Melantheraside A–E, five original triterpenes with natural chloride or oxime group from the aerial parts of Melanthera elliptica O. Hoffm.

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Abstract

Five new pentacyclic triterpenoids, Melantheraside A-E (**1-5**) together with eleven known compounds (**6-16**) were isolated from the methanol extract of the aerial parts of *Melanthera elliptica* O. Hoffm. Their structures were established by extensive analysis of 1D- (^{1}H, ^{13}C), 2D- (COSY, ROESY, HSQC and HMBC) NMR data experiments in conjunction with mass spectrometry (TOFESIMS and HR-TOFESIMS) and by comparison with data reported in the literature. The structures of the new compounds were established as: 12*α*-chloro,3*β*,13*β*-dihydroxyoleanan-28, 13-olide (**1**), 3-*O*-acetyl-12*α*-hydroxytaraxer-14-en-28-oic acid (**2**), 12-oxime-3*β*-hydroxytaraxeran-28,14-olide (**3**), 12-oxime-3-*O*-acetyl-taraxeran-28,14-olide (**4**) and 3-oxo-2*β*-carboxyamino-12*α*-chloro,13*β*-hydroxy-1-*nor*-oleanan-28,13-olide (**5**). The 3-*O*-acetyl-12*α*-chloro,13*β*-hydroxyoleanan-28,13-olide (**6**) was isolated for the first time as natural product.

Keywords: *Melanthera elliptica*, pentacyclic triterpenoids, Melantheraside

1. Introduction

The genus *Melanthera* (Asteraceae), contains 39 species of flowering plants native to North and South America, as well as Africa, Asia and Oceania **(Parks, 1973).** Many species of this genus such as *M. elliptica* and *M. scandens* are used traditionally for the treatment of various diseases (**Agyare et al., 2009; Ajibesin et al 2008; Guede et al., 2010).** Previous studies on plants of this genus revealed the presence of flavonoids, alkaloids, tannins, reducing sugars, diterpenes, terpenes and saponins (**Omoyeni et al., 2012, Slimenstad et al., 1996).** In the course of our continuing search for secondary metabolites with potentially interesting bioactivity, we investigated the EtOAc and *n*-BuOH soluble fractions from MeOH extract of aerial parts of *Melanthera elliptica* O. Hoffm.. We reported here the isolation and structure elucidation of five new pentacyclic triterpenoids with natural chloride and oxime substituent in addition to eleven known compounds.

2. Results and discussion

The crude MeOH extract of the aerial parts of *Melanthera elliptica,* was partition against EtOAc and *n*-BuOH by liquid-liquid partition. Purification on silica gel column chromatographies of EtOAc soluble fractions led to the isolation of five new compounds, Melantheraside A-E (**1-5**) and three known compounds (**6-9**). Similarly, purification on silica gel column chromatography and Sephadex LH-20 of *n*-BuOH soluble fractions gave the identification of six known compounds (**10-16**).

Melantheraside A (**1)** was obtained as a white powder which reacted positively with Liebermann-Burchard reagent. Its TOFESIMS spectrum exhibited a sodium adduct peak at *m/z* 513.3120 $[M+Na]^+$ (calcd. for $C_{30}H_{47}O_3ClNa$ 513.3111). The intensity of isotopic ion at *m/z* 515.3085 $[M+Na+2]^+$ was about one third comparing with $[M+Na]^+$ ion, which was attributed to the presence of element chlorine. These MS data and ^{13}C NMR spectrum (Table 1) corresponded to a molecular formula $C_{30}H_{47}O_3Cl$ for compound **1**. Comparison of the ^1H and ^{13}C NMR data with related triterpenoids indicated that **1** was a typical oleanane-type triterpenoid with one hydroxyl group (Monte et al., 1998, Ogawa et al., 2007). The ^1H NMR spectrum (Table 1) of **1** exhibited seven tertiary methyl groups at δ_H: 0.91 (H-23, s), 0.68 (H-24, s), 0.83 (H-25, s), 1.11 (H-26, s), 1.34 (H-27, s), 0.96 (H-29, s) and 0.87 (H-30, s), an oxygenated methine at δ_H 3.03 (H-3, *dd*, 11.9, 4.8 Hz), a chloro-methine at δ_H 4.41 (H-12, *dd*, 3.8, 2.5 Hz) and one methine at δ_H 2.14 (H-18, *m*). This was confirmed by its ^{13}C NMR spectrum (Table 1) on which, carbon signals of seven methyl groups were at δ_C: 28.5 (C-23), 16.3 (C-24), 16.9 (C-25), 19.0 (C-26), 20.2 (C-27), 33.4 (C-29) and 24.1 (C-30) with one hydroxymethine at δ_C 77.1 (C-3) and a methine at δ_C 50.8 (C-18) characteristic of oleanane-type triterpenes (**Mahato and Kundu, 1994**). The HSQC spectrum located the carbon of the chloro-methine at δ_C: 65.3 (C-12) as in compound **6**, obtained by synthesis (Ogawa et al., 2007) and two natural chloro-saponin (Monte et al. 1998, Xu et al., 2010). The extensive analysis of the HSQC spectrum led us to attribute the signals at δ_C 91.3 to a quaternary sp^3 hydroxylated carbon. The correlation in HMBC spectrum between the oxymethine proton signal at δ_H 3.03 (H-3) and the two methyl groups at δ_C: 28.5 (C-23) and 16.3 (C-24), confirme the hydroxyl group position C-3. The coupling constant of 8.5 and 6.8 Hz with both protons H-2 indicated an α-axial orientation for H-3 and thus that hydroxyl was β-orientated. Moreover, signal of H-3 showed correlation in COSY spectrum with the proton of the hydroxyl group at δ_H 4.32 (d, J= 5.2 Hz) indicating that this hydroxyl group was free and not acetylated as in compound **6** (Ogawa et al., 2007). According to HMBC, the proton at δ_H 2.12 was correlated with carbons at δ_C 178.4 (C-28), 91.3 (C-13), 65.3 (C-12), 45.7 (C-17) and 43.0 (C-14), indicating that this proton was attributed to H-18. Comparing with a serie of oleanane-type lactone (Fu et al., 2005, Ogawa et al., 2007, Xu et al., 2010), compound **1** was established as an oleane13,28 lactone (Table 1). The chlorinated-proton at

δ_H 4.41 (H-12, *dd*, 3.8, 2.5 Hz) was correlated in the HMBC spectrum with the carbons at δ_C 91.3 (C-13), 50.8 (C-18), 44.8 (C-9), 43.0 (C-14), and 29.4 (C-11) confirming its position at C612 as in compound **6**. The small coupling constant of H-12 with the methylene protons H-11 suggested a β-equatorial position of H-12 and an a-orientation for chlorure. This is confirmed by the ROESY correlation between H-12 and H-11*β*/H-18 also indicated the alpha orientation of 12-Cl. The ROESY correlations observed between H-3 and H-23/H-5; H-5 and H-9, H-27 and H-9/H-19 (*α*), H-16 (*α*) and H-21 (*α*)/H-22 (*α*), H-25 and H-26, H-18 and H-12/H-30, H-30 and H-22 (*β*) (Figure 2) provided evidence for the triterpenoid ring fusion of A/B *trans*, B/C *trans*, C/D *trans*, D/E *cis* and 13*R* configuration. Accordingly, the structure of compound **1**, melantheraside A was established as 12*α*-chloro,3*β*,13*β*-dihydroxyoleanan-28,13-olide.

Compound **6**, 3-*O*-acetyl-12*α*-chloro,13*β*-hydroxyoleanan-28,13-olide, was previously described in the literature as a synthetic triterpenoid (Ogawa et al., 2007), and was identified as the 3-*O*-acetyl derivatives of compound **1**. In the ^{1}H and ^{13}C NMR spectra, the signals of an acetyl group were observed at δ_H 2.00 (3H, s) and δ_C 170.7 and 21.5 (Table 1). Its position at C-3 was determined through an HMBC experiment in which the oxymethine proton signal at δ_H 4.42 (H-3) showed $^3J_{C-H}$ correlation with the carbonyl at δ_C 170.7. Compound **6**, melantheraside F, was firstly isolated here as a natural product.

Melantheraside B (**2)** was isolated as a white amorphous powder and also reacted positively with Liebermann-Burchard reagent. Its positive TOFESIMS exhibited a sodium adduct at *m/z* 537 $[M+Na]^+$. Its positive HR-TOFESIMS exhibited a sodium adduct peak at *m/z* 519.3456 $[M+Na-H_2O]^+$ (calcd. for $C_{32}H_{48}O_4Na$ 519.3450) indicating the loss of an hydroxyl group. The molecular formula was also identified as $C_{32}H_{50}O_5$. The ^{1}H NMR spectrum of **2** (Table 2) exhibited resonances for seven tertiary methyl groups at δ_H: 0.79 (H_3-23, s), 0.81 (H_3-24, s), 0.89 (H_3-25, s), 0.87 (H_3-26, s), 1.02 (H_3-27, s), 0.91 (H_3-29, s) and 0.91 (H-30, s), a methine at δ_H 2.62 (H-18, *dd*, 13.4, 4.6) and an olefinic proton at δ_H 5.68 (H-15, *dd*, 8.2, 3.3) characteristic of taraxer-14-ene triterpenes with one oxidated methyl group (**Mahato and Kundu, 1994**). The correlation observed in its HMBC spectrum between the proton at δ_H 2.62 (H-18) and carbonyl group at δ_C 179.1 confirmed the oxidative nature of methyl C-28. In the same spectrum, correlations observed between the proton at δ_H 1.02 (H_3-27) and the carbons at δ_C 40.0 (C-18), 43.4 (C-13), 71.1 (C-12), and 157.3 (C-14) confirmed the taraxastane type skeleton of this compound. The ^{13}C NMR data combined with HSQC spectrum indicated the presence of trisubstituted double bond at δ_C 157.3 (C-14) and 121.2 (C-15), a carbonyl group at δ_C 179.1 (C-28) and two oxymethine signals at δ_C 80.2 (C-3) and 71.1 (C-12). The position C_{14}-C_{15} of double bond was confirmed by the HMBC correlation between the proton at δ_H 5.68 (H-15) and the carbons at δ_C 39.3 (C-8), 43.4 (C-13), 29.3 (C-16) and 50.4 (C-17). The β configuration of proton H-12 and H-18 were confirmed with the ROESY experiment that showed the correlations between H-12 and H-18 on the one hand, H-18 and H_3-26/30 on the other. In addition, the ROESY correlations between H_3-25/H_3-26 β-orientated, H-3/H-5/H-9, and H_3-27/H-16 α-oriented (Figure 3) provide evidence of the triterpenoid ring fusion of A/B *trans*, B/C *trans*, D/E *cis* and 13*S* configuration (Kuroda et al., 2006). In addition to the proton and carbon resonances assigned to the portion of compound **2**, we note the presence of an acetyl group [δ_C 170.6 (CO), 21.5 (CH_3)] on the ^{13}C NMR spectrum (Table 2). Its position was deduced on the basis of the HMBC correlation between oxymethine proton at δ_H 4.38 (H-3) and the carbonyl carbon at δ_C 170.6. The configuration of the acetyl group at C-3 was assigned as β on the basis of the interactions observed between H-3 and CH_3-23/CH_3-25 in the ROESY experiment. Thus, compound **2**, melantheraside B is, 3-*O*-acetyl-12α-hydroxytaraxer-14-en-28-oic acid.

Melantheraside C (**3**), obtained as white powder gave the same result as the previous compounds with Liebermann-Burchard reagent and had a molecular formula $C_{30}H_{47}NO_4$ deduced from a sodium adduct peak at *m/z* 508.3410 $[M+Na]^+$ (calcd. for $C_{30}H_{47}NO_4Na$ 508.3403) on its positive HR-TOFESIMS spectrum. The ^1H and ^{13}C NMR spectra (Table 2) of compound **3,** exhibited signals attributed to the taraxerane triterpenoid with one oxidated methyl (**Mahato and Kundu, 1994**). Its ^1H NMR spectrum exhibited a signal at δ_H: 10.33 (1H, *s*) attributed to a hydroxyl of oxime group in addition to the signal observed in the ^{13}C NMR spectrum at δ_C 162.6 attributed to the quaternary sp^2 carbon atom of this oxime group (Nian et al., 2017). From the same ^{13}C NMR spectrum, the signal at δ_C 77.1 was attributed to C-3; this was comforted by its HMBC correlation with the methyls signals H_3-23 and H_3-24. This carbon C-3 was hydroxylated. In ^1H-^1H COSY spectrum, correlation observed between the proton H-3 at δ_H 3.00 and that at δ_H 4.32 (3-OH) indicated that this hydroxyl group was not involved in any ether bond. The deshielding of carbon C-14 (δ_C 91.1) suggested that, this carbon is linked to a hydroxy group, probably coming from hydroxylation of the olefinic carbons of the $\Delta^{14\text{-}15}$ functionality of taraxerane skeleton. The correlation observed in its HMBC spectrum between the proton at δ_H 2.07 (H-18), 1.32 (H_3-27) and the oxime carbon atom allowed us to locate this oxime group at position C-12. This spectrum also showed the correlation between the oxygenated carbon at δ 91.1 (C-14) involved in the lactone ring and the protons H-9, H-15, H-16 and H_3-27. The correlation between H-21 (α) and H-16 (α); H-22 (α) and H-16 (α); H_3-27 and H-19 (α), observed in ROESY spectrum indicated the *cis*-fusion of D-E ring and consequently the β-orientation of the γ-lactone ring (C-28,14) (figure 4). The same ROESY experiment had confirmed the beta configuration of the proton H-18 and the hydroxy group at C-3. Therefore, compound **3**, melantheraside C, is 12-oxime-3β-hydroxytaraxeran-28,14-olide.

Melantheraside D (**4**) was isolated as a white powder and gave a positive test with Liebermann-Burchard reagent. Its molecular formula $C_{32}H_{49}NO_5$ was deduced from its positive HR-TOFESIMS spectrum, exhibiting a sodium adduct peak at *m/z* 550.3502 $[M+Na]^+$ (calcd. for $C_{32}H_{49}NO_5Na$ 550.3508). A comparison with compound **3** (*m/z* 508.3410) suggested the presence of an additional acetyl group. The ^1H NMR and ^{13}C NMR (Table 2) spectra of compound **4** were almost imposable to those of compound **3**, excepted the supplementary acetyl group [δ_C 170.6 (CO), 21.5 (CH_3)] and the chemical shift of protons H-3, H_3-23 and H_3-24 (Table 2). The deshielding signals of H-3 at δ_H 4.43 (Δ + 1.43 ppm) and C-3 δ_c 80.0 (Δ + 2.9 ppm) suggested the position of esterification. This was readily confirmed by the HMBC experiment in which the oxymethine proton at δ_H 4.43 (H-3α) showed the correlation with the carbonyl at δ_C 170.6. From the above information, compound **4**, melantheraside D, is 12-oxime-3-*O*-acetyltaraxeran-28,14-olide.

Melantheraside E (**5)** obtained as white powder gave the same result as compounds (**1-4**) with Liebermann-Burchard reagent. The positive TOFESIMS spectrum gave a sodium adduct peak at *m/z*: 556.2816 $[M+Na]^+$ (calcd. for $C_{30}H_{44}NO_5ClNa$ 556.2806), indicating a chlorinated triterpenoid with a molecular formula of $C_{30}H_{44}NO_5Cl$. The ^1H and ^{13}C NMR spectra (Table 1) of compound **5** were very similar to those of compound **1** in the high field, except for signals of ring A. This observation confirmed the oleanane-type triterpene of compound **5** (**Mahato and Kundu, 1994**). Signals at δ_H 12.20 and 5.56 on its ^1H NMR spectrum (Table 1) were attributed to acid and amide groups while those at δ_H 4.42 was attributed to chloromethine as in compound **1**. A supplementary signal at δ_H 4.80 (d, *J*=1.7 Hz), showed cross-peak correlation on ^1H-^1H COSY spectrum with the amide proton at δ_H 5.56 suggesting an aminomethine for this proton. Its ^{13}C NMR spectrum showed signals at three carbonyl groups at δ_C 203.5, 178.3 and 156.2 assigned to the carbonyl of ketone, lactone and amide group, respectively. This ^{13}C NMR also showed the signals of an oxygenated quaternary carbon at δ_C 91.4 (C-13), and a chloromethine carbon at δ_C 64.7 (C-12), as in compounds **1** and **6** (Table 1). In addition, signal at δ_C 64.6 was attributed to the aminomethine carbon by its correlation in the HSQC spectrum with the proton at δ_H 4.80. The HMBC correlation between the proton at δ_H 4.80 and the carbonyl at δ_C 156.2, confirmed the existence of the carboxyamino group in the structure of compound **5**. In the HMBC spectrum, the correlation between the methyl protons at δ_H 1.09 (H_3-23) and 0.96 (H_3-24) with the carbon at δ_C 203.5 indicated the position C-3 for the ketone. The extensive analysis of HMBC correlations between proton at δ_H 4.80 (H-2) and carbons C-3 (δ_C 203.5), C-4 (δ_C 47.3), C-5 (δ_C 44.7), C-9 (δ_C 33.6), and C-10 (δ_C 40.3) indicated the 3-oxo-cyclopentanic type of ring A (He et al., 2009). Thus compound **5** is a 1-*nor*oleanane type skeleton and the carboxamino group was attached to position C-2. The *β*-orientation of the carboxamino group was deduced from the ROESY correlations between H-2/H-9, H-5/H-9, H_3-27/H-9 α-oriented. The relative 13*β*-configuration of hydroxy group, and *β*-configuration of protons H-12 and H-18 were determined using the ROESY experiment as in compounds **1-4**. Thus compound **5,** melantheraside E, is 3-oxo-2*β*-carboxyamino-12*α*-chloro,13*β*-hydroxy-1-*nor*oleanan-28,13olide.

The known compounds were identified by comparison of their spectroscopic data with literature values as: oleanolic acid (**7**) (**Mahato and Kundu, 1994**), auriantiamide (**8**) **(Avijit and Rita, 1981)**, auranamide (**9**) (**Catalan et al., 2003**), Didymin (**10**) (**Gattuso et al., 2007),** 3-*O*-*β*-D-glucuronopyranosyl-oleanolic acid (**11**) (**Vidal-Ollivier et al., 1989**), oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**12**) (**Min et al., 2012**), 3-*O*-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**13**) (**Vidal-Ollivier et al., 1989**), 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl oleanolic acid (**14**) (**Paphassarang et al., 1989)**, 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**15**) (**Lee et al., 2013**) and mannitol (**16**) (**Kim et al., 2008**).

In conclusion, compounds **11** and **13** were previously isolated from *Melanthera scandens* (Penders and Delaude, 1994). All saponins are oleanolic acid derivatives. Compounds **1**, **5** and **6** with a chloride are original and rare in the nature, similar compounds were identified in the kiwifruit *Actinidia chinensis* (Xu et al., 2010). To our knowledge, compounds **3** and **4** are the first triterpenoid with an oxime group at C-12 obtained as natural product. Only triterpenoids with an oxime group at C-15 were recently identified in *Cimicifuga frigida* (Nian et al., 2017).

3. Experimental

3.1. General experimental procedures

Melting points were recorded with a Schorpp Gerätetechnik apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 spectrometer equipped with a cryoprobe (^{1}H at 600 MHz and ^{13}C at 150 MHz). 2D NMR experiments were recorded by means of standard Bruker microprograms (Xwin-NMR version 2.1 software TopSpin 3.2). Chemical shifts (δ) are reported in parts per million (ppm) using the residual solvent signals as secondary reference relatively to TMS (δ = 0), while the coupling constants (J values) are given in Hertz (Hz). TOF-ESIMS and HR-TOFESIMS spectra were recorded using a Micromass Q-TOF micro instrument (Manchester, UK) equipped with an electrospray source. The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 mL min^{-1}. Column chromatography was run on Merck silica gel 60 (70–230 mesh) and gel permeation on Sephadex LH-20 while TLC was carried out on silica gel GF254 pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C, or by visual inspection under UV lamp at 254 and 365 nm.

3.2. Plant material

The aerial parts of *Melanthera elliptica* were collected at FOTO Village (Menoua Division, Western region of Cameroon) in January 2014. Authentication was done by Mr. Fulbert Tadjouteu, a Botanist of the Cameroon National Herbarium, Yaoundé, where a voucher specimen (N°8002/HNC) has been deposited.

3.3. Extraction and isolation

The air-dried plant material (4 kg) was powdered and extracted at room temperature with methanol (3 x 20 L, 72 h). The solvent was evaporated under reduced pressure, leaving an extract (220 g). Part of this extract (210 g) was suspended in water (300 mL) and successively extracted with equal volumes (500 mL) of ethyl acetate (EtOAc) and *n*-BuOH yielding respectively 65 g and 30 g fractions after evaporation to dryness.

A part of the *n*-BuOH fraction (25 g) was subjected to silica gel column chromatography using EtOAc-MeOH (10:0→4:6) gradient as eluent. 47 fractions of 250 mL each were collected and combined on the basis of their TLC profiles to give 5 fractions: A (1-7), B (8-12), C (13-15), D (16-47) and E (24-47). Fraction A (3.5 g) was purified on silica gel column chromatography eluted with *n*-hexane-EtOAc (2:8) to give compound **12** (35 mg). Fraction B (3 g) was also purified on silica gel column chromatography eluted with EtOAc to give compound **11** (75 mg). Fraction C (5 g) mainly yielded compounds **13** (30 mg), **14** (30 mg) and **10** (10 mg) after multiple chromatographic steps over sephadex LH-20 using MeOH as eluents and silica gel using EtOAc-MeOH-H_2O (95:5:2) and (9:0,5:0,5) as eluents. Fraction D (6 g) submitted on column chromatography separation over silica gel eluted with EtOAc-MeOH (85:15) yielded three sub-fractions (D_1-D_3). Compound **15** (30 mg) was obtain to fraction D_2 after multiple chromatography separation over silica gel using EtOAc-MeOH-H_2O (8:1:1) and (8:2:1) as eluents while compound **16** (50 mg) was obtain after purification of fraction E on silica gel column chromatography using EtOAc-MeOH-H_2O (8:2:1).

One part of EtOAc fraction (60 g) was subjected to silica gel column chromatography using *n*-Hexane-EtOAc (100:0→0:100) gradient as eluent. 60 fractions of 250 mL each were collected and combined on the basis of their TLC profiles to give 7 fractions: F (1-7), G (8-15), H (16-25), I (26-35), J (36-45), K (46-51) and L (52-60). Fraction G (5g) was purified on column chromatography using *n*-Hexane- EtOAc (95:5) as eluent to give compounds **6** (45 mg) and **2** (15 mg). Compound **1** (30 mg) was obtained after purification of fraction H (4 g) on silica gel column chromatography using the same eluent at the same polarity available to purify the fraction G. Fraction I (8 g) was purified on silica gel column chromatography with *n*-hexane-AcOEt (90-10) to give 2 sub-fractions (I_1-I_2). Sub-fraction I_1 (3 g) yielded compound **7** (60 mg) after filtration. Sub-fraction I2 (4 g) was purified on a silica gel column chromatography using n-Hexane-EtOAc (85:15) as eluent. The 10 mL sub-fractions were collected and pooled on the base of their TLC profiles in two sub-fractions I2-1 (1.2 g) and I2-2 (0.8 g). The sub-fraction I2-1 was recrystallized in ethyl acetate to give compound **4** (25 mg). The subfraction I2-2 was purified over silica gel column chromatography using *n*-Hexane-EtOAc (80:20) as eluent to give compound **3** (15 mg). Compound **5** (20 mg) was also obtained after purification of fraction J (4 g) by column chromatography on silica gel eluted with *n*-Hexane-EtOAc (75:25). The purification of fraction K (4 g) on silica gel column chromatography using *n*-Hexane-EtOAc (60: 40) give compounds **8** (20 mg) and **9** (25 mg).

3.4. New compounds information

Melantheraside A (1): white powder; m.p. = 254.6 °C; $[\alpha]_D^{20}$ +59.3 ($CHCl_3$ *c* 0.55); for ^1H and ^{13}C-NMR data, see Table 1; TOFESIMS (positive ion mode) *m/z*: 513.3120 $[M+Na]^+$ (calcd. for $C_{30}H_{47}O_3ClNa$ 513.3120), 515.3085 $[M+Na+2]^+$.
Melantheraside B (2): white amorphous powder; $[\alpha]_D^{20}$ +4.3 ($CHCl_3$ *c* 0.12); for ^1H and ^{13}C-NMR data, see Table 2; HR-ESIMS (positive ion mode) at *m/z* 519.3450 $[M+Na-H_2O]^+$ (calcd. for $C_{32}H_{48}O_4Na$ 519.3450).
Melantheraside C (3): white powder; m.p. = 257.4 °C; $[\alpha]_D^{20}$ +38.9 ($CHCl_3$ *c* 0.18); ^1H and ^{13}C-NMR data, see Table 2; HR-TOFESIMS (positive ion mode) *m/z*: 508.3410 $[M+Na]^+$ (calcd. for $C_{30}H_{47}NO_4Na$ 508.3403).
Melantheraside D (4): white powder; m.p. = 249.5 °C; $[\alpha]_D^{20}$ +79.2 ($CHCl_3$ *c* 0.36); ^1H and ^{13}C-NMR data, see Table 2; HR-TOFESIMS (positive ion mode) *m/z*: 550.3502 $[M+Na]^+$ (calcd. for $C_{32}H_{49}NO_5Na$ 508.3503).
Melantheraside E (5): white powder; m.p. = 251.2 °C; $[\alpha]_D^{20}$ -6.0 ($CHCl_3$ *c* 0.12); ^1H and ^{13}C-NMR data, see Table 1; TOFESIMS (positive ion mode) *m/z*: 556.2816 $[M+Na]^+$ (calcd. for $C_{30}H_{44}NO_5ClNa$, 556.2806).
Melantheraside F (6): white powder; m.p. = 247.6 °C; $[\alpha]_D^{20}$ +54.9 ($CHCl_3$ *c* 0.92); ^1H and ^{13}C-NMR data, see Table 1; TOFESIMS (positive ion mode) *m/z*: 555.3210 $[M+Na]^+$ (calcd. for $C_{32}H_{49}NO_4ClNa$, 555.3217).

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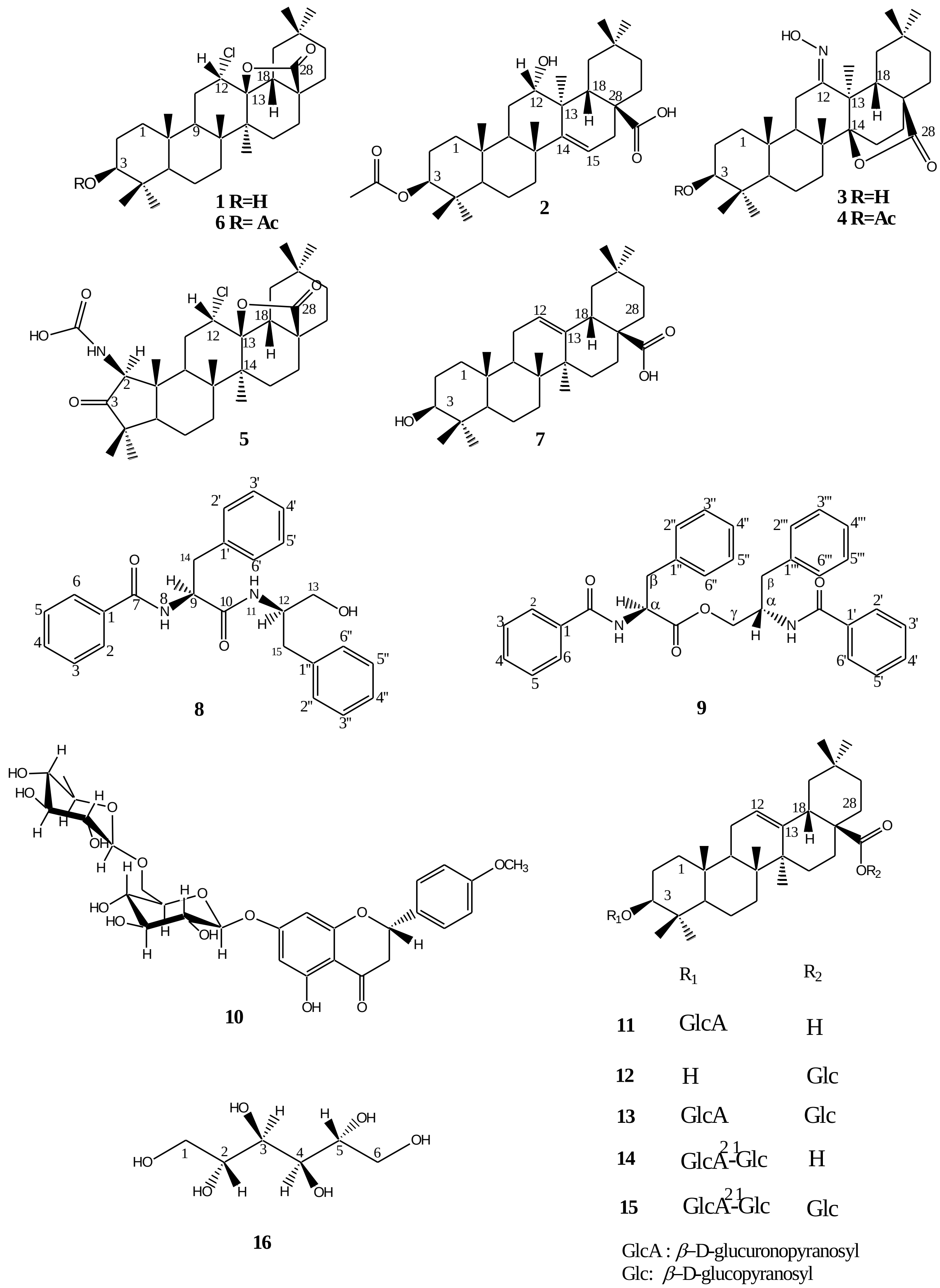

Fig.1 Structures of compounds **1-16** isolated from EtOAc and *n*-BuOH soluble extract of *Melanthera elliptica.*

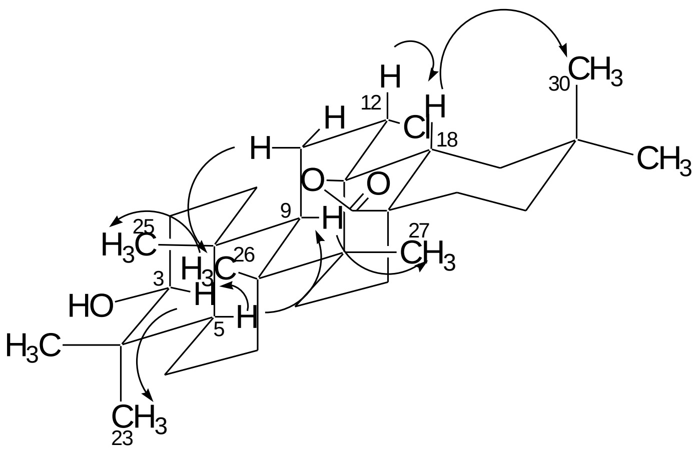

Fig.2 Key ROE correlations of compound **1**

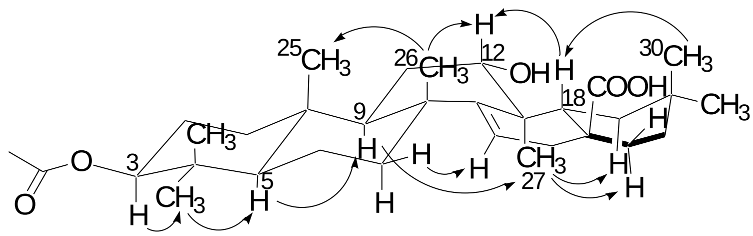

Fig.3 Key ROE correlations of compound **2**

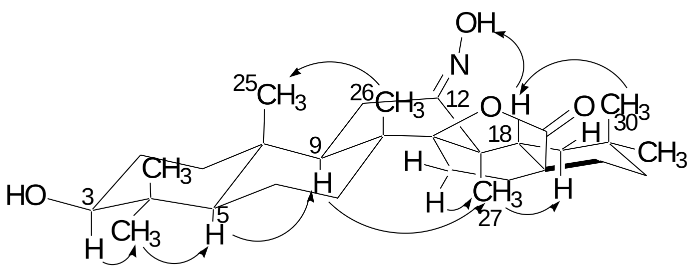

Fig.4 Key ROE correlations of compound **3**

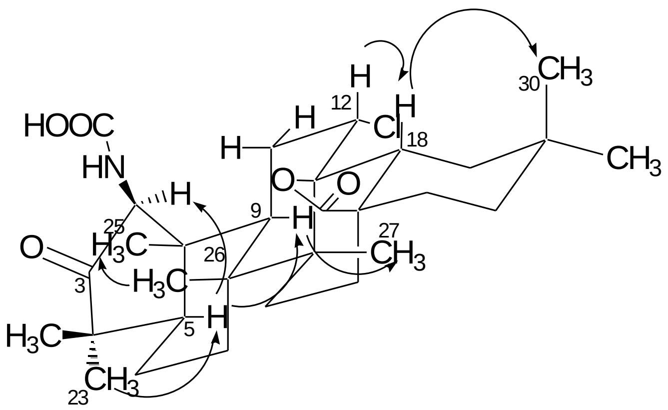

Fig.5 Key ROE correlations of compound **5**

Table 1: ^{1}H RMN (600 MHz) and ^{13}C NMR (150 MHz) data of compounds (**1, 5, 6)** in DMSO-d_6

N°	**1**		**5**		**6**	
	δ_H, m (J Hz)	δ_C	δ_H, m (J Hz)	δ_C	δ_H, m (J Hz)	δ_C
1 α	1.62 dt (12.9, 3.7)	38.7	-	-	1.68 m	38.2
β	0.90 m				0.99 td (13.2, 3.9)	
2 α	1.49 m	27.4	4.80 d (1.7)	64.6	1.57 td (13.1, 3.1)	23.6
β	1.49 m				1.62 dd (12.4, 2.6)	
3	3.03 ddd (8.5, 6.8, 5.2)	77.1	-	203.5	4.42 dd (11.9, 4.8)	80.2
4	-	39.0	-	47.3	-	37.8
5	0.74 dd (11.9, 1.8)	55.2	1.97 dd (11.7, 2.7)	44.7	0.93 dd (12.0, 1.8)	54.8
6 α	1.37 m	17.8	1.54 m	18.6	1.43 m	17.6
β	1.47 m		1.45 m		1.40 m	
7 α	1.54 td (12.7, 3.2)	34.4	1.27 m	33.4	1.57 td (12.8, 3.1)	34.2
β	1.24 dt (12.7, 3.2)		1.52 m		1.24 dt (12.8, 3.1)	
8	-	42.4	-	42.1	-	42.4
9	1.65 dd (12.5, 2.2)	44.8	2.72 dd (12.6, 1.5)	33.6	1.71 d (13.1)	44.7
10	-	36.5	-	40.3	-	36.4
11α	1.68 ddd (14.8, 12.5, 3.8)	29.4	1.71 dt (14.7, 1.5)	29.4	1.68 m	29.4
β	2.10 dt (14.8, 2.5)		2.14 m		2.12 m	
12β	4.41 dd (3.8, 2.5)	65.3	4.42 t (2.9)	64.7	4.41 brs	65.2
13	-	91.2	-	91.4	-	91.3
14	-	43.0	-	43.3	-	43.0
15 α	1.17 m	28.9	1.78 dt (13.5, 6.0)	28.8	1.18 m	28.9
β	1.77 td (13.3, 6.1)		1.23 m		1.76 td (13.7, 5.8)	
16 α	1.15 m	21.2	1.17 m	21.2	1.13 dd (13.5, 5.8)	21.1
β	2.21 td (13.3, 5.7)		2.23 td (13.4, 5.7)		2.21 td (13.5, 5.8)	
17	-	45.1	-	45.1	-	45.1
18 β	2.14 dd (12.6, 3.9)	50.8	2.15 dd (12.7, 3.9)	50.9	2.12 m	50.8
19 α	2.01 m	39.6	2.08 t (12.7)	39.4	2.05 m	39.4
β	2.05 t (12.6)		2.05 m		2.01 t (12.5)	
20	-	32.0	-	32.0	-	32.0
21 α	1.19 m	33.8	1.38 d (13.4, 5.4)	33.8	1.19 m	33.8
β	1.37 m		1.18 m		1.37 m	
22 α	1.48 m	27.6	1.49 m	27.6	1.42 m	27.5
β	1.53 td (13.7, 3.8)		1.52 m		1.50 td (13.5, 3.8)	
23	0.91 s	28.5	1.09 s	26.7	0.82 s	28.0
24	0.68 s	16.3	0.96 s	22.5	0.81 s	16.8
25	0.83 s	16.9	0.81 s	15.3	0.87 s	16.9
26	1.11 s	19.0	1.17 s	18.9	1.11 s	19.0
27	1.34 s	20.2	1.38 s	19.9	1.35 s	20.2
28	-	178.3	-	178.3	-	178.4
29	0.96 s	33.4	0.97 s	33.4	0.96 s	33.4
30	0.87 s	24.1	0.88 s	24.1	0.87 s	24.1
Ac					-	170.7
					2.00 s	21.5
C=O				156.2		
OH	4.32 d (5.2)					
NH			5.55 d (3.5)			
COOH			12.19 s			

Table 2: [1]H RMN (600 MHz) and ^{13}C NMR (150 MHz) data of compounds (**2-4**) in DMSO-d_6

N°	**2**		**3**		**4**	
	δ_H, m (J Hz)	δ_C	δ_H, m (J Hz)	δ_C	δ_H, m (J Hz)	δ_C
1 α	1.45 td (13.2, 3.3)	37.0	1.56 m	38.5	1.61 m	38.3
β	1.04 dd (12.5, 3.8)		0.86 dd (12.9, 3.8)		0.99 dd (12.9, 3.8)	
2 α	1.52 m	23.5	1.49 m	27.4	1.58 m	23.7
β	1.57 m		1.53 m		1.63 m	
3	4.38 dd (11.6, 4.7)	80.2	3.00 dt (10.6, 5.4)	77.1	4.43 dd (11.7, 4.7)	80.0
4	-	37.7	-	38.9	-	37.9
5	0.91 m	54.9	0.82 d (11.6)	55.4	1.06 dd (11.6, 3.5)	54.7
6 α	1.51 m	18.6	1.58 m	19.9	1.57 m	19.4
β	1.41 td (12.9, 3.4)		1.58 m		1.57 m	
7 α	1.96 dt (13.1, 3.1)	40.9.	1.34 m	36.4	1.41 td (12.1, 4.5)	37.2
β	1.18 td (13.2, 3.3)		1.57 m		1.64 m	
8	-	39.3	-	43.5	-	43.3
9	1.74 dd (10.1, 9.8)	50.2	1.59 m	51.8	1.80 dd (13.9, 1.9)	48.8
10	-	37.6	-	37.5	-	37.7
11α	2.07 m	30.9	2.20 d (14.7)	27.4	1.91 dd (16.2, 13.9)	19.9
β	1.83 dt (14.0, 9.6)		2.23 d (14.7)		2.85 dd (16.2, 1.9)	
12β	4.65 dd (10.2, 8.5)	71.1	-	162.6	-	165.5
13	-	43.4	-	47.8	-	46.3
14	-	157.3	-	91.1	-	90.6
15 α	5.68 dd (8.2, 3.3)	121.2	1.90 m	20.9	1.99 td (14.5, 2.9)	23.1
β			1.73 td (14.6, 6.3)		1.67 m	
16 α	2.08 m	29.3	1.11 m	20.3	1.12 m	21.0
β	1.99 dd (13.1, 3.1)		2.31 td (12.6, 5.4)		2.28 ddd (13.4, 10.5, 6.4)	
17	-	50.4	-	39.2	-	39.2
18 β	2.62 dd (13.4, 4.6)	40.0	2.07 dm (12.1)	41.3	2.45 dd (13.0, 4.0)	41.5
19 α	1.53 dd (13.4, 3.6)	37.1	1.90 t (12.1)	35.4	1.51 t (13.0)	36.5
β	1.18 t (13.4)		1.38 d (12.1)		1.32 dd (13.0, 4.0)	
20	-	30.9	-	31.4	-	30.9
21 α	1.10 m	33.7	1.20 m	33.4	1.17 m	33.4
β	1.08 m		1.17 m		1.21 td (12.9, 3.9)	
22 α	1.59 ddd (12.6, 8.1, 4.1)	31.5	1.29 dt (14.4, 3.3)	27.6	1.27 dt (14.3, 3.6)	27.3
β	1.42 m		1.74 td (13.9, 5.4)		1.82 td (13.9, 5.8)	
23	0.79 s	28.0	0.85 s	28.2	0.79 s	27.8
24	0.81 s	16.8	0.69 s	15.8	0.83 s	16.6
25	0.89 s	15.7	0.85 s	17.7	0.92 s	17.4
26	0.87 s	27.1	1.09 s	19.1	1.02 s	18.5
27	1.02 s	19.1	1.32 s	18.0	1.37 s	23.6
28	-	179.1	-	177.9	-	177.8
29	0.91 s	32.3	0.94 s	33.8	0.96 s	33.6
30	0.91 s	26.6	0.78 s	23.8	0.83 s	24.3
OH			4.32 d (5.4)			
NOH					10.51 s	
Ac	-	170.6			-	170.6
	2.00 s	21.5			2.01 s	21.5