.56 (H-29 and 29', s). The ^{13}C NMR and DEPT spectra revealed 30 carbon signals, which were shown by DEPT experiment to be 6 methyl groups, 12 methylene groups, 6 methine carbons, 6 quaternary carbons and 2 olefinic carbons.

In the down-field region of the spectrum, there are five proton-signals at δ 4.66 (s, H-29), 4.56 (s, H-29'), 3.79 (dd, $J = 10.7, 4.3$, H-28), 3.31 (dd, $J = 10.7, 3.7$, H-28') and 3.18 (dd, $J = 11.1, 5.58$, H-3). The former two signals (H-29, 29') correlated in the HMQC spectrum, with an olefinic carbon at δ 109.67. Cross peaks between H-29, 29' and H-28, 28' are observed in ^1H - ^1H COSY and correlation between protons 29, 29' with carbons 30, 19 is observed in HMBC Spectrum (Table 3). The Signal at δ 3.18 (H-3) in the proton spectrum showed a cross peak with a carbon peak at δ 78.96 in the HMQC spectrum and long-range correlation with the carbon signals at 38.69 (C-1), 27.37 (C-2) and 15.34 (C-24), indicating the location of hydroxyl group at C-3. The relative configuration of H-3 was further supported by a NOESY experiment, wherein NOE enhancement was observed between H-3 and H-5 and H-23. The structure and configuration of 1 were confirmed by comparing the NMR data with those of betulin reported by (Tino et al., 1992; Hayek et al., 1989). The NOESY spectrum supported the proposed configuration of 1.

HR EI-MS spectrum of 2 showed the molecular ion at

Table 1. ^1H and ^{13}C -NMR data of Betulin.

| Proton(s) or carbon | ^{13}C -NMR | Dept | ^1H -NMR |
|---------------------|----------------------|------|---|
| 1 | 38.69 | CH2 | 0.88, 1.63 (m) |
| 2 | 27.37 | CH2 | 1.57, 1.52 (m) |
| 3 | 78.96 | CH | 3.18 (dd, $J = 11.1, 5.58$ Hz) |
| 4 | 38.86 | C | |
| 5 | 55.28 | CH | 0.67 (m) |
| 6 | 18.28 | CH2 | 1.51, 1.37 (m) |
| 7 | 34.22 | CH2 | < 1.38 >(m) |
| 8 | 40.94 | C | |
| 9 | 50.3 | CH | 1.26 (m) |
| 10 | 37.14 | C | |
| 11 | 20.81 | CH2 | 1.42, 1.24 (m) |
| 12 | 25.19 | CH2 | 1.01, 1.63 (m) |
| 13 | 37.29 | CH | 1.62 (m) |
| 14 | 42.73 | C | |
| 15 | 27.03 | CH2 | 1.67, 1.03 (m) |
| 16 | 29.15 | CH2 | 1.92, 1.19 (m) |
| 17 | 47.76 | C | |
| 18 | 48.75 | CH | 1.56 (m) |
| 19 | 47.83 | CH | 2.37 (ddd, $J=5.7, 11$ Hz) |
| 20 | 150.46 | C | |
| 21 | 29.73 | CH2 | 1.9, 1.42 (m) |
| 22 | 33.95 | CH2 | 1.02, 1.82 (m) |
| 23 | 27.96 | CH3 | 0.95, (s) |
| 24 | 15.34 | CH3 | 0.74, (s) |
| 25 | 16.09 | CH3 | 0.8, (s) |
| 26 | 15.97 | CH3 | 1, (s) |
| 27 | 14.74 | CH3 | 0.96, (s) |
| 28 | 60.5 | CH2 | a:3.79 (dd, $J=10.7, 4.3$ Hz), b:3.31(dd, $J=10.7, 3.7$ Hz) |
| 29 | 109.67 | CH2 | 4.66, 4.56 (s,s) |
| 30 | 19.06 | CH3 | 1.66, (s) |

m/z 654.2582 $[\text{M}]^+$ in agreement with the molecular formula $\text{C}_{35}\text{H}_{42}\text{O}_{12}$ and indicated fifteen degrees of unsaturation. The base peak of EIMS at $m/z = 105$ and the fragment at 121 ($\text{C}_6\text{H}_5\text{COO}$) indicated the presence of benzoate ester group in the molecule. The IR spectrum showed peaks for OH group at 3500 cm^{-1} , carbonyl groups at $1710\text{-}1750\text{ cm}^{-1}$ and at 1600 and 700 cm^{-1} for benzene ring. The ^1H -NMR spectrum showed four singlets for acetate methyl groups at 1.73, 1.94, 2.06 and 2.11.

The terminal olefinic protons (H-18) overlapped with H-7, were observed as a multiplet at δ 4.88. The vicinal olefinic protons showing signals at δ 5.7 (dd, $J = 9.5, 4.5$ Hz, H-9) and at δ 5.94 (ddd, $J = 7.9, 7.3, 1.15$ Hz, H-8) and separated by a methine proton at δ 3.28 (br. t, $J = 5.5$ Hz, H-11) from H-18. Aromatic protons showed signals at δ 7.8 (d, $J = 7.4$ Hz, H-2', 6'), δ 7.3 (t, $J = 7.4$ Hz, H-3', 5') and δ 7.5 (t, $J = 7.4$ Hz, H-4') in ^1H -NMR spectrum. A couple of doublets at δ 3.97 ($J = 11.8$ Hz), δ 4.34 ($J = 11.8$ Hz) are observed in

the ^1H -NMR that belong to protons of methylene group (H-17, 17') and was deduced with ^1H - ^1H COSY. Because of the chirality of carbone 6 (δ 48.13), two protons 17, 17' appeared in different chemical shifts.

The ^{13}C NMR of 2 showed 33 signals (35 carbons) comprising of seven CH_3 groups, three CH_2 groups, twelve CH groups and eleven quaternary carbons. Having 15 degrees of unsaturation for compound 2 and taking the total number of double bonds, carbonyl groups and benzene ring in to account, the remaining three degrees of unsaturation indicates the presence of 3 rings in the structure of this compound.

The correlation of protons with the corresponding carbons has been established by HMQC. The correlation between H-2',6' and H-3',5', H-8 and H-9, H-11 and H-12 were deduced by observing cross peaks in ^1H - ^1H COSY. Also correlations between germinal protons H-17, 17' and H-1, 1' (δ 3.17, δ 1.62) were observed in ^1H - ^1H COSY. Cross peaks between C7' (δ 165), H-5 (δ 6.37) and

Table 2. ^1H and ^{13}C -NMR data of decipinone.

| Proton(s) or carbon | ^1H -NMR | ^{13}C -NMR |
|---------------------|--|-----------------------|
| 1 | 3.17 (dd, $J=9.28, 14.2$ Hz) 1.62 (dd, $J=9.28, 14.2$ Hz) | 44.9 CH ₂ |
| 2 | 2.09 (m) | 37.59 CH |
| 3 | 5.3 (t, $J=3.26$ Hz) | 79.27 CH |
| 4 | 2.36 (dd, $J=11.5, 3.5$ Hz) | 53.03 CH |
| 5 | 6.37 (d, $J=11.6$ Hz) | 70.8 |
| 6 | | 48.13 C |
| 7 | 4.88 (m) | 67.74 CH |
| 8 | 5.94 (ddd, $J=7.9, 7.3, 1.15$ Hz) | 122.03 CH |
| 9 | 5.7 (dd, $J=9.5, 4.5$ Hz) | 135.9 CH |
| 10 | | 146.9 C |
| 11 | 3.28 (br. t, $J=5.5$ Hz) | 45.8 CH |
| 12 | 4.08 (d, $J=7.9$ Hz) | 40.9 CH |
| 13 | | 86.14 C |
| 14 | | 205.6 C |
| 15 | | 86.36 C |
| 16 | 0.89 (d, $J=6.7$ Hz) | 14.3 CH ₃ |
| 17 | 3.97 (d, $J=11.8$ Hz) 4.34 (d, $J=11.8$ Hz) | 62.28 CH ₂ |
| 18 | 4.88 (m) | 113.1 CH ₂ |
| 19 | 1.76 (s) | 20.1 CH ₃ |
| 20 | 1.67 (s) | 23.4 CH ₃ |
| 1' | | 129.78 C |
| 2', 6' | 7.8 (d, $J=7.4$ Hz) | 129.61 CH |
| 3', 5' | 7.3 (t, $J=7.6$ Hz) | 128.33 CH |
| 4' | 7.5 (t, $J=7.4$ Hz) | 133.1 CH |
| 7' | | 165.15 C |
| OCOCH ₃ | 1.73 (s) | 20.7 CH ₃ |
| OCOCH ₃ | 1.94 (s) | 20.8 CH ₃ |
| OCOCH ₃ | 2.06 (s) | 21.03 CH ₃ |
| OCOCH ₃ | 2.11 (s) | 21.07 CH ₃ |
| OCO | | 169.9 C |
| OCO | | 170 C |
| OCO | | 170.4 C |
| OCO | | 170.9 C |
| OH | 4.14 | |

carbon of acetate group (δ 169.9 - 170.9) with H-3, 7 and 17 in HMBC confirmed the location of acetate and benzoate groups. The relative stereochemical structure of 2 was confirmed by NOESY spectra (Figure 2).

Betulin can be used as such or after chemical modification as a starting compound for other useful materials and compounds, which possess various interesting

pharmacological properties. Different biological activities of betulin have been reported in literatures. Among derivatives of ursane, oleanane and lupane series of triterpenes, betulin and betulinic acid are the most effective compounds in skin inflammation and ear edema induced by mezerin, DPT (12-deoxyphorbol-13-tetra-decanoate) or TPA (12-O-tetradecanoylphorbol-13-acetate) in mice

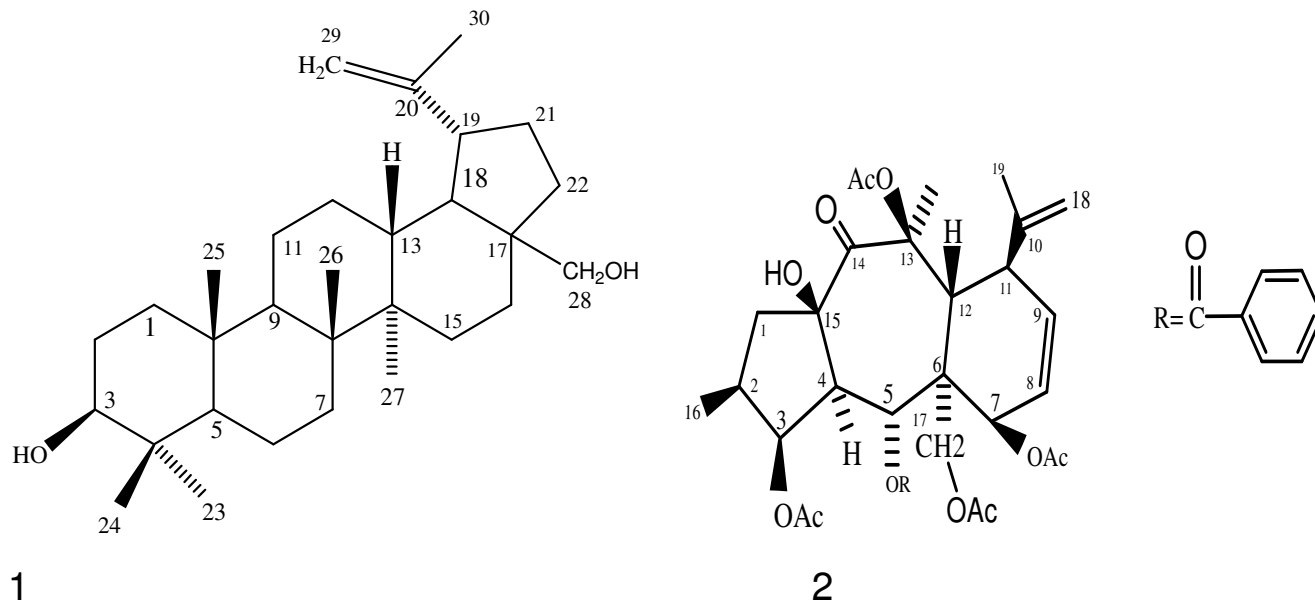


Figure 1. Structures of Betulin (1) and Decipinone (2).

Table 3. The important correlations of compound 1 in HMBC spectrum.

| H-atom | Correlated with C-atom (J) | H-atom | Correlated with C-atom (J) |
|--------|---|--------|---|
| H-29 | 30 (³ J), 19(³ J). | H-28 | 22 (³ J), 16 (³ J), 17 (² J). |
| H-30 | 20(² J), 29(³ J) | H-26 | 9 (³ J), 14 (³ J), 7(³ J). |
| H-3 | 23(³ J), 24(³ J) | H-23 | 24 (³ J), 3 (³ J), 5 (³ J). |
| H-19 | 20(² J), 29(³ J) | H-27 | 8 (³ J), 14 (² J), 13(³ J). |
| H-24 | 23 (³ J), 5 (³ J), 3 (³ J). | H-25 | 5 (³ J), 9 (³ J), 1 (³ J). |

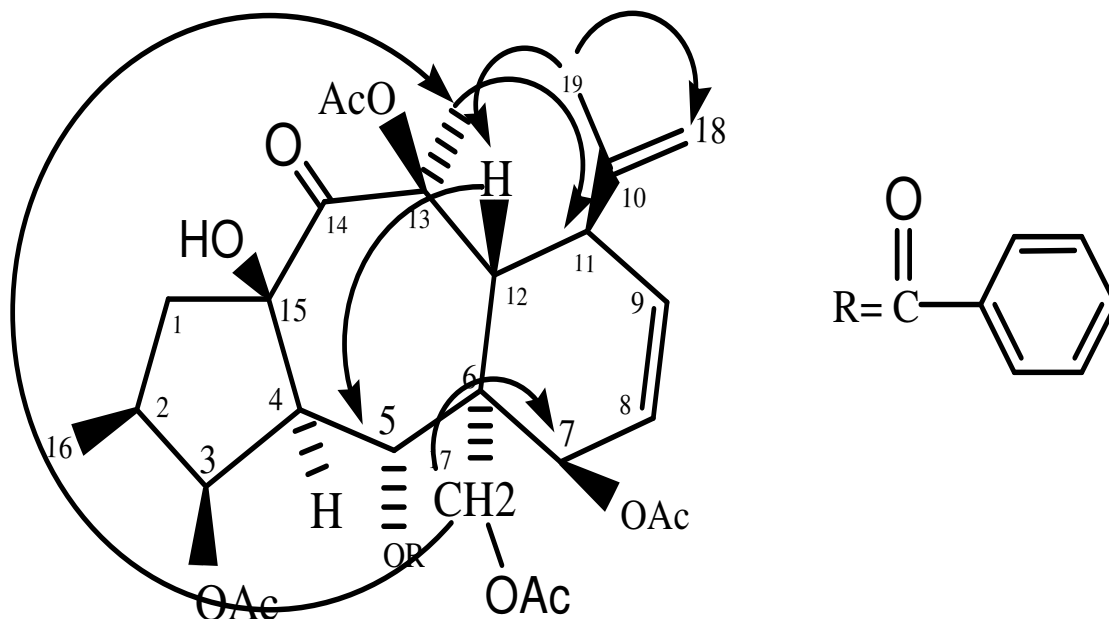


Figure 2. NOESY correlations of compound 2.

(Sami et al., 2006). Pavlova, N.I. et al., reported that Betulin showed antiviral activity against herpes simplex (Pavlova et al., 2003).

Isolation and purification of other fractions of this plant is being carried out. Some of these fractions contain terpenoids or flavonoids that may have new structures.

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