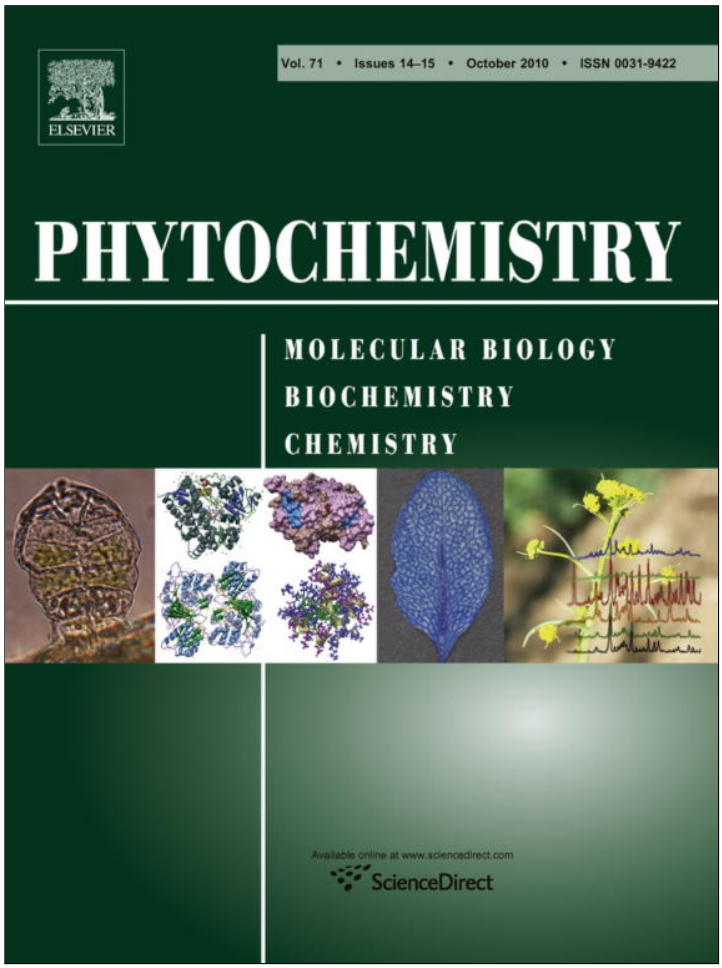

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Antiproliferative terpenoids and alkaloids from the roots of *Maytenus vitis-idaea* and *Maytenus spinosa*

María Tereza Rojo de Almeida [a,1], Carla Ríos-Luci [b], José M. Padrón [b], Jorge A. Palermo [a,*]

[a] *UMYMFOR, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428 Buenos Aires, Argentina*

[b] *BioLab, Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG), Universidad de La Laguna, Spain*

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ABSTRACT

Investigation of the organic extracts of the roots of *Maytenus vitis-idaea* and *Maytenus spinosa*, collected in the province of Salta, Argentina, led to isolation of eighteen compounds belonging to several classes. From *M. vitis-idaea*, eight methylenequinone celastroids (**1**–**8**) were isolated, four of which (**4**–**7**) were hitherto unknown. Additionally, from *M. spinosa*, two known celastroids, a known celastroid dimer (**9**), three pentacyclic triterpenoids (**10**–**12**) and six β-dihydroagarofuran sesquiterpenoid alkaloids (**13**–**18**) were identified. Compounds **4**–**7** were active against six solid tumor cell lines at micromolar concentrations.

1. Introduction

The family Celastraceae comprises more than 90 genera and nearly 1300 species (Simmons et al., 2008), the vast majority of which are exclusively tropical, with the exceptions of the widely distributed genera *Celastrus* and *Euonymus*. Many species belonging to this family have been extensively studied due to their worldwide use in agriculture and traditional medicine (Muñoz et al., 1995; González et al., 2000; Alvarenga and Ferro, 2006).

The genus *Maytenus*, which includes more than 225 worldwide distributed species, is one of the most representative of this family. Compounds isolated from *Maytenus* typically belong to the following classes of secondary metabolites: maytansinoids, β-dihydroagarofuran sesquiterpene alkaloids (Gao et al., 2007) or methylenequinone triterpenoids (Gunatilaka, 1996). The latter can also take the form of dimers (Shirota et al., 1998), or trimers (González et al., 1999). The alkaloids are typically found in the leaves, while the triterpenoids are generally extracted from the roots.

Methylenequinone triterpenoids are well known for their antitumor activities, and abundant compounds of this family have been used as scaffolds for the preparation of analogues in the course of a structure–activity relationship study (Ravelo et al., 2004).

The organic extracts of the roots of two species found in Argentina: *Maytenus spinosa* and *Maytenus vitis-idaea*, were thus investigated as part of a survey for new bioactive celastroids. *M. vitis-idaea* (Griseb, 1874) is an endemic shrub from northern Argentina, and the south of Bolivia and Paraguay. There is only one previous study of this species, which was collected in Paraguay (Alvarenga et al., 2001). That contribution reported the isolation of pristimerine, tingenone and 20β-hydroxytingenone, as well as the insecticidal and antifeedant activities of these compounds against the moth *Cydia pomonella*.

The second species, *M. spinosa* (Griseb.) Lourteig and O'Donell (1955), is an endemic shrub from Argentina, and its root bark is used in tanneries as a red pigment. There are no previous studies on the chemistry of this species.

From the roots of *M. vitis-idaea*, four new methylenequinone triterpenoids were isolated, together with four known compounds of the same class. The extract of the roots of *M. spinosa* yielded two new pentacyclic triterpenoids, together with tingenone, scutione, and a known methylenequinone triterpenoid dimer. From *M. spinosa*, minor amounts of six β-dihydroagarofuran sesquiterpenoid

* Corresponding author. Tel./fax: +54 11 4576 3385.
E-mail address: palermo@qo.fcen.uba.ar (J.A. Palermo).
[1] Present address: Departamento de Ciências Farmacêuticas, Universidade Federal de Santa Catarina, Campus Trindade, 88040-970 Florianópolis, SC, Brazil.

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alkaloids were also isolated, two of them being previously unreported. The four new compounds from *M. vitis-idaea* were active against a panel of six solid tumor cell lines.

2. Results and discussion

The crude extract of the roots of *M. vitis-idaea* was fractionated by vacuum flash chromatography on silica gel (see Section 4). Purification of the obtained fractions by reversed-phase HPLC and Sephadex LH-20 afforded compounds **1**–**8** (Fig. 1).

Compounds **1**–**3** and **8** were readily identified as scutione (González et al., 1996), tingenone (Delle Monache et al., 1973), pristimerin (Harada et al., 1962), and celastrol (Nakanishi et al., 1955), respectively, by comparison of both their NMR spectroscopic data with literature values (Ngassapa et al., 1994; Gunatilaka et al., 2005), and reference spectra. Compound **4** was isolated as a red lacquer and showed an exact mass 462.2777 in its HR-ESIMS spectrum, which accounted for a molecular formula $C_{30}H_{38}O_4$, this being indicative of 12 unsaturations. Inspection of the ^{13}C NMR spectrum (Table 1) established the presence of two carbonyls and five double bonds, indicating the structure of a pentacyclic skeleton. Analysis of the ^1H NMR spectrum (Table 2) showed characteristic signals of A, B rings of pristimerin, as well as the presence of a methyl ester. HMBC correlations identified this carboxyl moiety as C-29, as well as additional similarities with pristimerin in ring E. The main difference between **4** and pristimerin was the presence of an additional unsaturation (disubstituted double bond). Taking into account that this double bond had to be located either on the C or D rings, Δ11 or Δ15 structures were the only possibilities. COSY correlations showed a pair of coupled isolated methylenes which displayed HMBC correlations with Me-25 and Me-27. An HMBC correlation was also observed between one of the methylene signals (δ^1H: 1.92) and C-10 ($\delta^{13}C$: 164.4). Taken together, this evidence clearly indicated that these methylenes were C-11 and C-12, thus ruling out the possibility of a Δ11 double bond. On the other hand, the additional double bond protons showed HMBC correlations with the quaternary carbons C-14 and C-17 as well as with Me-26 and Me-28 (Fig. 2). These data, together with a ROESY correlation between H-15 and Me-28, confirmed the presence of a Δ15 structure. In this way compound **4** was determined as the new compound 15-dehydropristimerin.

Compound **5** was isolated as a red amorphous powder and had also a molecular formula $C_{30}H_{38}O_4$ obtained by HR-ESIMS. Its 12 unsaturations were deduced by inspection of the NMR spectra as two carbonyls, five double bonds and five rings. As in compound **4**, the ^1H NMR spectrum showed the presence of a methyl ester which was again identified as C-29 by HMBC correlations. Rings A and B were also similar to those of compound **4**, but the chemical shift of Me-26 (δ^1H: 1.73) suggested that ring D was similar to that of scutione, and that the Me-26 was bound to a Δ14 double bond. A complete set of 2D NMR spectra confirmed the tentative structure of this new compound, for which we propose the name vitideasin. In particular, HMBC correlations of Me-26 with the double bond carbons C-14 and C-15, and with C-16 methylene, which in turn correlated with Me-28, and the observed NOE between Me-26 and H-7 confirmed the structure of ring D.

The high-resolution mass spectrum of **6** indicated a molecular formula $C_{28}H_{34}O_4$, which suggested a bis-nortriterpenoid structure. The ^1H NMR spectrum, especially the chemical shift of Me-26 clearly determined that **6** was also a derivative of scutione. As for the four oxygen atoms, three were clearly assigned by inspec-

Table 1
^{13}C NMR spectroscopic data for compounds **4**, **5**, **6**, **7**, **11** and **12** in $CDCl_3$, δ in ppm.

No. C	4	5	6	7	11	12
C-1	119.8	119.9	120.0	119.8	40.3	40.8
C-2	178.4	178.0	178.1	178.3	34.4	34.4
C-3	146.0	146.2	146.3	146.2	218.0	218.3
C-4	117.0	116.7	116.8	117.5	47.7	47.6
C-5	127.5	127.5	127.9	128.5	55.5	55.3
C-6	133.8	134.9	134.4	132.1	19.8	19.7
C-7	117.9	121.6	121.9	118.6	33.9	33.2
C-8	165.7	159.7	158.6	164.9	42.9	42.7
C-9	43.0	44.5	44.6	43.0	50.4	50.9
C-10	164.4	159.7	159.7	164.2	37.7	37.6
C-11	34.3	37.5	37.3	32.5	76.3	77.0
C-12	27.5	35.6	35.9	31.1	121.6	124.2
C-13	40.3	43.1	42.4	39.0	149.3	143.3
C-14	47.3	135.3	136.9	49.6	42.0	42.4
C-15	129.4	128.3	128.6	73.0	26.2	26.6
C-16	135.6	37.8	40.5	41.4	26.8	27.9
C-17	33.7	33.7	39.8	37.6	32.4	33.8
C-18	42.6	43.9	43.7	43.7	47.2	58.8
C-19	30.7	33.9	40.7	31.9	46.4	39.3
C-20	41.0	42.6	75.0	41.8	31.1	39.5
C-21	29.4	28.6	213.0	213.7	34.7	31.1
C-22	32.3	36.1	50.5	54.2	36.9	41.3
C-23	10.3	10.3	10.4	10.3	26.7	26.9
C-24	–	–	–	–	21.5	21.4
C-25	37.4	29.4	29.6	41.0	16.4	16.6
C-26	28.8	21.9	22.1	23.6	18.1	18.2
C-27	18.0	24.0	23.7	23.5	25.2	22.5
C-28	27.4	31.5	30.4	32.9	28.5	28.7
C-29	178.3	179.3	–	–	33.2	17.5
C-30	31.3	19.8	24.8	15.0	23.6	21.3
OMe	51.6	51.8	–	–	53.7	54.4

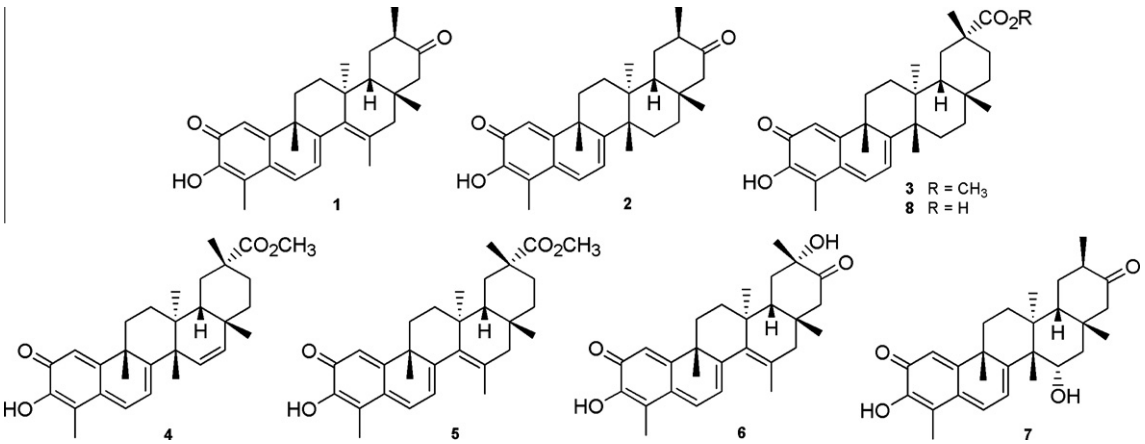
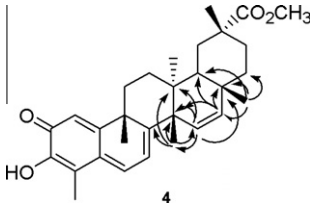


Fig. 1. Compounds isolated from *Maytenus vitis-idaea*.

Table 2
^{1}H NMR spectroscopic data for compounds **4**, **5**, **6**, **7**, **11** and **12** in $CDCl_3$, δ in ppm, *J* (Hz).

H	4	5	6	7	11	12
H-1	6.55 *s*	6.58 *s*	6.59 *s*	6.53 *s*	2.30 *ddd* (14.0, 7.5, 4.0) 1.66 *m*	2.32 *ddd* (14.0, 7.5, 4.0) 1.67 *ddd* (14.0, 10.0, 8.0)
H-2	–	–	–	–	2.53 *ddd* (16.0, 10.5, 7.5) 2.39 *ddd* (16.0, 7.5, 4.0)	2.52 *ddd* (16.0, 10.0, 7.5) 2.41 *ddd* (16.0, 8.0, 4.0)
H-5	–	–	–	–	1.38 *m*	1.40 *m*
H-6	7.04 *d* (7.0)	7.18 *d* (7.0)	7.16 *d* (7.0)	7.00 *d* (7.0)	1.51 *m* 2H	1.48 *m* 1.43 *m*
H-7	6.57 *d* (7.0)	6.17 *d* (7.0)	6.19 *d* (7.0)	6.52 *d* (7.0)	1.53 *m* 1.36 *m*	1.54 *m* 1.38 *m*
H-9	–	–	–	–	1.81 *d* (9.4)	1.77 *d* (9.5)
H-11	2.19 *m* 1.92 *m*	1.97 *m* 1.93 *m*	1.93 *m* 2H	2.14 *m* 2H	3.93 *dd* (9.4, 3.0)	3.89 *dd* (9.5, 3.0)
H-12	1.93 *m* 1.77 *m*	2.54 *ddd* (14.0, 14.0, 5.6) 1.31 *m*	2.32 *m* 1.40 *bd*	1.90 *bd* (13.0) 1.82 *dd* (13.0, 6.0)	5.36 *d* (3.0)	5.30 *d* (3.0)
H-15	6.15 *d* (10.0)	–	–	4.38 *bs*	1.70 *ddd* (13.5, 13.5, 4.5) 1.01 *ddd* (13.5, 4.0, 3.5)	1.78 *ddd* (13.5, 13.5, 5.0) 1.03 *ddd* (13.5, 4.7, 2.0)
H-16	5.28 *d* (10.0)	2.65 *d* (15.0) 1.26 *d* (15.0)	2.27 *d* (15.0) 1.63 *d* (15.0)	2.13 *dd* (15.5, 3.5) 1.71 *dd* (15.5, 2.0)	2.02 *m* 0.85 *m*	2.03 *ddd* (13.5, 13.5, 4.7) 0.91 *m*
H-18	1.94 *d* (8.3)	1.43 *d* (13.5)	1.93 *m* 1H	1.75 *m* 1H	2.01 *m*	1.39 *m*
H-19	2.51 *d* (16.0) 1.63 *dd* (16.0, 8.3)	1.69 *bd* (13.5) 1.54 *dd* (13.5, 13.5)	2.07 *dd* (14.3, 3.5) 1.70 *dd* (14.3, 14.3)	2.24 *dd* (14.0, 7.0) 1.81 *d* (14.0)	1.67 *dd* (13.5, 13.5) 1.09 *m*	1.37 *m*
H-20	–	–	–	2.58 *m*	–	0.92 *m*
H-21	2.16 *bd* (14.0) 1.32 *ddd* (14.0, 14.0, 3.5)	1.89 *ddd* (14.0, 14.0, 4.0) 1.46 *d* (14.0)	–	–	1.35 *m* 1.11 *m*	1.41 *m* 1.28 *m*
H-22	1.77 *m* 1.17 *m*	1.63 *ddd* (14.0, 14.0, 4.0) 1.29 *m*	2.81 *d* (13.0) 2.19 *d* (13.0)	3.47 *d* (14.7) 1.98 *d* (14.7)	1.45 *ddd* (13.5, 13.5, 3.8) 1.24 *dt* (13.5, 3.0)	1.45 *m* 1.30 *m*
Me-23	2.22 *s* 3H	2.26 *s* 3H	2.26 *s* 3H	2.22 *s* 3H	1.11 *s* 3H	1.11 *s* 3H
Me-24	–	–	–	–	1.08 *s* 3H	1.07 *s* 3H
Me-25	1.43 *s* 3H	1.29 *s* 3H	1.27 *s* 3H	1.53 *s* 3H	1.16 *s* 3H	1.15 *s* 3H
Me-26	1.40 *s* 3H	1.73 *s* 3H	1.77 *s* 3H	1.31 *s* 3H	1.07 *s* 3H	1.10 *s* 3H
Me-27	0.48 *s* 3H	0.82 *s* 3H	0.88 *s* 3H	1.26 *s* 3H	1.22 *s* 3H	1.16 *s* 3H
Me-28	1.13 *s* 3H	1.20 *s* 3H	1.23 *s* 3H	1.02 *s* 3H	0.85 *s* 3H	0.81 *s* 3H
Me-29	–	–	–	–	0.89 *s* 3H	0.88 *d* (6.5) 3H
Me-30	1.21 *s* 3H	1.21 *s* 3H	1.31 *s* 3H	1.01 *d* (6.5) 3H	0.90 *s* 3H	0.92 *bs* 3H
OMe	3.58 *s* 3H	3.68 *s* 3H	–	–	3.24 *s* 3H	3.29 *s* 3H
OH	6.96 *bs*	7.11 *bs*	7.07 *bs*	6.94 *bs*		



4

Fig. 2. Diagnostic HMBC correlations for compound **4**.

tion of the ^{13}C NMR spectrum as C-2 and C-21 carbonyls and C-3 hydroxyl, while the presence of a quaternary carbon at δ 75.0 indicated an additional carbinolic carbon. HMBC correlations of Me-30 with the latter carbon, C-21 carbonyl and C-19 clearly placed this hydroxyl at C-20. NOESY correlations between Me-30 and both C-19 protons established the relative configuration of C-20, and in this way **6** was clearly determined as the previously unreported 20-α-hydroxyscutione.

The NMR spectroscopic features of compound **7** showed that it was a derivative of tingenone, with additional oxygenation. Besides the characteristic Me-23, there were no other methyls bound to sp^2 carbons, and there was a methyl doublet at δ 1.01 (Me-30) which correlated in the HMBC spectrum with the C-21 carbonyl. A signal at δ_C 73.0 and δ_H 4.38 (*bs*) was indicative of the presence of an hydroxyl group on a tertiary carbon. The molecular formula $C_{28}H_{36}O_4$, obtained by high-resolution mass spectrometry, confirmed the tentative structure. The identification of Me-26, Me-27 and Me-28 was necessary to determine the site of hydroxylation. A methyl singlet at δ_H 1.02 showed HMBC correlations with a methylene (δ_C 54.2, C-22) which was vicinal to C-21 ketone and with a methine at δ_C 43.7 (C-18), and was assigned as Me-28. A methyl singlet at δ_H 1.26 was assigned as Me-27 due to its HMBC correlation with C-18, with the quaternary carbons at δ_C 39.0 (C-13) and 49.6 (C-14), and with a methylene at δ_C 31.1 (C-12). Finally, Me-26 (δ_H 1.31) was clearly identified by its HMBC correlation with the sp^2 quaternary carbon at δ_C 164.9 (C-8). A neat HMBC correlation of Me-26 with the oxidized carbon at δ_C 73.0 located the hydroxyl at C-15. The broad singlet pattern of its ^{1}H NMR signal indicated that H-15 had to be equatorial. ROESY correlations of H-15 (δ_H 4.38) with Me-26 and H-7 (δ_H 6.52), as well as with both C-16 protons (δ_H 1.71, δ_H 2.13) clearly confirmed the relative stereochemistry at C-15 as 15-α-OH (Fig. 3). Compound **7** was thus determined as the previously unreported 15-α-hydroxytingenone.

The crude extract of the roots of *M. spinosa* was fractionated by vacuum chromatography on reversed-phase silica gel (see Section 4). Further chromatographic separations and final purification by reversed-phase HPLC yielded compounds **1**, **2**, **9**–**12** (Fig. 4), to-

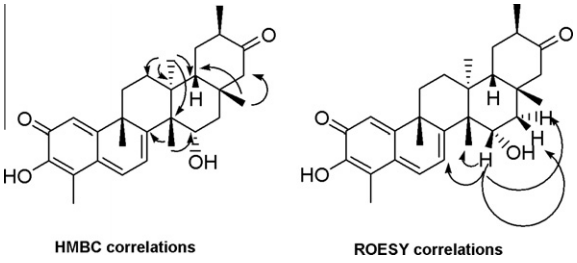


Fig. 3. Diagnostic HMBC and ROESY correlations for compound **7**.

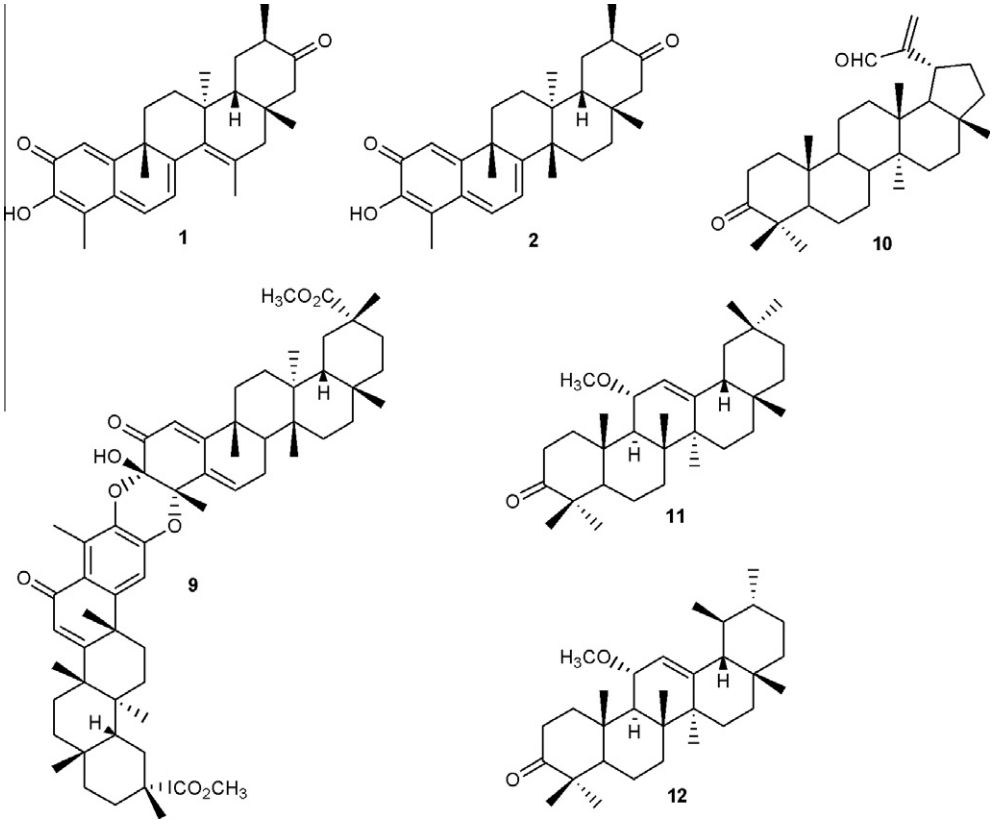


Fig. 4. Terpenoids isolated from *Maytenus spinosa*.

gether with the β-dihydroagarofuran sesquiterpene alkaloids **13**–**18** (Fig. 5).

Compound **9** was characterized as a celastroid dimer by NMR spectroscopic analyses. This being composed by two pristimerin units, one in a quinoid form and the other in an aromatic form, and was identified as 7,8-dihydroisoxuxuarine-Eα by comparison with reference data (Shirota et al., 1998). The remaining terpenoids of this extract were pentacyclic triterpenoids lacking a methylen-equinone functionality. Compound **10** was identified as the known triterpenoid 3-oxolup-20(29)-en-30-al by interpretation of its NMR spectra (De Souza e Silva et al., 2005).

The NMR spectra of compounds **11** and **12** were very similar, and both substances had identical molecular formulae $C_{31}H_{50}O_2$ established by high-resolution mass spectrometry. In both cases the seven double bond equivalents could be assigned to a ketone, a trisubstituted double bond and five rings. Another common feature was the presence of a methoxy group, namely a methyl ether. All of these data indicated that **11** and **12** were pentacyclic triterpene ketones with a methoxyl substituent. Compound **11** showed eight methyl singlets by ^{1}H NMR. An HMBC experiment indicated the presence of two geminal pairs of methyls thus suggesting a possible oleanane skeleton. A complete set of 2D experiments (COSY, HSQC, HMBC, ROESY) clearly defined the structure of this compound. HMBC correlations of the geminal methyls Me-23 and Me-24 with the signal at δ_C 218.0 placed the ketone at C-3. Correlations observed in the COSY and HMBC experiments placed the methoxyl group at C-11, vicinal to the Δ12 double bond. The remaining correlations from the 2D NMR spectra confirmed the oleanane skeleton. The large coupling constant (9.4 Hz) between H-9 and H-11 indicated that the latter was pseudoaxial, thus establishing **11** as an 11α-methoxy triterpenoid. ROESY correlations of H-11 with Me-25 and Me-26, as well as correlations of H-18 with

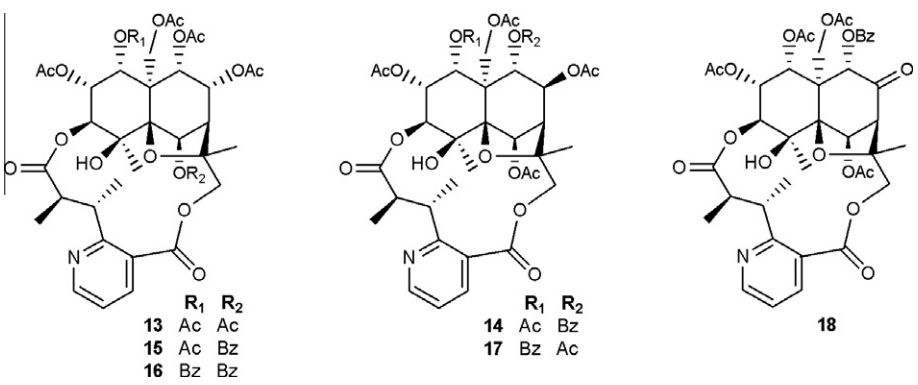


Fig. 5. Alkaloids isolated from *Maytenus spinosa*.

Me-28 and Me-30, and H-9 with H-5 and Me-25 confirmed the relative stereochemistry. In this way, compound **11** was determined as the new triterpenoid 3-oxo-11α-methoxyolean-12-ene.

Inspection of a complete set of NMR spectra confirmed that compound **12** shared with **11** the following features: a Δ12 double bond, a 11α-methoxyl and the 3-keto group, while the pattern of the methyl groups was different, suggesting another skeleton. The presence of a methyl doublet at δ 0.87 (*d*, *J* = 6.5 Hz) suggested a possible ursane skeleton; however, only one of the doublets was detected. Its methyl group was assigned as Me-29 due to its HMBC correlation with C-18. Analysis of all the 2D NMR spectra confirmed that the A–D rings in **11** and **12** were identical, leaving only ring E to be elucidated. In the ^{1}H NMR spectrum, a broad signal could be observed at δ 0.92, which integrated for five protons. Careful inspection of the HSQC spectrum showed that these protons corresponded to the missing methyl group (δ_C 21.4), one of C-16 (δ_C 27.9) protons and a methine at δ_C 39.5, which was the only methine that remained unidentified and was tentatively assigned as C-20. The proton signal at δ 0.92 gave COSY correlations with protons at δ 1.28 (H-21) and δ 1.37 (H-19) confirming this last assignment. The coincidence in ^{1}H chemical shift of Me-30 and H-20 generated a strongly coupled system which led to the loss of the doublet structure of Me-30. An ursane structure was thus confirmed, and **12** was finally identified as the new triterpenoid 3-oxo-11α-methoxyurs-12-ene.

Other compounds isolated from this extract were a series of β-dihydroagarofuran sesquiterpene alkaloids **13**–**18**, obtained in small amounts. This was an unexpected result since these compounds are typically found in aerial parts. In compounds **13**–**18**, the diacid fragment was readily identified as evoninic acid by the typical ^{1}H NMR signals: a 2,3 disubstituted pyridine system, two methyl doublets and two quartet methines which were adjacent but with no visible coupling between them. In all cases the positions of the sesquiterpenoid moiety esterified by this diacid were C-3 and C-13. The main differences between these compounds were a variable number of acetyl and benzoyl substituents. Compounds **13**–**16** were identified as the known alkaloids euonine (Sugiura et al., 1973), horridine (González et al., 1986), cangorinine E-I (Shirota et al., 1994) and ebenifoline E-II (Han et al., 1990) respectively by comparison of their NMR spectra with literature data.

Compound **17** was isolated as a white amorphous solid, and had a molecular formula $C_{42}H_{49}NO_{18}$ obtained by HRMS. Five acetates and a benzoate group could be readily identified by ^{1}H NMR spectroscopic analysis (Table 3), together with the typical evoninic acid signals. HMBC correlations placed these acetates at C-2, C-6, C-8, C-9 and C-15, while an HMBC correlation between the carbonyl at δ_C 164.3 and the proton at δ 5.88 (H-1) placed the benzoate group at C-1. Analysis of the coupling constants showed small couplings between H-1 and H-2, H-2 and H-3, H-7 and H-8, while a large diaxial coupling (10 Hz) was observed for H-8 and H-9. A ROESY experiment showed correlations of H_2-15 with Me-14, H-8, H-7 and H-6. Taking into consideration all this information, the substituents were established as 1α, 2α, 6β, 8β and 9α. Compound **17** is, to the best of our knowledge, a new isoeuonyminol-type alkaloid, 8β-acetoxy-*O*^{1}-benzoyl-*O*^{1}-deacetyl-8-deoxoevonine.

Analysis of the ^{1}H NMR of compound **18** indicated the presence of four acetate and one benzoate moieties, and an evoninic acid group. An AMX system was observed, with resonances at δ 5.76 (1H, *d*, *J* = 3.0 Hz), δ 5.27 (*t*, 1H, *J* = 3.0 Hz) and δ 4.81 (1H, *d*, *J* = 3.0 Hz), which were assigned by COSY and HMBC correlations as H-1, H-2 and H-3, respectively. The ^{1}H NMR signals of the other ring showed a different pattern, since in compound **18** both H-9 (δ 5.86, 1H) and H-7 (δ 3.09, 1H) resonances were singlets. A carbonyl group was detected at δ_C 195.6 which showed HMBC correlations to both H-7 and H-9. This carbonyl was assigned as C-8 and explained the relatively large chemical shift of H-7 and H-9. An HMBC correlation between the benzoate carbonyl at δ_C 164.8 and H-9 placed this group at C-9 and explained the low chemical shift (δ_H 1.47, *s*, 3H) of the C-15 acetate methyl. Analysis of the coupling constants, as well as ROESY correlations allowed the elucidation of the substitution pattern as 1α, 2α, 6β and 9α. Therefore, the structure of compound **18** was determined as the previously unreported *O*^{9}-benzoyl-*O*^{9}-deacetylevonine.

Table 3
NMR spectroscopic data for alkaloids **17** and **18** in $CDCl_3$, δ in ppm, *J* (Hz).

17			**18**		
No.	^{1}H	^{13}C	No.	^{1}H	^{13}C
1	5.88 *d* (4.0)	72.3	1	5.76 *d* (3.0)	71.5
2	5.37 *dd* (4.0, 3.0)	68.6	2	5.27 *t* (3.0)	68.6
3	4.80 *d* (3.0)	74.9	3	4.81 *d* (3.0)	74.8
4	–	69.9	4	–	70.5
5	–	92.9	5	–	95.2
6	6.65 *s*	74.7	6	6.85 *s*	73.5
7	2.47 *d* (3.0)	49.4	7	3.09 *bs*	61.8
8	5.54 *dd* (10.0, 3.0)	73.4	8	–	195.6
9	5.79 *d* (10.0)	73.9	9	5.86 *s*	79.2
10	–	51.4	10	–	52.5
11	–	84.7	11	–	86.0
12	1.76 *s* 3H	19.3	12	1.73 *s* 3H	19.2
13	5.98 *d* (11.5)	70.1	13	6.09 *d* (11.5)	70.0
	3.70 *d* (11.5)			3.76 *d* (11.5)	
14	1.62 *s* 3H	23.7	14	1.65 *s* 3H	23.4
15	4.93 *d* (13.0)	60.4	15	4.98 *d* (13.0)	60.5
	4.88 *d* (13.0)			4.68 *d* (13.0)	
2′	–	165.1	2′	–	165.4
3′	–	125.0	3′	–	n.o.
4′	8.08 *dd* (8.0, 2.0)	137.9	4′	8.11 *dd* (8.0, 2.0)	137.6
5′	7.28 *dd* (8.0, 5.0)	120.9	5′	7.29 *dd* (8.0, 5.0)	121.2
6′	8.71 *dd* (5.0, 2.0)	151.6	6′	8.72 *dd* (5.0, 2.0)	151.7
7′	4.67 *q* (7.0)	36.3	7′	4.72 *q* (7.0)	35.9
8′	2.64 *q* (7.0)	44.8	8′	2.60 *q* (7.0)	44.5
9′	1.42 *d* (7.0) 3H	12.0	9′	1.43 *d* (7.0) 3H	11.9
10′	1.25 *d* (7.0) 3H	9.7	10′	1.22 *d* (7.0) 3H	9.8
11′	–	173.0	11′	–	173.8
12′	–	168.4	12′	–	n.o.
BzO-1	–	164.3	AcO-1	–	169.0
	7.78 *bd* (8.0) 2H	129.3		2.25 *s* 3H	21.4
	7.53 *t* (8.0) 1H	133.2			
	7.40 *t* (8.0) 2H	128.9			
AcO-2	–	169.7	AcO-2	–	168.2
	1.95 *s* 3H	20.7		2.17 *s* 3H	21.1
AcO-6	–	169.5	AcO-6	–	169.9
	2.23 *s* 3H	21.4		2.08 *s* 3H	20.4
AcO-8	–	170.0		–	–
	2.33 *s* 3H	21.17			
AcO-9	–	165.1	BzO-9	–	164.8
	1.40 *s* 3H	20.3		7.98 *bd* (8.0) 2H	129.7
				7.60 *t* (8.0) 2H	133.6
				7.46 *t* (8.0) 1H	128.6
AcO-15	–	168.0	AcO-15	–	170.1
	2.14 *s* 3H	20.8		1.47 *s* 3H	20.0

n.o. = not observed.

2.1. Biological activity

The biological activity of all new compounds: **4**-**7**, **11**–**12**, and **17**–**18** was studied in a representative panel of human solid tumor cell lines. As a model for the antiproliferative activity, the following human solid tumor cell lines were used: A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon). The *in vitro* antiproliferative activity was evaluated after 48 h of drug exposure using the sulforhodamine B (SRB) assay (Miranda et al., 2006). The results expressed as TGI (total growth inhibition, Monks et al., 1991) values are shown in Table 4. Four positive control substances were included

Table 4
TGI values (μM) for the *in vitro* screening against human solid tumor cells.

Compound	Cell line					
	A2780 (ovarian)	HBL-100 (breast)	HeLa (cervix)	SW1573 (lung)	T-47D (breast)	WiDr (colon)
4	6.9	3.2	4.2	4.0	15	4.5
5	5.4	2.7	3.3	3.9	3.5	3.1
6	7.0	1.1	3.1	1.7	3.4	3.2
7	4.0	1.0	3.1	1.0	3.4	2.6
11	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100
17	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100
Camptothecin	1.7	2.5	64	2.2	71	84
Etoposide			12	85	25	>100
Cisplatin	16	14	15	47	99	>100
5-Florouracil	>100	88	>100	>100	>100	>100

in Table 4: camptothecin (DNA Topoisomerase I inhibitor), etoposide (DNA Topoisomerase II inhibitor), cisplatin (DNA alkylating agent) and 5-fluorouracyl (antimetabolite).

3. Conclusions

This work describes the secondary metabolite profile of the root extracts of *M. vitis-idaea* and *M. spinosa*, with a total of eighteen isolated and completely characterized compounds. In summary, in the present work, eight new compounds (four celastroids, two pentacyclic triterpenoids and two β-dihydroagarofuran sesquiterpene alkaloids) were identified and described.

As expected, the four new celastroids were highly active against a panel of six solid tumor cell lines, all of them at micromolar concentrations. This is another example of the importance of celastroids as potential antitumor agents, and as scaffolds for the preparation of more active derivatives. The similar TGI values obtained indicate that the particular structural features of these compounds (mostly on D and E rings) do not play a significant role on the biological activity. On the other hand, the pentacyclic triterpenoids and the β-dihydroagarofuran sesquiterpenoid alkaloids were not active against the same solid tumor cell lines. The presence of the sesquiterpene alkaloids in the roots of *M. spinosa*, even at nearly trace amounts, is a rare finding, since it is well known that these compounds are generally located in the aerial parts of Celastraceae, where they usually display insecticidal activity. In this work, great care was taken to extract only the root material of the plant, so the possibility of contamination with aerial parts material is highly unlikely. This result may bring some interesting questions on the true location of the biosynthetic processes that give rise to this class of compounds.

4. Experimental

4.1. General

NMR experiments were performed on a Bruker Avance 2 (500 MHz) instrument at 500.13 MHz for ^{1}H and 125.13 MHz for ^{13}C. All spectra were recorded in $CDCl_3$ using TMS as internal standard. All 2D NMR experiments (COSY, HSQC, HMBC, ROESY, NOESY) were performed using standard sequences. HR-EIMS electron-impact mass spectra were determinated on a VG Auto Speccon mass spectrometer at IUBO-AG, Universidad de La Laguna, Tenerife, Spain. Optical rotations were measured on a Perkin-Elmer 343 polarimeter. UV spectra were obtained on a Hewlett Packard 8453 spectrophotometer and IR spectra were recorded on a Nicolet Magna 550 spectrophotometer. Vacuum flash chromatography was carried out either on silica gel (Aldrich Chemical Co.) or reversed-phase silica gel (Aldrich Chemical Co.). All solvents were distilled prior to use. HPLC separations were performed using a Thermo Separations SpectraSeries P100 pump, a Thermo Separations Refractomonitor IV RI detector and a Thermo Separations SpectraSeries UV 100 UV detector, HPLC grade solvents and YMC RP-18 (5 μm, 20 mm × 250 mm; 5 μm, 10 mm × 250 mm) columns. UV detection was performed at 220 nm. Sephadex LH-20 was obtained from Pharmacia Inc.; TLCs were carried out on Merck Sílicagel 60 F_{254} plates, using CH_2Cl_2/EtOAc mixtures as mobile phase. TLC plates were sprayed with 2% vanillin in concentrated H_2SO_4.

4.2. Plant material

Roots of *M. vitis-idaea* and *M. spinosa* were collected by Dr. Juan Carlos Oberti (Universidad de Córdoba, Argentina) at Osma, Salta province, Argentina, in February 2005 and October 2005, respectively. Voucher specimens were identified by Prof. Lázaro Novoa (Universidad Nacional de Salta, Argentina) and were deposited at the herbarium (UNS, Salta, Argentina). Voucher numbers: 12001 for *M. vitis-idaea* and 12281 for *M. spinosa*.

4.3. Extraction and Isolation of compounds from M. vitis-idaea

Dried roots of *M. vitis-idaea* (375 g) were diced and extracted with a mixture of Et_2O/cyclohexane (1:1, 2 × 1 L) for two days at room temperature. The combined extracts were concentrated under reduced pressure and then triturated with CH_2Cl_2 in order to separate an insoluble latex (0.7 g). The supernatant was evaporated to dryness, giving a crude extract (2.9 g). The latter was subjected to vacuum flash chromatography on silica gel using a cyclohexane/EtOAc gradient ranging from 100% cyclohexane to 100% EtOAc, and seven fractions (MV1–MV7) were obtained. TLC analysis showed that fractions MV3 and MV5 contained the compounds of interest. Fraction MV3 (250 mg, eluted with cyclohexane/EtOAc 80:20) was purified by reversed-phase HPLC using the 20 mm × 250 mm column described in Section 4.1, CH_3CN/H_2O (85:15) as eluant and a flow rate of 5 mL/min yielding the following compounds (in order of elution): **1** (Rt: 24 min, 30 mg); **2** (Rt: 25 min, 24 mg); **5** (Rt: 60 min, 20 mg); **4** (Rt: 72 min, 5.2 mg) and **3** (Rt: 96 min, 11 mg). Fraction MV5 (950 mg, eluted with cyclohexane/EtOAc 50:50) was permeated through a Sephadex LH-20 column (80 × 4 cm) using MeOH as eluant, and 14 fractions (150 mL) were collected. Fractions 6 and 7 were combined (280 mg) and then subjected to reversed-phase HPLC (same column, flow rate: 4 mL/min, eluant: MeOH/H_2O (85:15)) to yield pure compound **8** (Rt: 12 min, 11 mg) and a mixture (Rt: 18 min, 12 mg) which was separated by preparative TLC on silica gel (eluant: CH_2Cl_2/MeOH (20:1)) to obtain **6** (2.3 mg) and **7** (1.7 mg).

4.4. Extraction and Isolation of compounds from M. spinosa

Dried roots of *M. spinosa* (400 g) were diced and extracted with a mixture of Et_2O/cyclohexane (1:1, 2 × 1 L) for two days at room temperature, which yielded, after evaporation at reduced pressure, a crude extract (2.7 g). Reversed-phase vacuum chromatography was used for fractionation of the crude extract, using a H_2O/MeOH gradient ranging from 50% MeOH to 100% MeOH, obtaining four fractions. Fraction 2 (600 mg, eluted with H_2O/MeOH (40:60)) was permeated through a Sephadex LH-20 column (60 × 2 cm) using MeOH as eluant, and 30 fractions (20 mL) were collected. TLC inspection and combination resulted in nine fractions, S1–S9. Fraction S4 was purified by reversed-phase HPLC using the 10 mm × 250 mm column, MeOH/H_2O (70:30) as eluant and a flow rate of 2.5 mL/min to yield compound **17** (Rt: 32 min, 1.5 mg). HPLC of fraction S5 under the same conditions, yielded compounds (in order of elution): **13** (Rt: 26 min, 1.1 mg), **14** (Rt: 27 min, 1.5 mg), **18** (Rt: 30 min, 0.9 mg), **15** (Rt: 36 min, 2.8 mg) and **16** (Rt: 60 min, 1 mg). Fraction 4 (2 g), eluted with MeOH from reversed-phase VLC, was permeated twice through a Sephadex LH-20 column (80 × 4 cm) using MeOH as eluant, and 100 mL fractions were collected. TLC inspection and combination resulted in three fractions, M1–M3 which contained the compounds of interest. Reversed-phase HPLC of fraction M1 (10 mm × 250 mm column, MeOH as eluant, flow rate of 2.5 mL/min) yielded compound **10** (Rt: 72 min, 2.8 mg). Fraction M2 was purified by reversed-phase HPLC (10 mm × 250 mm column, MeOH/H_2O (98:2)) and compounds **11** (Rt: 48 min, 8 mg) and **12** (Rt: 60 min, 6.2 mg) were obtained, while HPLC purification of fraction M3 (20 mm × 250 mm column, MeOH/H_2O (98:2), flow rate: 6 mL/min), yielded compound **9** (Rt: 60 min, 6.2 mg).

4.4.1. 15-Dehydropristimerin **4**

Red lacquer, $[\alpha]_D$ −53.6 (*c* 2.60, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.00), 424 (3.64), HR-EIMS *m*/*z* 462.2777, $C_{30}H_{38}O_4$ requires 462.2770; IR ν_{max} (KBr) 3300; 2924; 1726; 1594; 1550; 1444; 1379; 1288; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.2. Vitideasin **5**

Red powder, $[\alpha]_D$ −87.9 (*c* 1.60, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 446 (3.97); HR-EIMS *m*/*z* 462.2693, $C_{30}H_{38}O_4$ requires 462.2770; IR ν_{max} (KBr) 3310; 2931; 1724; 1596; 1507; 1441; 1248; 1205; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.3. 20-β-Hydroxyscutione **6**

Red powder, $[\alpha]_D$ −64.8 (*c* 1.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (3.69), 444 (3.48): HR-EIMS *m*/*z* 434.2459, $C_{28}H_{34}O_4$ requires 434.2457; IR ν_{max} (KBr) 3358; 2922; 1715; 1589; 1505; 1442; 1283; 1206; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.4. 15-α-Hydroxytingenone **7**

Orange-red amorphous solid, $[\alpha]_D$ −91.6 (*c* 0.85, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (3.72), 424 (3.55); HR-EIMS *m*/*z* 434.2588, $C_{28}H_{36}O_4$ requires 434.2614; IR ν_{max} (KBr) 3378; 2925; 1701; 1595; 1443; 1266; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.5. 3-Oxo-11α-methoxyolean-12-ene **11**

Colourless oil, $[\alpha]_D$ 35.5 (*c* 4.00, $CHCl_3$); HR-EIMS *m*/*z* 454.3820, $C_{31}H_{50}O_2$ requires 454.3811; IR ν_{max} (film) 2948; 1705; 1462; 1384; 1083; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.6. 3-Oxo-11α-methoxyurs-12-ene **12**

Colourless oil, $[\alpha]_D$ 13.8 (*c* 3.10, $CHCl_3$); HR-EIMS *m*/*z* 454.3796, $C_{31}H_{50}O_2$ requires 454.3811; IR ν_{max} (film) 2924; 1705; 1456; 1384; 1082; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.7. 8β-Acetoxy-O^1-benzoyl-O^1-deacetyl-8-deoxoevonine **17**

Colourless oil, $[\alpha]_D$ 13.8 (*c* 3.10, $CHCl_3$); ESI-APCI-MS *m*/*z* 890.2856, $C_{43}H_{49}NO_{18}Na$ requires 890.2847; IR ν_{max} (film) 3443; 2948; 1742; 1386; 1240; 1018; 758; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 3.

4.4.8. O^9-Benzoyl-O^9-deacetylevonine **18**

Colourless oil, $[\alpha]_D$ 13.8 (*c* 3.10, $CHCl_3$); HR-EIMS *m*/*z* 846.2595, $C_{41}H_{45}NO_{17}Na$ requires 846.2585; IR ν_{max} (film) 3461; 2925; 1748; 1386; 1248; 1090; 766; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 3.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2010.06.023.

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