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Triterpenoid Glycosides from Leaves of *Medicago arborea* L.

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Eighteen triterpene saponins (**1**−**18**) from *Medicago arborea* leaves have been isolated and their structures elucidated by spectroscopic, spectrometric (1D and 2D NMR, FAB-MS, ESI-MS/MS), and chemical methods. They have been identified as glycosides of medicagenic, zanhic, and 2β-hydroxyoleanolic acids, soyasapogenol B, bayogenin, and 2β,3β-dihydroxyolean-12-en-23-al-28-oic acid. Twelve of them, identified as 3-*O*-β-D-glucopyranosyl-28-*O*-[α-L-arabinopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside] zanhic acid (**3**), 3-*O*-β-D-glucopyranosyl-28-*O*-{β-D-xylopyranosyl(1→4)-[α-L-arabinopyranosyl-(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside} zanhic acid (**4**), 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→2)-β-D-glucopyranosyl]-2β-hydroxyoleanolic acid (**5**), 3-*O*-β-D-glucuronopyranosyl-28-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]medicagenic acid (**6**), 3-*O*-β-D-glucuronopyranosyl-28-*O*-[β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]bayogenin (**9**), 3-*O*-β-D-glucuronopyranosyl-28-*O*-[β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]-2β,3β-dihydroxyolean-12-en-23-al-28-oic acid (**10**), 3-*O*-β-D-glucuronopyranosyl-28-*O*-{β-D-xylopyranosyl(1→4)-[β-D-apiofuranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}zanhic acid (**12**), 3-*O*-β-D-glucuronopyranosyl-28-*O*-{β-D-xylopyranosyl(1→4)-[α-L-arabinopyranoside(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}zanhic acid (**13**), 3-*O*-β-D-glucuronopyranosyl-28-*O*-[β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]zanhic acid (**14**), 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-28-*O*-{β-D-xylopyranosyl(1→4)-[β-D-apiofuranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}zanhic acid (**16**), 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-28-*O*-{β-D-xylopyranosyl(1→4)-[α-L-arabinopyranosyl(1→3)]-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside}zanhic acid (**17**), and 3-*O*-β-D-glucuronopyranosyl-28-*O*-{β-D-xylopyranosyl(1→4)-[β-D-apiofuranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}medicagenic acid (**18**), are reported as new natural compounds. The presence of the aldehydic group on the sapogenin moiety of saponin **10** is discussed in the framework of a possible elucidation of the biosynthesis of these metabolites.

KEYWORDS: ***Medicago arborea*** **L.; saponins; chemical structure; triterpene glycosides; FAB-MS; ESI-MS/MS; NMR**

INTRODUCTION

As a continuation of our previous studies on different species of *Medicago*, we have characterized the saponins from *M. arborea* L., commonly known as tree medic. This plant is a woody shrub growing in many Mediterranean areas, showing good regrowth ability after summer stasis. It represents one of the most interesting plants used to prevent soil erosion in semiarid locations and to increase forage availability. Agronomic investigations in Italy allowed the selection of the best-performing phenotypes of *M. arborea* in terms of biomass production. The presence of antinutritional compounds such as saponins might, however, constitute a negative trait for the breeder (*1*, *2*).

The occurrence of saponins in *M. arborea* has been known since 1940 (*3*), but their detailed stuctures are still largely unknown. Only sparse data on secondary metabolites isolated from this species are, in fact, reported (*4*, *5*). Saponins from *M. sativa* (alfalfa) have been particularly studied due to the

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importance of this species for animal feeding, and several papers have been published on the subject (*6*−*24*). However, knowledge of the occurrence and chemical structures of saponins in other *Medicago* species is very limited, and only some chemical investigations on *M. lupulina* (*25*), *M. hispida* (*26*, *27*), *M. polymorpha* (*28*), *M. arabica* (*29*), and *M. truncatula* (*30*, *31*) have been previously reported.

Saponins from *Medicago* species are high molecular weight triterpenic glycosides, with medicagenic acid, hederagenin, zanhic acid, bayogenin, and soyasapogenols A and B as the predominant aglycone moieties. They are reported to be responsible for various biological and physiological effects such as antimicrobial, fungistatic, insecticidal, allelopathic, cholesterol-binding, membrane depolarizing, and hemolytic properties (*32*, *33*). A cytotoxic activity has also been observed (*34*). Recently, results on the in vitro antimicrobiotic activity of saponins from *M. sativa*, *M. arborea*, and *M. arabica* against a selection of medically important yeasts and Gram-positive and Gram-negative bacteria have also been described (*35*).

Due to the increasing interest in these natural compounds, the present study reports on the structural characterization of saponins from *M. arborea* leaves. Saponins from this species have never been extensively investigated before.

MATERIALS AND METHODS

Plant Material. *M. arborea* L. was grown at the C.R.A. Aerial parts were collected at the beginning of flowering, dried at 40 °C, and ground for successive extractions of saponins.

Extraction and Purification. Powdered leaves (700 g) were defatted with $CHCl_3$ in a Soxhlet apparatus (fats = 5.5% DM). Defatted material (500 g) was refluxed with 80% MeOH for 24 h. The solvent was removed under reduced pressure, and the residue was suspended in water. The solution was applied onto a 100 × 60 mm, 40−63 μm LiChroprep RP-18 column (Merck, Darmstadt, Germany), preconditioned with water. Elution was carried out with water (500 mL) and successively with 40% MeOH (500 mL) to remove sugars and some phenolics. Total saponins were then eluted with MeOH (300 mL) and dried under vacuum. The yield was 4.5% (22.5 g of crude saponins).

Fractionation. Total saponins (10 g) were dissolved in *n*-BuOH saturated with water and submitted to a 400 × 35 mm, 40−60 μm silica gel column (Merck). Fractions were eluted with *n*-BuOH saturated with water and checked with either or both silica gel 60H, developed with AcOEt/acetic acid/water (7:2:2), or RP-18 TLC plates (Merck), developed with 75% MeOH. Chromatograms were sprayed with 10% sulfuric acid in MeOH and heated at 120 °C. Separation on silica gel column gave six fractions: I (400 mg), II (280 mg), III (870 mg), IV (1.64 g), V (890 mg), and VI (1.07 g).

Separation. Single saponins were purified from fractions I−VI by means of reversed-phase chromatography on a 400 × 25 mm, 40−63 μm, LiChroprep RP-18 (Merck) column and eluted with diluted MeOH. Fraction I, eluted with 60% MeOH, afforded pure saponin **1** (35 mg); fraction II, 70% MeOH, yielded saponin **2** (80 mg); fraction III, 55% MeOH, gave a mixture of saponins **3** and **4** (165 mg), and subsequent elution with 70% MeOH provided saponin **5** (5 mg). Fraction IV purified with 50% MeOH afforded saponin **6** (110 mg), further elution with 65% MeOH gave saponin **7** (70 mg), and still further elution with 70% MeOH provided saponin **8** (154 mg). A mixture of saponins **9** and **10** (96 mg) was obtained from fraction V with 50% MeOH. The last fraction, VI, eluted with 50% MeOH, gave a mixture of saponins **11**−**14** (215 mg), and elution with 55% MeOH gave a mixture of saponins **15**−**18** (308 mg).

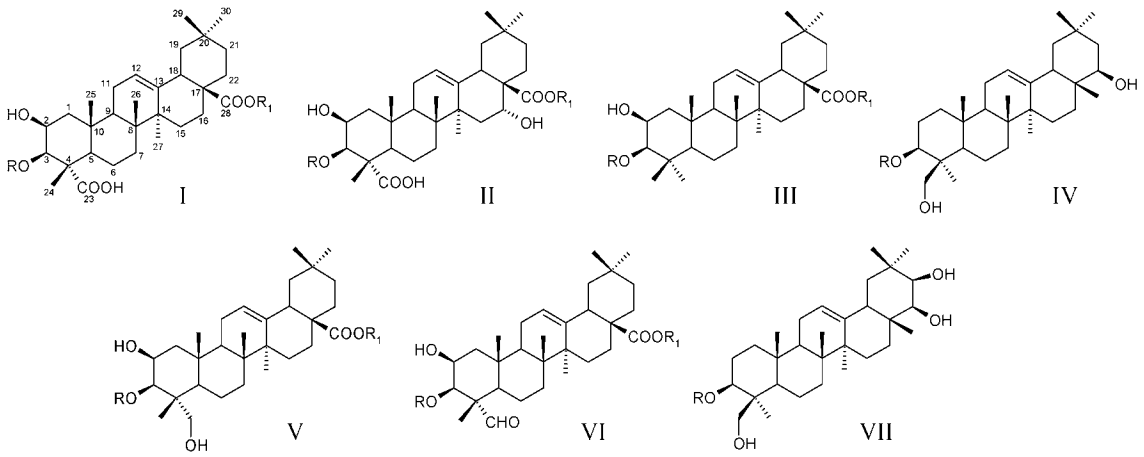
HPLC Analyses. All of the fractions obtained by reversed phase chromatography were analyzed by HPLC (Perkin-Elmer, Norwalk, CT) equipped with an LC250 binary pump and a DAD 235 detector. Separation was performed on a 250 × 4.6 mm i.d., 5 μm, Discovery C18 column (Supelco, Bellefonte, PA) with the following mobile phase: solvent A, CH_3CN/0.05% CF_3COOH; solvent B, H_2O/1% MeOH/0.05% CF_3COOH. Chromatographic runs were carried out under gradient elution from 25% (5 min isocratic condition) to 90% of solvent A in 50 min. Ten microliters of MeOH solutions (1 mg/mL) of all samples was injected. Saponins were eluted at 1.0 mL/min and detected by UV monitoring at 215 nm.

Pure saponins **3**, **4**, and **9**−**18** were obtained from the prepurified fractions by preparative HPLC using a 250 × 10 mm i.d., 5 μm, Discovery C18 column (Supelco) and the same chromatographic conditions as described above, flow rate =2.0 mL/min, injecting 100 μL of MeOH solutions of 10 mg/mL saponins. Saponins **3** (28 mg) and **4** (9 mg) were obtained from the first fraction eluted from fraction III; saponins **9** and **10** (10 mg each) from fraction V; and saponins **11** (9 mg), **12** (28 mg), **13** (25 mg), **14** (12 mg), **15** (7 mg), **16** (21 mg), **17** (10 mg), and **18** (16 mg) were obtained as pure compounds from the two fractions eluted from VI.

Hydrolysis of Saponins. Saponin crude mixtures (5 mg) and each individual pure saponin (1 mg) were treated with 0.5 mL of 2 N HCl in 50% aqueous MeOH in a stoppered test tube and stirred at 80 °C for 8 h. After cooling, MeOH was eliminated with a stream of N_2 and aglycones extracted with AcOEt (2 × 0.5 mL). Both the organic solution, containing the aglycones, and the aqueous solution, containing sugars, were dried under N_2 and used for the successive analyses.

Analysis of Sapogenins. Aglycones were identified by TLC, GC-FID, and GC-MS. Sapogenins were compared to previously purified alfalfa sapogenins (*36*) by TLC (Merck, silica gel 60H ready to use) developed with petroleum ether/$CHCl_3$/acetic acid (7:2:1) or benzene/MeOH (9:1). Spots were visualized by spraying with MeOH/acetic anhydride/H_2SO_4 (10:1:1 v/v) followed by heating at 120 °C. Sapogenins were also analyzed by GC-FID and GC-MS as their methyl-peracetyl/silyl derivatives. Aglycones were dissolved in 0.5 mL of MeOH and treated with CH_2N_2. This solution was divided in two subsamples and the solvent eliminated under a stream of N_2. Acetylation was performed by using 0.2 mL of pyridine/acetic anhydride/4-(dimethylamino)pyridine (1:1:0.1). After overnight stirring, water was added and methylated-peracetylated compounds were extracted with AcOEt (3 × 1 mL). The solvent was dried over anhydrous Na_2SO_4, concentrated under a stream of N_2, and used for GC-FID and GC-MS analyses. Silylation was performed on the methylated sapogenin subsamples using 0.2 mL of a mixture of pyridine/hexamethyldisilazane/chlorotrimethylsilane (2:1:1) at 70 °C for 10 min. Samples were diluted with isooctane and used for GC-FID and GC-MS analyses. GC-FID analyses of both methylated-acetylated and methylated-silylated sapogenins were carried out using a Perkin-Elmer (Norwalk, CT) model 8500 GC equipped with a 30 m × 0.32 mm i.d., 0.25 μm, DB-5 capillary column. Injector and detector temperatures were set at 350 °C, and the oven temperature program was as follows: 90 °C for 5 min, increased at 20 °C/min to 250 °C for 1 min and then increased at 4 °C/min to 350 °C for 15 min. Samples (1 μL) were injected in the splitless mode. He was the carrier gas with a head pressure of 12.2 psi. GC-MS analyses were carried out using a Perkin-Elmer Clarus 500 GC equipped with a MS detector and a 30 m × 0.25 mm i.d., 0.25 μm, Elite-5MS capillary column using the same chromatographic conditions as for GC-FID. Mass spectra were acquired over the 50−850 amu range at 1 scan/s with an ionizing electron energy of 70 eV. Transfer line temperature was 300 °C, and the carrier gas was He at 1.2 mL/min. Retention times and MS spectra were compared to those of previously identified sapogenins, except for the aglycone of saponin **10**, and further characterized by NMR experiments.

Analyses of Sugars and Determination of Absolute Configuration. Sugars were separated on Merck cellulose plates with benzene/ButOH/pyridine/water (1:5:3:3), made visible with a silver nitrate spray and identified by comparison with authentic reference compounds. The determination of sugar absolute configurations was carried out by GC-FID using a 30 m × 0.32 mm i.d., 0.25 μm Chirasil-Val column (Alltech, Deerfield, IL). Sugars were suspended in MeOH (0.5 mL) and treated with CH_2N_2. Solvent was eliminated under a stream of N_2 and the residue dissolved in 1-(trimethylsilyl)imidazole (Tris-Z) and pyridine (1:1; 0.3 mL), on stirring at 60 °C for 5 min. After the solution had been dried under N_2, the residue was separated by water and CH_2Cl_2 (1 mL, 1:1). The organic layer was used for GC analyses. GC temperature gradient was as follows: 60 °C for 3 min, raised to 200 °C at 5 °C/min; injector and detector temperatures were set at 200 and



Compound	Aglycone	R	R_1
1	I	β-D-Glc	α-L-Rha-(1→2)-α-L-Ara
2	I	β-D-Glc	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara
3	II	β-D-Glc	α-L-Ara -(1→3)-α-L-Rha-(1→2)-α-L-Ara
4	II	β-D-Glc	β-D-Xyl -(1→4)-α-L-Rha-(1→2)-α-L-Ara α-L-Ara -(1→3)-┘
5	III	α-L-Rha-(1→2)-α-L-Ara-(1→2)-β-D-Glc	-
6	I	β-D-GlcA	α-L-Rha-(1→2)-α-L-Ara
7	I	β-D-Glc-(1→2)-β-D-Glc	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara
8	IV	α-L-Rha-(1→2)-β-D-Gal-(1→2)-β-D-GlcA	-
9	V	β-D-GlcA	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara
10	VI	β-D-GlcA	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara
11	VII	α-L-Rha-(1→2)-β-D-Gal-(1→2)-β-D-GlcA	α-L-Rha
12	II	β-D-GlcA	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara β-D-Api-(1→3)-┘
13	II	β-D-GlcA	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara α-L-Ara-(1→3)-┘
14	II	β-D-GlcA	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara
15	I	β-D-GlcA	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara
16	II	α-L-Ara-(1→2)-β-D-Glc-(1→2)-β-D-Glc	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara β-D-Api-(1→3)-┘
17	II	β-D-Glc-(1→2)-β-D-Glc	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara α-L-Ara-(1→3)-┘
18	I	β-D-GlcA	β-D-Xyl-(1→4)-α-L-Rha-(1→2)-α-L-Ara β-D-Api-(1→3)-┘

Figure 1. Structures of saponins **1**–**18** (I, medicagenic acid; II, zanhic acid; III, 2β-hydroxyoleanolic acid; IV, soyasapogenol B; V, bayogenin; VI, 2β,3β-dihydroxyolean-12-en-23-ale-28-oic acid; VII, soyasapogenol A).

250 °C, respectively. He was the carrier gas with a head pressure of 12 psi; samples (0.2 μL) were injected in the splitless mode. Authentic reference compounds from Sigma-Aldrich, treated in the same way as reported above, were used for sugar identifications. Co-injection of each hydrolysate with the standards gave single peaks. Sugar identification was also carried out by GC-MS as described in Tava et al. (*36*).

Hydrolysis of Saponin 10. Pure saponin **10** (8.0 mg) was treated with 1 mL of 2 N HCl in 50% aqueous MeOH and stirred at 80 °C for 8 h. After cooling, MeOH was eliminated under N_2 and the aglycone extracted with AcOEt (3 × 1 mL). Solvent evaporation gave 3 mg of pure sapogenin VI (**Figure 1**).

NMR Analyses. ^{1}H and ^{13}C NMR were measured on a Bruker AV-300 spectrometer at the operating frequencies of 300.13 and 75.13 MHz, respectively. The samples were examined as solutions in pyridine-d_5 (5−10 mg/0.5 mL for saponins, 3 mg/0.5 mL for sapogenins) in 5 mm tubes at 25 °C. ^{1}H and ^{13}C chemical shifts were expressed in parts per million relative to pyridine signals at 7.2 and 123.5 ppm, respectively. 2D NMR experiments (H,H DQF-COSY; H,H TOCSY; H,C HSQC;

H,C HMBC) were carried out on all compounds using the phase sensitive method. On the basis of 2D NMR analyses, assignments of ^{1}H and ^{13}C signals were obtained. NMR analyses of saponins **3**, **4**, and **12**−**18** were also recorded in CD_3OD (data not shown).

FAB-MS Analyses. FAB-MS spectra were run on a Finnigan MAT spectrometer. The samples were homogeneously mixed with glycerol and bombarded with 13-kV cesium-beam atoms. Mass spectra were recorded in the range of *m*/*z* 100−1500 by scanning the magnetic field in 10 s with mass resolution 1000.

ESI-MS/MS. Analyses were performed on a 1100 series Agilent LC/MSD Trap-System VL. An Agilent Chemstation was used for data acquisition and processing (LC/MSD TrapSoftware 4.1). All of the analyses were carried out using an ESI ion source in the negative mode with the following settings: capillary voltage, 4000 V; nebulizer gas (N_2), 15 psi; drying gas (N_2), heated at 350 °C and introduced at a flow rate of 5 L/min. Full-scan spectra were acquired over the range of *m*/*z* 100−2200 with a scan time of 13000 *m*/*z*/s. Automated MS/MS was performed by isolating the base peaks (molecular ions) using an isolation width of 4.0 *m*/*z*, a fragmentation amplitude of 1.0 V, a threshold set at 100, and the ion charge control on, with a maximum acquire time set at 300 ms. Samples were dissolved in MeOH/H_2O (9:1) at the concentration of 20−30 ppm and injected by direct infusion at a flow rate of 10 μL/min with a syringe pump (KDScientific).

Melting points were determined using a Buchi (Flawil, Switzerland) apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded in KBr with a Spectrum 2000 instrument. Elemental analyses were carried out on a Carlo Erba instrument. Spectroscopic data here reported equally refer to all of the analyzed saponins: IR ν_{max}^{KBr} cm^{-1}, 3200−3700 (large, OH), 2940 (CH), 1735 (C═O), 1644 (C═C), 1000−1250 (large, C−O). Molecular formulas obtained by elemental analysis of saponins were in agreement with data obtained by MS analysis.

RESULTS AND DISCUSSION

Preliminary TLC investigations of saponin extracts from *M. arborea* showed a different composition from those of *M. sativa* (*24*) and *M. arabica* (*29*). GC-FID and GC-MS analyses of derivatized sapogenins confirmed that medicagenic acid was the major aglycone, representing ∼34% of the total aglycones, followed by zanhic acid (22%) and soyasapogenols, altogether amounting to 14%. Identification and quantification of zanhic acid were achieved on the basis of all the peaks, identified as artifacts (unpublished results), obtained after acid hydrolyses of pure zanhic acid saponin **3** analyzed by GC and GC-MS. Bayogenin (5%), oleanolic acid (1%), 2β-hydroxyoleanolic acid (1%), and hederagenin (<1%) were also identified together with a novel sapogenin (2%) identified as 2β,3β-dihydroxyolean-12-ene-23-ale-28-oic acid and named medicagenic aldehyde.

Crude saponins obtained from the defatted leaves of *M. arborea* (4.5%) were fractionated by a combination of silica gel, RP-18 open column chromatography, and preparative HPLC to afford 18 saponins (**1**−**18**) in a pure form.

To elucidate their chemical structures, spectroscopic and chemical techniques were employed. Aglycone moieties obtained from each of the pure saponins were identified by TLC R_f values, GC-FID, GC-MS, and NMR data compared to those of reference compounds and to literature data (*24*, *29*). The presence of medicagenic acid in saponins **1**, **2**, **6**, **7**, **15**, and **18**, zanhic acid in saponins **3**, **4**, **12**−**14**, **16**, and **17**, soyasapogenol A in saponin **11**, soyasapogenol B in saponin **8**, bayogenin in saponin **9**, and 2β-hydroxyoleanolic acid in saponin **5** could be established. A new aglycone, containing an aldehydic group, was detected in saponin **10**.

Sugar moieties were identified by means of TLC, comparing the R_f values with those of reference compounds. The absolute configuration of the sugar residues was obtained from GC analysis on a chiral column.

The assignment of all ^{1}H and ^{13}C signals for each pure saponin was obtained by 2D NMR experiments. Their structure elucidation was performed by NMR and MS (FAB-MS and ESI-MS/MS). The molecular weights were obtained from elemental analyses and MS spectra and evaluated from NMR signals in which all carbons were revealed. MS fragmentation ions were also used to establish sugar chains in the molecule. The chemical structure of the identified saponins **1**−**18** are reported in **Figure 1**, ^{13}C NMR chemical shifts are reported in **Tables 1** and **2**, and ^{1}H NMR data are reported in **Tables 3** and **4**. All of the monosaccharides but apiose were determined to be in the pyranose form from their ^{13}C NMR data. Sugar configurations were also established by comparison of their spectroscopic data with literature values (*37*, *38*).

Compound **1**, 35 mg, MW 942, after acid hydrolysis released medicagenic acid and arabinose, rhamnose, and glucose, in the ratio 1:1:1. Its spectroscopic characteristics were identical to those of 3-*O*-[β-D-glucopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]medicagenic acid, previously identified in *M. sativa* (*16*, *24*).

Compound **2**, 80 mg, MW 1074, after acid hydrolysis gave medicagenic acid and arabinose, rhamnose, xylose, and glucose, in the ratio 1:1:1:1, and had spectral characteristics identical to those of 3-*O*-[β-D-glucopyranosyl]-28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]medicagenic acid. This saponin was previously found in *M. sativa* (*11*, *14*, *24*) and *M. truncatula* (*31*).

Compound **3**, 28 mg, was isolated as an amorphous solid: mp 268−270 °C, $[\alpha]^{25}_{D}$ −24.6 (MeOH, *c* 0.18). The molecular formula was determined to be $C_{52}H_{82}O_{24}$ (MW 1090) by elemental analysis. On acid hydrolysis it gave zanhic acid and arabinose, rhamnose, and glucose, in the ratio 2:1:1. The ^{13}C NMR spectrum shows the presence of four anomeric signals at δ 107.14, 104.93, 101.13, and 93.75, confirmed in the ^{1}H spectrum in which four anomeric protons (δ 6.45, 5.94, 5.21, and 5.18) were observed. The structure of the saccharide units was determined by 2D NMR spectroscopy. A DQF-COSY experiment allowed the sequential assignment of most resonances for each sugar unit, starting from the well-isolated proton signals at δ 6.45, 5.94, 5.21, and 5.18. Complete assignments of all the proton resonances in each sugar unit were achieved by a combination of DQF-COSY and TOCSY results. In the HMBC experiment the anomeric signal at δ 5.21 (H-1_{GlcI}) showed a long-range correlation with the signal at δ 86.72 (C-3), indicating that glucose is directly linked to the triterpenic structure at C-3. The 2D NMR experiments revealed the presence of a sugar chain at the C-28 position. The resonance of the C-28 carboxylic group at δ 175.85 indicated the presence of a sugar linked in this position. This carbon gave a clear correlation in the HMBC experiment with the anomeric proton at δ 6.45 (H-1_{AraII}), indicating that arabinose was esterified at the C-28 position as the first sugar in the chain. The sugar linked to arabinose was determined as rhamnose. The position of this sugar was indicated in the HMBC spectra in which the two anomeric protons at δ 6.45 (H-1_{AraII}) and δ 5.94 (H-1_{RhaII}) correlate with the same carbon (C-2_{AraII}) at δ 74.99. The third sugar of the chain should then be arabinose, and consistently its anomeric proton at δ 5.18 (H-1_{AraIV}) gave a correlation with C-3_{RhaII} at δ 83.25. These findings were also deduced from the mass spectra. In the FAB-MS, the molecular ion $[M - H]^-$ at *m*/*z* 1089 (100%) corresponds to zanhic acid plus two pentoses, one hexose, and rhamnose. The peak at *m*/*z* 957 (1%) corresponds to the loss of a pentose unit. The other observed ions were *m*/*z* 679 (8%) [M − H − 2 pentoses − rhamnose]$^-$

Table 1. ^{13}C NMR Data (δ) of the Aglycone Moieties of Saponins **3**–**6**, **9**, **10**, **12**–**14**, and **16**–**18**

C	3	4	5	6	9	10	12	13	14	16	17	18
1	44.49	44.66	44.65	43.87	45.75	43.37	43.88	43.75	43.27	43.71	43.25	43.90
2	69.85	69.38	71.12	69.66	70.39	71.78	69.19	70.03	70.35	69.94	70.32	69.73
3	86.72	87.06	87.05	86.95	83.01	82.78	86.51	86.74	86.12	87.10	86.93	86.84
4	53.16	53.37	38.98	53.06	42.11	54.07	52.87	53.21	53.42	53.65	53.67	53.37
5	52.43	52.53	56.05	51.71	47.31	47.87	51.92	51.88	52.11	52.24	52.23	52.61
6	21.42	21.62	18.31	20.51	18.22	19.89	20.60	20.89	21.03	20.45	20.90	20.81
7	32.15	33.66	32.94	33.48	33.58	33.82	31.13	33.04	31.95	32.57	31.78	31.60
8	40.42	40.72	39.93	39.59	39.47	39.69	39.81	40.02	40.35	39.96	40.09	39.87
9	47.61	47.90	48.57	47.80	47.46	49.06	47.02	47.75	47.52	47.41	47.12	47.53
10	36.77	37.05	37.48	35.97	36.26	35.76	36.09	35.78	35.89	36.56	36.91	36.03
11	23.93	24.13	23.68	22.63	22.65	22.41	23.41	23.25	23.03	23.60	23.51	23.34
12	122.79	122.69	122.98	122.24	122.76	122.74	122.20	122.34	122.29	122.34	122.41	122.37
13	144.58	144.83	144.49	143.80	143.80	143.83	143.84	143.75	143.77	144.10	143.65	143.89
14	42.09	42.31	41.96	41.69	41.73	41.86	41.59	41.92	41.68	41.73	41.58	41.65
15	35.92	36.04	28.43	27.46	27.75	27.82	35.21	35.89	35.64	35.48	35.77	27.82
16	72.68	72.50	23.88	23.39	23.53	23.96	72.71	72.53	72.15	72.17	72.83	23.34
17	49.36	49.68	46.94	46.79	46.90	47.21	48.98	48.94	49.09	49.14	49.25	46.98
18	41.06	41.30	42.48	41.10	41.24	41.28	40.71	40.98	41.10	40.84	40.59	40.88
19	47.00	47.24	46.15	45.74	43.69	45.82	46.54	47.15	46.82	46.68	46.13	45.93
20	30.77	30.84	30.12	30.23	30.34	30.95	30.18	30.24	30.12	30.50	30.31	30.57
21	36.10	36.38	33.96	32.23	32.38	32.41	35.68	36.24	35.96	35.48	35.57	32.61
22	32.09	32.00	32.91	32.07	32.16	31.20	31.15	32.07	31.58	31.60	31.82	31.45
23	183.40	183.74	30.98	184.46	66.55	207.80	183.83	183.51	182.78	181.09	180.72	180.63
24	14.77	14.87	18.98	14.21	14.44	11.10	14.07	14.04	14.36	13.68	13.16	13.89
25	17.09	17.25	16.74	16.38	16.80	16.30	16.52	16.47	17.03	16.44	16.58	16.61
26	17.44	17.64	17.88	16.83	17.18	17.07	17.06	17.12	17.57	17.14	16.95	17.08
27	27.23	27.53	26.36	25.69	25.70	25.91	26.73	27.46	27.34	26.80	26.34	25.79
28	175.85	175.90	180.23	176.20	176.18	176.15	175.65	175.92	175.83	175.77	175.95	176.19
29	33.15	33.20	33.18	32.71	32.74	32.81	32.63	32.89	33.15	32.86	32.74	33.04
30	24.60	24.90	23.48	23.12	23.22	23.20	24.17	24.51	24.16	24.41	24.51	24.38

and *m*/*z* 517 (3%) [M − H − 2 pentoses − rhamnose − hexose]$^-$, corresponding to zanhic acid. The peak at *m*/*z* 455 (89%) originated from zanhic acid by loss of H_2O and CO_2 was also observed: ESI-MS (negative ion mode), *m*/*z* (relative intensity) 1089.6 (100%) [M ($C_{52}H_{82}O_{24}$) − H]$^-$, which fragmented in the MS/MS giving 865.4 (2%) [M − H − 162 (Glu) − CO_2 − H_2O]$^-$; 679.3 (3%) [M − H − 132(Ara) − 146(Rha) − 132(Ara)]$^-$, 455.3 (100%) [518(Zhan) − CO_2 − H_2O − H]$^-$. On the basis of these data, saponin **3** was established to be 3-*O*-β-D-glucopyranosyl-28-*O*-[α-L-arabinopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]zanhic acid.

Saponin **4**, 9 mg, was isolated as an amorphous solid: mp 281−283 °C, $[\alpha]_D^{25}$ −8.2 (MeOH, *c* 0.02). The molecular formula was determined to be $C_{57}H_{90}O_{28}$ (MW 1222). Its molecular weight was 132 mass units higher than that of **3**, which indicated the presence of an additional pentose unit. After acid hydrolysis, it released zanhic acid and arabinose, rhamnose, xylose, and glucose in the ratio 2:1:1:1. The ^1H and ^{13}C NMR spectra of **4** exhibited five anomeric protons at δ 6.50, 5.78, 5.35, 5.16, and 5.07 and carbons at δ 107.20, 105.34, 104.99, 101.22, and 94.00. NMR data for the sugar chain allowed the deductions as for compound **3**. The presence of a glucose unit at C-3 was established from the HMBC correlation between H-1_{GlcI} (δ 5.16) and C-3 (δ 87.06) of the aglycone. The sequence of the sugar chain at C-28 was determined by the following HMBC correlations: H-1_{RhaIII} (δ 5.78) with C-2_{AraII} (δ 75.22), H-1_{XylIV} (δ 5.35) with C-4_{RhaIII} (δ 78.62), and H-1_{AraV} (δ 5.07) with C-3_{RhaIII} (δ 82.86). The attachment of the tetrasaccharide moiety to C-28 of the aglycone was based on an HMBC correlation of H-1_{AraII} (δ 6.50) with C-28 (δ 175.90) of the aglycone. In the FAB-MS, the molecular ion [M − H]$^-$ at *m*/*z* 1221 (93%) corresponds to zanhic acid plus three pentoses, one hexose, and one rhamnose. The peak at *m*/*z* 1089 (100%) corresponds to the loss of a pentose unit, from which the loss of a second pentose unit gave *m*/*z* 957 (15%). The other observed ions were *m*/*z* 679 (28%) [M − H − 3 pentoses − rhamnose]$^-$ and *m*/*z* 517 (12%) [M − H − 3 pentoses − rhamnose − hexose]$^-$ corresponding to zanhic acid. The peak at *m*/*z* 455 (75%) originated from zanhic acid by loss of H_2O and CO_2 was also observed. ESI-MS (negative ion mode), *m*/*z* (relative intensity) 1221.6 (100%) [M ($C_{57}H_{90}O_{28}$) − H]$^-$, which fragmented in the MS/MS giving 1089.6 (5%) [M − H − 132 (Ara)]$^-$, 977.7 (5%) [M − H − 162(Glu) − CO_2 − H_2O]$^-$, 679.4 (6%) [M − H − 132(Ara) − 146(Rha) − 132 (Xyl)]$^-$, 455 (65%) [518(Zhan) − CO_2 − H_2O − H]$^-$. This compound was identified as 3-*O*-β-D-glucopyranosyl-28-*O*-{β-D-xylopyranosyl(1→4)-[α-L-arabinopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}zanhic acid.

Saponin **5**, 5 mg, was isolated as an amorphous solid: mp 227−229 °C, $[\alpha]_D^{25}$ 3.4 (MeOH, *c* 0.10). The molecular formula was calculated as $C_{47}H_{76}O_{17}$ (MW 912). After acid hydrolysis, it released 2β-hydroxyoleanolic acid and arabinose, rhamnose, and glucose in the ratio 1:1:1. The ^{13}C NMR spectrum shows the presence of three anomeric signals at δ 105.15, 102.07, and 103.70, confirmed in the ^1H spectrum in which the corresponding three anomeric protons were detected at δ 5.70, 5.15, and 5.04. The C-28 signal at δ 180.23 in the ^{13}C NMR spectrum clearly indicated that the 28-COOH function of the sapogenin is free. The sugar directly linked at the C-3 position of the sapogenin is glucose: its anomeric signal at δ 5.15 showed, as expected, a long-range correlation with C-3 at δ 87.05 in the HMBC experiments. The position of the second saccharide unit was indicated in the HMBC experiment in which the two anomeric protons at δ 5.15 (H-1_{GlcI}) and δ 5.04 (H-1_{AraII}) give a correlation with the same carbon (C-2_{GlcI}) at δ 83.86. The third sugar in the chain is rhamnose, and its anomeric proton (H-1_{RhaIII}) at δ 5.70 correlates with C-2_{AraII} at δ 75.67. The base peak in the FAB-MS spectrum is detected at *m*/*z* 911 (100%), [M − H]$^-$, which corresponds to 2β-hydroxyoleanolic acid plus a hexose, a pentose, and a rhamnose. Loss of rhamnose gave the peak at *m*/*z* 765 (14%); its fragmentation with the loss of a

Table 2. ^{13}C NMR Data (δ) of the Saccharide Moieties of Saponins **3**–**6**, **9**, **10**, **12**–**14**, and **16**–**18**

C	**3**	**4**	**5**	**6**	**9**	**10**	**12**	**13**	**14**	**16**	**17**	**18**
	Glc (I)	Glc (I)	Glc (I)	Gluc Ac (I)	Gluc Ac (I)	Gluc Ac (I)	Gluc Ac (I)	Gluc Ac (I)	Gluc Ac (I)	Glc (I)	Glc (I)	Gluc Ac (I)
1	104.93	104.99	105.15	103.62	103.52	103.34	103.68	103.89	103.71	104.38	104.81	103.45
2	74.45	74.70	**83.86**	73.80	73.88	73.92	73.82	73.85	73.81	**84.12**	**83.91**	73.90
3	77.48	77.73	78.12	75.86	76.10	76.31	77.39	77.57	76.95	78.21	78.14	77.21
4	71.22	71.62	71.41	72.57	72.75	72.50	73.01	73.28	72.88	71.47	71.33	72.95
5	78.10	78.12	77.38	75.60	75.23	75.65	75.81	76.06	75.73	78.45	78.41	76.10
6	62.28	62.65	62.30	175.89	175.70	175.67	175.41	175.05	175.23	62.21	62.35	175.54
			Ara (II)							Glc (II)	Glc (II)	
1			103.70							105.17	104.97	
2			**75.67**							**85.16**	76.51	
3			70.92							77.48	77.64	
4			66.34							71.15	71.02	
5			63.15							78.16	78.09	
6										62.36	62.43	
			Rha (III)							Ara (III)		
1			102.07							107.09		
2			72.10							73.57		
3			72.35							74.61		
4			71.28							70.29		
5			69.58							67.33		
6			18.12									
	Ara (II)	Ara (II)		Ara (II)	Ara (II)	Ara (II)	Ara (II)	Ara (II)	Ara (II)	Ara (IV)	Ara (III)	Ara (II)
1	93.75	94.00		92.52	92.82	92.69	92.73	93.05	92.81	92.98	92.51	92.77
2	**74.99**	**75.22**		**74.69**	**74.58**	**74.90**	**74.62**	**74.51**	**74.95**	**75.17**	**74.91**	**75.06**
3	70.41	70.41		69.22	71.06	71.20	70.94	70.90	71.15	70.63	70.41	71.16
4	66.78	66.38		65.39	65.80	65.41	66.07	65.94	65.48	65.53	65.72	66.12
5	62.50	63.07		62.14	62.69	62.15	63.34	63.62	62.76	62.31	62.28	62.57
	Rha (III)	Rha (III)		Rha (III)	Rha (III)	Rha (III)	Rha (III)	Rha (III)	Rha (III)	Rha (V)	Rha (IV)	Rha (III)
1	101.13	101.22		100.66	100.57	100.57	100.08	100.25	100.18	100.50	100.71	100.94
2	71.61	72.03		72.36	72.04	71.98	72.12	72.45	71.94	72.31	72.09	72.17
3	**83.25**	**82.86**		71.08	71.56	71.23	**81.38**	**81.49**	71.31	**81.53**	**81.42**	**81.65**
4	72.68	**78.62**		71.32	**82.05**	**82.27**	**79.12**	**79.08**	**82.31**	**78.96**	**79.03**	**79.27**
5	69.85	70.55		69.21	69.40	69.13	69.30	69.90	69.33	68.97	69.12	69.08
6	18.33	18.38		17.70	17.83	17.64	17.72	17.87	17.96	17.75	17.88	17.83
	Ara (IV)	Xyl (IV)			Xyl (IV)	Xyl (IV)	Xyl (IV)	Xyl (IV)	Xyl (IV)	Xyl (VI)	Xyl (V)	Xyl (IV)
1	107.14	105.34			106.09	105.99	105.72	105.14	106.12	106.15	106.23	105.78
2	73.75	75.89			74.11	74.35	75.21	74.48	74.85	74.85	74.55	74.92
3	74.18	77.95			77.46	77.63	77.01	77.18	77.23	77.41	77.12	77.67
4	70.38	72.04			71.78	71.25	72.31	72.45	71.88	71.15	71.03	71.28
5	67.08	66.78			66.55	66.82	66.28	66.41	66.37	66.63	66.89	66.41
		Ara (V)					Api (V)	Ara (V)		Api (VII)	Ara (VI)	Api (V)
1		107.20					110.49	106.77		110.04	107.18	110.98
2		73.32					77.61	73.23		78.34	73.31	78.08
3		74.75					80.75	75.50		80.43	75.68	80.54
4		70.51					75.48	70.81		74.93	70.53	75.17
5		67.24					65.26	68.02		65.52	67.96	65.11

pentose gives the peak at *m*/*z* 633 (16%), and from this last ion the loss of a hexose originates the peak at *m*/*z* 471 (4%) corresponding to 2β-hydroxyoleanolic acid. ESI-MS/MS confirmed the above data. ESI-MS (negative ion mode), *m*/*z* (relative intensity): 911.7 (100%) $[M(C_{47}H_{76}O_{17}) - H]^-$, which fragmented in the MS/MS giving 893.7 (100%) $[M - H - H_2O]^-$, 849.6 (25%) $[M - H - CO_2 - H_2O]^-$, 703.6 (36%) $[M - H - 146$ (Rha) $- CO_2 - H_2O]^-$, 615.5 (84%) $[M - H - 18 - 146$(Rha) $- 132$(Ara)$]^-$, 453.4 (40%) [472(OHOlea) $- H - H_2O]^-$. This compound was identified as 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→2)-β-D-glucopyranosyl]-2β-hydroxyoleanolic acid.

Compound **6**, 110 mg, was isolated as an amorphous solid: mp 240−242 °C, $[\alpha]^{25}_D$ −10.0 (MeOH, *c* 0.07). The molecular formula was calculated as $C_{47}H_{72}O_{20}$ (MW 956). After acid hydrolysis, it released medicagenic acid and arabinose, rhamnose, and glucuronic acid in the ratio 1:1:1. Three anomeric signals were evident at δ 103.62, 100.66, and 92.52 in the ^{13}C NMR spectrum and at δ 6.51, 5.60, and 4.96 in the ^1H NMR spectrum. In the HMBC experiment the anomeric signal at δ 4.96 (H-1_{GluAcI}) showed a long-range correlation with C-3 at δ 86.95, indicating that glucuronic acid is directly linked to the sapogenin at this position. The C-28 carboxylic group at δ 176.20 clearly suggested the presence of a sugar linked to this carbon atom, whereas the signal at δ 184.46 indicated that the C-23 carboxylic group is free. In the HMBC experiment a correlation between the C-28 and the anomeric proton at δ 6.51 (H-1_{AraII}) was observed. The sugar linked to arabinose is rhamnose, and its anomeric proton at δ 5.60 (H-1_{RhaIII}) gave a correlation with C-2_{AraII} at δ 74.69. The structure was also confirmed by MS (FAB-MS and ESI-MS/MS). In the FAB-MS spectrum, the molecular ion $[M - H]^-$ at *m*/*z* 955 (33%) corresponds to medicagenic acid plus uronic acid, plus a pentose, plus a rhamnose. From the molecular ion the loss of rhamnose gave the peak at *m*/*z* 809 (1%), and the loss of uronic acid gave the ion at *m*/*z* 779 (3%). The other observed peaks were *m*/*z* 677 (6%) $[M - H -$ rhamnose $-$ pentose$]^-$, *m*/*z* 633 (4%) $[M - H -$ uronic acid $-$ rhamnose$]^-$, and *m*/*z* 501 (3%), which corresponds to medicagenic acid. The peak at *m*/*z* 439 (89%), originating from medicagenic acid by loss of H_2O and CO_2,

Table 3. ^{1}H NMR Data of the Aglycone Moieties of Saponins **3**–**6**, **9**, **10**, **12**–**14**, and **16**–**18**[a]

proton	3	4	5	6	9	10
H-1a	1.55, 1H, dd (14.0, 4.0)	1.58, 1H, dd (14.0, 4.0)	1.14, 1H, dd (14.0, 4.0)	1.71, 1H, dd (14.0, 4.0)	1.38, 1H, dd (14.0, 4.0)	1.45, 1H, dd (14.0, 4.0)
H-1b	2.53, 1H, dd (14.0, 4.0)	2.49, 1H, dd (14.0, 4.0)	1.58, 1H, dd (14.0, 4.0)	2.64, 1H, dd (14.0, 4.0)	2.41, 1H, dd (14.0, 4.0)	2.52, 1H, dd (14.0, 4.0)
H-2	4.88, 1H, m	4.91, 1H, m	4.81, 1H, m	4.93, 1H, m	4.85, 1H, m	4.25, 1H, m
H-3	4.38, 1H, d (4.0)	4.35, 1H, d (4.0)	4.31, 1H, d (4.0)	4.27, 1H, d (4.0)	4.52, 1H, d (4.0)	4.46, 1H, d (4.0)
H-5	1.60, 1H, dd (12.0, 4.0)	1.63, 1H, dd (12.0, 4.0)	1.50, 1H, dd (12.0, 4.0)	1.75, 1H, dd (12.0, 4.0)	1.71, 1H, dd (12.0, 4.0)	1.52, 1H, dd (12.0, 4.0)
H-6	1.20 e 1.74, 2H, m	1.25 e 1.73, 2H, m	1.22 e 1.67, 2H, m	1.18 e 1.74, 2H, m	1.18 e 1.70, 2H, m	1.20 e 1.68, 2H, m
H-7	1.15 e 1.35, 2H, m	1.14 e 1.38, 2H, m	1.10 e 1.36, 2H, m	1.20 e 1.44, 2H, m	1.14 e 1.38, 2H, m	1.15 e 1.40, 2H, m
H-9	1.77, 1H, m	1.72, 1H, m	1.65, 1H, m	1.81, 1H, m	1.78, 1H, m	1.73, 1H, m
H-11	1.95–2.20, 2H, m	1.95–2.20, 2H, m	1.94–2.10, 2H, m	2.00–2.15, 2H, m	1.97–2.20, 2H, m	1.85–2.10, 2H, m
H-12	5.69, 1H, t (3.0)	5.73, 1H, t (3.0)	5.78, 1H, t (3.0)	5.85, 1H, t (3.0)	5.82, 1H, t (3.0)	5.73, 1H, t (3.0)
H-15	2.00 e 2.35, 2H, m	2.05 e 2.35, 2H, m	1.90 e 2.35, 2H, m	1.15 e 2.28, 2H, m	1.18 e 2.32, 2H, m	1.80 e 2.10, 2H, m
H-16	4.53, 1H, m	4.58, 1H, m	1.85–2.10, 2H, m	2.15–2.25, 2H, m	2.05 e 2.19, 2H, m	1.95 e 2.20, 2H,m
H-18	3.31, 1H, dd (14.0, 4.0)	3.39, 1H, dd (14.0, 4.0)	3.24, 1H, dd (14.0, 4.0)	3.30, 1H, dd (14.0, 4.0)	3.32, 1H, dd (14.0, 4.0)	3.35, 1H, dd (14.0, 4.0)
H-19	1.30 e 1.75, 2H, m	1.35 e 1.75, 2H, m	1.27 e 1.73, 2H, m	1.35 e 1.70, 2H, m	1.32 e 1.73, 2H, m	1.27 e 1.70, 2H, m
H-21	1.80–1.95, 2H, m	1.80–1.90, 2H, m	1.75–1.85, 2H, m	1.80–1.95, 2H, m	1.81 e 1.89, 2H, m	1.78 e 1.85, 2H,m
H-22	1.58–1.70, 2H, m	1.60–1.73, 2H, m	1.30–1.45, 2H, m	1.72–1.84, 2H, m	1.71 e 1.83, 2H, m	1.40 e 1.52, 2H, m
H-23a					3.62, 1H, bd (12.0)	9.87, 1H, s
H-23b					4.35, 1H bd (12.0)	
Me-23			1.33, 3H, s			
Me-24	1.95, 3H, s	1.98, 3H, s	0.82, 3H, s	1.97, 3H, s	1.90, 3H, s	1.73, 3H, s
Me-25	1.68, 3H, s	1.61, 3H, s	1.18, 3H, s	1.62, 3H, s	1.61, 3H, s	1.65, 3H, s
Me-26	1.21, 3H, s	1.18, 3H, s	1.02, 3H, s	1.19, 3H, s	1.16, 3H, s	1.12, 3H, s
Me-27	1.36, 3H, s	1.38, 3H, s	1.24, 3H, s	1.38, 3H, s	1.20, 3H, s	1.35, 3H, s
Me-29	1.03, 3H, s	1.10, 3H, s	0.92, 3H, s	1.09, 3H, s	1.09, 3H, s	1.02, 3H, s
Me-30	0.97, 3H, s	0.95, 3H, s	0.86, 3H, s	0.98, 3H, s	1.04, 3H, s	0.95, 3H, s

proton	12	13	14	16	17	18
H-1a	1.53, 1H, dd (14.0, 4.0)	1.63, 1H, dd (14.0, 4.0)	1.57, 1H, dd (14.0, 4.0)	1.60, 1H, dd (14.0, 4.0)	1.58, 1H, dd (14.0, 4.0)	1.75, 1H, dd (14.0, 4.0)
H-1b	2.48, 1H, dd (14.0, 4.0)	2.49, 1H, dd (14.0, 4.0)	2.53, 1H, dd (14.0, 4.0)	2.54, 1H, dd (14.0, 4.0)	2.50, 1H, dd (14.0, 4.0)	2.71, 1H, dd (14.0, 4.0)
H-2	4.81, 1H, m	4.95, 1H, m	4.89, 1H, m	4.97, 1H, m	4.86, 1H, m	4.86, 1H, m
H-3	4.35, 1H, d (4.0)	4.41, 1H, d (4.0)	4.40, 1H, d (4.0)	4.39, 1H, d (4.0)	4.41, 1H, d (4.0)	4.32, 1H, d (4.0)
H-5	1.58, 1H, dd (12.0, 4.0)	1.63, 1H, dd (12.0, 4.0)	1.62, 1H, dd (12.0, 4.0)	1.65, 1H, dd (12.0, 4.0)	1.61, 1H, dd (12.0, 4.0)	1.77, 1H, dd (12.0, 4.0)
H-6	1.22 e 1.70, 2H, m	1.25 e 1.70, 2H, m	1.23 e 1.72, 2H, m	1.26 e 1.78, 2H, m	1.20 e 1.70, 2H, m	1.21 e 1.83, 2H, m
H-7	1.25 e 1.38, 2H, m	1.16 e 1.40, 2H, m	1.14 e 1.35, 2H, m	1.12 e 1.37, 2H, m	1.15 e 1.33, 2H, m	1.23 e 1.48, 2H, m
H-9	1.83, 1H, m	1.79, 1H, m	1.80, 1H, m	1.75, 1H, m	1.79, 1H, m	1.75, 1H, m
H-11	1.90–2.20, 2H, m	1.95–2.15, 2H, m	1.94–2.20, 2H, m	1.90–2.22, 2H, m	1.93–2.24, 2H, m	2.50–2.18, 2H, m
H-12	5.75, 1H, t (3.0)	5.73, 1H, t (3.0)	5.69, 1H, t (3.0)	5.74, 1H, t (3.0)	5.71, 1H, t (3.0)	5.88, 1H, t (3.0)
H-15	2.00 e 2.40, 2H, m	2.05 e 2.40, 2H, m	2.10 e 2.40, 2H, m	2.05 e 2.30, 2H, m	2.00 e 2.35, 2H, m	1.12 e 2.31, 2H, m
H-16	4.61, 1H, m	4.55, 1H, m	4.62, 1H, m	4.60, 1H, m	4.58, 1H, m	2.15–2.25, 2H, m
H-18	3.38, 1H, dd (14.0, 4.0)	3.41, 1H, dd (14.0, 4.0)	3.38, 1H, dd (14.0, 4.0)	3.43, 1H, dd (14.0, 4.0)	3.48, 1H, dd (14.0, 4.0)	3.35, 1H, dd (14.0, 4.0)
H-19	1.35 e 1.75, 2H, m	1.38 e 1.74, 2H, m	1.33 e 1.72, 2H, m	1.35 e 1.76, 2H, m	1.32 e 1.74, 2H, m	1.38 e 1.76, 2H, m
H-21	1.83–1.90, 2H, m	1.83–1.91, 2H, m	1.78–1.90, 2H, m	1.81–1.89, 2H, m	1.85–1.97, 2H, m	1.81–1.94, 2H, m
H-22	1.61–1.77, 2H, m	1.62–1.79, 2H, m	1.58–1.69, 2H, m	1.58–1.70, 2H, m	1.61–1.76, 2H, m	1.74–1.86, 2H, m
H-23a						
H-23b						
Me-23						
Me-24	1.92, 3H, s	1.95, 3H, s	1.91, 3H, s	1.88, 3H, s	1.90, 3H, s	1.93, 3H, s
Me-25	1.61, 3H, s	1.58, 3H, s	1.65, 3H, s	1.69, 3H, s	1.73, 3H, s	1.59, 3H, s
Me-26	1.24, 3H, s	1.18, 3H, s	1.26, 3H, s	1.23, 3H, s	1.28, 3H, s	1.21, 3H, s
Me-27	1.38, 3H, s	1.41, 3H, s	1.37, 3H, s	1.35, 3H, s	1.41, 3H, s	1.44, 3H, s
Me-29	1.10, 3H, s	1.08, 3H, s	1.12, 3H, s	1.02, 3H, s	1.05, 3H, s	1.02, 3H, s
Me-30	0.94, 3H, s	0.93, 3H, s	0.98, 3H, s	0.92, 3H, s	0.90, 3H, s	0.96, 3H, s

[a] Assignments were established by HSQC, DQF-COSY, and TOCSY spectra. *J* values (in hertz) are given in parentheses.

was also observed. ESI-MS/MS further confirmed the above data. ESI-MS (negative ion mode): *m/z* (relative intensity) 955.5 (100%) [M($C_{47}H_{72}O_{20}$) − H]$^-$, which generated in the MS/MS the following product ions: 779.4 (5%) [M − H − uronic acid]$^-$, 679.5 (5%) [M − H − 146(Rha) − 132(Ara)]$^-$, 501.4 (13%) [502(Med) − H]$^-$. In agreement with the above data, saponin **6** was identified as 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]medicagenic acid.

Saponin **7**, 70 mg, MW 1236, released after acid hydrolysis medicagenic acid and arabinose, rhamnose, xylose, and glucose in the ratio 1:1:1:2. Its spectroscopic characteristics were identical to those reported for 3-*O*-[*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-28-*O*-[*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]medicagenic acid, previously isolated in *M. sativa* (*14*, *24*).

Saponin **8**, 154 mg, MW 942, after acid hydrolysis afforded a small amount of soyasapogenol B and soyasapogenols C, D, and F as artifacts (*39*). Rhamnose, galactose, and glucuronic acid in the ratio 1:1:1 were identified as the sugar constituents after acid hydrolysis. On the basis of these characteristics and spectroscopic data, this compound was identified as 3-*O*-[α-L-rhamnopyranosyl(1→2)-*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranoside]soyasapogenol B, also known as soyasaponin I. It is often found as a constituent of saponins from Leguminosae, and it has been previously detected in *M. sativa* (*24*).

Compound **9**, 10 mg, was isolated as an amorphous solid: mp 230−233 °C, $[\alpha]_D^{25}$ 7.0 (MeOH, *c* 0.04). The molecular formula was calculated as $C_{52}H_{82}O_{23}$ (MW 1074). The acid hydrolysis of this saponin gave bayogenin and arabinose, rhamnose, xylose, and glucuronic acid in the ratio 1:1:1:1. Four anomeric signals were observed in the ^{13}C NMR spectrum at *δ*

Table 4. ^{1}H NMR Data of the Monoaccharide Moieties of Saponins **3**–**6**, **9**, **10**, **12**–**14**, and **16**–**18**[a]

C	3	4	5	6	9	10	12	13	14	16	17	18
	Glc (I)	Glc (I)	Glc (I)	Glu Ac (I)	Glu Ac (I)	Glu Ac (I)	Glu Ac (I)	Glu Ac (I)	Glu Ac (I)	Glc (I)	Glc (I)	Glu Ac (I)
1	5.21, d (7.5)	5.16 d (7.6)	5.15, d (7.3)	4.96, d (7.6)	4.92, d (7.5)	4.88, d (7.6)	4.90, d (7.6)	4.87, d (7.5)	4.95, d (7.4)	5.18, d (7.3)	5.15, d (7.3)	4.93, d (7.5)
2	4.11	4.04	4.03	4.25	4.22	4.23	4.27	4.23	4.20	4.03	4.02	4.26
3	4.16	4.08	4.12	4.21	4.29	4.21	4.20	4.15	4.17	4.08	4.06	4.21
4	4.22	4.18	4.20	3.98	4.03	4.09	4.04	4.01	3.95	4.12	4.09	4.15
5	3.82	3.83	3.78	4.34	4.41	4.38	4.45	4.36	4.41	3.83	3.85	4.42
6	4.41, 4.55	4.30, 4.48	4.32, 4.45							4.22, 4.51	4.26, 4.42	
			Ara (II)							Glc (II)	Glc (II)	
1			5.04 d (2.0)							5.07 d (7.4)	5.02 d (7.5)	
2			4.43							4.06	4.02	
3			4.02							4.10	4.08	
4			4.15							4.22	4.19	
5			3.65, 4.48							3.75	3.71	
6										4.28, 4.49	4.35, 4.51	
			Rha (III)							Ara (III)		
1			5.70, d (0.9)							5.11 d (2.1)		
2			4.82							4.41		
3			4.37							3.98		
4			4.50							4.18		
5			1.77, d (6.1)							3.82, 4.51		
	Ara (II)	Ara (II)		Ara (II)	Ara (II)	Ara (II)	Ara (II)	Ara (II)	Ara (II)	Ara (IV)	Ara (III)	Ara (II)
1	6.45, d (2.5)	6.50, brs		6.51, d (2.4)	6.48, brs	6.53, brs	6.50, brs	6.55, brs	6.47, brs	6.51, brs	6.54, brs	6.49, brs
2	4.52	4.55		4.54	4.48	4.53	4.55	4.43	4.51	4.55	4.46	4.45
3	4.49	4.48		4.48	4.45	4.49	4.53	4.42	4.57	4.47	4.39	4.42
4	4.38	4.35		4.31	4.30	4.34	4.36	4.35	4.31	4.31	4.30	4.28
5	3.93, 4.52	3.91, 4.53		3.87, 4.61	3.85, 4.65	3.90, 4.59	3.88, 4.64	3.76, 4.59	3.69, 451	3.93, 4.51	3.91, 471	3.90, 4.62
	Rha (III)	Rha (III)		Rha (III)	Rha (III)	Rha (III)	Rha (III)	Rha (III)	Rha (III)	Rha (V)	Rha (IV)	Rha (III)
1	5.94, brs	5.78, brs		5.60, d (0.9)	5.58, brs	5.65, brs	5.76, brs	5.64, brs	5.71, brs	5.69, brs	5.65, brs	5.68, brs
2	4.55	4.59		4.48	4.51	4.47	4.63	4.82	4.78	4.66	4.71	4.50
3	4.53	4.52		4.53	4.60	4.62	4.71	4.57	4.60	4.57	4.55	4.58
4	4.35	4.38		4.37	4.39	4.41	4.38	4.51	4.48	4.51	4.46	4.36
5	4.37	4.41		4.39	4.42	4.48	4.42	4.41	4.35	4.39	4.33	4.44
6	1.78, d (6.0)	1.76, d (6.0)		1.74, d (6.1)	1.76, d (6.0)	1.81, d (6.0)	1.78, d (6.1)	1.73 d (6.2)	1.75 d (6.0)	1.77 d (6.0)	1.80(6.0)	1.78, d (6.0)
	Ara (IV)	Xyl (IV)			Xyl (IV)	Xyl (IV)	Xyl (IV)	Xyl (IV)	Xyl (IV)	Xyl (VI)	Xyl (V)	Xyl (IV)
1	5.18, d (2.1)	5.35, d (7.6)			5.25, d (7.5)	5.19, d (7.6)	5.28, d (7.6)	5.30, d (7.5)	5.23, d (7.5)	5.25, d (7.5)	5.22, d (7.5)	5.29, d (7.6)
2	3.94	3.94			3.88	3.91	3.94	3.89	3.93	3.87	3.96	3.91
3	4.18	4.20			4.21	4.20	4.01	4.05	4.24	4.19	4.22	4.17
4	4.12	4.10			4.19	4.20	4.07	4.12	4.10	4.15	4.08	4.14
5	3.32, 4.14	3.38, 4.08			3.28, 4.15	3.36, 4,18	3.40, 4.20	3.42, 4.11	3.39, 4.09	3.35, 4.12	3.29, 4.13	3.29, 4.18
		Ara (V)					Api (V)	Ara (V)		Api (VII)	Ara (VI)	Api (V)
1		5.07, brs					6.12, d (2.3)	5.11, d (2.0)		6.23, d (2.3)	5.07, brs	6.18, d (2.5)
2		4.41					4.77	4.43		4.80	4.47	4.77
3		4.03						3.98			4.02	
4		4.12					4.25, 4.70	4.18		4.21, 4.68	4.23	4.18, 4.71
5		3.52, 4.10					4.18	3.50, 4.14		4.15	3.61, 4.22	4.08

[a] Assignments were established by HSQC, DQF-COSY, and TOCSY spectra. *J* values (in hertz) are given in parentheses.

106.09, 103.52, 100.57, and 92.82 and at δ 6.48, 5.58, 5.25, and 4.92 in the ^{1}H NMR spectrum. The C-3 position of bayogenin was glycosylated with a glucuronic acid: its anomeric proton at δ 4.92 correlated in fact with C-3 at δ 83.01 in the HMBC experiment. The resonance of the C-28 carboxylic group at δ 176.18 indicated the presence of a sugar linked in this position. The sequence of the saccharide chain at C-28 was also defined by HMBC experiment. A cross-peak between C-28 (δ 176.18) and H-1_{AraI} (δ 6.48) was revealed. Additionally, cross-peaks between C-2_{AraII} (δ 74.58) and H-1_{RhaIII} (δ 5.58) and between C-4_{RhaIII} (δ 82.05) and H-1_{XylIV} (δ 5.25), respectively, were observed, suggesting that the terminal xylose was linked at C-4 of rhamnose, which in turn was attached at C-2 of arabinose, directly linked to the sapogenin at 28-COOH. The structure of compound **9** was also confirmed by MS. In the FAB-MS, the molecular ion $[M - H]^-$ at *m*/*z* 1073 (74%) corresponds to bayogenin plus uronic acid, plus two pentoses, plus rhamnose. The loss, in order, of a pentose from the molecular ion gave the peak at *m*/*z* 941 (3%), then the loss of rhamnose gave the peak at *m*/*z* 795 (4%), the subsequent loss of a pentose generated the ion *m*/*z* 663 (23%), and finally the loss of uronic acid gave the peak at *m*/*z* 487 (11%), corresponding to bayogenin. From the molecular ion, a peak at *m*/*z* 897 (2%) attributable to the ion $[M - H -$ uronic acid$]^-$ was also observed. ESI-MS/MS confirmed the above data. ESI-MS (negative ion mode), *m*/*z* (relative intensity): 1073.8 (100%) $[M(C_{52}H_{82}O_{23}) - H]^-$, which fragmented in the MS/MS giving 663.4 (100%) $[M - H - 132$(Ara) $- 146$(Rha) $- 132$(Xyl)$]^-$, 487.4 (18%) $[488$(Bay) $- H]^-$. Compound **9** was then established to be 3-*O*-β-D-glucuronopyranosyl-28-*O*-[β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]-bayogenin.

Compound **10**, 10 mg, was isolated as an amorphous solid: mp 226−228 °C, $[\alpha]_D^{25}$ 17.9 (MeOH, *c* 0.03). The molecular formula was calculated as $C_{52}H_{80}O_{23}$ (MW 1072). On acid hydrolysis it gave medicagenic aldehyde and arabinose, rhamnose, xylose, and glucuronic acid in the ratio 1:1:1:1. Four anomeric signals related to the sugars were observed in the ^{13}C NMR spectrum at δ 105.99, 103.34, 100.57, and 92.69 and at δ 6.53, 5.65, 5.19, and 4.88 in the ^{1}H NMR spectrum. The 2D NMR analysis of the sugar portion of saponin **10** revealed the presence of the same monosaccharide at C-3 position and the same sugar chain at C-28 position of the sapogenin as in compound **9**. In the HMBC experiment H-1_{GluAcI} (δ 4.88) gave

a correlation with C-3 at δ 82.78. A cross-peak between C-28 (δ 176.15) and H-1_{AraII} (δ 6.53) was revealed, and cross-peaks between C-2_{AraII} (δ 74.90) and H-1_{RhaIII} (δ 5.65) and between C-4_{RhaII} (δ 82.27) and H-1_{XylIV} (δ 5.19) were observed. Differences were revealed in the aglycone moiety. The singlet signal at δ 9.87 in the ^{1}H NMR spectrum confirmed by the signal at δ 207.80 in the ^{13}C NMR spectrum clearly indicated the presence of an aldehydic group. In the HMBC experiment this signal correlated to the 24-position methyl group (δ 11.10), suggesting that the aldehydic group should be at C-23. Further evidence was derived from the GC-MS analyses of the methylated-peracetylated sapogenin from **10**. Although functionalization gave some artifacts, a peak corresponding to the methylated-peracetylated 2β,3β-dihydroxyolean-12-en-23-ale-28-oic acid was well separated and identified. Artifacts probably originated from a reaction involving the aldehydic group of the molecule under the alkaline conditions used for acetylation. The molecular ion $[M]^+$ was detected at *m*/*z* 584, which corresponds to $C_{35}H_{52}O_7$, from which the loss of CO gave *m*/*z* 556 and the loss of an acetic acid unit gave *m*/*z* 524; all of these peaks had an intensity of <1%. Another ion was detected at *m*/*z* 496 (1%) corresponding to $[M - 28 - 60]^+$. The typical retro-Diels−Alder fragmentation of the cyclic triterpene skeleton gave rise to the ions at *m*/*z* 321 (not detected) and *m*/*z* 262 (33%). From the ion at *m*/*z* 321 the loss of CO gave *m*/*z* 293 (2%) and the loss of acetic acid gave *m*/*z* 233 (4%). The loss of acetic acid from the ion at *m*/*z* 262 gave the fragment at *m*/*z* 203, which represented the base peak. This finding is a characteristic diagnostic tool for the presence of a 12−13 double bond in terpenes of the β-amyrin class (*40*). Additional ions from cleavage of the central ring of the β-amyrin structure were at *m*/*z* 247 (4%), originating from the ion *m*/*z* 335 (not detected) by loss of acetic acid plus CO, and at *m*/*z* 249 (5%). ESI-MS/MS gave the following information: ESI-MS (negative ion mode), *m*/*z* (relative intensity) 1071.8 (100%) $[M(C_{52}H_{82}O_{22}) - H]^-$, which fragmented in the MS/MS giving the following product ions, 661.4 (100%) [M − H − 132(Xyl) − 146(Rha) − 132(Ara)]$^-$, 485.4 (14%) [486(MedAld) − H]$^-$. On the basis of these data, saponin **10** was identified as 3-*O*-β-D-glucuronopyranosyl-28-*O*-[β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]-2β,3β-dihydroxyolean-12-en-23-ale-28-oic acid.

Saponin **11**, 9 mg, MW 1104, released after acid hydrolysis soyasapogenol A and rhamnose, galactose, and glucuronic acid in the ratio 2:1:1. Its spectroscopic characteristics were identical to those of 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-28-*O*-α-L-rhamnopyranosidesoyasapogenol A, previously isolated in *M. sativa* (*24*).

Compound **12**, 28 mg, was isolated as an amorphous solid: mp 291−295 °C, $[\alpha]_D^{25}$ −13.5 (MeOH, *c* 0.04). The molecular formula was calculated as $C_{57}H_{88}O_{29}$ (MW 1236). On acid hydrolysis it gave zanhic acid and apiose, arabinose, rhamnose, xylose, and glucuronic acid in the ratio 1:1:1:1:1. The ^{1}H and ^{13}C NMR of **12** exhibited five anomeric protons at δ 6.50, 6.12, 5.76, 5.28, and 4.90 and carbons at δ 110.49, 105.72, 103.68, 100.08, and 92.73. The presence of a glucuronic unit at C3 was established from the HMBC correlation between H-1_{GluAcI} (δ 4.90) and C3 (δ 86.51) of the aglycone. The sequence of the sugar chain at C-28 was determined by the following HMBC correlations: H-1_{RhaIII} (δ 5.76) with C-2_{AraII} (δ 74.62), H-1_{XylIV} (δ 5.28) with C-4_{RhaIII} (δ 79.12), and H-1_{ApiV} (δ 6.12) with C-3_{RhaIII} (δ 81.38). The attachment of the tetrasaccharide moiety at the C-28 of the aglycone was based on an HMBC correlation of H-1_{AraII} (δ 6.50) with C-28 (δ 175.65) of the aglycone. In the FAB-MS, the molecular ion $[M - H]^-$ at *m*/*z* 1235 (100%) corresponds to zanhic acid plus three pentoses, one uronic, and one rhamnose. The peak at *m*/*z* 1059 (12%) corresponds to the loss of the uronic unit, from which the loss of a pentose unit gave *m*/*z* 927 (7%). The other observed ions were *m*/*z* 693 (5%) [M − H − 3 pentoses − rhamnose]$^-$ and *m*/*z* 517 (4%) [M − H − 3 pentoses − rhamnose − uronic]$^-$, corresponding to zanhic acid. ESI-MS (negative ion mode), *m*/*z* (relative intensity): 1235.6 (100%) $[M(C_{57}H_{88}O_{29}) - H]^-$, which fragmented in the MS/MS giving 1059.8 (100%) [M − H − 176(GlcA)]$^-$, 927.7 (5%) [M − H − 132(Api) − H]$^-$, 825.6 (6%) [M − H − 132(Xyl) − 146(Rha) − 132(Ara)]$^-$, 694.4 (21%) [518 (Zanh) + GlcA]$^-$, 517.4 (24%) [518(Zanh) − H]$^-$. This compound was identified as 3-*O*-β-D-glucuronopyranosyl-28-*O*-{β-D-xylopyranosyl(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}zanhic acid.

Compound **13**, 25 mg, was isolated as an amorphous solid: mp 290−295 °C, $[\alpha]_D^{25}$ −18.8 (MeOH, *c* 0.05). The molecular formula was calculated as $C_{57}H_{88}O_{29}$ (MW 1236). The acid hydrolysis of this saponin gave zanhic acid and arabinose, rhamnose, xylose, and glucuronic acid in the ratio 2:1:1:1. Five anomeric signals were observed in the ^{13}C NMR spectrum at δ 106.77, 105.14, 103.89, 100.25, and 93.05 and at δ 6.55, 5.64, 5.30, 5.11, and 4.87 in the ^{1}H NMR spectrum. The 2D NMR analysis of the sugar portion of saponin **13** revealed the presence of the same monosaccharide at the C-3 position as in saponin **12** and the same sugar chain at the C-28 position of the sapogenin as in compound **4**. In the HMBC experiment H-1_{GluAcI} (δ 4.87) gave a correlation with C-3 at δ 86.51. A cross-peak between C-28 (δ 175.65) and H-1_{AraII} (δ 6.55) was revealed, and cross-peaks between C-2_{AraII} (δ 74.51) and H-1_{RhaIII} (δ 5.64), between C-4_{RhaIII} (δ 79.08) and H-1_{XylIV} (δ 5.30), and between C-3_{RhaIII} (δ 81.49) and H-1_{AraV} (δ 5.11) were observed. In the FAB-MS, the molecular ion $[M - H]^-$ at *m*/*z* 1235 (100%) corresponds to zanhic acid plus three pentoses, one uronic, and rhamnose. The peak at *m*/*z* 1059 (15%) corresponds to the loss of the uronic unit, from which the loss of a pentose unit gave *m*/*z* 927 (5%). The other observed ions were *m*/*z* 693 (8%) [M − H − 3 pentoses − rhamnose]$^-$ and *m*/*z* 517 amu (3%) [M − H − 3 pentoses − rhamnose − uronic]$^-$ corresponding to zanhic acid. ESI-MS (negative ion mode), *m*/*z* (relative intensity): 1257.5 (100%) $[M(C_{57}H_{88}O_{29}) - 2H + Na]^-$, which fragmented in the MS/MS giving 1125.5 (22%) [M − 2H + Na − 132(Ara)]$^-$, 715.4 (14%) [M − 2H + Na − 132(Ara) − 132-(Ara) − 146(Rha) − 132(Xyl)]$^-$, 455.4 (53%) [518(Zanh) − CO_2 − H_2O − H]$^-$. It was identified as 3-*O*-β-D-glucuronopyranosyl-28-*O*-{β-D-xylopyranosyl(1→4)-[α-L-arabinopyranoside-(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}-zanhic acid.

Compound **14**, 12 mg, was isolated as an amorphous solid: mp 273−275 °C, $[\alpha]_D^{25}$ −8.5 (MeOH, *c* 0.03). The molecular formula was calculated as $C_{52}H_{80}O_{25}$ (MW 1104). Its acid hydrolysis afforded zanhic acid and arabinose, rhamnose, xylose, and glucuronic acid in the ratio 1:1:1:1. Four anomeric signals were observed in the ^{13}C NMR spectrum at δ 106.12, 103.71, 100.18, and 92.81 and at δ 6.47, 5.71, 5.23, and 4.95 in the ^{1}H NMR spectrum. The 2D NMR analysis revealed the presence of glucuronic acid directly linked at the C-3 position: its anomeric proton H-1_{GluAcI} (δ 4.95) gave a correlation with C-3 (86.12) of the aglycone. The same monosaccharide chain at the C-28 position as in saponins **9** and **10** was revealed. A cross-peak between C-28 (δ 175.83) and H-1_{AraII} (δ 6.47) and a cross-peaks between C-2_{AraII} (δ 74.95) and H-1_{RhaIII} (δ 5.71) and between C-4_{RhaII} (δ 82.31) and H-1_{XylIV} (δ 5.23) were observed.

In the FAB-MS, the molecular ion $[M - H]^-$ at *m/z* 1125 (100%) corresponds to zanhic acid plus two pentoses, one uronic, and a rhamnose. The peak at *m/z* 949 (10%) corresponds to the loss of the uronic unit. The other observed ions were *m/z* 693 (7%) [M − H − 2 pentoses − rhamnose]$^-$, *m/z* 647 (12%) [M − H − pentose − rhamnose − uronic]$^-$, and *m/z* 517 (5%) [M − H − 2 pentoses − rhamnose − uronic]$^-$ corresponding to zanhic acid. ESI-MS (negative ion mode), *m/z* (relative intensity): 1125.5 (100%) $[M(C_{52}H_{80}O_{25}) - 2H + Na]^-$, which fragmented in the MS/MS giving 715.4 (75%) [M − 2H + Na − 132(Ara) − 146(Rha) − 132(Xyl)]$^-$, 455.4 (53%) [518(Zanh) − CO_2 − H_2O − H]$^-$. This compound was identified as 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-[*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]zanhic acid.

Saponin **15**, 7 mg, MW 1088, released after acid hydrolysis medicagenic acid and arabinose, rhamnose, xylose, and glucuronic acid in the ratio 1:1:1:1. ^{1}H and ^{13}C data of the aglycone moiety were superimposable with those reported for compound **1**; ^{13}C data of the sugars were superimposable with those of compound **9**. It was identified as 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-[*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]medicagenic acid, previously isolated in *M. sativa* (*24*).

Compound **16**, 21 mg, was isolated as an amorphous solid: mp 293−296 °C, $[\alpha]_D^{25}$ −14.3 (MeOH, *c* 0.06). The molecular formula was estimated as $C_{68}H_{108}O_{37}$ (MW 1516). On acid hydrolysis it gave zanhic acid and apiose, arabinose, rhamnose, xylose, and glucose in the ratio 1:2:1:1:2. The ^{1}H and ^{13}C NMR of **16** exhibited seven anomeric protons at *δ* 6.51, 6.23, 5.69, 5.25, 5.18, 5.11, and 5.07 and carbons at *δ* 110.04, 107.09, 106.15, 105.17, 104.38, 100.50, and 92.98. After all of the proton and carbon signals were assigned, the seven sugar units were identified as two arabinose, two glucose, one rhamnose, one xylose, and one apiose and were confirmed by GLC and TLC analysis. The linkage of the sugar unit at C-3 was established from the HMBC correlations of H-1_{AraIII} (*δ* 5.11) with C-2_{GlcII} (*δ* 85.16), H-1_{GlcII} (*δ* 5.07) with C-2_{GlcI} (*δ* 84.12), and the long-range HMBC correlation between H-1_{GlcI} (*δ* 5.18) and C-3 (*δ* 87.10) of the aglycone, which confirmed the attachment of the trisaccharide moiety to the C-3 of the aglycone. The sequence of the sugar chain at C-28 was the same as in saponin **12**, as determined by the following HMBC correlations: H-1_{RhaV} (*δ* 5.69) with C-2_{AraIV} (*δ* 75.17), H-1_{XylVI} (*δ* 5.25) with C-4_{RhaV} (*δ* 78.96), and H-1_{ApiVII} (*δ* 6.23) with C-3_{RhaIII} (*δ* 81.53). The attachment of the tetrasaccharide moiety to C-28 was based on an HMBC correlation of H-1_{AraIV} (*δ* 6.51) with C-28 (*δ* 175.77) of the aglycone. In the FAB-MS, the molecular ion $[M - H]^-$ at *m/z* 1515 (100%) corresponds to zanhic acid plus four pentoses, two hexoses, and a rhamnose. The other observed ions were *m/z* 1383 (65%) [M − H − pentose]$^-$, *m/z* 1221 (85%) [M − H − pentose − hexose]$^-$, *m/z* 1089 (21%) [M − H − 2 pentoses − hexose]$^-$, *m/z* 927 (5%) [M − H − 2 pentoses − 2 hexoses]$^-$, *m/z* 517 (4%) [M − H − 4 pentoses − 2 hexoses − rhamnose]$^-$ corresponding to zanhic acid. The peak at *m/z* 455 (15%) originated from zanhic acid by loss of H_2O and CO_2 was also observed. ESI-MS (negative ion mode), *m/z* (relative intensity): 1515.6 (94%) $[M(C_{68}H_{108}O_{37}) - H]^-$, which fragmented in the MS/MS giving 1383.6 (65%) [M − H − 132(Api)]$^-$, 1220.6 (10%) [M − H − 132 − 162(Glc)]$^-$, 973.5 (100%) [M − H − 132(Api) − 132(Ara) − 146(Rha) − 132(Xyl)]$^-$, 455.4 (43%) [518(Zhan) − CO_2 − H_2O − H]$^-$. This compound was identified as 3-*O*-[α-L-arabinopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-28-*O*-{*β*-D-xylopyranosyl(1→4)-[*β*-D-apiofuranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}zanhic acid.

Compound **17**, 10 mg, was isolated as an amorphous solid: mp 287−290 °C, $[\alpha]_D^{25}$ −18.7 (MeOH, *c* 0.04). The molecular formula was calculated as $C_{63}H_{100}O_{33}$ (MW 1384). The acid hydrolysis of this saponin gave zanhic acid and apiose, arabinose, rhamnose, xylose, and glucose in the ratio 1:2:1:1:2. From the ^{13}C NMR six anomeric signals were evidenced at *δ* 107.18, 106.23, 104.97, 104.81, 100.71, and 92.51, confirmed in the ^{1}H NMR spectra in which six anomeric protons were found at *δ* 6.54, 5.65, 5.22, 5.15, 5.07, and 5.02. The linkage of the sugar unit at C-3 was established from the HMBC correlations of H-1_{GlcII} (*δ* 5.02) with C-2_{GlcI} (*δ* 83.91) and the long-range HMBC correlation between H-1_{GlcI} (*δ* 5.15) and C-3 (*δ* 86.93) of the aglycone, which confirmed the attachment of the disaccharide moiety to the C-3. The sequence of the sugar chain at C-28 was the same as in **4** and **13**, as supported by the following HMBC correlations: H-1_{RhaIV} (*δ* 5.65) with C-2_{AraIII} (*δ* 74.91), H-1_{XylV} (*δ* 5.22) with C-4_{RhaIV} (*δ* 79.03), and H-1_{AraVI} (*δ* 5.07) with C-3_{RhaIV} (*δ* 81.42). The attachment of the trisaccharide moiety to C-28 of the aglycone was based on an HMBC correlation of H-1_{AraIII} (*δ* 6.54) with C-28 (*δ* 175.95). In the FAB-MS, the molecular ion $[M - H]^-$ at *m/z* 1383 (100%) corresponds to zanhic acid plus three pentoses, two hexoses, and a rhamnose. The other observed ions were *m/z* 1221 (72%) [M − H − pentose − hexose]$^-$, *m/z* 1089 (14%) [M − H − 2 pentoses − hexose]$^-$, *m/z* 927 (15%) [M − H − 2 pentoses − 2 hexoses]$^-$, *m/z* 517 (6%) [M − H − 4 pentoses − 2 hexoses − rhamnose]$^-$ corresponding to zanhic acid. The peak at *m/z* 455 (21%) originated from zanhic acid by loss of H_2O and CO_2 was also observed. ESI-MS (negative ion mode), *m/z* (relative intensity): 1383.6 (100%) $[M(C_{63}H_{100}O_{33}) - H]^-$, which fragmented in the MS/MS giving 1251.5 (3%) [M − H − 132(Api)]$^-$, 1220.6 (10%) [M − H − 132 − 162(Glc)]$^-$, 841.7 (13%) [M − H − 132(Ara) − 132(Ara) − 146(Rha) − 132(Xyl)]$^-$, 455.4 (100%) [518(Zhan) − CO_2 − H_2O − H]$^-$. It was identified as 3-*O*-[*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-28-*O*-{*β*-D-xylopyranosyl(1→4)-[α-L-arabinopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside}zanhic acid.

Compound **18**, 16 mg, was isolated as an amorphous solid: mp 271−275 °C, $[\alpha]_D^{25}$ −22.3 (MeOH, *c* 0.07). The molecular formula was calculated as $C_{57}H_{88}O_{28}$ (MW 1220). The acid hydrolysis of this saponin gave medicagenic acid and apiose, arabinose, rhamnose, xylose, and glucuronic acid in the ratio 1:1:1:1:1. From the ^{13}C NMR five anomeric signals were observed at *δ* 110.98, 105.78, 103.45, 100.94, and 92.77, confirmed in the ^{1}H NMR spectra in which five anomeric protons were found at *δ* 6.49, 6.18, 5.68, 5.29, and 4.93. The 2D NMR analysis revealed the presence of glucuronic acid directly linked at the C-3 position: its anomeric proton H-1_{GluAcI} (*δ* 4.93) correlated with C-3 (86.84) of the aglycone. The same monosaccharide chain at the C-28 position as in saponins **12** and **16** was revealed. A cross-peak between C-28 (*δ* 176.19) and H-1_{AraII} (*δ* 6.49) was revealed, and cross-peaks between C-2_{AraII} (*δ* 75.06) and H-1_{RhaIII} (*δ* 5.68), between C-4_{RhaIII} (*δ* 79.27) and H-1_{XylIV} (*δ* 5.29), and between C-3_{RhaIII} (*δ* 81.65) and H-1_{ApiV} (*δ* 6.18) were observed. In the FAB-MS, the molecular ion $[M - H]^-$ at *m/z* 1219 (100%) corresponds to zanhic acid plus three pentoses, one uronic, and a rhamnose. The loss of 132 gave *m/z* 1087 (24%) [M − H − pentose]$^-$, and the loss of 176 gave *m/z* 1043 (18%) [M − H − uronic]$^-$. The other observed ions were *m/z* 911 (12%) [M − H − pentose − uronic]$^-$, *m/z* 501 (1%) [M − H − 3 pentoses − uronic −

rhamnose]$^-$ corresponding to medicagenic acid and the peak at *m/z* 439 (10%) originated from medicagenic acid by loss of H_2O and CO_2. ESI-MS (negative ion mode), *m/z* (relative intensity): 1219.6 (100) [M($C_{57}H_{88}O_{28}$) − H]$^-$, which fragmented in the MS/MS giving 1043.6 (70%) [M − H − uronic acid]$^-$, 677.5 (10%) [M − H − 132(Api) − 132(Ara) − 146(Rha) − 132(Xyl)]$^-$, 501.4 (4%) [502(Med) − H]$^-$. Saponin **18** was identified as 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-{*β*-D-xylopyranosyl(1→4)-[*β*-D-apiofuranisyl(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}medicagenic acid.

The data reported here indicate that 12 of the 18 purified compounds from *M. arborea* are novel saponins, identified as glycosides of zanhic acid (**3**, **4**, **12**−**14**, **16**, and **17**), 2*β*-hydroxyoleanolic acid (**5**), medicagenic acid (**6** and **18**), bayogenin (**9**), and 2*β*,3*β*-dihydroxyolean-12-en-23-ale-28-oic acid (**10**). Saponins **1**, **2**, **7**, **8**, **11**, and **15** have been previously detected in *M. sativa* and *M. truncatula*. It is worth noting that saponins isolated in this work from *M. arborea* are glycosides of seven different aglycones, of which hederagenin, a common triterpene from *Medicago* spp., has only been found in trace amounts (<1% of total sapogenins) in this species. Although there are differences in the aglycone moieties, similarities are instead observed for the sugar moieties. The first sugar linked at the C-3 position is glucose, as in saponins **1**−**5**, **7**, **16**, and **17**, or its glucuronic acid derivative, as in saponins **6**, **8**−**15**, and **18**. Additionally, the same sugar chain Xyl(1→4)Rha(1→2)-Ara has been detected in all of the C-28 glycosylated saponins except for saponins **1** and **6**, in which the disaccharide chain Rha(1→2)Ara has been identified, and saponin **3**, in which Xyl is substituted by Ara in the side chain at C-28.

A previous investigation on *M. truncatula* (*31*) has shown that, in contrast to *M. sativa* (*24*), this species produces saponins with a specific (1→3) linkage between the two glucose units at C-3, suggesting the presence of a specific glucosyltransferase. Results obtained in this work show instead a closer similarity of *M. arborea* to *M. sativa* in that when more than one sugar is present in the side chain at C-3, the linkage is always (1→2). In contrast, side chains at C-28 with more than two sugars are always characterized by Ara, directly linked to C-28, and Rha in the central position, linked (1→4) with Xyl. Branching points are formed by Ara or Api linked (1→3) at Rha. These features have been also observed in saponins extracted from *M. sativa* and *M. truncatula* (*24*, *31*) and suggest a high enzymatic selectivity for the sugar position independent of the involved genin.

A very interesting feature of the saponins isolated from *M. arborea* is the presence of the aldehydic group at the C-23 position in the newly detected aglycone of saponin **10**. The compound has also been identified as a sapogenin moiety in *M. hybrida* root saponins (unpublished results). This metabolite might represent an interesting intermediate in the oxidative steps that, from a methyl group, lead to the corresponding carboxylic acid (*41*, *42*). That is, if we consider the following genins found in *M. arborea*, 2*β*-hydroxyoleanolic acid (III, **Figure 1**), bayogenin (V), medicagenic aldehyde (VI), and medicagenic acid (I), all of the oxidative products at C-23 can be observed. The above genins all possess the same stereochemistry (2*β*,3*β*) in the hydroxylated triterpene carbons with the different functional groups at the C-23 position. The presence of the aldehydic group in the genin **10**, identified for the first time in *Medicago* spp., indicates a possible biosynthetic mechanism for the sapogenins of this genus. Accordingly, medicagenic acid may originate from bayogenin by subsequent oxidative enzymatic steps involving the formation of medicagenic aldehyde, whereas bayogenin may originate by a selective oxidative demethylation at C-23 from 2*β*-hydroxyoleanolic acid.

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LITERATURE CITED

(1) Martiniello, P.; Baviello, G. Preliminary evaluation for agronomic traits in accessions of *Atriplex* and *Medicago* shrubs. *REUR Tech. Ser. FAO* **1993**, *28*, 95−99.

(2) Stringi, L.; Sarno, R.; Amato, G.; Leto, G.; Pristina, L.; Corrao, A. Shrubs utilization in semi-arid environment: bio-agronomic aspects and their use in sheep husbandry. *World Rev. Anim. Prod.* **1987**, *23*, 79−90.

(3) Jaretzy, R. The occurrence of saponins in species of genus *Medicago. Angew. Bot.* **1940**, *22*, 147−156.

(4) Torck et al. Some polyphenols of *Medicago arborea. Plantes Med. Phytoter.* **1980**, *14*, 20−25.

(5) Torck et al. Flavonoid heterosides of *Medicago arborea. Pharmazie* **1983**, *38*, 783.

(6) Morris, R. J.; Dye, W. B.; Gisler, P. S. Isolation, purification and structural identity of an alfalfa root saponin. *J. Org. Chem.* **1961**, *26*, 1241−1243.

(7) Morris, R. J.; Hussey, E. W. A natural glycoside of medicagenic acid. An alfalfa blossom saponin. *J. Org. Chem.* **1965**, *30*, 166−168.

(8) Gestetner, B. Structure of a saponin from lucerne (*Medicago sativa*). *Phytochemistry* **1971**, *10*, 2221−2223.

(9) Timbekova, A. E.; Abubakirov, N. K. Triterpene glycosides of alfalfa. I. Medicoside G—a novel bisdesmoside from *Medicago sativa*. *Khim. Prir. Soedin.* **1984**, 451−458.

(10) Timbekova, A. E.; Abubakirov, N. K. Triterpene glycosides of alfalfa. II. Medicoside C. *Khim. Prir. Soedin.* **1985**, 805−808.

(11) Timbekova, A. E.; Abubakirov, N. K. Triterpene glycosides of alfalfa. III. Medicoside I. *Khim. Prir. Soedin.* **1986**, 607−610.

(12) Timbekova, A. E.; Abubakirov, N. K. Triterpene glycosides of alfalfa. IV. Medicoside J. *Khim. Prir. Soedin.* **1986**, 610−613.

(13) Massiot, G.; Lavaud, C.; Guillaumen, D.; Le Men-Olivier, L. Reinvestigation of saponins and prosapogenins from alfalfa (*Medicago sativa*). *J. Agric. Food Chem.* **1988**, *36*, 902−909.

(14) Massiot, G.; Lavaud, C.; Le Men-Olivier, L.; Binst, G.; Miller, S. F.; Fales, H. M. Structural elucidation of alfalfa root saponins by MS and NMR analysis. *J. Chem. Soc., Perkin Trans.* **1988**, 3071−3079.

(15) Levy, M.; Zehavi, U.; Naim, M.; Polacheck, J. Isolation, structure determination and antifungal activity of a new native alfalfa root saponin. *Carbohydr. Res.* **1989**, *193*, 115−123.

(16) Timbekova, A. E.; Larin, M. F.; Yagudaev, M. R.; Abubakirov, N. K. Triterpene glycosides of alfalfa. V. Medicoside H. *Khim. Prir. Soedin.* **1989**, 673−677.

(17) Oleszek, W.; Price, K. R.; Colquhoun, I. J.; Jurzysta, M.; Ploszynski, M.; Fenwick, G. R. Isolation and identification of alfalfa (*Medicago sativa* L.) root saponins: their activity in relation to a fungal bioassay. *J. Agric. Food Chem.* **1990**, *38*, 1810−1817.

(18) Timbekova, A. E.; Verechagin, A. L.; Semenov, A. A.; Abubakirov, N. K. Triterpene glycosides of alfalfa. VI. Medicoside L. *Khim. Prir. Soedin.* **1990**, 221−227.

(19) Massiot, G.; Lavaud, C.; Besson, V.; Le Men-Olivier, L.; Van Binst, G. Saponins from aerial parts of alfalfa (*Medicago sativa*). *J. Agric. Food Chem.* **1991**, *39*, 78−82.

(20) Massiot, G.; Lavaud, C.; Benkhaled, M.; Le Men-Olivier, L. Soyasaponin VI, a new maltol conjugate from alfalfa and soybean. *J. Nat. Prod.* **1992**, *55*, 1339−1342.
(21) Oleszek, W.; Jurzysta, M.; Ploszynski, M.; Coloquhoun, I. J.; Price, K. R.; Fenwick, G. R. Zahnic acid tridesmoside and other dominant saponins from alfalfa (*Medicago sativa* L.) aerial parts. *J. Agric. Food Chem*. **1992**, *40*, 191−196.
(22) Timbekova, A. E.; Shashkov, A. S.; Abubakirov, N. K. Triterpene glycosides from alfalfa. VII. Medicosides E and F. *Khim. Prir. Soedin.* **1993**, *5*, 701−705.
(23) Timbekova, A. E.; Shashkov, A. S.; Abubakirov, N. K. Triterpene glycosides from alfalfa. VIII. Medicoside E from the leaves. *Khim. Prir. Soedin.* **1993**, *5*, 705−710.
(24) Bialy, Z.; Jurzysta M.; Oleszek, W.; Piacente, S.; Pizza C. Saponins in alfalfa (*Medicago sativa* L.) root and their structural elucidation. *J. Agric. Food Chem*. **1999**, *47*, 3185−3192.
(25) Oleszek, W.; Price, K. R.; Fenwick, G. R. Triterpene saponins from the roots of *Medicago lupulina* L. (black medic trefoil). *J. Sci. Food Agric*. **1988**, *43*, 289−297.
(26) Walter, E. D. Isolation of a saponin, hederin and its sapogenin hederagenin from Bur clover (*Medicago hispida*). *J. Am. Pharm. Assoc.* **1957**, *47*, 466−467.
(27) Mahato, S. B. Triterpenoid saponins from *Medicago hispida*. *Phytochemistry* **1991**, *30*, 3389−3393.
(28) Konjo, J.; Uemara, H.; Nakamura, M.; Nohara, T. Two novel triterpenoidal glycosides from *Medicago polymorpha* L. *Chem. Pharm. Bull*. **1994**, *42*, 1339−1341.
(29) Bialy, Z.; Jurzysta, M.; Mella, M.; Tava, A. Triterpene saponins from aerial parts of *Medicago arabica* L. *J. Agric. Food Chem*. **2004**, *52*, 1095−1099.
(30) Huhman, D. V.; Sumner, L. W. Metabolic profiling of saponins in *Medicago sativa* and *Medicago truncatula* using HPLC coupled to an electrospray ion-trap mass spectrometer. *Phytochemistry* **2002**, *59*, 347−360.
(31) Kapusta, I.; Stochmal, A.; Perrone, A.; Piacente, S.; Pizza, C.; Oleszek, W. Triterpene saponins from barrel medic (*Medicago truncatula*) aerial parts. *J. Agric. Food Chem*. **2005**, *53*, 2164−2170.
(32) Oleszek, W. Alfalfa saponins: structure, biological activity and chemotaxonomy. In *Saponins Used in Food and Agriculture*; Waller, G. R., Yamasaki, K., Eds.; Plenum Press: New York, 1996; pp 155−170.
(33) Jurzysta, M.; Waller, G. R. Antifungal and haemolytic activity of aerial parts of alfalfa (*Medicago*) species in relation to saponin composition. In *Saponins Used in Traditional and Modern Medicine*; Waller, G. R., Yamasaki, K., Eds.; Plenum Publishing: New York, 1996; pp 565−574.
(34) Tava, A.; Odoardi, M. Saponins from *Medicago* spp.: chemical characterization and biological activity against insects. In *Saponins Used in Food and Agriculture*; Waller, G. R., Yamasaki, K., Eds.; Plenum Publishing: New York, 1996; pp 97−109.
(35) Avato, P.; Bucci, R.; Tava, A.; Vitali, C.; Rosato, A.; Bialy, Z.; Jurzysta, M. Antimicrobial activity of saponins from *Medicago* sp. *Abstract Book*, 3rd International Symposium on Natural Drugs, Napoli, Italy, 2003; p 73.
(36) Tava, A.; Oleszek, W.; Jurzysta, M.; Berardo, N.; Odoardi, M. Alfalfa saponins and sapogenins: isolation and quantification in two different cultivars. *Phytochem. Anal*. **1993**, *4*, 269−274.
(37) Nagao, T.; Tanaka, R.; Shimokawa, H.; Okabe, H. Studies on the constituents of *Aster scaber* Thunb. II. Structures of echinocystic acid glycosides isolated from the roots. *Chem. Pharm. Bull.* **1991**, *39*, 1719−1725.
(38) Jia, Z.; Koike, K.; Nikaido, T. Major triterpenoid saponins from *Saponaria officinalis*. *J. Nat. Prod.* **1998**, *61*, 1368−1373.
(39) Jurzysta, M. Transformation of soyasapogenol B into soyasapogenols C, D and F under acidic conditions. *Proceedings of the 14th International Symposium on Natural Products*; 1984; pp 127.
(40) Budzikiewicz, H.; Wilson, J. M.; Djerassi, C. Mass spectrometry in structural and stereochemical problems. XXXII. Pentacyclic triterpenes. *J. Am. Chem. Soc.* **1963**, *85*, 3688−3699.
(41) Dewick, P. M. In *Medicinal Natural Products—A Biosynthetic Approach*, 2nd ed.; Wiley: Chichester, U.K., 2001.
(42) Torsell, K. B. G. In *Natural Product Chemistry—A Mechanistic, Biosynthetic and Ecological Approach*; Swedish Pharmaceutical Society: Stockholm, Sweden, 1997.

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