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Triterpene Saponins from Aerial Parts of *Medicago arabica* L.

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Eight major triterpene saponins have been isolated from the aerial parts of *Medicago arabica* and their structures elucidated by FAB-MS and NMR analysis. Three of them are new compounds and are identified as 3-*O*-(α-L-arabinopyranoside) bayogenin, 3-*O*-(α-L-arabinopyranosyl), 28-*O*-(β-D-glucopyranoside) bayogenin, and 3-*O*-[α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl], 28-*O*-β-D-glucopyranoside 2-β-hydroxyoleanolic acid. Two saponins, identified as 3-*O*-(α-L-arabinopyranoside) hederagenin and 3-*O*-(α-L-arabinopyranosyl), 28-*O*-(β-D-glucopyranoside) hederagenin are known compounds but not previously reported as saponin constituents of *Medicago* species, while three other saponins, being mono- and bidesmosides of hederagenin, have been previously isolated from roots of *M. sativa*.

KEYWORDS: ***Medicago arabica* L.; triterpene saponins; chemical structure; FAB-MS; NMR**

INTRODUCTION

During the past few years, more attention has been paid to the possibility of using saponins, a group of secondary metabolites detected in several plant families, as natural fungicides that could replace harmful synthetic compounds. It is well-known that saponins from *M. sativa*, identified as a complex mixture of triterpenic glycosides that are derivatives of medicagenic acid, zanthic acid, bayogenin, hederagenin, and soyasapogenols (*1*−*18*), possesses a broad spectrum of biological properties such as antinutritional, antifungal, insecticidal, phytotoxic, and hemolytic activities (*19*). In this respect, in a search for a new rich source of saponins, the aerial parts of twenty-nine *Medicago* species have been tested by *Trichoderma viride* bioassay (*20*−*21*). Results showed that few of the tested species were very biologically active, of which *M. arabica* shows fifty times higher fungistatic activity in comparison with *M. sativa*. Successive investigations have indeed also confirmed that saponin extract from *M. arabica* was strongly active both toward plant pathogenic fungi (*22*) and as a dermatophyte (*23*).

M. arabica is a wild annual species; plants are 40−65 cm long and profusely branched from the base, usually with an anthocyanin-colored patch in the middle (*24*). Although its origin is Mediterranean, this grass is one of the most widespread species of *Medicago* in the world, as it is cultivated in continental Europe and used in Australia and in the United States as a fodder crop (*25*).

The aim of this study was to investigate the saponin composition from the aerial parts of *M. arabica* and to isolate its main glycosides to elucidate their structures. Saponins from *Medicago* species have been studied in *M. sativa* due to its practical importance for animal feeding. However, the knowledge of saponins from other *Medicago* species, especially their structure, is very limited, and chemical investigations only on *M. lupulina* (*26*), *M. hispida* (*27*, *28*), and *M. polymorpha* (*29*) have been reported.

MATERIAL AND METHODS

Plant Material. *Medicago arabica* L. (SA:7,746) seeds, received from the Australian Medicago Genetic Resource Centre, Adelaide (Australia) were sown in Pulawy (Poland). Aerial parts of plants were cut at the beginning of flowering. The plant material was dried at 40 °C, ground, and used for the successive extraction.

Extraction and Purification. The powdered plant material was defatted with $CHCl_3$ in a Soxhlet apparatus and then extracted with 80% MeOH under reflux. 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 preconditioned with water. The column was washed with water and then with 40% MeOH. Total saponins (10.3 g) were eluted with MeOH and dried.

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, Darmstadt, Germany). The column was washed with *n*-BuOH saturated with water, and the fractions were checked by TLC on silica gel (Merck), developed with ethyl acetate/acetic acid/water (7:2:2) and RP-18, developed with 75% MeOH. Chromatograms were sprayed with 10% sulfuric acid in MeOH and heated at 130 °C. Column chromatography gave three fractions: I (3.9 g), II (2.6 g), and III (1.2 g).

Separation. Single saponins were separated from the fractions by means of reversed-phase chromatography on a 40−63 mm, LiChroprep RP-18 column, eluted with diluted MeOH. Fraction I eluted with 60%

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MeOH afforded saponins **1** (1.2 g) and **2** (270 mg); further elution with 70% MeOH gave saponins **3** (570 mg) and **4** (220 mg). Fraction II eluted with 70% MeOH yielded saponin **5** (380 mg), and after elution with 75% MeOH saponin **6** (12 mg) was obtained. Fraction III eluted with 50% MeOH gave saponin **7** (170 mg), and 65% MeOH allowed saponin **8** (190 mg) to be obtained.

Analysis of Hydrolysis Products. The saponin mixture and each individual pure saponin were hydrolyzed with 2 N HCl in 50% acqueous methanol, and thereafter, sugars and aglycones were separated and identified by TLC, GC/MS, and NMR methods.

Sapogenins were compared to the standards (*30*) by TLC on Merck silica gel 60 plates. The plates were developed with petroleum ether/chloroform/acetic acid (7:2:1) or benzene/methanol (9:1), and spots were visualized by spraying with methanol/acetic anhydride/sulfuric acid (50:5:5 v/v) followed by heating at 120 °C.

Sugars were separated on Merck cellulose plates with benzene/butanol/pyridine/water (1:5:3:3), made visible with a silver nitrate spray and identified by comparison with authentic reference compounds.

GC/MS of sapogenins and sugars were recorded after methylation and silylation or acetylation as previously reported (*31*).

^{1}H and ^{13}C NMR spectra of sapogenins were recorded after dissolving the sample in pyridine-d_5 (5 mg/0.5 mL).

MS Analyses. FAB-MS spectra were conducted 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 *m*/*z* 100−1500 by scanning the magnetic field in 10 s with mass resolution 1000.

NMR Analyses. ^{1}H and ^{13}C NMR were measured on a Bruker AV-300 spectrometer at operating frequencies of 300.13 and 75.13 MHz, respectively. The samples were examined as solutions in pyridine-d_5 (10 mg/0.5 mL) in 5-mm tubes at 25 °C. ^{1}H and ^{13}C chemical shifts were expressed in parts per million relative to pyridine signal at 7.2 and 123.5 ppm, respectively. Two-dimensonal (2D) NMR experiments (H,H DQF-COSY; H,H TOCSY; H,H NOESY; H,C HSQC; HMBC) were carried out on all compounds using the phase sensitive method. On the basis of these 2D NMR analyses, assignment of the ^{1}H and ^{13}C signals were obtained.

Melting points were determined using a Dr. Tottotoli (Buchi, Switzerland) apparatus and are uncorrected. *Optical rotations* were conducted on a Jasco model P-1020 colorimeter. *IR* spectra were recorded with a Spectrum 2000 instrument in KBr. *Elemental analyses* were carried out on a Carlo Erba instrument.

RESULTS AND DISCUSSION

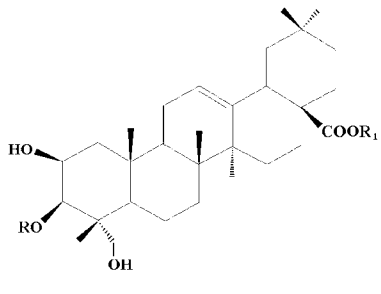
Preliminary investigation of *M. arabica* saponins, performed by TLC, showed a different composition compared to the well-known saponins from *M. sativa*. GC and GC/MS investigation of derivatized sapogenins, obtained after acid hydrolyses of saponins, confirmed hederagenin as the major aglycone, representing about 35% of the total aglycones, followed by bayogenin (30%). Oleanolic acid (5%) and another aglycone, further identified as 2-β-hydroxy oleanolic acid (7%) were also identified.

The crude saponins, obtained from the defatted green material in 4.35% yield, were fractionated by a combination of silica gel and RP-18 column chromatography to afford eight major compounds.

To elucidate the chemical structure of saponins **1**−**8**, spectroscopic and chemical techniques were employed. The TLC analyses of aglycones showed that compounds **1** and **3** had the same genin as saponins **2**, **4**, **5**, **6**, and **8**. Only saponin **7** showed a different aglycone moiety. R_f values, GC/MS, and NMR data, compared to that of reference compounds, revealed the presence of hederagenin in saponins **2**, **4**, **5**, **6**, and **8**, and bayogenin in compounds **1** and **3**. The aglycone moiety of saponin **7** was identified as 2-β-hydroxyoleanolic acid. The assignment of all ^{1}H and ^{13}C signals was obtained on the basis of 2D NMR (H,H DQF−COSY; H,H TOCSY; H,H NOESY; H,C HSQC; HMBC) experiments. The structure elucidation of all saponins were performed by analyzing NMR and MS data. The molecular weights were obtained from elemental analyses and evaluated from MS data and ^{13}C NMR spectra in which all carbons were revealed. MS fragmentation ions were also used to establish sugar chain in the molecule. The chemical structure of the identified saponins **1**−**8** are reported in **Figure 1**, while ^{13}C NMR chemical shift data are reported in **Table 1**. ^{1}H NMR data of sugar moieties were reported in **Table 2**.

Compound **1** was isolated as an amorphous solid, mp 213−215 °C, $[\alpha]_D^{25}$ +51.67° (MeOH, c = 1.0). IR ν_{max}^{KBr} cm^{-1}: 3200−3700 (large, OH), 2945 (CH), 1730 (C=O), 1642 (C=C), 1000−1250 (large, C−O). Anal. calcd for C_{41} H_{66} O_{14}: C, 62.90; H, 8.50. Found: C, 62.88; H, 8.52. The molecular formula was estimated as $C_{41}H_{66}O_{14}$ (MW 782). The acid hydrolysis of this saponin gave bayogenin, glucose, and arabinose. The ^{1}H NMR spectrum show the presence of several signals attributed to the aglycone moiety as follows: δ 5.53 (1H, t, J = 3.0 Hz, H-12), δ 4.56 (1H, bq, J = 4.0 Hz, H-2), δ 4.41 (1H, d, J = 4.0 Hz, H-3), δ 4.32 (1H, d, J = 11.0 Hz, H-23a), δ 3.75 (1H, d, J = 11.0 Hz, H-23b), δ 3.27 (1H, dd, J = 14.0 and 4.0 Hz, H-18), δ 2.00−2.20 (2H, m, H-11), δ 2.40 (1H, m, H-1a), δ 1.35 (1H, m, H-1b), δ 1.85 (1H, m, H-9), δ 1.68 (3H, s, Me-25), δ 1.44 (3H, s, Me-24), δ 1.34 (3H, s, Me-27), δ 1.18 (3H, s, Me-26), δ 1.08 (3H, s, Me-30), δ 1.00 (3H, s, Me-29). The stereochemistry of C-2, C-3, and C-4 of the bayogenin was confirmed by 2D-NOESY experiments. A cross-peak between H-2 (δ 4.56) and H-3 (δ 4.41) was observed, confirming the presence of the 2β,3β-dihydroxyoleanane skeleton; additionally, no correlation between H-3 (δ 4.41) and Me-24 indicated the β configuration of the 23-hydroxymethyl group. The ^{13}C NMR spectrum showed the presence of two anomeric signals at δ 106.82 and 95.75. The ^{1}H NMR spectrum confirms the presence of two anomeric protons at δ 6.38 (d, J = 8.0 Hz) and 5.15 (d, J = 7.3 Hz). The structure of saccharide units was evaluated by 2D NMR spectroscopy. DQF-COSY and TOCSY experiments allowed the sequential assignment of resonances for each sugar unit, starting from the well-isolated anomeric proton signals at δ 6.38 and 5.15. Complete assignments of all proton resonances in each sugar were achieved. The shifts of the sugar resonances were attributed to α-L-arabinopyranoside (anomeric proton at δ 5.15) and β-D-glucopyranoside (anomeric proton at δ 6.38) units. The HSQC experiment showed that the proton at δ 6.38 was correlated with the carbon at δ 95.75, while the hydrogen at δ 5.15 was correlated with the carbon at δ 106.82. In the ^{13}C NMR spectrum, the signal at δ 176.42 (C-28) indicated that the 28-COOH function of bayogenin was esterified with a sugar. The chemical shift of H-1$_{gluII}$ (6.38 δ) and C-1$_{gluII}$ (95.7 δ) indicated this sugar to be involved in an ester linkage with the C-28 carboxylic group. Additionally, in the HMBC experiments, a correlation between C-28 and the anomeric proton at δ 6.38 was observed. Evidence of linkage of arabinose to the C-3 of bayogenin were obtained from correlation between arabinose anomeric proton at δ 5.15 and C-3 (δ 82.66) in the HMBC spectra.

These findings were also obtained from the mass spectrum. The molecular ion $[M - H]^-$ at *m*/*z* 781 (3% relative intensity) corresponded to bayogenin plus hexose plus pentose. The 100% peak at *m*/*z* 619 corresponded to the loss of hexose sugar from the C-28, giving a stable ion due to delocalization of the negative charge on the carboxylic unit because the ester linkage is easier to break compared to an ether linkage. A peak due to the loss of a pentose unit was also observed in low amount (*m*/*z* 487, 9% relative intensity). On the basis of these spectroscopic data



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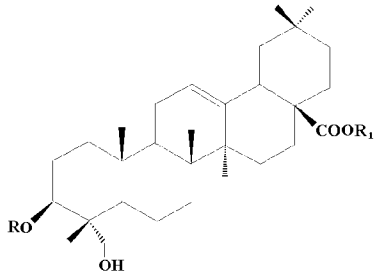

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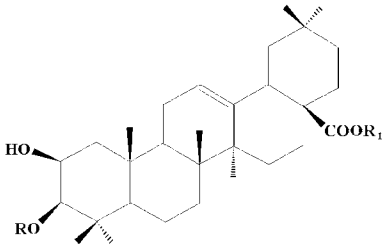

III

Compound	Aglycone	R	R_1
1	I	*α*-L-arabinopyranosyl	*β*-D-glucopyranosyl
2	II	*α*-L-arabinopyranosyl	*β*-D-glucopyranosyl
3	I	*α*-L-arabinopyranosyl	H
4	II	*α*-L-arabinopyranosyl	H
5	II	*β*-D-glucopyranosyl(1→2)-*α*-L-arabinopyranosyl	*β*-D-glucopyranosyl
6	II	*β*-D-glucopyranosyl(1→2)-*α*-L-arabinopyranosyl	H
7	III	α-L-arabinopyranosyl (1→2- β-D-glucuronopyranosyl	*β*-D-glucopyranosyl
8	II	*α*-L-arabinopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*α*-L-arabinopyranosyl	*β*-D-glucopyranosyl

Figure 1. Structure of saponins **1**–**8** (I, bayogenin; II, hederagenin; III, 2-*β*-hydroxyoleanolic acid).

saponin **1** was tentatively established as 3-*O*-(α-L-arabinopyranosyl) 28-*O*-(*β*-D-glucopyranosyl) bayogenin, and it was a new compound.

Compound **2** released hederagenin, glucose, and arabinose after acid hydrolysis. Its spectroscopic characteristics were identical to those of 3-*O*-(α-L-arabinopyranosyl),28-*O*-(*β*-D-glucopyranosyl) hederagenin. This saponin occurs rarely in nature and has been found before in *Hedera nepalensis* (*32*) and *Chenopodium quinoa* (*33*).

Saponin **3**, an amorphous solid, mp 232−233 °C, $[\alpha]_D^{25}$ +65.82° (MeOH, $c = 1.0$). IR ν_{max}^{KBr} cm^{-1}: 3200−3700 (large, OH), 2946 (CH), 1698 (C═O), 1648 (C═C), 1000−1250 (large, C−O). Anal. calcd for C_{35} H_{56} O_9: C, 67.71; H, 9.09. Found: C, 67.67; H, 9.12. It had an estimated molecular formula of $C_{35}H_{56}O_9$ (MW 620). Acid hydrolysis of this saponin gave bayogenin and arabinose. The ^{1}H NMR spectrum showed the presence of several signals attributed to the aglycone moiety as follows: *δ* 5.53 (1H, t, $J = 3.0$ Hz, H-12), *δ* 4.45 (1H, bq, $J = 4.0$ Hz, H-2), *δ* 4.28 (1H, d, $J = 4.0$ Hz, H-3), *δ* 4.16 (1H, d, $J = 11.0$ Hz, H-23a), *δ* 3.75 (1H, d, $J = 11.0$ Hz, H-23b), *δ* 3.38 (1H, dd, $J = 14.0$ and 4.0 Hz, H-18), *δ* 2.00−2.20 (2H, m, H-11), *δ* 2.40 (1H, m, H-1a), *δ* 1.35 (1H, m, H-1b), *δ* 1.80 (1H, m, H-9), *δ* 1.65 (3H, s, Me-25), *δ* 1.44 (3H, s, Me-24), *δ* 1.33 (3H, s, Me-27), *δ* 1.16 (3H, s, Me-26), *δ* 1.09 (3H, s, Me-30), *δ* 0.98 (3H, s, Me-29). The stereochemistry of C-2, C-3, and C-4 of the bayogenin was confirmed by 2D-NOESY experiments. A cross-peak between H-2 (*δ* 4.45) and H-3 (*δ* 4.28) was observed, confirming the presence of the 2*β*,3*β*-dihydroxyoleanane skeleton; additionally, no correlation between H-3 (*δ* 4.28) and Me-24 indicate the *β* configuration of the 23-hydroxymethyl group. Both in the ^{1}H and ^{13}C spectra of this saponin, only one anomeric proton was evident at *δ* 5.08 and *δ* 106.76, respectively. The C-28 chemical shift was *δ* 180.15, indicating that this carboxylic group was free. The HMBC experiments clearly showed a long-range correlation between C-3 (*δ* 82.73) and the only anomeric proton at *δ* 5.08. The base peak in the MS spectrum was detected at *m*/*z* 619, $[M - H]^-$, that corresponds to bayogenin plus a pentose unit. Loss of the pentose unit gave a peak at *m*/*z* 487 (22% relative intensity). The structure of this compound was tentatively established to be 3-*O*-(α-L-arabinopyranosyl) bayogenin. This saponin is a new glycoside.

Compound **4** afforded after acid hydrolysis hederagenin and arabinose and its spectroscopic characteristics was identical to those of 3-*O*-(α-L-arabinopyranoside) hederagenin. This saponin is one of the widely occurring hederagenin glycoside in plants but has never previously been found in *Medicago* spp.

Hydrolysis of saponin **5** and saponin **6** released hederagenin, glucose, and arabinose. On the basis of spectroscopic data and TLC comparison of authentic samples, saponin **5** was established to be 3-*O*-[*β*-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl],-28-*O*-*β*-D-glucopyranosyl hederagenin, and saponin **6** was identified as 3-*O*-[*β*-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl] hederagenin. Both of these saponins were previously detected in *M. sativa* (*34*).

Compound **7** was isolated as an amorphous solid, mp 240 °C (decomp), $[\alpha]_D^{25}$ +22.79° (MeOH, $c = 1.0$). IR ν_{max}^{KBr} cm^{-1}: 3200−3200 (large, OH), 2945 (CH), 1732 (C═O), 1612 (C═C), 1000−1250 (large, C−O). Anal. calcd for C_{47} H_{74} O_{19}: C, 59.86; H, 7.91. Found: C, 59.90; H, 7.95. Its molecular formula was estimated as $C_{47}H_{74}O_{19}$ (MW 942). MS *m*/*z* (relative intensity): 941 (100) $[M - H]^-$, 809 (5) $[M - H -$

Table 1. ^{13}C NMR Spectroscopic Assigments (δ) of the Aglycone and Monosaccharide Moieties of Saponins **1**, **3**, and **7**

aglycone				monosaccharide			
C	1	3	7	C	1	3	7
					arabinose	arabinose	glucuronic acid
1	46.11	46.42	45.39	1	106.82	106.76	106.35
2	70.90	70.89	69.72	2	72.89	72.92	81.98
3	82.66	82.73	90.06	3	74.62	74.62	78.22
4	42.87	42.86	39.02	4	69.65	69.62	73.98
5	48.57	48.59	56.76	5	67.24	67.20	75.51
6	17.96	17.98	18.93	6			176.48
7	33.96	34.23	32.87				
8	40.05	39.91	40.40				arabinose
9	47.73	47.78	48.76	1			103.32
10	37.00	37.02	37.38	2			73.18
11	23.37	23.70	23.74	3			74.57
12	123.17	122.77	123.08	4			69.08
13	144.09	144.81	144.41	5			67.68
14	42.27	42.33	42.09				
15	28.20	28.27	28.52		glucose		glucose
16	24.00	24.01	24.38	1	95.75		96.06
17	46.98	46.66	47.32	2	74.14		74.43
18	41.74	42.01	42.62	3	78.90		79.17
19	44.39	44.33	46.52	4	71.08		71.46
20	30.74	30.93	30.32	5	79.32		79.57
21	32.88	33.23	34.34	6	62.17		62.55
22	32.55	32.99	32.89				
23	65.23	65.32	31.06				
24	14.99	14.97	18.91				
25	17.31	17.26	17.14				
26	17.59	17.97	18.00				
27	26.13	26.23	26.49				
28	176.42	180.15	176.70				
29	33.09	33.24	33.46				
30	23.64	23.77	23.99				

Table 2. ^1H NMR Chemical Shift Data (δ_H, J, Hz) of the Monosaccharide Moieties of Saponins **1**, **3**, and **7**

	1	3	7
	arabinose	arabinose	glucuronic acid
1	5.15 d (7.0)	5.08 d (7.0)	5.01 d (7.0)
2	4.60 dd (7.0, 9.0)	4.58 dd (7.0, 9.0)	4.21 m
3	4.15 m	4.14 m	4.30 m
4	4.28 m	4.25 m	4.20 m
5	4.30 m	4.29 m	4.20 m
6	3.80 m	3.80 m	
			arabinose
1			5.30 d (7.0)
2			4.70 m
3			4.20 m
4			4.32 m
5			4.41 m
			3.82 m
	glucose		glucose
1	6.38 d (7.5)		6.37 d (7.5)
2	4.28 m		4.25 m
3	4.35 m		4.37 m
4	4.43 m		4.40 m
5	4.12 m		4.14 m
6	4.45 m		4.45 m
	4.50 m		4.50 m

pentose]$^-$, 780 (10) [M − H − hexose]$^-$, 647 (3) [M − H − hexose − pentose]$^-$, 471 (5) [M−H − hexose − pentose − uronic]$^-$. After acid hydrolysis, saponin **7** afforded glucose, arabinose, and glucuronic acid as the sugar constituents and an aglycone moiety identified as 2-β-hydroxy oleanolic acid on the basis of NMR and MS spectra (*35*, *36*). The ^1H NMR spectrum show the presence of several signals attributed to the aglycone moiety as follows: δ 5.52 (1H, t, J = 3.0 Hz, H-12), δ 4.45 (1H, bq, J = 4.0 Hz, H-2), δ 4.33 (1H, d, J = 4.0 Hz, H-3), δ 3.31 (1H, dd, J = 14.0 and 4.0 Hz, H-18), δ 2.00−2.20 (2H, m, H-11), δ 2.40 (1H, m, H-1a), δ 1.35 (1H, m, H-1b), δ 1.80 (1H, m, H-9), δ 1.57 (3H, s, Me-25), δ 1.47 (3H, s, Me-24), δ 1.44 (3H, s, Me-27), δ 1.34 (3H, s, Me-23), δ 1.21 (3H, s, Me-26), δ 1.09 (3H, s, Me-29), δ 0.97 (3H, s, Me-30). The stereochemistry of C-2 and C-3 of the 2-β-hydroxy oleanolic acid was confirmed by 2D-NOESY experiments. A cross-peak between H-2 (δ 4.45) and H-3 (δ 4.33) was observed, confirming the presence of the 2β,3β-dihydroxyoleanane skeleton. A signal at δ 176.70 in the ^{13}C NMR spectrum suggested the occurrence of a COOH group at C-28.

Saponin **7** showed three anomeric signals at δ 106.05, δ 103.09, and δ 95.75 in the ^{13}C NMR spectrum and δ 6.37, δ 5.30, and δ 5.01 in the ^1H NMR spectrum. The carboxylic group at δ 176.41 indicated the presence of sugar linked in this position. The ^1H NMR chemical shift observed (δ 6.37) correlated with the carbon at δ 95.75 is characteristic of glucose linked at the C-28 position. In the HMBC experiment, a correlation between the anomeric proton at δ 6.37 and this carboxylic group was found. 2D NMR experiments indicated the presence of a disaccharide chain made up of one β-D-glucuronopyranose directly linked to the C-3 of aglycone and an α-L-arabinopyranoside linked at C2 of glucuronic acid. In the HMBC spectrum, a clear correlation between C-3 (δ 90.06) and anomeric proton of glucuronic acid was observed. The position of arabinose was indicated in the HMBC experiment: the two anomeric carbons at δ 106.35 (C-1_{gluAcI}) and δ 103.32 (C-1_{araII}) gave a correlation with the same proton (δ 4.21, H-2_{gluAcI} of glucuronic acid). The structure of saponin **7** was also confirmed by MS. In this case, the molecular ion [M − H]$^-$ (*m*/*z* 941 amu), represents the base peak. The free COOH group on the molecule gave the stable negative ion. Loss of a hexose unit (*m*/*z* 780, 10% relative intensity) and loss of a pentose unit (*m*/*z* 647, 3% relative intensity) was observed. Loss of a glucuronic unit, giving the corresponding aglycone (*m*/*z* 471), confirms that glucuronic acid is directly linked to the triterpene. On the basis of these data, saponin **7** was established to be 3-*O*- [α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl],28-*O*-β-D-glucopyranosyl 2-β-hydroxyoleanolic acid. This saponin has been isolated from plant material for the first time.

Compound **8** after acid hydrolysis gave hederagenin, glucose, and arabinose and showed spectroscopic and chromatographic characteristics identical to those of 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl],28-*O*-β-D-glucopyranosyl hederagenin, previously identified in *M. sativa* (*34*).

In conclusion, we have isolated eight saponins from the aerial parts of *M. arabica* that represent the major compounds and identified their chemical structure as glycosides of bayogenin, hederagenin, and 2-β-hydroxyoleanolic acid. Saponins **1**, **3**, and **7** are new compounds; the others are known saponins, of which two (saponins **2** and **4**) have never been reported in *Medicago* species before. Only two saponins (compounds **1** and **3**) are glycosides of bayogenin, while five saponins (compounds **2**, **4**, **5**, **6**, and **8**) of the eight identified are hederagenin derivatives. This indicates that glycosylation in *M. arabica* is more selective toward bayogenin than to hederagenin. It is also interesting to note that in all these saponins, except for compound **7**, a glycoside of 2-β-hydroxy oleanolic acid, the first sugar in the C-3 position of the triterpene, is arabinose, and when present, the sugar in position C-28 is glucose. This indicates a higher enzymatic selectivity for the sugar position.

Three minor saponins have also been detected by TLC in

the total saponin extract but were not present in sufficient quantity to enable their isolation and structural elucidation in this study. Additionally, it has also to be noted that neither medicagenic acid nor zanhic acid glycosides have been found in aerial part of *M. arabica* (*20*), although these compounds represent the main saponins in *M. sativa*. This result can have a significant chemotaxonomical meaning in the further studies for classification of the *Medicago* species (*37*).

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