Article

Chemical Constituents from *Aspidosperma illustre* (Apocynaceae)

*Lara F. Barbosa, Leda Mathias, Raimundo Braz-Filho and Ivo J. Curcino Vieira**

Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense Darcy Ribeiro,
28013-602 Campos dos Goytacazes-RJ, Brazil

A partir de *Aspidosperma illustre*, foi obtido como produto natural um novo triterpeno da série oleanano, o olean-12-en-11α-metóxi-3β-acetato (**10**), além dos triterpenos β-amyrina (**3**), lupeol (**4**), acetato de β-amyrina (**5**), acetato de lupeol (**6**), olean-12-en-28-hidróxi-3β-tetradecanoato (**7**), olean-12-en-28-carboxi-3β-hexadecanoato (**8**), ácido ursólico (**9**) e dois alcalóides indólicos monoterpênicos, β-ioimbina (**1**) e 1,2-desidroaspidospermidina (**2**). As substâncias isoladas foram identificadas através de métodos espectroscópicos, principalmente uni (RMN ^{1}H, ^{13}C, APT) e bidimensionais (^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, HMQC e HMBC) e de massas, envolvendo também comparação com dados de literatura.

A new natural product oleanane-type triterpene, olean-12-ene-11α-methoxy-3β-acetate (**10**) was isolated from *Aspidosperma illustre*, together with β-amyrin (**3**), lupeol (**4**), β-amyrin acetate (**5**), lupeol acetate (**6**), olean-12-ene-28-hydroxy-3β-tetradecanoate (**7**), olean-12-ene-28-carboxy-3β-hexadecanoate (**8**), ursolic acid (**9**) triterpenes, and two monoterpenic indole alkaloids, β-yoimbine (**1**) and 1,2-dehydroaspidospermidine (**2**). These compounds were characterized on their spectral data basis, mainly one- (^{1}H, ^{13}C, APT) and two-dimensional (^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, HMQC and HMBC) NMR, and mass spectra, involving also comparison with data from the literature.

Keywords: *Aspidosperma illustre,* Apocynaceae, oleanane triterpenes, alkaloids

Introduction

The *Aspidosperma* (Apocynaceae) genus is endemic to Americas and is found mainly in regions between Mexico and Argentina.[1] *Aspidosperma* genus continues to be fascinating as an expressive source of indole alkaloids with novel skeletons,[2] which are interesting from a biosynthetic perspective and reported biological properties.[2,3] Species of the *Aspidosperma* genus are applied broadly by popular medicine as potential antimalarial agents, leishmaniasis treatment, uterus and ovary inflammation, as contraceptive, in diabetes, in stomach problems, against cancer, fever and rheumatism.[3]

Aspidosperma illustre, commonly known as "Tambu-Pequiá" in Atlantic forests in the North of Espírito Santo State, appears as a tree of 5-20 m. This species is not reported on studies of chemical composition described in the literature.

In the present paper, we describe the isolation and characterization of a novel oleanane-type triterpene,

olean-12-ene-11α-methoxy-3β-acetate (**10**), along with seven known triterpenes, β-amyrin (**3**), lupeol (**4**), β-amyrin acetate (**5**), lupeol acetate (**6**), olean-12-ene-28-hydroxy-3β-tetradecanoate (**7**), olean-12-ene-28-carboxy-3β-hexadecanoate (**8**), ursolic acid (**9**) and two known monoterpenic indole alkaloids, β-yoimbine (**1**) and 1,2-dehidroaspidospermidine (**2**). The structures of known and new compound are being mentioned for the first time in this species and were established on the basis of spectral data, mainly ^{1}H and ^{13}C (1D and 2D) NMR spectra, mass spectrometry and by comparison with literature data.

Results and Discussion

The hexane and MeOH extracts of stem bark and leaves of *A. illustre* were subjected to a classical chromatographic methods to yield the a new oleanane-type triterpene, olean-12-ene-11α-methoxy-3β-acetate (**10**), in addition to known, β-amyrin (**3**),[4] lupeol (**4**),[4] β-amyrin acetate (**5**),[4] lupeol acetate (**6**),[4] olean-12-ene-28-hydroxy-3β-tetradecanoate (**7**),[4] olean-12-ene-28-carboxy-3β-hexadecanoate (**8**),[5,6] ursolic acid (**9**),[4] β-yoimbine (**1**)[7-9]

*e-mail: curcino@uenf.br

and 1,2-dehydroaspidospermidine (**2**),[10] were identified on the basis of ^{1}H and ^{13}C NMR spectral data, including ^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, HSQC and HMBC NMR experiments,[11,12] which were also used to complete unambiguous ^{1}H and ^{13}C chemical shift assignments of the triterpenoid **10**, the new natural product.

The pentacyclic triterpene (**10**) was obtained as an amorphous solid. Comparative analysis of $\{^1H\}$- and APT-^{13}C NMR spectra (Table 1), involving the corroboration of ^{1}H NMR spectra (1D ^{1}H NMR and 2D ^{1}H-^{1}H-COSY), allowed to recognize the presence of 33 signals corresponding to eight nonhydrogenated [(C)$_8$: six sp^3, two sp^2 (including one carbonyl groups at δ_C 171.0 and one sp^2 olefinic at δ_C 149.7], six methine [(CH)$_6$: five sp^3 (including two oxygenated linked to acetyl group at δ_C 80.8 and to methoxyl function at δ_C 75.7) and one sp^2 olefinic at δ_C 121.8], nine methylene [(CH$_2$)$_9$, all sp^3] and ten methyl [(CH$_3$)$_{10}$: including two oxygenated attributed to acetate (δ_C 21.4/δ_H 2.06, s) and methoxyl groups (δ_C 53.4/δ_H 3.21, s)] carbon atoms, allowing to deduce the expanded molecular formula (C)$_7$(C=O)(CH)$_6$(CH$_2$)$_9$(CH$_3$)$_9$(OCH$_3$) for **10**.

The LREI-MS (70 eV) spectrum of **10** showed of molecular peak [M$^+$] at *m/z* 498 Daltons, allowing in conjugation with the ^{13}C NMR spectral data to propose molecular formula C$_{33}$H$_{54}$O$_3$ (**10**), containing seven degrees of unsaturation and consistent with the presence of one acetyl group and one double bond in a pentacyclic triterpenoid acetylated and methoxylated, compatible with the structure triterpenic sustaining an acetate group at carbon atom CH-3 [δ_C 80.8/δ_H 4.51 (dd, *J* 8.8 and 7.6 Hz)] in an β-amyrin skeleton (**3**, β-amyrin).[4] In fact, heteronuclear long-range coupling ($^3J_{CH}$) of this carbon atom (δ_C 80.8) with H-5 (δ_H 0.87, value approximated deduced through of HMQC spectrum) and both methyl groups linked to quaternary carbon C-4 [δ_H 0.85 (3H-23) and 0.88 (3H-24)], as shown in Table 1.

The ^{13}C NMR spectrum of **10** revealed signals at δ_C 121.8 (CH-12) and at δ_C 149.7 (C-13), indicating the presence of a trisubstituted double bond. In the HMQC spectrum a cross-peak correlation $^1J_{CH}$ was observed between the CH-12 at δ_C 121.8 and the singlet signal at δ_H 5.30, which was assigned to the vinylic hydrogen.

The stereochemistry of the stereogenic carbons CH-3 and CH-11 of **10** was determined from the coupling constants of relevant hydrogens and from the observed [1]H-[1]H-NOESY. The values corresponding to *vicinal* interaction ($^3J_{H,H}$) between the hydrogen atoms H-3 and H-2 suggested axial-axial interaction, since the H-3 signal (δ_H 4.51, dd, *J* 8.8 and 7.6 Hz) revealed axial-axial coupling (*J* 8.8 Hz, Table 1); similarly the multiplicity observed in the signal of the hydrogen H-11 (δ_H 3.88, dd, *J* 9.4 and 3.5 Hz)

Table 1. [1]H (400 MHz) and ^{13}C (100 MHz) NMR data of triterpene (**10**), in CDCl$_3$ as solvent and TMS used as internal reference. Chemical shifts (δ, ppm) and coupling constants (*J*, Hz, in parenthesis)*

		10		
	HMQC		HMBC	
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
C				
4	38.0	-	3H-23; 3H-24	
8	43.1	-	H-9; 3H-26	3H-27
10	38.0	-	H-5; 3H-25	
13	149.7	-		3H-27
14	41.7	-	3H-27	H-9; 3H-26
17	32.3	-	3H-28	
20	31.1	-	3H-29; 3H-30	
1'	171.0	-	3H-2'	
CH				
3	80.8	4.51 (dd, 8.8, 7.6)		H-5; 3H-23; 3H-24
5	55.3	0.87		3H-23; 3H-24; 3H-25
9	51.0	1.72		3H-25; 3H-26
11	75.7	3.88 (dd, 9.4, 3.5)	H-9	MeO-11
12	121.8	5.30 (d, 3.5)		3H-28
18	46.9	2.35 (m)		
CH$_2$				
1	39.1	1.42, 1.22		H-9; 3H-25
2	23.8	1.72, 1.60		
6	18.3	1.98 (t, 13.5), 1.69	H-5	
7	33.2			3H-26
15	26.6			3H-27
16	26.7			3H-28
19	46.5	1.60, 1.30		3H-29; 3H-30
21	34.7			3H-29; 3H-30
22	37.0			3H-28
CH$_3$				
23	28.2	0.85 (s)		3H-24
24	16.7	0.88 (s)		3H-23
25	16.9	1.07 (s)		H-1b; H-9
26	18.2	1.00 (s)		
27	25.1	1.21 (s)		
28	28.5	0.83 (s)		
29	33.1	0.88 (s)		3H-30
30	23.7	0.88 (s)		3H-29
MeO-11	53.4	3.21 (s)		
Me-2'	21.4	2.05 (s)		

*Number of hydrogens bound to carbon atoms deduced by comparative analysis of $\{^1H\}$- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (*J*) were obtained of 1D ^{1}H NMR spectrum. ^{1}H-^{1}H-COSY and [1]H-[1]H-NOESY experiments were also used to these assignments. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC and HMBC spectra.

and both H-12 (δ_H 5.30, d, *J* 3.5 Hz) and H-9 (δ_H 1.72) allowed to recognize axial-axial interaction of H-11ax with H-9ax, since the H-11 signal revealed axial-axial coupling (*J* 9.4 Hz, Table 1) and H-11ax and H-12eq by = 3.5 Hz, consistent with the relative configuration shown in **10a**. Consistent with these observations, the ¹H-¹H-NOESY spectrum of **10** showed cross-peaks assigned to dipolar interaction (spatial proximity, *vide* **10a**) of H-11 (δ_H 3.88) with 3H-25 (δ_H 1.07) and 3H-26 (δ_H 1.00); hydrogen atoms of the methyl group present in the 3β-*O*-acetyl (δ_H 2.05) with both 3H-23 (δ_H 0.85) and 3H-24 (δ_H 0.88).

The oxidation at CH-11 of **10** is adequate to introduce significant modification in fragmentation of this pentacyclic triterpene containing double bond between the carbon

atoms CH-12 e C-13 in the mass spectrometer, revealing the absence of peaks produce by reaction Retro-Diels-Alder (RDA), as observed in other triterpenes with such characteristics.[13,14]

Confirmation of structure **10** was done by comparison with authentic sample of derivative monoacetate of triterpene 11-α-methoxy-β-amyrin isolated from *Myroxylon balsamum* (Fabaceae).[15]

According to the literature, the triterpene **10** was also obtained as an intermediate reaction by allylic oxidation by *N*-bromosuccimide with β-amyrin acetate.[16]

Thus, the pentacyclic isolated from *Aspidosperma illustre* was characterized as olean-12-ene-11α-methoxy-3β-acetate (**10**), a new natural product.

3. R¹=R²=R⁵= H; R³=R⁴= CH₃
5. R¹= Ac; R²=R⁵= H; R³=R⁴= CH₃
7. R¹= CO(CH₂)₁₂CH₃ ; R²=R⁵= H; R³=CH₂OH; R⁴= CH₃
9. R¹=R²=R⁴= H; R³= COOH; R⁵= CH₃
11. R¹=R²=R⁵= H; R³= COOH; R⁴= CH₃

4. R= H
6. R= Ac
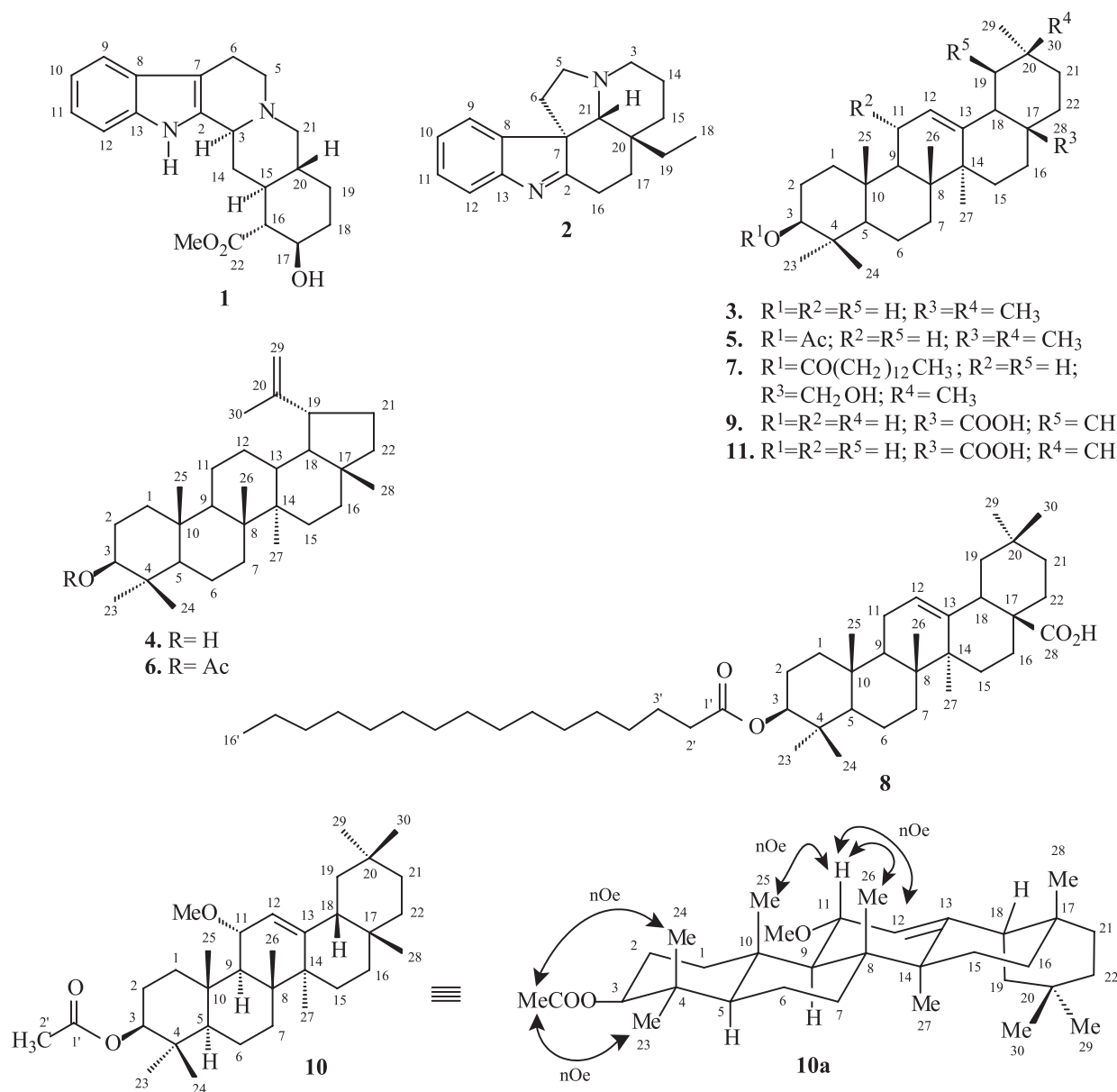
Figure 1. Structure of the compounds isolated from *A. illustre*.

The results of the extensive application of 1D and 2D NMR spectral techniques were also used to confirm the structure and to establish the ^{1}H and ^{13}C resonance assignments of **10** (Table 1).

Experimental

General procedures

Optic rotation measures were obtained on a Perkin Elmer 343 digital polarimeter. Melting points were obtained on a Microquímica MQRPF and are uncorrected. FTIR spectra were recorded on a FTIR-8300 Shimadzu spectrometer using KBr disk. ESI-MS (high resolution) mass spectra were obtained on a MICROMASSUltrOTOF-Q (Brüker Daltonics, Billerica, MA) mass spectrometer, using the negative ion mode of analysis and EI-MS (low resolution) mass spectra were obtained on Shimadzu QP5050A mass spectrometer. Column chromatographic purifications were carried out over silica gel (70-230 mesh). Silica gel 60F$_{254}$ was used in thin layer chromatography analysis.

^{1}H and ^{13}C NMR spectra were measured on a Jeol Eclipse 400 spectrometer, operating at 400 (^{1}H) and 100 (^{13}C) MHz. CDCl$_3$ was used as solvent and TMS as internal reference. Chemical shifts are given in the δ scale (ppm) and coupling constants J in Hz. One dimensional (1D) ^{1}H and ^{13}C NMR spectra were acquired under standard conditions by using a direct detection 5 mm ^{1}H/^{13}C dual probe. Standard pulse sequences were used for two dimensional spectra by using a multinuclear inverse detection 5 mm probe with field gradient.

Plant materials

The stem barks and leaves of *Aspidosperma illustre* (Vell.) Kuhlm. & Piraja were collected in November 2004 at Reserva Florestal de Linhares, Linhares, Espírito Santo State, Brazil. A voucher specimen (CVRD 338) is deposited at the Reserva Florestal Vale do Rio Doce Herbarium, Linhares, Espírito Santo State.

Extraction and isolation

Dried and powdered stem bark (3.08 kg) from *A. illustre* (Vell.) Kuhlm. and Piraja were extracted with hexane and methanol at room temperature, furnishing, after solvent evaporation, 10.1 g and 46.6 g of crude hexane and methanol extracts, respectively.

The hexane extract (10.1 g) from stem bark was chromatographed over silica gel column with a gradient of hexane/ethyl acetate to afford five fractions. Fraction 1 (1.83 g) was rechromatographed over a silica gel column with a gradient of hexane/ethyl acetate yielding of a mixture **5** and **6** (15.0 mg). Fraction 2 (1.0 g) and 5 (848.7 mg) was rechromatographed over a silica gel column with a gradient of hexane/ethyl acetate supplying of a mixture **3** and **4** (42.0 mg). Fraction 3 (3.26 g) presented as an amorphous white solid identified as compound (**3**). Fraction 4 (682.4 mg) was rechromatographed over a silica gel column with a gradient of hexane/ethyl acetate yielding of a compound (**10**) (7.0 mg).

A portion of the methanol extract (10.0 g) was chromatographed over silica gel column with a gradient of CH$_2$Cl$_2$/MeOH supplying four fractions. Fraction 1 (589.0 mg) was rechromatographed over silica gel column with a gradient of CH$_2$Cl$_2$/MeOH yielding of a compound (**7**, 9.0 mg) and mixture of compounds (**3**) e (**4**) (53.0 mg). Fraction 3 (360.0 mg) was rechromatographed over silica gel column with a gradient of CH$_2$Cl$_2$/MeOH furnishing two compounds (**1**, 319.0 mg) and (**2**, 10.0 mg).

Dried and powdered leaves (1.78 kg) were extracted with methanol at room temperature, furnishing, after solvent evaporation, 159.5 g of crude methanol extract.

A portion of the methanol extract (40.0 g) was partitioned with CH$_2$Cl$_2$/H$_2$O, supplying CH$_2$Cl$_2$ phase (23.2 g). A portion of the CH$_2$Cl$_2$ phase (5.0 g) was chromatographed over a silica gel column with a gradient of hexane/ethyl acetate supplying seven fractions. The fractions 4 (603.3 mg) and 5 (4.1 g) after successive chromatography's furnishing of compound (**8**) (138.4 mg). The fraction seven (963.7 mg) was rechromatographed over a silica gel column with a gradient of hexane/ethyl acetate yielding of compound (**9**) (83.8 mg).

The water phase (23.2 g) was extracted with ethyl acetate furnishing the ethyl acetate phase (5.35 g). The portion of the ethyl acetate phase (2.0 g) was chromatographed over silica gel column with a gradient of CH$_2$Cl$_2$/MeOH supplying thirteen fractions. The fractions 3 (16.6 mg) and 5 (12.7 mg) were submitted to a preparative TLC with hexane/ethyl acetate supplied by compounds **7** (7.0 mg) and **3** and **4** mixture (4.0 mg), respectively.

Transesterification reaction

After purification, the triterpene **8** was submitted to transesterification reaction with boron trifluorate methanol (BF$_3$-MeOH). In a flask were added 20 mg of triterpene **8** and 5 mL of BF$_3$-MeOH 20%. The solution was heated to 90 °C for 10 h. After was added 10 mL of H$_2$O to the reaction and the aqueous phase separated from the organic phase by liquid-liquid extraction with hexane. The organic

phase was concentrated, dried with Na_2SO_4 and evaporated for subsequent analysis of gas chromatography.

*Olean-12-ene-11α-methoxy-3β-acetate (**10**)*

Amorphous solid, mp 148-152 °C; $[\alpha]_D^{23}$ = + 8.06° ($CHCl_3$, *c* 0.062); LREI-MS (rel. int.) 498 (29, M$^{+\bullet}$), 483 (7, M - Me$^\bullet$), 466 (21, M - MeOH), 451 (9, M - MeOH - Me$^\bullet$), 391 (8, M - MeOH - Me$^\bullet$ - AcOH), 293 (32), 255 (58), 253 (20), 191 (41); ^{1}H and ^{13}C NMR: see Table 1.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

Acknowledgments

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Chemical Constituents from *Aspidosperma illustre* (Apocynaceae)

Lara F. Barbosa, Leda Mathias, R. Braz-Filho and Ivo J. Curcino Vieira*

Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 28013-602 Campos dos Goytacazes-RJ, Brazil
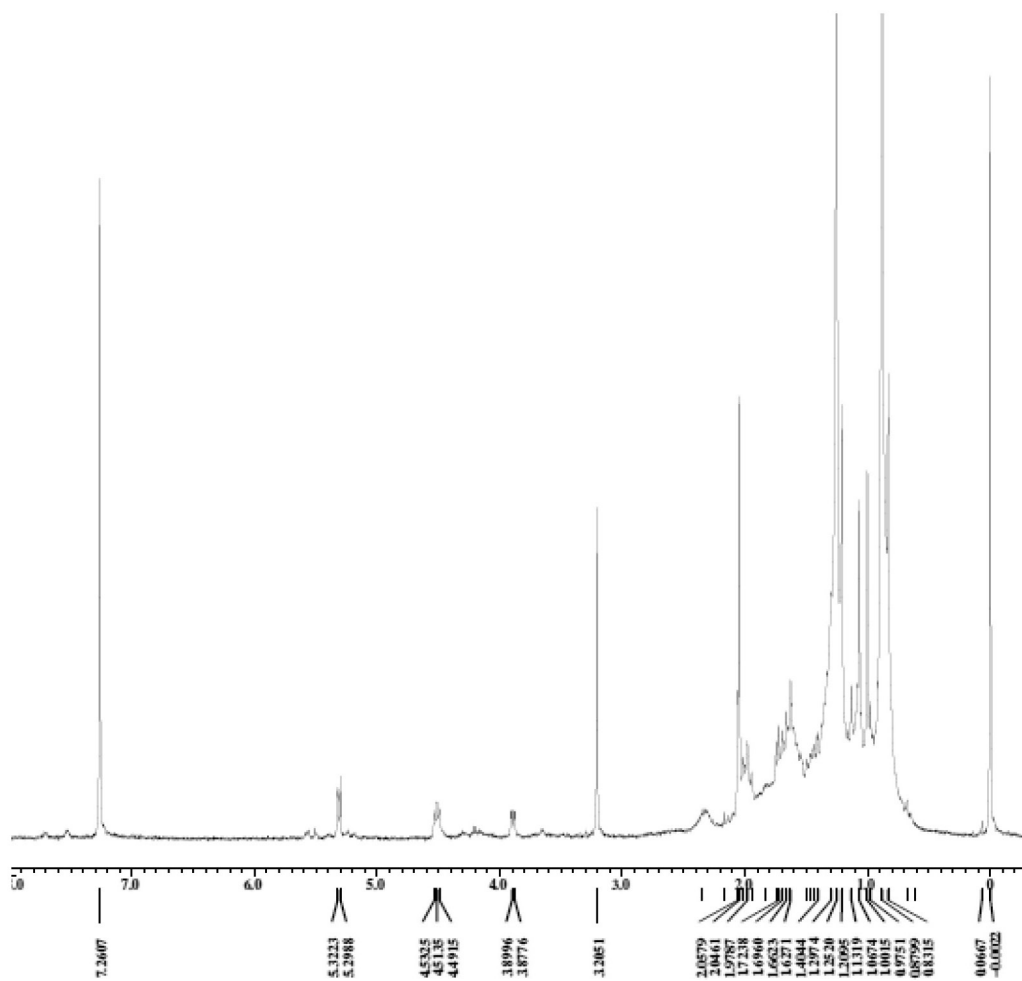
Figure S1. ¹H NMR spectrum of triterpene **10** (400 MHz, CDCl₃).
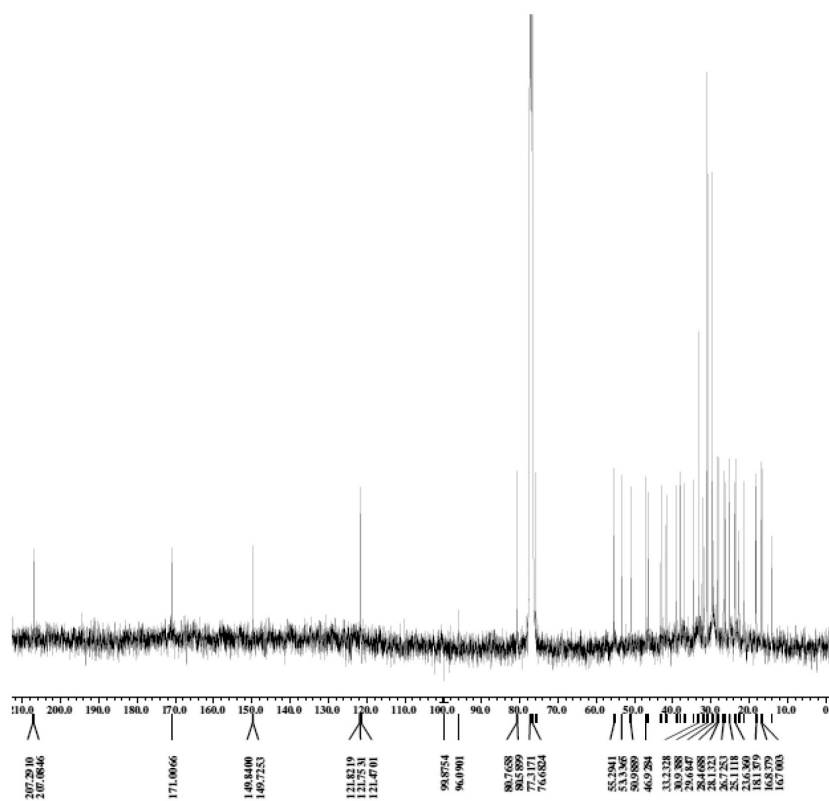
*e-mail: curcino@uenf.br

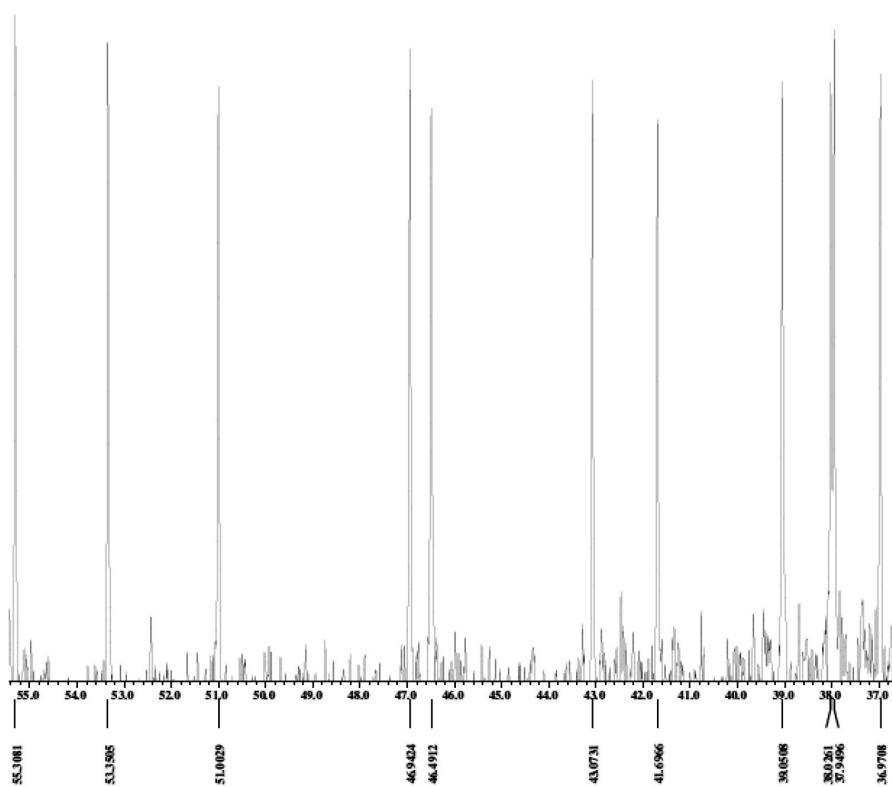**Figure S2.** ^{13}C NMR spectrum of triterpene **10** (100 MHz, CDCl$_3$).

Figure S3. Expansion of ^{13}C NMR spectrum of triterpene **10** (100 MHz, CDCl$_3$).
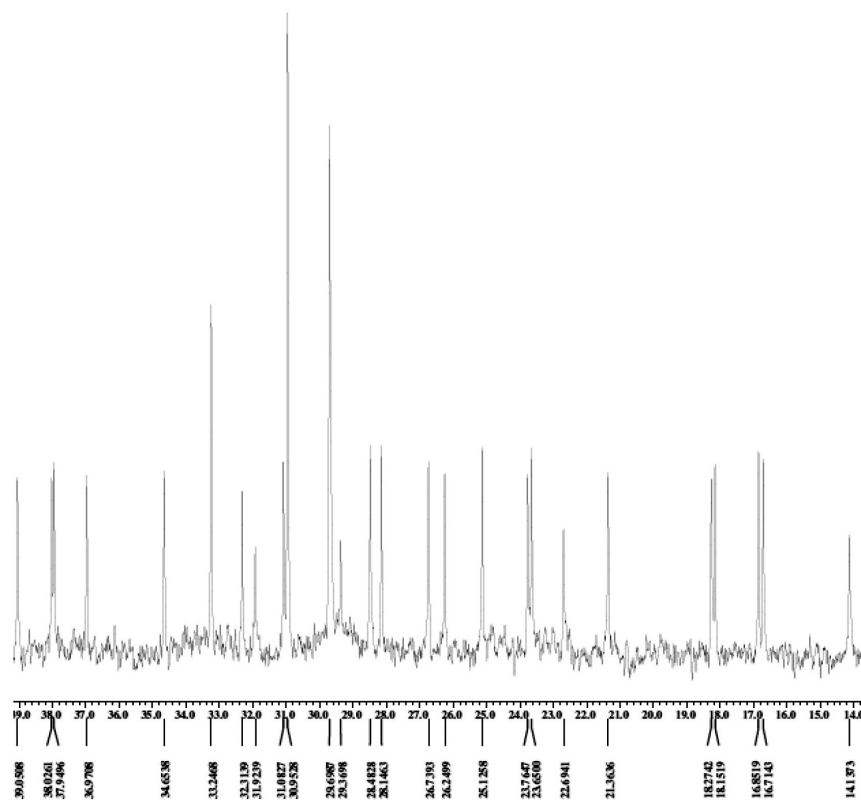
Figure S4. Expansion of ^{13}C NMR spectrum of triterpene **10** (100 MHz, CDCl$_3$).
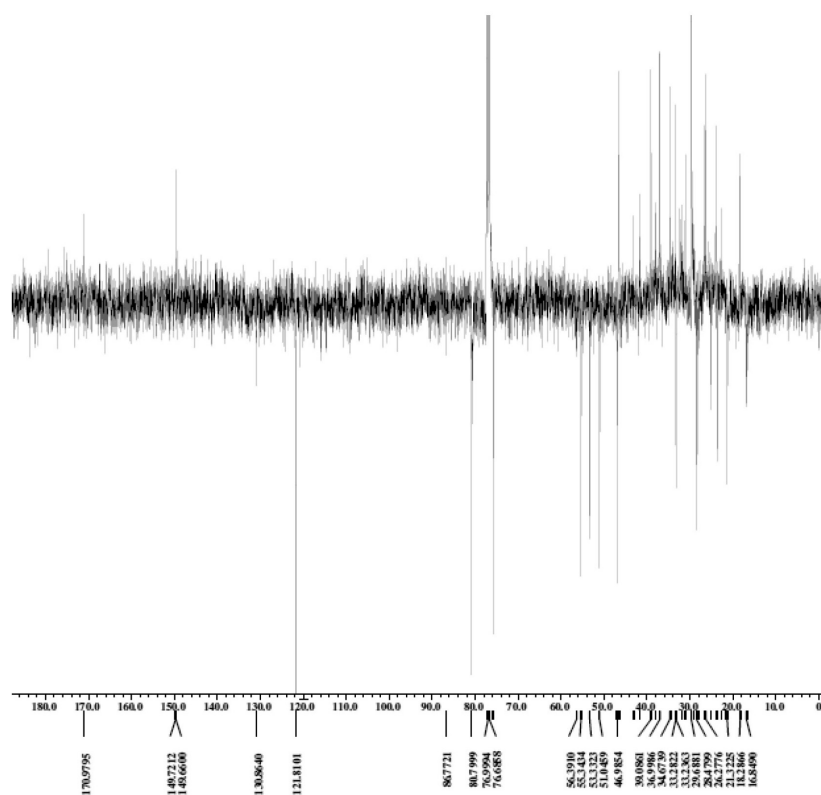
Figure S5. ^{13}C NMR-APT spectrum of triterpene **10** (100 MHz, CDCl$_3$).
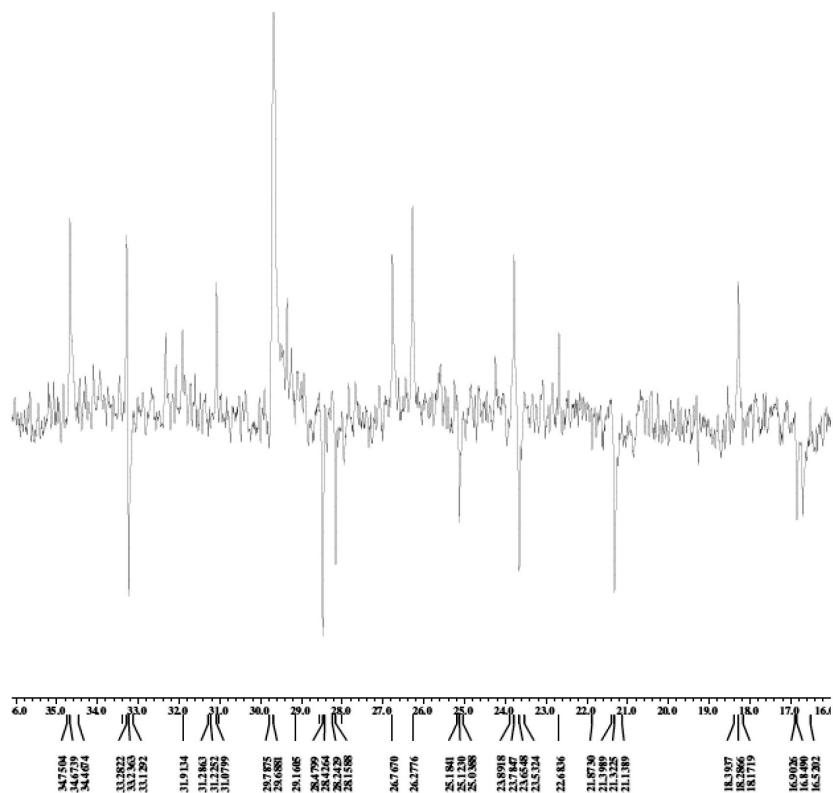
Figure S6. Expansion ^{13}C NMR-APT spectrum of triterpene **10** (100 MHz, CDCl$_3$).
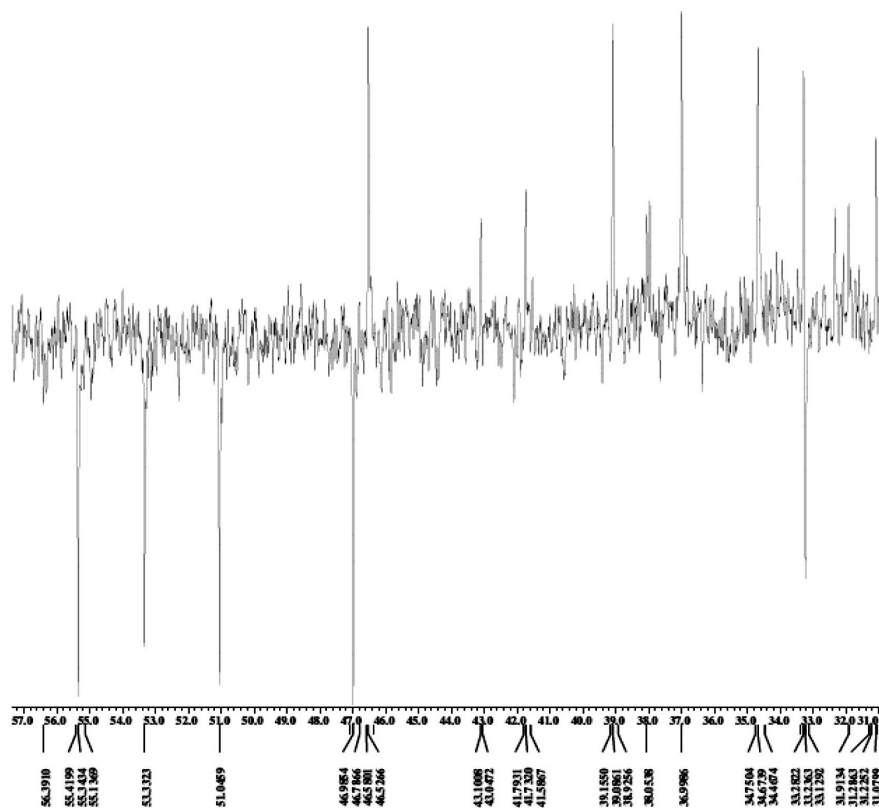
Figure S7. Expansion ^{13}C NMR-APT spectrum of triterpene **10** (100 MHz, CDCl$_3$).
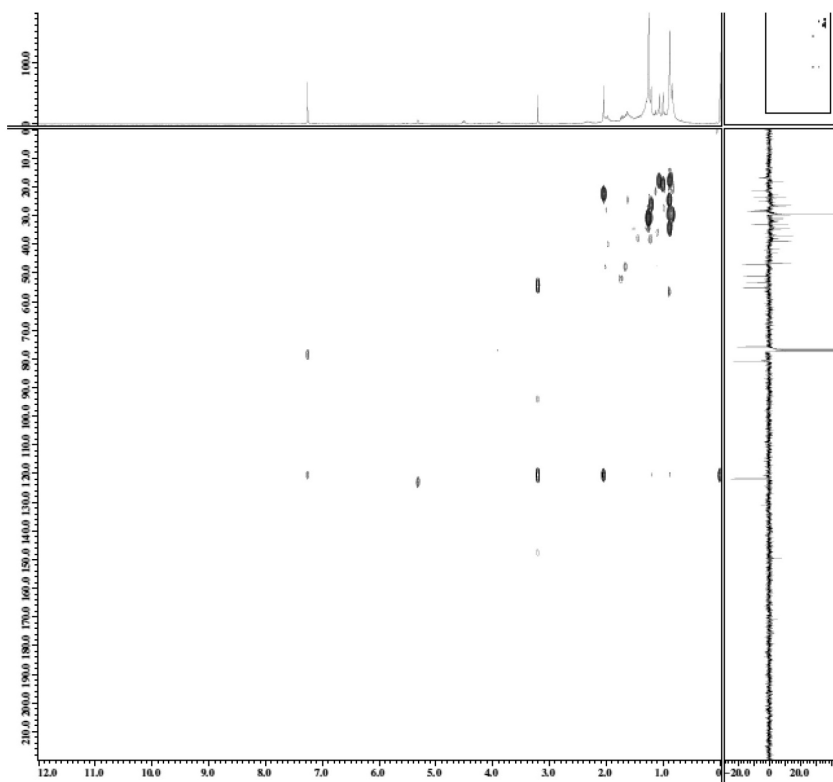
Figure S8. HMQC spectrum of triterpene **10** (400 MHz, CDCl$_3$).
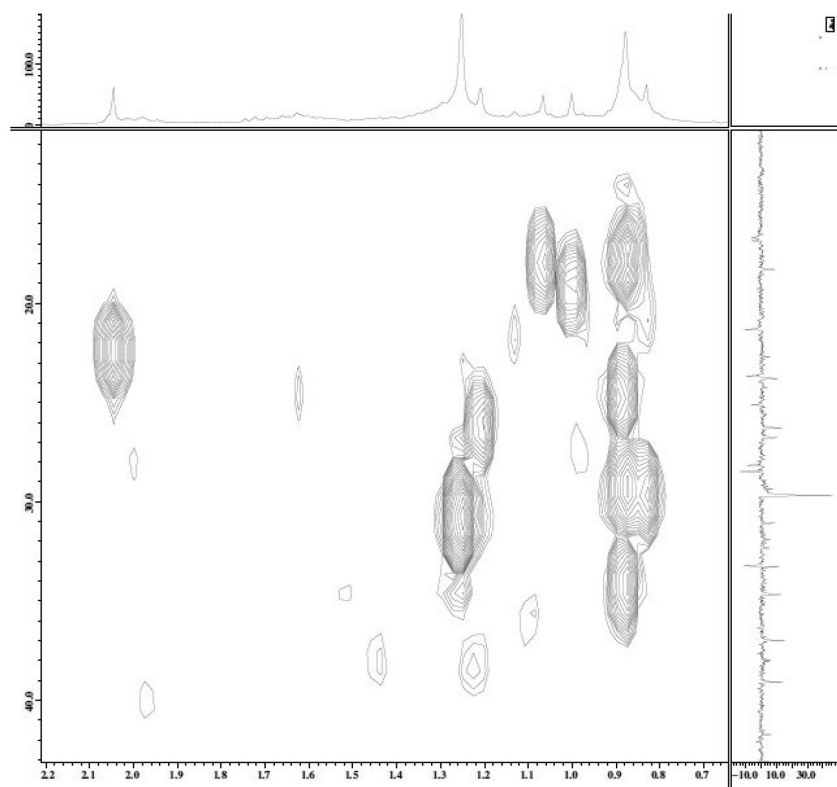
Figure S9. Expansion of HMQC spectrum of triterpene **10** (400 MHz, CDCl$_3$).
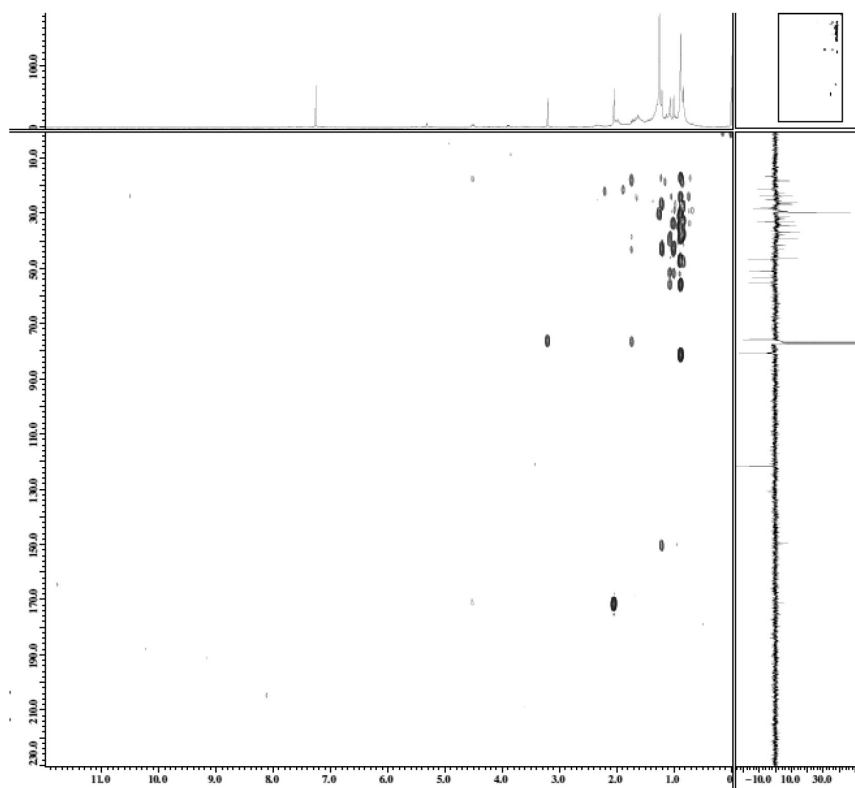
Figure S10. HMBC spectrum of triterpene **10** (400 MHz, CDCl₃).
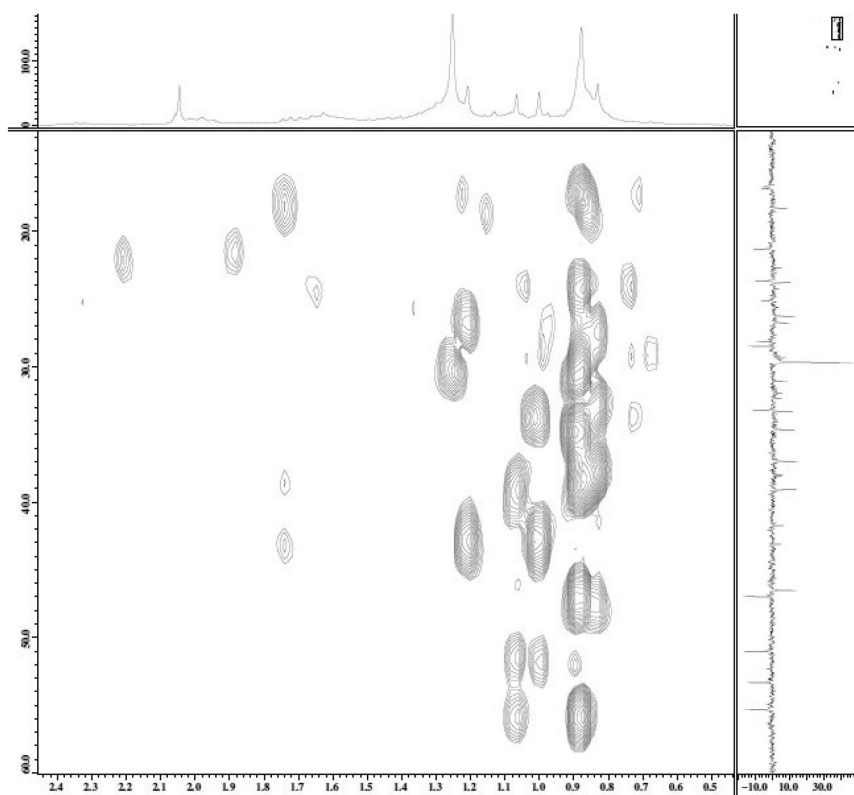
Figure S11. Expansion of HMBC spectrum of triterpene **10** (400 MHz, CDCl₃).
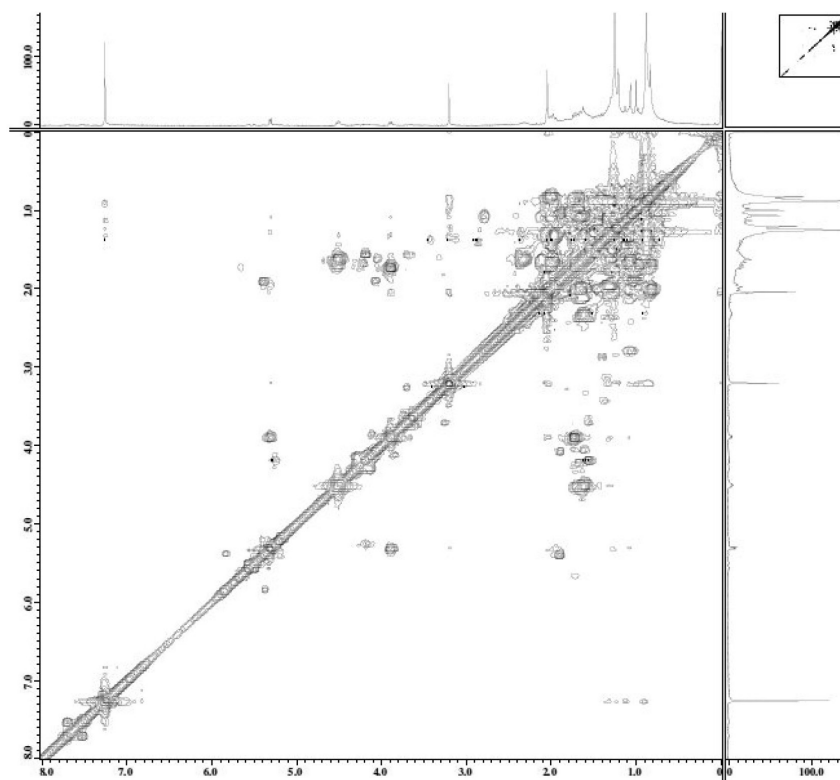
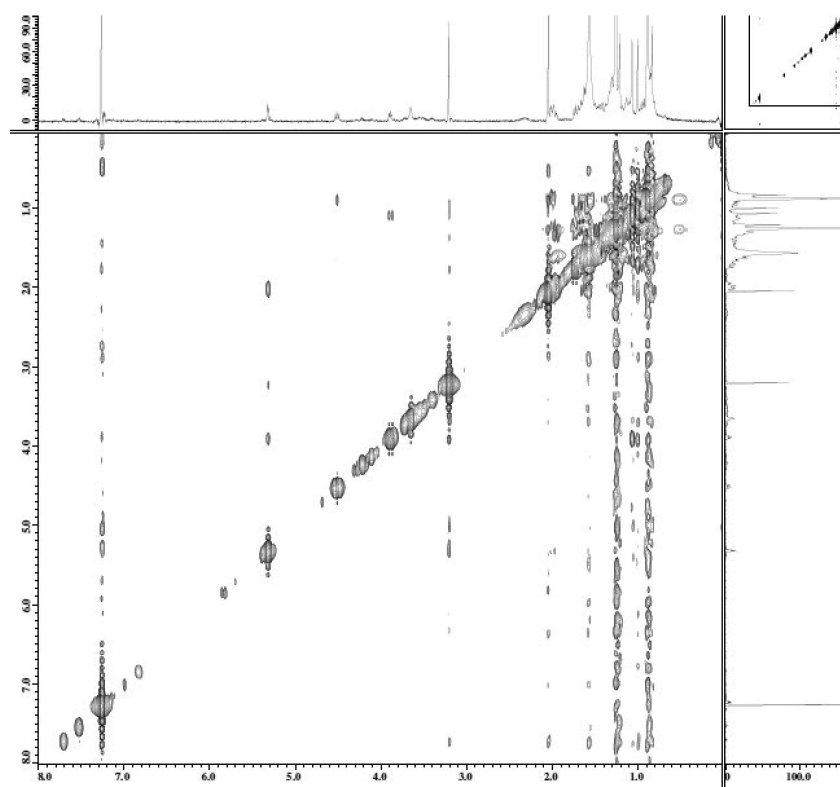**Figure S12.** ^{1}H-^{1}H-COSY spectrum of triterpene **10** (400 MHz, CDCl$_3$).

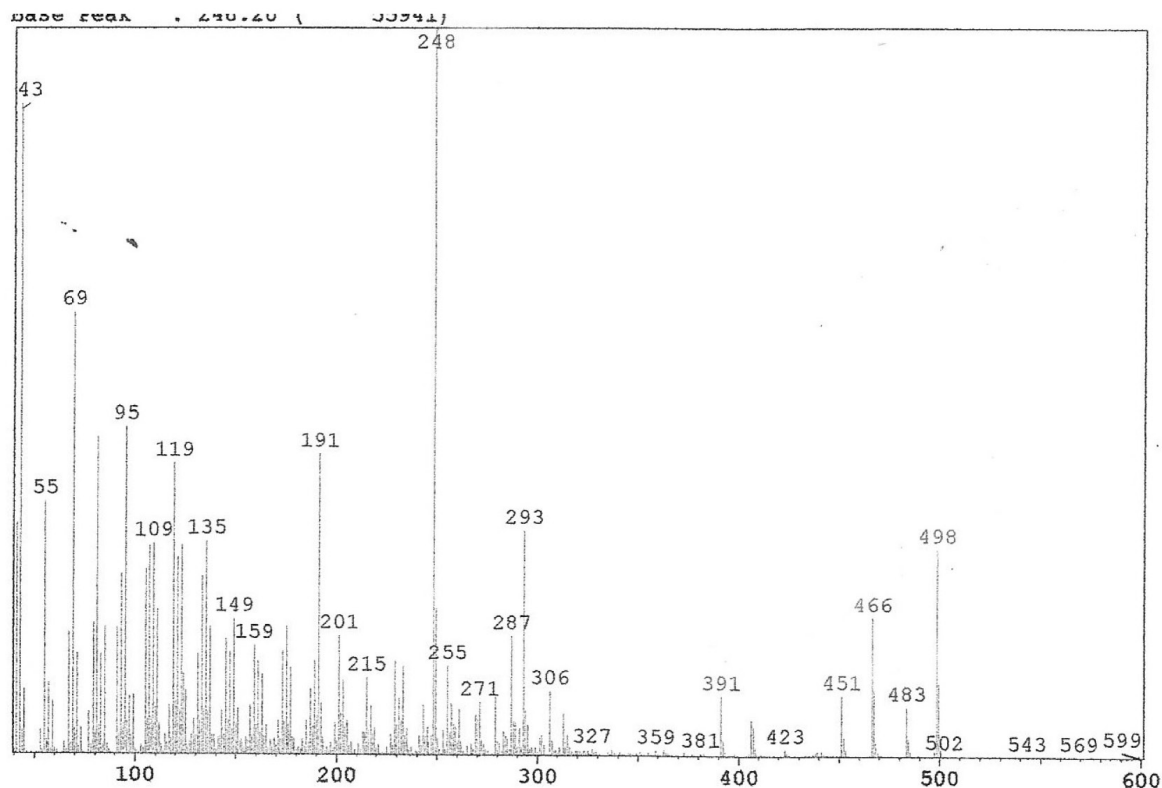**Figure S13.** ^{1}H-^{1}H-NOESY spectrum of triterpene **10** (400 MHz, CDCl$_3$).

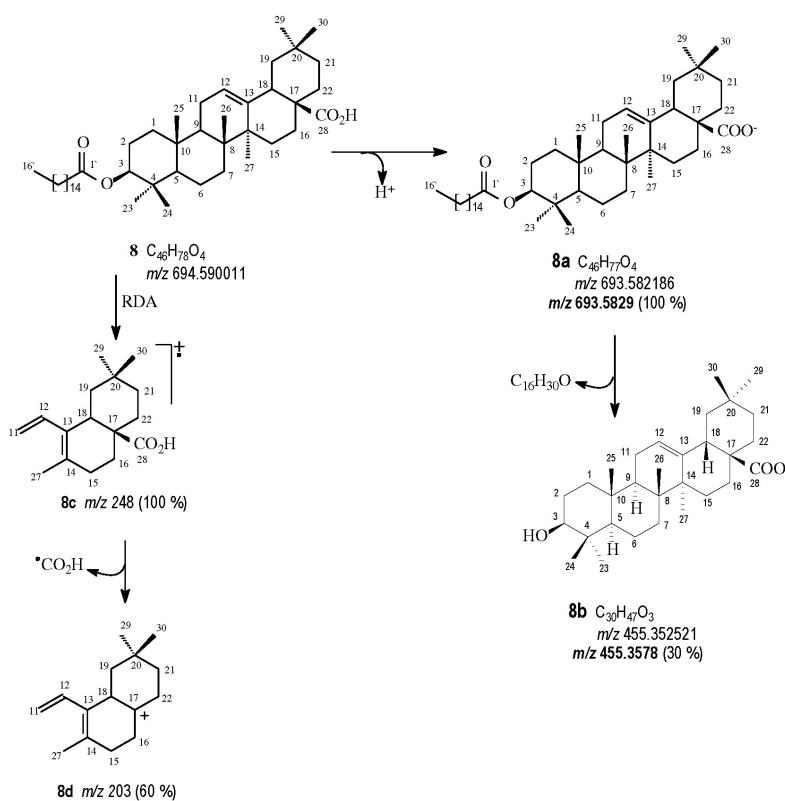**Figure S14.** LREIMS spectrum of triterpene **10** (70 eV).

Figure S15. Proposed fragmentation mechanisms of triterpene **8** by MS/MS (HRESIMS) of the peaks at *m/z* **693.5829** ([M-H$^+$]$^-$) with production of fragments **8a** and **8b** and EIMS (70 eV) to furnish **8c** and **8d**, only peaks classified as principals.
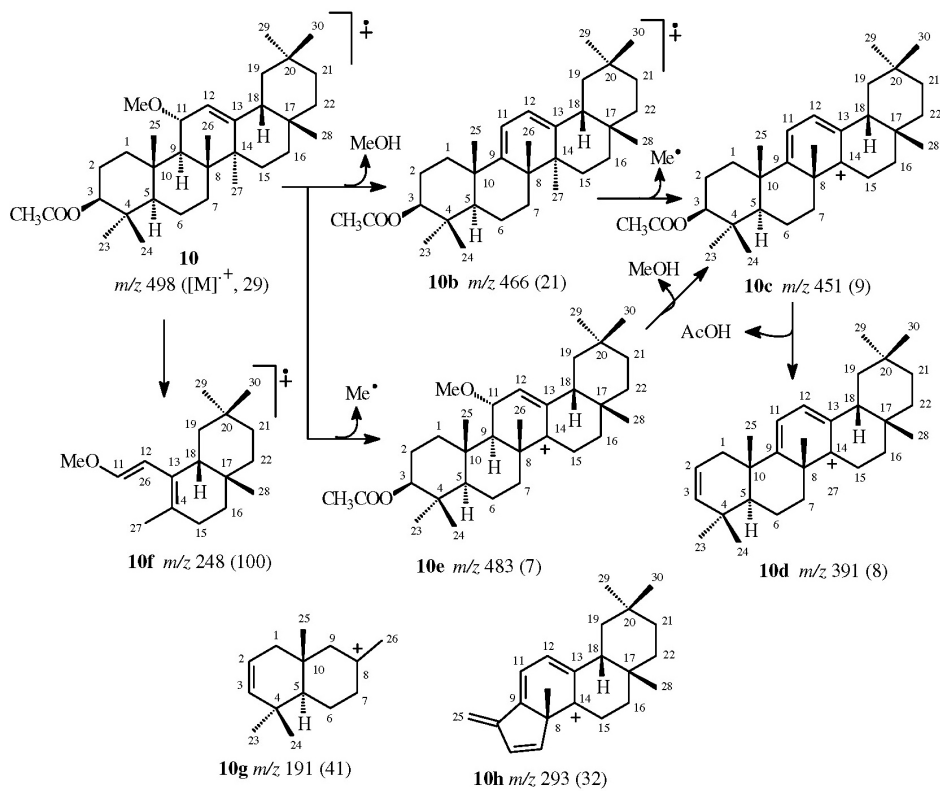
Figure S16. Proposed fragmentation mechanisms to justify principal peaks observed in the mass spectrum (LREIMS, 70 eV) of **10** (in parenthesis percentage of relative abundance).