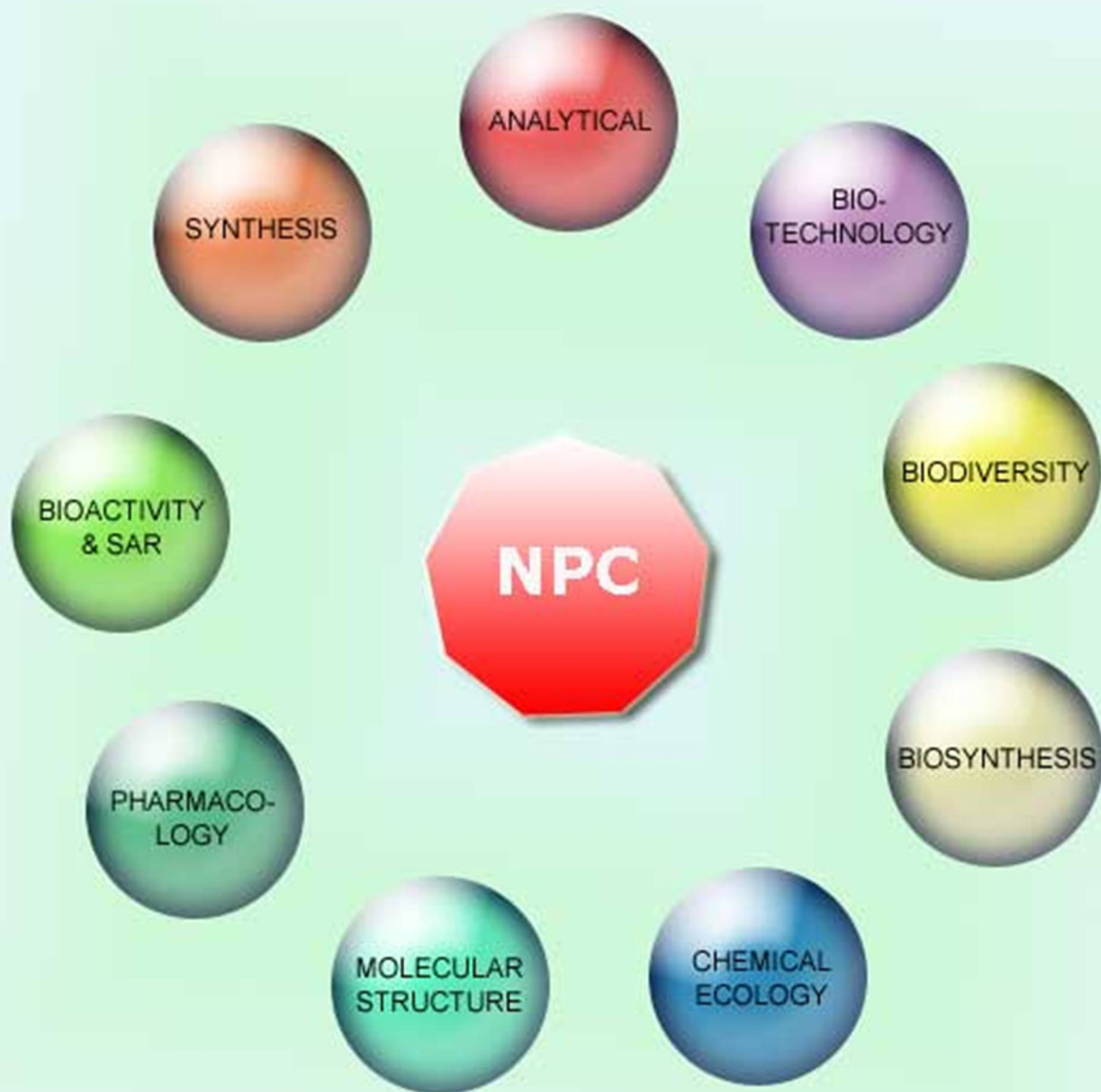


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Triterpene Derivatives from *Abies spectabilis* Leaves of Nepalese Origin

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Our ongoing studies of Nepalese medicinal plants has led to the isolation and characterization of five new triterpenes, two known triterpenes and two phenolic derivatives from *Abies spectabilis* (D.Don) Mirb leaves grown in the high mountain. The structures of the isolated compounds were characterized by means of 1D and 2D NMR spectroscopic and MS techniques.

Keywords: *Abies spectabilis*, Pinaceae, triterpene, isolation, NMR.

Abies spectabilis (Pinaceae), an evergreen conifer found in the sub-alpine forests of the Himalayan region, grows mainly in central and eastern Nepal at an altitude from 2600 to 4000 m, especially in humid and rainy zones [1]. The leaves are used in the traditional medicine of Nepal as a tonic, as an antispasmodic and carminative, a cough remedy and for other pulmonary diseases [2]. In the Manang region (Central Nepal), the needles are used as an adjuvant in the treatment of bone fractures [3]. The decoction of the leaves, considered helpful for bone repair, is consumed orally, but also applied topically to the site of fracture twice a day. Despite the relatively large amount of literature on the constituents of different *Abies* species [4], only limited information is available on *A. spectabilis*. To the best of our knowledge, a biflavonoid named abiesin and two phenolic derivatives (betuloside and methylbetuloside) [4,5] are the only compounds previously reported for this species.

In our ongoing study of Nepalese medicinal plants as a source of potentially bioactive compounds [6-9], we investigated the leaves of *A. spectabilis*. Here we report the isolation and structural elucidation of five new triterpene derivatives (**1-5**), along with two known lanostane derivatives, kaempferol 3,5,7-trimethyl ether, and *p*-benzoic acid.

Compound **1** was isolated as a white amorphous powder. The HR-API-TOF-MS showed a deprotonated molecular ion [M-H]⁻ at *m/z* 499.3063, corresponding to a molecular formula of C₃₀H₄₃O₆. In the ¹H NMR spectrum, five singlet

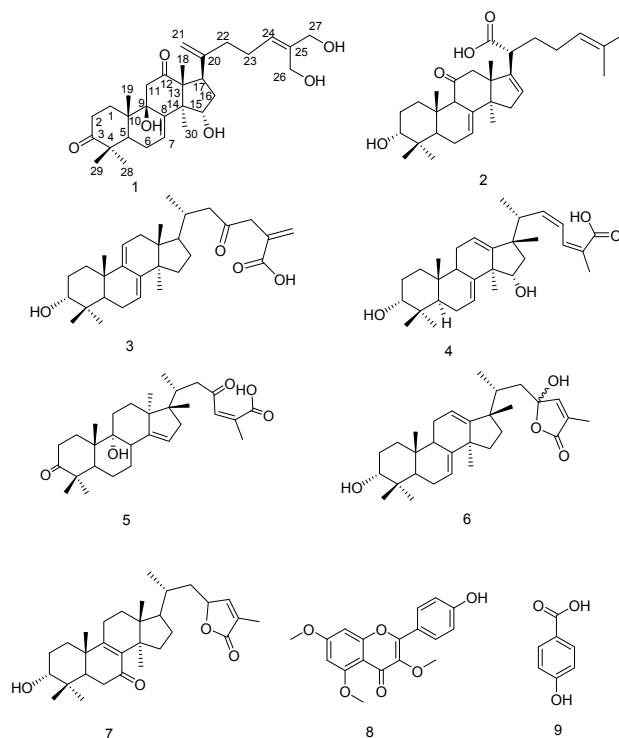


Figure 1: Structures of isolated compounds.

signals, each integrating for three protons, were observed at δ 0.94, 1.03, 1.15, 1.25 and 1.53 suggesting the presence of five quaternary methyl groups. From the HMQC spectrum the chemical shift of non quaternary carbon resonances were obtained. Eight methylenes were identified, namely at δ_{H} 1.76-2.00 and δ_{C} 30.0 (C-1),

δ_{H} 2.01-2.58 and δ_{C} 35.0 (C-2), δ_{H} 1.33 and δ_{C} 29.3 (C-6), δ_{H} 1.53-0.93 and δ_{C} 41.9 (C-11), δ_{H} 1.08-1.30 and δ_{C} 30.0 (C-16) and δ_{H} 2.39 and δ_{C} 29.7 (C-24), δ_{H} 4.15 and δ_{C} 64.6 (C-27), and δ_{H} 3.58 δ_{C} 61.6 (C-27). Connections between CH_2 and CH were established through COSY and TOCSY spectra. The following spin systems were observed: H-1 and H-2, H-5 (δ 2.14) with H-6 (δ 1.33) and H-7 (δ 5.67); and H-15 (δ 3.52) with H-16 (δ 1.08-1.30) and H-17 (δ 1.73). Diagnostic long-range (HMBC) correlations were observed between the singlet at δ 1.15 and 1.03 (CH_3 -28 and 29), and carbon resonances at δ 220.3 (C-3), 47.0 (C-4) and 51.2 (C-5). Furthermore, HMBC correlations were also observed between methyl 28 and C-29 (δ 28.6) and between methyl 29 and C-28 (δ 22.0), supporting the geminal position of these two groups. Further diagnostic long-range correlations were observed between the singlet at δ 1.53 (CH_3 -19) and the carbon resonances at δ 53.3 (C-10) and 65.1 (C-9), supporting the presence of a functional group containing an electronegative atom at C-9. A second ketonic function was revealed from the HMBC correlation between the methyl group assigned to position 18 (δ 1.25) and the carbon resonance at δ 215.5. Further HMBC correlation between the singlet at δ 0.94 (CH_3 -30) and carbon resonances at δ 77.3 (C-15) and 156.8 (C-8) were seen. This finding suggested the presence of an electron attractive group at C-15, as well as the presence of a double bond at 8. An exocyclic methylene group at C-21 was established on the basis of HMBC correlation between H-22 and H-23, as well as on the basis of NOESY correlations between the signal at δ 5.35 and the methyl group at δ 1.25 (CH_3 -18). Three more signals due to olefin protons were detected at δ 5.64 (H-23) and 6.04-5.35 (H-21). Their relative positions were deduced on the basis of HMBC and COSY correlations. Thus, the side chain of the compound was established with an exocyclic methylene at 21 and a double bond at 24-25. With all this spectral evidence, we established the structure of compound **1**, which is reported for the first time. Similar compounds have been isolated previously from *Ganoderma lucidum* Reishi, a bitter fungus widely used in the folk medicine of many oriental countries [10]. Recently, similar triterpenes isolated from the same mushroom were evaluated for their ability to inhibit 5α -reductase [11].

The HR-API-TOF-MS of compound **2** showed a deprotonated molecular ion $[\text{M-H}]^-$ at m/z 467.3113, corresponding to a molecular formula $\text{C}_{30}\text{H}_{44}\text{O}_4$. The ^1H NMR spectrum showed seven singlets (δ 0.83, 0.89, 0.92, 0.93, 0.97, 1.15 and 1.16) integrating for three protons each, thus supporting the presence of seven methyl groups. Diagnostic long range correlations were observed in the HMBC spectrum between the methyl groups at δ 0.93 and 0.97 (CH_3 -28 and 29) and carbon resonances at δ 76.6 (C-3), 38.7 (C-4), and 51.1 (C-5), the singlet at δ 0.93 (CH_3 -28) and the carbon resonance at δ 29.0, the singlet at δ 0.97 and the carbon at δ 23.0 (C-28) indicating the presence of two geminal methyl groups at position 4. The

chemical shift values (δ_{H} 3.46, δ_{C} 76.6) for position 3 indicated the presence of an electron attractive group. The NOESY correlations for H-3 with H-5 supported a β -orientation for the hydroxyl group [12]. The COSY spectrum allowed us to establish several spin systems, namely H-1 (δ 1.27), H-2 (δ 1.58-1.65), H-3 (δ 3.46); H-5 (δ 1.37), H-6 (δ 1.92), H-7 (δ 5.62), H-15 (δ 1.85-2.12); and H-16 (δ 5.16), H-22 (δ 1.80-1.28), H-23 (δ 1.93), and H-24 (δ 5.46). Diagnostic long range correlations were observed between the singlet at δ 1.15 (CH_3 -30) and the carbon at δ 146.3 (C-8). CH_3 -18 (δ 1.16) HMBC correlations were observed with C-17 (δ 157.6) and C-12 (δ 38.9) supporting a double bond at position 16-17. This is further confirmed by the NOESY correlations between H-16 (δ 5.15) and the methyl groups at C-26 and C-27. A keto function was revealed by the HMBC correlation between H-12 (δ 1.27) and the carbon at δ 213.5 (C-11). The side chain was found to be formed by two methyl groups linked to a sp^2 carbon, as evidenced by the HMBC correlations between the signals at δ 0.83 (C-26) and δ 0.89 (C-27) with the carbon resonances at δ 132.4 (C-25) and 123.0 (C-24). Diagnostic long range correlation was also observed between CH_2 -22 (δ 1.26-1.80) and a carboxyl function at δ 187.0, supporting the presence of a carboxylic acid at position 21. The presence of the carboxy function was also confirmed by an ESI-MS/MS experiment performed by ion trap spectrometry. The fragmentation of the molecular ion at m/z 467 yielding a fragment at m/z 423 supported the loss of CO_2 . The structure of compound **2** was established as a lanostane derivative related to tramemenolic acid [13], but with a double bond at position 16-17 and a carbonyl function at 11. Because of the presence of these structural features, compound **2** represented a new structure. These types of compounds are produced by fungal species such as *Formitropis pinicola* that grow as saprophytes on Pinaceae and related botanical species.

Compound **3** was isolated as amorphous powder. The HR-MS resulted in a molecular ion $[\text{M-H}]^-$ at m/z 467.3149, corresponding to a molecular formula of $\text{C}_{30}\text{H}_{44}\text{O}_4$. Six methyl groups were observed in the ^1H NMR spectrum at δ 0.82 (3H, d, $J = 6.5$ Hz), 0.86 (3H, s), 0.91 (3H, s), 1.00 (3H, s), 1.14 (3H, s), and 1.25 (3H, s). The presence of a methine proton bearing a functional group containing an electron attracting atom was observed at δ 3.34 (dd, $J = 4.0, 9.0$ Hz). Signals ascribable to double bonds were revealed at δ 5.38, 5.40, 5.54 and 5.83. HMQC and HMBC spectra allowed us to establish a large part of the molecular skeleton. Diagnostic long-range correlations were observed between the proton signal at δ 1.24 (CH_3 -18) and carbon resonances at δ 155.0 (C-9), 28.5 (C-1) and 49.7 (C-10). Furthermore, the singlet at δ 1.14 (CH_3 -30) showed a correlation with a carbon resonance at δ 146.8 (C-8). These correlations supported the position of two double bonds at positions 7-8 and 9-11. COSY and TOCSY experiments established the connection of signals at δ 1.97 (H-1) and 1.04-2.00 (H-2) and δ 3.34 (H-3); at

δ 1.36 (H-5) and 1.75-2.17 (H-6); δ 5.40 (H-11) and 1.28-1.91 (H-12); and δ 0.82 (CH_3 -21) and 1.96 (H-20). An exocyclic methylene was revealed by a pair of doublets ($J = 3.10$) at δ 5.54 and 5.83. The long-range correlations between the proton signal at δ 1.98 (brs) and the carbon resonances at δ 207.6 (C-23), 154.0 (C-24) and δ 177.1 (C-26) revealed the presence of keto and carboxylic acid functions in the side chain of the compound. Thus the structure of compound **3** was established as a derivative of isofirmanoic [14] acid, namely 9,7-dehydroisofirmanoic acid, a new natural compound.

Compound **4** was isolated as amorphous powder. The HR-MS resulted in a molecular ion $[M-H]^-$ at m/z 467.3122 corresponding to a molecular formula of $C_{30}H_{44}O_4$, thus being an isomer of compounds **2** and **3**. Five olefinic proton signals were observed in the 1H NMR spectrum at δ 6.82, 6.73, 5.73, 5.58 and 5.48. Six signals supporting the presence of methyl groups were detected at δ 0.93 (3H, s), 0.98 (3H, s), 0.95 (3H, s), 1.03 (3H, d, $J = 6.5$), 1.13 (3H, s) and 1.89 (3H, s). Two signals at δ 3.48 (1H, m) and 3.51 (1H, s) supported the presence of two methine protons linked to functional groups containing an electronegative atom. Extensive analysis of HMQC and HMBC spectra revealed that the structure of compound **4** was similar to that of compound **3**. The main differences were observed at the side chain methyl group 18 and the position of the double bonds in the lanostane skeleton. Diagnostic long range correlations were observed between the singlet at δ 0.93 (CH_3 -18) and 1.13 (CH_3 -30) and carbon resonances at δ 157.0 (C-13) supporting the presence of a double bond at position 12-13. The methyl group at C-18 also showed long range correlations with carbon resonances δ 51.0 (C-17), 38.2 (C-16), and 47.5 (C-20), indicating the presence of a methyl group at C-17. HMBC correlations were also observed between the methyl group at δ 1.89 (CH_3 -27) and carbon resonances C-26 (δ 172.6), 137.5 (C-25), and 147.1 (C-24), and the proton signal at δ 6.73 (H-24) and carbon C-27 (δ 10.2). The side chain was thus found to contain two double bonds (δ 5.58, 6.82 and 6.73) in the positions 22-23 and 24-25, and a carboxyl function at C-26. NOESY correlations between signals at δ 5.58 and 6.82 supported the *cis* geometry of this double bond. NOESY correlation between δ 6.73 and 1.89 allowed us to establish the *cis* geometry of the 24-25 double bond. A further double bond was assigned at positions 7-8 on the basis of COSY and HMBC data. Thus compound **4** was deduced as a lanostane derivative with a novel structure. Compounds with a side chain similar to compound **4** were previously reported from *A. mariesii* and *A. firma* [14]. Similar structures were also recently isolated from *Garcinia hombroniana* [15].

Compound **5** was isolated as amorphous powder. The HR-MS exhibited a molecular ion $[M-H]^-$ at m/z 483.2994, corresponding to a molecular formula of $C_{30}H_{44}O_5$. The 1H NMR spectrum showed the presence of seven methyl

groups at δ 1.89 (3H, d, $J = 6.7$), 1.16 (3H, d, $J = 6.3$), 1.09 (3H, s), 1.07 (3H, s), 0.98 (3H, s), 0.95 (3H, s) and 0.88 (3H, s). A signal ascribable to an olefinic proton is observed at δ 7.38 (1H, brs). A keto function was assigned at position 3 on the basis of HMBC correlations observed between the singlets at δ 1.07 and 1.09 (CH_3 -28 and -29) and the carbon resonance at δ_C 216.7 (C-3). Moreover, the geminal methyl groups at C-28 and C-29 showed long range correlations with C-4 (δ 48.2) and C-5 (δ 50.4). The presence of a hydroxyl function at position 9 was supported by the long range correlations between the methyl group at C-19 (δ 0.98) and the carbons at δ 30.0 (C-1), 39.5 (C-10) and 67.1 (C-9). The methyl group at C-30 (δ 0.88) showed long range correlations with C-17 (δ 41.6), 30.0 (C-12) and 138.6 (C-14) supporting the presence of a double bond at position 14-15. A second keto group was revealed by the HMBC correlation between the signal at δ 1.26 (H-22) and the carbon resonance at 215.0 (C-23). The deshielded proton signal at δ 7.39 and its HMBC with the resonances at δ 138.0 (C-25) and 179.5 (C-26) allowed us to establish the presence of a double bond at position 24-25, and a carboxylic function at position 26. The orientation of the side chain linked at 17 was assumed as α on the basis of NOESY correlations and literature data for similar compounds. This derivative is similar to other friedolanostane type triterpenes previously isolated from *Garcinia hombroniana* [15] except for the keto groups at positions 3 and 23. Because of these differences, compound **5** was deduced to be a new natural product.

Compound **6**, 18-nordammara-7,12,24-trien-26-oic acid, 3,23,23-trihydroxy-17-methyl-, γ -lactone, ($3\alpha,9\beta,17\alpha$), was previously reported for *A. sibirica*, and compound **7**, lanosta-8,24-dien-26-oic acid, 3,23-dihydroxy-7-oxo-, γ -lactone, ($3\alpha,23R$), for *A. mariesii* [14-18]. Kaempferol 3,5,7-trimethyl ether (**8**) and *p*-hydroxybenzoic acid (**9**) were also isolated and characterized.

The structures of the isolated triterpenes appear to be unusual for high altitude conifers, such as *A. spectabilis*. These types of compounds are usually reported from fungi. For example, several lanostane type triterpenes akin to compound **1** were produced by species of *Ganoderma* and *Anthrodia* mycetes. There are some reports that Nepalese mountain trees are invaded by different types of fungi [19-21]. Fungi are known to affect secondary metabolite production processes in plants [22,23]. As an example, a large number of similar lanostane derivatives have been reported from both *Pisolithus tinctorius* isolates and its *Pinus sylvestris* ectomycorrhizas [24]. For this reason, further investigations are required in order to establish if the isolated triterpenes from *Abies spectabilis* leaves are secondary metabolites derived from the plant, from the invading fungal species, or from the mutualistic interaction of the plant and the fungus.

Experimental

Plant materials: The aerial parts of *A. spectabilis* were collected from Sagarmatha National Park at 3300 m asl in October 2007. A voucher specimen is deposited at Department of Pharmaceutical Sciences of University of Padova with no. ASL1107.

Extraction and isolation of compounds: Dried leaves (70 g) were sequentially extracted at room temperature by ultrasound assisted extraction (UAE) with solvents of increasing polarity, light petroleum, chloroform and methanol, yielding 3 different extracts. The extractive yields were 1.35, 11.2 and 15.0% for light petroleum, chloroform and methanol, respectively. The chloroform extract (2 g) was subjected to silica gel column chromatography (450 mL) using as eluents cyclohexane-ethyl acetate (4:1) for 2 column volumes, then chloroform-methanol in increasing ratio (from 0% to 15 %). Fractions were pooled in 18 groups on the basis of their chromatographic behaviour. Further separation was performed by semi-preparative HPLC using either methanol-water (90:10) or (70:30) as eluents on a Licrocart C-18 (10 μ x 250 mm) column. The chromatographic separation led to the isolation of the new terpene derivatives **1** (12.0 mg), **2** (11.0 mg), **3** (11.0 mg), **4** (15.1 mg), and **5** (14.0 mg), the two known terpenes **6** (12.2 mg) and **7** (28 mg), the flavonoid, kaempferol 3,5,7-trimethyl ether (**8**) (25 mg), and *p*-hydroxybenzoic acid (**9**) (12 mg). Structures of the isolated compounds were elucidated on the basis of 1D and 2D NMR spectroscopic techniques as well as HR-MS.

5 α -lanostan-8,20,22-trien-15,26,27-trihydroxy-3,12-dione [9,15-Dihydroxy-17-(6-hydroxy-5-hydroxymethyl-1-methylene-hex-2-enyl)-4,4,10,13,14-pentamethyl-1,4,5,6,9,10,11,13,14,15,16,17-dodecahydro-2H-cyclopenta[a]phenanthrene-3,12-dione (**1**)]

$[\alpha]_D$: +1.4 (*c* 1.10, CHCl₃).

¹H NMR (300 MHz, CDCl₃): 2.00-1.76 (2H, m, H-1), 2.52-2.01 (2H, m, H-2), 2.14 (1H, m, H-5), 1.33 (1H, m, H-6), 5.66 (1H, brs, H-7), 1.53-0.93 (2H, m, H-11), 3.52 (1H, dd, *J* = 8.0; 3.0 Hz, H-15), 1.08-1.30 (2H, m, H-16), 1.73 (2H, m, H-17), 1.53 (3H, s, H-18) 1.25 (3H, s, H-19), 6.04-5.35 (2H, brs, H-21), 2.64 (1H, m, H-22), 2.39 (1H, m, H-23), 5.51 (2H, m, H-24), 4.51 (4H, dd, *J* = 11.0; H-26 H-27), 1.15 (3H, s, H-28), 1.03 (3H, s, H-29), 0.94 (3H, s, H-30).

¹³C NMR (100 MHz CDCl₃): 30.0 (CH₂, C-1), 35.0 (CH₂, C-2), 220.3 (C, C-3), 47.0 (C, C-4), 51.2 (CH, C-5), 29.3 (C-6), 122.3 (C-7), 156.8 (C-8), 65.1 (C-9), 53.3 (C-10), 41.9 (C-11), 215.5 (C-12), 44.1 (C-13), 43.2 (C-14), 77.3 (C-15), 30.0 (C-16), 50.2 (C-17), 18.7 (C-18), 18.9 (C-19), 141.0 (C-20), 123.2 (C-21), 30.0 (C-22), 23.6 (C-23), 127.0 (C-24), 140.8 (C-25), 64.6 (C-26) 64.6 (C-27), 22.0 (C-28), 28.6 (C-29), 27.9 (C-30).

HRMS-API-TOF: *m/z* [M-H]⁻ calcd for C₃₀H₄₃O₆: 499.3054; found: 499.3063.

3 β -hydroxylanosta-7,16,24-trien-11-oxo-21-oic acid [2-(3-Hydroxy-4,4,10,13,14-pentamethyl-11-oxo-2,3,4,5,6,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-6-methyl-hept-5-enoic acid (**2**)]

$[\alpha]_D$: +2.6 (*c* 0.350, CHCl₃).

¹H NMR (300 MHz, CDCl₃): 1.27 (2H, m, H-1), 1.58-1.65 (2H, m, H-2), 3.46 (1H, dd, *J* = 7.80, 3.10, H-3), 1.37 (1H, m, H-5), 1.92 (1H, m, H-6), 5.62 (1H, brs, H-7), 1.45 (1H, m, H-9), 1.30 (1H, m, H-12), 1.85-2.12 (2H, m, H-15), 5.16 (1H, brs, H-16), 1.16 (3H, s, CH₃-18), 0.92 (3H, s, CH₃-19), 2.27 (1H, t, H-20), 1.28-1.80 (2H, m, H-22), 1.93 (2H, m, H-23), 5.46 (1H, brd, H-24), 0.83 (3H, s, CH₃-26), 0.89 (3H, s, CH₃-27), 0.93 (3H, s, CH₃-28), 0.97 (3H, s, CH₃-29), 1.15 (3H, s, CH₃-30).

¹³C NMR (100 MHz CDCl₃): 29.7 (CH₂, C-1), 25.8 (CH₂, C-2), 76.6 (CH, C-3), 38.7 (C, C-4), 51.1 (CH, C-5), 23.7 (CH₂, C-6), 119.1 (CH, C-7), 146.3 (C, C-7), 38.6 (C, C-9), 39.9 (C, C-10), 213.5 (C, C-11), 38.9 (CH₂, C-12), 50.2 (C, C-13), 49.3 (C, C-14), 37.3 (CH₂, C-15), 115.9 (CH, C-16), 157.5 (C, C-17), 26.3 (CH₃, C-18), 22.7 (CH₃, C-19), 48.4 (CH, C-20), 187.0 (C, C-21), 27.3 (CH₂, C-22), 24.1 (CH₂, C-23), 123.2 (CH, C-24), 132.4 (C, C-25), 16.0 (CH₃, C-26), 25.0 (CH₃, C-27), 23.0 (CH₃, C-28), 29.0 (CH₃, C-29), 26.3 (CH₃, C-30).

HRMS-API-TOF: *m/z* [M-H]⁻ calcd for C₃₀H₄₃O₆: 467.3161; found: 467.3143.

6-(3-Hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,10,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-17-yl)-2-methylene-4-oxo-heptanoic acid (**3**)

$[\alpha]_D$: +1.6 (*c* 1.350, CHCl₃).

¹H NMR (300 MHz, CDCl₃): 1.97 (2H, m, H-1), 1.94-2.00 (2H, m, H-2), 3.34 (1H, dd, *J* = 8.0; 3.0, H-3), 1.36 (1H, m, H-5), 1.75-2.17 (2H, m, H-6), 5.38 (1H, dd, *J* = 7.0, 2.0, H-7), 5.40 (1H, dd, *J* = 7.0, 3.0, H-11), 1.28-1.91 (2H, m, H-12), 2.00-2.45 (2H, m, H-15), 1.23 (2H, m, H-16), 2.66 (1H, m, H-17), 0.86 (3H, s, CH₃-18), 1.25 (3H, s, CH₃-19), 1.96 (1H, m, H-20), 0.82 (3H, d, *J* = 6.5, CH₃-21), 1.82 (3H, m, CH₂-22), 1.98 (2H, s, CH₂-24), 5.54 (1H, d, *J* = 3.1, H-27-a) 5.83 (1H, d, *J* = 3.1, H-27-b), 1.00 (3H, s, CH₃-28), 0.91 (3H, s, CH₃-29), 1.14 (3H, s, CH₃-30).

¹³C NMR (100 MHz CDCl₃): 28.5 (CH₂, C-1), 25.5 (CH₂, C-2), 77.3 (CH, C-3), 41.0 (C, C-4), 52.0 (CH, C-5), 28.3 (CH₂, C-6), 118.7 (CH, C-7), 146.8 (C, C-8), 155.0 (C, C-9), 49.7 (C, C-10), 122.3 (CH, C-11), 25.7 (CH₂, C-12), 48.0 (C, C-13), 51.3 (C, C-14), 38.3 (CH₂, C-15), 22.1 (CH₂, C-16), 47.4 (CH, C-17), 29.3 (CH₃, C-18), 19.8 (CH₃, C-19), 28.0 (CH, C-20), 16.2 (CH₃, C-21), 48.0 (CH₂, C-22), 207.6 (C, C-23), 31.4 (CH₂, C-24), 144.0 (C, C-25), 177.1 (C, C-26), 121.2 (CH, C-27), 29.2 (CH₃, C-28), 22.9 (CH₃, C-29), 26.3 (CH₃, C-30).

HRMS-API-TOF: *m/z* [M-H]⁻ calcd for C₃₀H₄₃O₆: 467.3149; found: 467.3143.

6-(3,15-Dihydroxy-4,4,10,14,17-pentamethyl-2,3,4,5,6,9,10,11,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-17-yl)-2-methyl-hepta-2,4-dienoic acid (4)[α]_D: +0.6 (c 1.100, CHCl₃).¹H NMR (300 MHz, CDCl₃): 1.97 (2H, m, H-1), 1.19-1.61 (2H, m, H-2), 3.48 (1H, dd *J* = 8.2, 2.6, H-3), 1.39 (1H, m, H-5), 1.82-2.21 (2H, m, H-6), 5.48 (1H, dd, *J* = 7.0, 2.0, H-7), 1.90 (2H, m, H-11), 5.63 (1H, m, H-12), 3.51 (2H, m, H-15), 1.88 (2H, m, H-16), 0.93 (3H, s, CH₃-18), 0.95 (3H, s, CH₃-19), 1.97 (1H, m, H-20), 1.03 (3H, d, *J* = 6.4, CH₃-21), 5.65 (1H, m, H-22), 6.82 (1H, d, H-23), 6.73 (1H, H-24), 1.89 (3H, s, CH₃-27), 0.95 (3H, s, CH₃-28), 0.99 (3H, s, CH₃-29), 1.13 (3H, s, CH₃-30).¹³C NMR (100 MHz CDCl₃): 30.0 (CH₂, C-1), 25.7 (CH₂, C-2), 77.9 (CH, C-3), 39.6 (C, C-4), 51.2 (CH, C-5), 24.9 (CH₂, C-6), 123.0 (CH, C-7), 146.7 (C, C-8), 38.1 (C, C-9), 44.6 (C, C-10), 38.8 (CH, C-11), 118.8 (CH₂, C-12), 157.0 (C, C-13), 50.9 (C, C-14), 68.9 (CH₂, C-15), 50.8 (CH₂, C-16), 51.1 (CH, C-17), 25.6 (CH₃, C-18), 21.2 (CH₃, C-19), 47.7 (CH, C-20), 18.1 (CH₃, C-21), 144.0 (CH₂, C-22), 123.9 (C, C-23), 137.1 (CH₂, C-24), 126.7 (C, C-25), 172.6 (C, C-26), 10.8 (CH, C-27), 29.2 (CH₃, C-28), 22.9 (CH₃, C-29), 15.6 (CH₃, C-30).HRMS-API-TOF: *m/z* [M-H]⁻ calcd for C₃₀H₄₃O₆: 467.3122; found: 467.3143.**6-(9-Hydroxy-4,4,10,13,17-pentamethyl-3-oxo-2,3,4,5,6,7,8,9,10,11,12,13,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-2-methyl-4-oxo-hept-2-enoic acid (5)**[α]_D: -0.2 (c 1.100, CHCl₃).¹H NMR (300 MHz, CDCl₃): 1.80 (2H, m, H-1), 2.58 (2H, m, H-2), 1.49 (1H, m, H-5), 2.01 (2H, m, H-6), 2.16-1.48 (2H, m, H-7), 1.54 (1H, m, H-8), 1.34 (2H, m, H-11), 1.25-2.50 (2H, m, H-12), 5.76 (1H, brd, H-16), 0.88 (3H, s, CH₃-18), 0.99 (3H, s, CH₃-19), 2.10 (1H, m, H-20), 1.16 (3H, d, *J* = 6.3, CH₃-21), 1.26 (2H, m, H-22), 7.39 (1H, s, H-24), 1.89 (3H, s, CH₃-27), 1.07 (3H, s, CH₃-28), 1.09 (3H, s, CH₃-29), 0.95 (3H, s, CH₃-30).¹³C NMR (100 MHz CDCl₃): 34.0 (CH₂, C-1), 30.0 (CH₂, C-2), 216.7 (CH, C-3), 48.2 (C, C-4), 50.4 (CH, C-5), 23.2 (CH₂, C-6), 21.3 (CH, C-7), 43.4 (CH, C-8), 67.1 (C, C-9), 39.5 (C, C-10), 23.1 (CH₂, C-11), 31.0 (CH₂, C-12), 39.6 (C, C-13), 138.6 (C, C-14), 122.1 (CH, C-15), 31.3 (CH₂, C-16), 41.6 (C, C-17), 15.4 (CH₃, C-18), 19.2 (CH₃, C-19), 49.7 (CH, C-20), 14.6 (CH₃, C-21), 30.1 (CH₂, C-22), 210.0 (C, C-23), 138.7 (CH, C-24), 147.1 (CH, C-25), 178.6 (C, C-26), 10.8 (CH, C-27), 29.2 (CH₃, C-28), 23.9 (CH₃, C-29), 24.8 (CH₃, C-30).HRMS-API-TOF: *m/z* [M-H]⁻ 483.2994 calcd for C₃₀H₄₄O₅; 483.3189**Acknowledgments** –Authors thanks MIUR for financial support. Field visit by BBS and PKJ to collect plant material was supported by HKKH Partnership Project, EVK-2-CNR, Italy.**References**

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