STUDY ON ISOLATION AND STRUCTURE ELUCIDATION OF OLEANANE-TYPE TRITERPENOIDS FROM *AGEMONE MEXICANA* L.

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ABSTRACT

Phytochemical investigation of the dichloromethane-soluble fraction of the non-alkaloid extract of *A. mexicana* afforded four known oleanane-type triterpenoids, namely macrocapoic acid B (**1**), anaphalisoleanenoic acid (**2**), 20-epi-katonic acid (**3**), and 3*α*,22*α*-dihydroxyolean-12-en-30-oic acid methyl ester (**4**). Their structures were elucidated by 1D and 2D NMR spectroscopic analyses and comparison with reported data. This is the first report of compounds **1**-**4** from *A. mexicana*.

Keywords: *Argemone mexicana*, *acidic-base extraction, triterpenoid, oleanane skeleton.*

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1. INTRODUCTION

Argemone mexicana Linn., belonging to the family Papaveraceae, is an indigenous plant of Mexico and the West Indies, but has become a widespread exotic weed in many tropical and subtropical countries around the world. In Vietnam, this is the only species of the *Argemone* genus found growing wild, and it is distributed mainly in the northeastern regions of the country [1]. *A. mexicana* is regarded as an important plant in traditional medicine. In India and several other countries, different parts of the plant have traditionally been used to treat edema, ophthalmia, and various chronic skin diseases. Its seeds and seed oil are ingredients in remedies for intestinal disorders, while the flowers and leaves have been used in the treatment of coughs and for maintaining cholesterol levels. Because of these diverse ethnomedicinal uses, *A. mexicana* has attracted considerable scientific interest for its pharmacological potential [2].

Figure 1. *Argemone mexicana* Linn. (Papaveraceae)

Phytochemical investigations of *A. mexicana* have been conducted since 1902, and to date, nearly 100 compounds have been isolated. Among them, more than half are alkaloids, while others include flavonoids, long-chain fatty acids, steroids, triterpenes, glycosides, and other phenolic compounds. Notably, only one oleanane-type triterpenoid, *β*-amyrin, has been reported from this species [2].

The crude methanolic and ethanolic extracts, as well as isolated alkaloids from *A. mexicana,* have been reported to exhibit various biological activities as anticancer, antibacterial, and antifungal properties [3-5]. However, in Vietnam, despite its potential bioactivities, research on this species remains limited in both phytochemical and biological aspects. Recent

publications on *A. mexicana* in Vietnam have mainly focused on evaluating the biological activities of crude extracts, particularly their acetylcholinesterase inhibitory and antioxidant effects [6, 7].

In this study, four triterpenoids namely macrocapoic acid B (**1**), anaphalisoleanenoic acid (**2**), 20-*epi*-katonic acid (**3**), and 3*α*,22*α*-dihydroxyolean-12-en-30-oic acid methyl ester (**4**) were isolated from the dichloromethane-soluble fraction of the non-alkaloid extract of *A. mexicana*. To the best of our knowledge, this is the first report on the isolation of these oleanane-type triterpenoids from *A. mexicana*.

2. EXPERIMENTAL

2.1. General experimental procedures

One- and two-dimensional nuclear magnetic resonance (1D and 2D NMR) spectra were recorded on a Bruker Avance 600 MHz spectrometer (Bruker, Germany). Tetramethylsilane (TMS) served as an internal standard for referencing the chemical shifts (δ, ppm). Column chromatography (CC) was carried out on silica gel 60 (230 - 400 mesh, Merck KGaA, Darmstadt, Germany), reversed-phase silica gel RP-C18 (100 mesh, YMC Co., Ltd., Japan), and Sephadex LH-20 (25 - 100µm, Sigma-Aldrich, St. Louis, MO, USA). Thin-layer chromatography (TLC) were performed on silica gel 60 F_{254} and RP-18 $F_{254}S$ plates (0.25mm, Merck KGaA, Darmstadt, Germany). Chromatographic spots were visualized under UV light (254nm) and by spraying with a vanillin-H_2SO_4 reagent in methanol, followed by heating.

2.2. Plant material

The plant material was collected in Thai Binh Province, Vietnam, in January 2024. It was identified as *Argemone mexicana* Linn. (Papaveraceae family) by botanist Prof. Tran Huy Thai from Vietnam Academy of Science and Technology (VAST). Voucher specimen (HUST.AM.06) has been stored at the laboratory of the Organic Chemistry Group, School of Chemistry and Life sciences, Hanoi University of Science and Technology (HUST), Vietnam.

2.3. Extraction and isolation

The aerial parts of *A. mexicana* (1kg) were powdered and repeatedly extracted with MeOH 80% (5 × 5L) at 40°C for 1h under sonication. The slovent was then removed under reduced pressure to yield the crude extract (AM-M). This extract was suspended in MeOH/H_2O (1:2, v/v, 500mL) and stirred with 400mL of 2N aqueous hydrochloric acid for 3h. The pH of the solution was adjusted to 3 - 4 to ensure that all alkaloids were converted into their salt forms. The acidic solution was successively partitioned with dichloromethane (3 × 500mL) to obtain a non-alkaloid fraction (AM-D, 26g) after solvent removal. The remaining aqueous layer was neutralized with 200mL of 25% ammonia (pH ~ 8 - 9) and then thoroughly extracted with dichloromethane (3 × 500mL) to afford the total alkaloid fraction (AM-A, 2g) after solvent evaporation under vacuum.

The dichloromethane-soluble fraction of the non-alkaloid extract (26g, AM-D) was subjected to silica gel column chromatography, eluted with dichloromethane/ethyl acetate (from 100:0 to 1:1, v/v). The chromatographic process was monitored by TLC analysis, affording six fractions (AM1-AM6). Fraction AM1 (7.33g) was further separated on a silica gel column with the elution of *n*-hexane/acetone (from 20:1 to 10:1, 5:1, and 2.5:1, v/v) to yield six subfractions (AM11-AM16). Fraction AM12 (0.34g) was further chromatographed on a Sephadex LH-20 column eluted with methanol, followed by RP-C18 silica gel column chromatography eluted with water/methanol (1:5, v/v), and finally purified on a silica gel column eluted with *n*-hexane/ethyl acetate (5:1, v/v) to afford compound **4** (35mg). Fraction AM14 (0.58g) was fractionated on a RP-C18 silica gel column eluted with water/acetone (1:4, v/v) to obtain two subfractions (AM141 and AM142). Compound **2** (52.8mg) was recrystallized from fraction AM141 using acetone. Fraction AM142 was purified by silica gel column chromatography eluted with *n*-hexane/EtOAc (5:1, v/v) to give compound **3** (10.1mg). Fraction AM16 (0.15g) was separated on a RP-C18 silica gel column eluted with water/methanol/ethyl acetate (1:1:1, v/v/v) and subsequently purified by silica gel column chromatography eluted with *n*-hexane/dichloromethane/ethyl acetate (2:2:0.1, v/v/v) to yield compound **1** (23.4mg).

Macrocapoic acid B (**1**): colorless crystal; ^{1}H NMR (600MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data are shown in Tables 1 and 2.

Anaphalisoleanenoic acid (**2**): colorless crystal; ^{1}H NMR (600MHz, $CDCl_3$) and ^{13}C NMR (150MHz, $CDCl_3$) data are shown in Tables 1 and 2.

20-epi-katonic acid (**3**): colorless crystal; ^{1}H NMR (600MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data are shown in Tables 1 and 2.

3α,22α-dihydroxyolean-12-en-30-oic acid methyl ester (**4**): colorless crystal; ^{1}H NMR (600MHz, CD_3OD) and ^{13}C NMR (150MHz, CD_3OD) data are shown in Tables 1 and 2.

3. RESULTS AND DISCUSSION

Structural elucidation of isolated compounds from *A. mexicana*

Compound **1** was obtained as a colorless crystals. Its molecular fomula, $C_{30}H_{46}O_4$, was confirmed by 1D and 2D NMR analyses and by comparison with literature data [9].

The ^{13}C NMR and DEPT spectra (Table 2) revealed 30 carbon signals corresponding to seven methyl groups, nine methylene groups, five methine groups, and nine quaternary carbons. Among these, one carbonyl carbon signal appeared at δ_C 217.7 (C-3), a carboxylic carbon signal at δ_C 181.4 (C-30), two olefinic carbons at δ_C 143.0 (C-13) and 123.2 (C-12), and an oxygenated methine carbon at δ_C 76.5 (C-22). The seven methyl carbons resonated at δ_C 28.6 (C-29), 26.5 (C-23), 26.1 (C-27), 24.5 (C-28), 21.5 (C-24), 16.7 (C-26), and 15.2 (C-25). The remaining aliphatic carbons appeared in the high-field region from δ_C 55.4 to 19.5ppm.

The ^1H NMR spectrum of **1** (Table 1) indicated a triplet signal for an olefinic proton at δ_H 5.34 (1H, t, J = 3.6Hz, H-12) and a doublet of doublets for an oxygenated methine proton at δ_H 3.50 (1H, dd, J = 12.6, 4.2Hz, H-22). In addition, seven singlet methyl protons appeared at δ_H 1.25 (s, H_3-29), 1.17 (s, H_3-27), 1.10 (s, H_3-23), 1.08 (s, H_3-25), 1.06 (s, H_3-24), 1.03 (s, H_3-26), 0.98 (s, H_3-28), together with signals of methylene and methine protons in the upfield region from δ_H 2.58 to 1.08ppm. The ^1H and ^{13}C NMR data of compound **1** suggested that it possesses an oleanane-type triterpenoid skeleton. Further evidence for this structure was provided by the ^1H-^1H COSY and HMBC correlations.

The oleanane-type framework of compound **1** was elucidated based on the HMBC spectrum (Figure 3), which indicated HMBC correlations from H_3-29 (δ_H 1.25) to C-19 (δ_C 41.9), C-20 (δ_C 43.5), and C-21 (δ_C 38.8); from H_3-27 (δ_H 1.17) to C-13 (δ_C 143.0), C-8 (δ_C 39.9), C-14 (δ_C 42.3), and C-15 (δ_C 25.6); from H_3-23 (δ_H 1.10) to C-4 (δ_C 47.5) and C-5 (δ_C 55.4); from H_3-25 (δ_H 1.08) to C-1 (δ_C 39.3), C-5 (δ_C 55.4), C-9 (δ_C 46.9), and C-10 (δ_C 36.7); from H_3-26 (δ_H 1.03) to C-7 (δ_C 32.1), C-8 (δ_C 39.9), C-9 (δ_C 46.9), and C-14 (δ_C 42.3); and from H_3-28 (δ_H 0.98) to C-16 (δ_C 19.5), C-17 (δ_C 37.5), and C-18 (δ_C 47.9). The HMBC correlations from H_2-1 (δ_H 1.89, 1.41), H_2-2 (δ_H 2.58, 2.39), H_3-23 (δ_H 1.10), and H_3-24 (δ_H 1.06) to C-3 (δ_C 217.7) confirmed the presence of a ketone group at C-3. Meanwhile, the HMBC cross-peaks from H_2-21 (δ_H 2.17 and 1.40) and H_3-28 (δ_H 0.98) to C-22 (δ_C 76.5) revealed the position of a hydroxyl group at C-22. The large coupling constant of H-22 ($^3J_{axial/axial}$ = 12.6Hz) suggested an *α*-orientation for this hydroxyl group. Based on the analysis of the NMR data and comparison with those of macrocarpoic acid B, compound **1** (Figure 2) was identified as 22*α*-hydroxyolean-12-en-3-oxo-30-oic acid ($C_{30}H_{46}O_4$), commonly known as macrocarpoic acid B. This compound was first isolated from the bark of *Maytenus macrocarpa* (Celastraceae) and exhibits potential anti-HIV activity with an EC_{50} value of 10μg/mL [9].

Compound **2** was obtained as colorless crystal. Comparison of its NMR spectra with those of compound **1** (Tables 1 and 2), revealed a similar oleanane skeleton with 30 carbon atoms, bearing an olefinic bond and a carboxylic group. In particular, the ^1H NMR spectrum of **2** displayed characteristic features of an oleanane-type triterpene, indicating aliphatic proton signals from methyl, methylene, and methine groups, as well as an olefinic proton appearing in the downfield region. Moreover, the proton signal at δ_H 3.23 (1H, dd, J = 11.4, 4.8Hz, H-3) suggested the presence of an oxygenated methine group in the structure of **2**. Consistently, the ^{13}C NMR spectrum of **2** showed carbon signals typical of an oleanane skeleton, with 30 carbons similar to those of compound **1**. The signal at δ_C 71.9 indicated the presence of an oxygenated methine carbon. However, the ^{13}C NMR data of **2** revealed the disappearance of the ketone carbon signal observed in compound **1**, as evidenced by the absence of a characteristic carbonyl resonance in the downfield region. Analysis of the ^1H and ^{13}C NMR spectra, together with comparison to compound **1**, indicated that compound **2** is an oleanane-type triterpenoid containing an olefinic bond, a carboxylic group, and a hydroxyl group. The position of the hydroxyl group was confirmed by HMBC and COSY correlations. In the HMBC spectrum of **2** (Figure 3) cross peaks from the singlet methyl protons H_3-23 (δ_H 1.00) and H_3-24 (δ_H 0.79) to C-3 (δ_C 79.1), together with the spin system H_2-2 (δ_H 1.60)/H-3 (δ_H 3.23) observed in the COSY spectrum, confirmed that the hydroxyl group is attached to C-3. Detailed analysis of the oxygenated methine proton at δ_H 3.23, which appeared as a doublet of doublets with coupling constants of $^3J_{axial/axial}$ = 11.4Hz and $^3J_{axial/equatorial}$ = 4.8Hz, indicated axial-axial and axial-equatorial interactions between H-3 and the two non-equivalent H_2-2 protons. These data demonstrated the *β*-orientation of the hydroxyl group at C-3. Based on above NMR spectral data, the structure of compound **2** (Figure 2) was identified as 3*β*-hydroxyolean-12-en-30-oic acid ($C_{30}H_{48}O_3$), commonly

known as anaphalisoleanenoic acid. This compound was originally isolated from the roots of *Anaphalis araneosa* [10].

Table 1. ^{1}H NMR data (δ in ppm, J in Hz, 600MHz) of compounds **1-4**

C position	1[a]	2[a]	3[b]	4[b]
1	1.89m 1.41m	1.62m 0.98m	1.36m	1.36m
2	2.58 ddd (18.6, 11.4, 7.2) 2.39 ddd (10.2, 6.6, 3.6)	1.60m	2.00m 1.55m	1.99m 1.54m
3	-	3.23 dd (4.8, 11.4)	3.33m	3.34m
4	-	-	-	-
5	1.33m	0.75m	1.33m	1.35m
6	1.54m	1.54m 1.41m	1.48m	1.48m
7	1.56m 1.41m	1.52m 1.32m	1.63m 1.36m	1.61m 1.36m
8	-	-	-	-
9	1.65m	1.57m	1.79m	1.81m
10	-	-		-
11	1.99m 1.93m	1.88 m	1.94m	1.95m
12	5.33 t (3.6)	5.29m	5.29 brt (3.0)	5.28 t (3.6)
13	-	-	-	-
14	-	-	-	-
15	1.72m 1.08m	1.80m 0.98m	1.85m 1.06m	1.74m 1.08m
16	1.68m 1.37m	1.96m 0.90m	2.07 td (13.2, 4.2) 0.92m	1.73m 1.36m
17	-	-	-	-
18	1.98m	1.98m	2.00m	1.87m
19	1.86m 1.72m	1.84m 1.64m	1.84m 1.69m	1.83m 1.77m
20	-	-	-	-
21	2.17m 1.41m	1.94m 1.36m	1.91m 1.36m	2.12m 1.44m
22	3.50 dd (12.0, 3.6)	1.36m	1.36m	3.28m
23	1.10s	1.00s	0.96s	0.96s
24	1.06s	0.79s	0.87s	0.87s
25	1.07s	0.94s	1.00s	1.00s
26	1.02s	0.96s	1.03s	1.02s
27	1.17s	1.14s	1.22s	1.23s
28	0.97s	0.81s	0.82s	0.92s
29	1.24s	1.20s	1.16s	1.17s
30	-	-	-	-
30-O$\underline{C}H_3$				3.70s

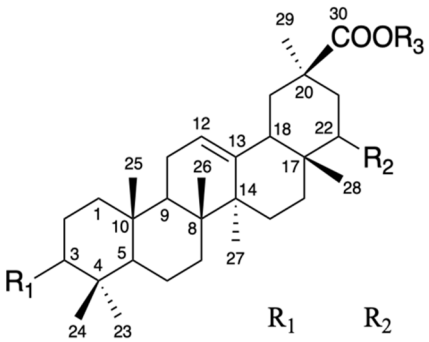
a: in $CDCl_3$, b: in CD_3OD

Table 2. ^{13}C NMR data for compounds **1-4** (δ in ppm, 150MHz)

C position	1[a]	2[a]	3[b]	4[b]
1	39.3	38.6	34.3	34.3
2	34.2	27.3	26.2	26.2
3	217.7	79.1	76.9	76.9
4	47.5	38.8	38.3	38.3
5	55.4	55.2	50.0	50.0
6	19.6	18.4	19.4	19.3
7	32.1	32.7	33.8	33.7
8	39.9	39.8	41.2	41.3
9	46.9	47.7	48.5	48.6
10	36.7	37.0	38.1	38.1
11	23.6	23.5	24.5	24.6
12	123.2	122.8	123.9	124.4
13	143.0	144.3	145.9	144.8
14	42.3	41.6	42.8	43.5
15	25.6	26.2	27.3	26.7
16	19.5	27.0	28.1	20.7
17	37.5	32.0	33.0	38.7
18	47.9	48.1	49.8	49.5
19	41.9	42.7	44.2	43.4
20	43.5	44.0	45.1	45.0
21	38.8	31.2	32.3	39.8
22	76.5	38.3	39.7	77.3
23	26.5	28.2	29.0	29.0
24	21.5	15.6	22.9	22.9
25	15.2	15.5	15.9	15.9
26	16.7	16.8	17.5	17.4
27	26.1	26.0	26.6	26.8
28	24.5	28.1	28.9	25.3
29	28.6	28.6	29.2	28.8
30	181.4	180.4	181.2	179.0
30-O$\underline{C}H_3$	-	-	-	52.3

a: in $CDCl_3$, b: in CD_3OD

Compound **3** was obtained as colorless crystal. The ^{1}H and ^{13}C NMR spectra of **3** closely resembled those of compound **2** (Tables 1 and 2), sharing the same molecular formula $C_{30}H_{48}O_3$, which indicated that it also possesses an oleanane-type triterpenoid skeleton. In particular, the spectral data suggested that compound **3** is 3-hydroxyolean-13-en-30-oic acid, as most of its chemical shifts were comparable to those of compound **2**, except for the carbon signals associated with the A-ring of the oleanane skeleton. Detailed analysis of the NMR data of **3** revealed distinct differences in the chemical shifts of C-3 and C-5 compared with compound **2** (Table 2), with both signals shifted upfield by approximately $\Delta\delta_C \sim 2$ and 5ppm, respectively. These observations suggested that the 3-hydroxyl group in 3 has an *α*-configuration [11]. Further confirmation was obtained from the NOE spectrum (Figure 3), which displayed the correlations between H-3 (δ_H 3.33) and H_3-23 (δ_H 0.96) as well as H_3-24 (δ_H 0.87), but no cross peak between H-3 (δ_H 3.33) and H-5 (δ_H 1.33), indicating the *β*-orientation of H-3. Moreover, the absence of a NOE cross peak between H-18 (δ_H 2.00) and H_3-30 provided evidence that the carboxyl group (–COOH) is located at C-30. Based on comprehensive NMR spectral analysis and comparison with literature data [11], the structure of compound **3** (Figure 2) was identified as 3*α*-hydroxyolean-12-en-30-oic acid ($C_{30}H_{48}O_3$), commonly known as 20-*epi*-katonic acid. This compound has previously been isolated from *Bocconia arborea* (Papaveraceae) [11].



	R_1	R_2	R_3
1	O	α-OH	H
2	β-OH	H	H
3	α-OH	H	H
4	α-OH	α-OH	CH_3

Figure 2. Structures of triterpenoids **1-4** isolated from *A. mexican*

Compound **4** was obtained as colorless crystal. The ^{1}H and ^{13}C NMR spectral data of **4** showed a close resemblance to those of compound **3** (Tables 1 and 2), indicating that it also possesses an oleanane-type triterpenoid skeleton. However, compound **4** contains two hydroxyl groups, one more than compound **3**, as evidenced by proton signals at δ_H 3.34 (1H, m, H3) and 3.28 (1H, m, H-22), together with oxygenated methine carbons at δ_C 76.9 (C-3) and 77.3 (C-22). In addition, the occurrence of an oxymethyl group, with proton and carbon signals at δ_H/δ_C 3.70 (3H, s)/52.3 (OCH_3), suggested methyl esterification of the carboxylic acid group in the structure of **4**. Analysis of the ^{1}H and ^{13}C NMR spectral data indicated that **4** is an oleanane-type triterpene bearing two hydroxyl groups and one ester functional group. Furthermore, the structure of **4** was confirmed by HMBC and ^{1}H-^{1}H COSY spectra (Figure 3). The HMBC correlations between H_3-28 (δ_H 0.92) and C-22 (δ_C 77.3), together with the spin system H-22 (δ_H 3.28)/H_2-21 (δ_H 2.12 and 1.44) observed in the COSY spectrum, established the attachment of the hydroxyl group at C-22. Meanwhile, the HMBC correlations from H_3-23 (δ_H 0.96), H_3-24 (δ_H 0.87) to C-3 confirmed the hydroxyl substitution at C-3. The position of the methyl ester group at C-30 was identified *via* the HMBC correlation between H_3-29 (δ_H 1.17) and C-30 (δ_C 179.0), as well as between the oxymethyl proton (δ_H 3.70) and C-30 (δ_C 179.0). The relative configuration of **4** was established from the NOE spectrum (Figure 3). In particular, the NOE correlations from H_3-28 (δ_H 0.92) to H-22 (δ_H 3.28), from H_3-28 (δ_H 0.92) to H-18 (δ_H 1.87), and from H-12 (δ_H 5.29) to H-18 (δ_H 1.87) indicated the *α*-configuration of the hydorxyl group at C-22. Additionally, NOE correlations between H-3 (δ_H 3.34) and H_3-23 (δ_H 0.96), H_3-24 (δ_H 0.87), together with the absence of NOE correlation from H-3 to H-5, confirmed the *β*-orientation of H-3, corresponding to the *α*-orientation of the hydroxyl group at C-3. Comparison of the spectroscopic data of **4** with those of compounds **1** and **3** (Tables 1 and 2) established the presence of *α*-oriented hydroxyl groups at both C-3 and C-22. Based on NMR spectral analysis and comparison with the reported data for 3*α*,22*α*-dihydroxyolean-12-en-30-oic acid, the structure of compound **4** (Figure 2) was identified as the methyl ester derivative of this compound, namely 3*α*,22*α*-dihydroxyolean-12-en-30-oic acid methyl ester ($C_{31}H_{50}O_4$) [12].

A. mexicana is known to be rich in alkaloids. To date, only one oleanane-type triterpenoid, *β*-amyrin, has been reported from this species [2]. In this study, four additional known oleanane-type triterpenoids (**1**-**4**) are described for the first time from *A. mexicana*.

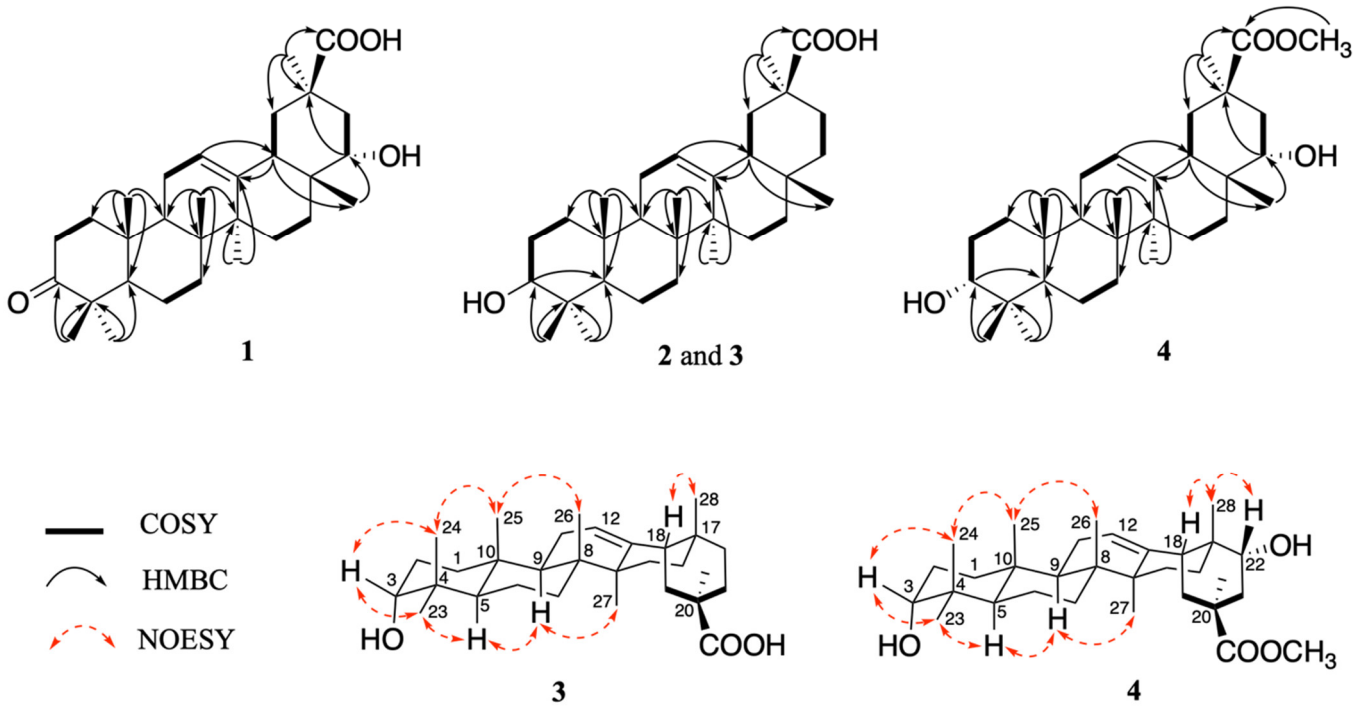


Figure 3. ^{1}H-^{1}H COSY, key HMBC, and NOESY correlations of compounds **1**-**4** from *A. mexicana*

4. CONCLUSIONS

In conclusion, four known oleanane-type triterpenoids, including macrocarpoic acid B (**1**), anaphalisoleanenoic acid (**2**), 20-*epi*-katonic acid (**3**), and 3*α*,22*α*-dihydroxyolean-12-en-30-oic acid methyl ester (**4**), were isolated from the AM-D fraction of *A. mexicana*. This study represents the first report on the isolation of these oleanane-type triterpenoids from *A. mexicana*. These findings expand the chemical diversity known for this species and highlight its potential as a promising source of bioactive triterpenoid compounds for further pharmacological investigation.

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