Cycloartane Glycosides from Three Species of *Astragalus* (Fabaceae)

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Nine cycloartane-type glycosides were isolated from three species of the genus *Astragalus* (Fabaceae): From the aerial parts of *A. cicer* L., two new saponins, cicerosides A and B (**1** and **2**, resp.), *i.e.*, a tetradesmosidic and tridesmosidic cycloartane-type glycosides besides one known compound, from the roots of *A. sempervirens* LAM., one known saponin, and from the roots of *A. ptilodes* BOISS. var. *cariensis* BOISS., five known compounds. Their structures were established mainly by 600-MHz 2D-NMR techniques (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, and HMBC) and mass spectroscopy.

Introduction. – The genus *Astragalus* contains more than 2000 species and represents one of the largest genus in the family of Fabaceae. It is widely distributed in the world of which *ca.* 70 species have been found in the Mediterranean region [1]. Some of them are well-known for their pharmacological properties, particularly hepatoprotective, immunostimulant, and antiviral activities [2]. Many phytochemical studies on *Astragalus* genus were realized bringing out the presence of saponins with cycloartane-type genins [1][3]. In our continuing search for bioactive saponins from the genus *Astragalus* [3], we report in this article the isolation and characterization of two new together with seven known cycloartane-type saponins from the aerial parts of *A. cicer* L., from the roots of *A. sempervirens* LAM., and from the roots of *A. ptilodes* BOISS. var. *cariensis* BOISS. Up to now, no phytochemical investigation of the species *A. cicer* L. and *A. ptilodes* BOISS. var. *cariensis* BOISS. has been made with the aim to search for saponins. *A. sempervirens* LAM. has already been the object of a phytochemical work, as we recently reported [3], bringing out the known compound trojanoside H previously isolated from *A. trojanus* STEV. [4].

Results and Discussion. – The air-dried and powdered aerial parts of *A. cicer* L. were subjected to a maceration with MeOH and then to reflux in MeOH. After filtration, the solvent was evaporated, the residue dissolved in H_2O, and the solution successively partitioned with petroleum ether, CH_2Cl_2, and BuOH saturated with H_2O.

The BuOH fraction was purified by column chromatography (CC *Sephadex LH-20*, with MeOH), reversed-phase flash chromatography (FC; SiO_2 *RP-18*; *CombiFlash*), and several normal- and reversed-phase medium-pressure liquid chromatographies (MPLC) to give **1** and **2** (see *Fig.*) and the known compound eremophiloside B (= (3*β*,6*α*,16*β*,24*R*)-16-(acetyloxy)-24-hydroxy-25-(*β*-D-xylopyranosyloxy)-9,19-cyclolanostane-3,6-diyl bis[6-deoxy-*α*-L-mannopyranoside]) [5], isolated previously from *A. eremophilus* BOISS.

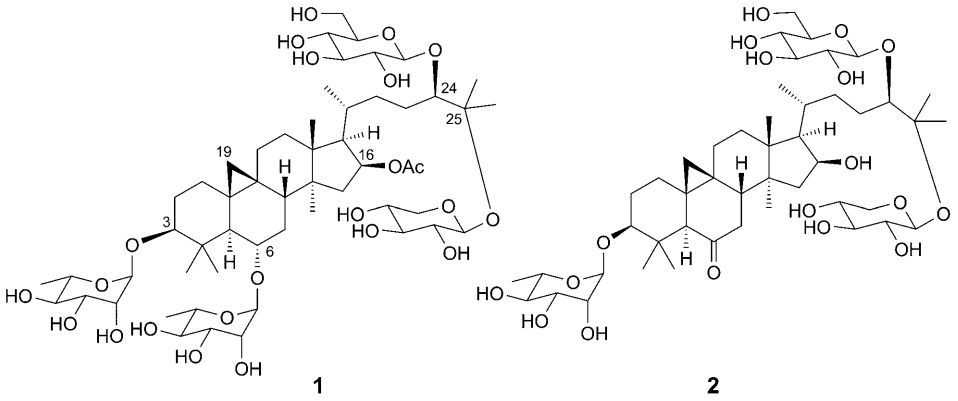

Figure. *Compounds* **1** *and* **2**, *isolated from* Astragalus cicer L.

The dried and powdered roots of *A. sempervirens* LAM. were extracted in the same way as *A. cicer* L. A part of the BuOH fraction was purified by successive chromatographic steps on normal- and reversed-phase SiO_2 affording the known saponin 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-25-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane (= (3*β*,6*α*,16*β*,20*R*,24*S*)-20,24-epoxy-25-(*β*-D-glucopyranosyloxy)-6,16-dihydroxy-9,19-cyclolanostan-3-yl 2-*O*-*α*-L-arabinopyranosyl-*β*-D-xylopyranoside) [6], which has been isolated before from *A. campylosema* BOISS. ssp. *campylosema*.

The dried and powdered roots of *A. ptilodes* BOISS. var. *cariensis* BOISS. were extracted with MeOH at room temperature, the extract evaporated, the residue dissolved in H_2O, and the solution successively partitioned with hexane, $CHCl_3$, and BuOH saturated with H_2O. A part of the BuOH fraction was submitted to vacuum liquid chromatography (VLC; polyamide), further purified by a reversed-phase FC (SiO_2 *RP-18*) and several separations by normal-phase MPLC (SiO_2) to give the five known compounds astragaloside I [7] and astragaloside VII [8], both previously isolated from *A. membranaceus* BUNGE, cyclosiversioside A [9], and cyclosiversiosides E and F [10][11] which have been isolated before from *A. sieversianus* PALL. All these known compounds were isolated for the first time in the three studied species.

The structures of **1** and **2** were determined by spectroscopic methods including 1D- and 2D-NMR experiments (^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HSQC, HMBC, COSY, TOCSY, and ROESY) in combination with ESI- and HR-ESI-MS. The monosaccharides obtained by acid hydrolysis of each compound were identified by comparison on TLC with authentic samples as glucose, xylose, and rhamnose for both

molecules. The absolute configurations of the sugars were determined to be D for glucose and xylose, and L for rhamnose, by GC analysis (see *Exper. Part*). In the 1D- and 2D-NMR spectra of compounds **1** and **2**, the relatively large ^3J(H–C(1),H–C(2)) values of the anomeric H-atom signals of glucose and xylose in their pyranose form (8.1 and 7.3, or 7.7 Hz, resp.), indicated a β-orientation [12]. The broad *s* of the anomeric H-atoms and the large ^1J(H–C(1),C(1)) values of the rhamnose (165–168 Hz), confirmed that the anomeric H-atoms were equatorial (α-pyranoid anomeric form) [12].

Table 1. *^{13}C- and ^{1}H-NMR Data* (150 and 600 MHz, resp. (D_5)pyridine) *of the Aglycone Maoiety of Compounds* **1** *and* **2***, from 1D- and 2D-NMR Experiments.* δ in ppm, *J* in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	29.8	1.44[a]), 2.03[a])	[b])	[b])
CH_2(2)	29.3	1.77[a]), 2.15[a])	[b])	[b])
H–C(3)	88.2	3.30 (*dd*, *J* = 11.3, 4.0)	83.8	4.17[a])
C(4)	41.9		41.7	
H–C(5)	51.8	1.55[a])	52.2	3.31 (*s*)
H–C(6) or C(6)	79.4	3.52 (*dt*, *J* = 6.0, 2.0)	212.4	
CH_2(7)	32.2	1.19[a]), 1.55[a])	41.9	2.33[b])
H–C(8)	45.9	1.37 (br. *dd*, *J* = 14, 3.4)	42.9	2.65 (*dd*, *J* = 7.3, 4.0)
C(9)	20.9		[b])	
C(10)	28.7		[b])	
CH_2(11)	25.9	1.19[a]), 1.90[a])	26.5	1.95[a]), 1.98[a])
CH_2(12)	32.7	1.59[a]), 1.66[a])	33.2	1.65[a]), 2.07[a])
C(13)	46.5		46.2	
C(14)	47.1		47.8	
CH_2(15)	46.0	1.70[a]), 2.37 (*dd*, *J* = 13.3, 8.5)	46.5	1.60[a]), 1.99[a])
H–C(16)	75.9	5.57–5.61 (*m*)	71.5	4.82[a])
H–C(17)	55.6	1.98[a])	56.4	1.84 (*dd*, *J* = 10.7, 7.5)
Me(18)	18.7	1.19 (*s*)	16.0	1.30 (*s*)
CH_2(19)	30.7	0.36, 0.49 (2*d*, *J* = 3.2)	[b])	[b])
H–C(20)	31.7	1.92[a])	32.3	2.17–2.20 (*m*)
Me(21)	18.5	1.04 (*d*, *J* = 6.0)	18.6	1.01 (*d*, *J* = 6.4)
CH_2(22)	33.4	1.15[a]), 2.30[a])	33.8	1.35[a]), 2.53[a])
CH_2(23)	34.4	1.44[a]), 2.01[a])	29.2	1.57[a]), 2.02[a])
H–C(24)	86.1	3.92 (*d*, *J* = 9.7)	87.2	3.99[a])
C(25)	82.2		82.1	
Me(26)	24.6	1.51 (*s*)	24.6	1.46 (*s*)
Me(27)	20.0	1.67 (*s*)	19.9	1.62 (*s*)
Me(28)	17.0	1.00 (*s*)	15.3	1.16 (*s*)
Me(29)	28.6	1.10 (*s*)	27.3	1.55 (*s*)
Me(30)	20.0	0.90 (*s*)	18.5	1.22 (*s*)
*Ac*O–C(16)	170.7, 21.5	2.10 (*s*)		

[a]) Overlapped signals are reported without designated multiplicity. [b]) Not determined.

Ciceroside A (**1**) was obtained as a white amorphous powder. The molecular formula of **1** was deduced as $C_{55}H_{92}O_{23}$ on the basis of HR-ESI-MS (positive-ion mode) exhibiting the $[M+Na]^+$ peak at *m*/*z* 1143.5932 (calc. 1143.5927). It was confirmed by

Table 2. *^{13}C-NMR and ^{1}H-NMR Data* (150 and 600 MHz, resp.; (D_5)pyridine) *of the Sugar Moieties of* **1** *and* **2**, *from 1D- and 2D-NMR Experiments*[a]). δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
3-O-*Rha* (Rha I):				
H–C(1)	104.6	5.28 (br. *s*)	104.7	5.35 (br. *s*)
H–C(2)	72.3	4.56 (br. *s*)	72.3	4.58 (*dd*, $J = 3.2, 1.6$)
H–C(3)	72.6	4.47[a])	72.8	4.47[a])
H–C(4)	74.2	4.29[a])	74.1	4.27[a])
H–C(5)	69.9	4.32[a])	69.8	4.29[a])
Me(6)	18.5	1.68[a])	18.4	1.57 (*d*, $J = 5.6$)
6-O-*Rha* (Rha II):				
H–C(1)	103.7	5.34 (br. *s*)		
H–C(2)	72.8	4.48 (br. *s*)		
H–C(3)	72.9	4.42[a])		
H–C(4)	73.9	4.28[a])		
H–C(5)	70.2	4.28[a])		
Me(6)	18.5	1.66[a])		
24-O-*Glc:*				
H–C(1)	104.7	5.37 (*d*, $J = 8.1$)	104.8	5.45 (*d*, $J = 8.1$)
H–C(2)	75.3	3.99 (*t*, $J = 8.3$)	75.7	4.01[a])
H–C(3)	78.3	4.15[a])	78.8	4.17[a])
H–C(4)	72.1	4.24[a])	71.7	4.16[a])
H–C(5)	77.7	3.76–3.80 (*m*)	77.9	3.82–3.86 (*m*)
CH_2(6)	63.5	4.39 (H_α)[a]), 4.47 (H_β)[a])	63.0	4.35 (*dd*, $J = 11.7, 5.2$, H_α), 4.47 (*dd*, $J = 8.9, 2.8$, H_β)
25-O-*Xyl:*				
H–C(1)	99.2	4.98 (*d*, $J = 7.3$)	99.1	4.96 (*d*, $J = 7.7$)
H–C(2)	75.6	3.97 (*d*, $J = 8.5$)	75.2	3.97[a])
H–C(3)	78.9	4.14[a])	78.4	4.13[a])
H–C(4)	71.3	4.22[a])	71.2	4.21[a])
CH_2(5)	67.1	3.69 (*t*, $J = 10.7$, H_α), 4.30 (H_β)[a])	67.0	3.68 (*t*, $J = 10.7$, H_α), 4.30 (H_β)[a])

[a]) Overlapped signals are reported without designated multiplicity. [b]) Not determined.

the negative-ion-mode ESI-MS experiment, which exhibited a quasimolecular-ion peak at m/z 1119 ($[M-H]^-$) indicating a M_r of 1120. Other fragment-ion peaks were observed at m/z 1076 ($[M-H-43]^-$), 987 ($[M-H-132]^-$), 914 ($[M-H-43-162]^-$), 843 ($[M-H-162-132+H_2O]^-$), 713 ($[M-H-146-146-132+H_2O]^-$), and 654 ($[M-H-43-162-146-132+H_2O]^-$). These results revealed the sucessive loss of one Ac, one pentosyl, one hexosyl, and two deoxyhexosyl moieties, respectively. The ^{1}H-NMR spectrum of **1** showed seven caracteristic Me signals typical for cycloartane-type saponins, at δ(H) 0.90, 1.00, 1.10, 1.19, 1.51, 1.67 (*s*, each) and 1.04 (*d*, $J = 6.0$ Hz) as well as the two characteristic H-atom signals of the cyclopropane ring at δ(H) 0.36 and 0.49 (each *d*, $J = 3.2$ Hz) (*Table 1*). The NMR data, based on correlations observed in the COSY, ROESY, HSQC, and HMBC spectra, allowed the identification of the aglycone of **1** as cycloasgenin C (=(3β,6α,16β,24*R*)-9,19-cyclo-

lanostane-3,6,16,24,25-pentol), previously isolated from *Astragalus taschkendicus* BUNGE, but only partial NMR assignments were reported [13]. The presence of an AcO function was shown by a signal in the ^{13}C-NMR spectrum (*Table 1*) at δ(C) 170.7, an additional *s* in the ^{1}H-NMR spectra at δ(H) 2.10, and a cross-peak in the HMBC plot between δ(H) 2.10 and δ(C) 170.7. The position of the AcO group was deduced from the chemical shift of C(16) (δ(C) 75.9) compared to that of the OH-substituted C(16) in **2** (δ(C) 71.5) and examples from the literature [5], as well as from the δ(H) of H–C(16) at lower field in **1** than in **2**. In the literature, the ^{13}C-NMR chemical shift of the OH-substituted C(24) has been used for the determination of the absolute configuration at this position [14–16]. While a (24*R*) configuration gives a resonance at δ(C) 80.0–80.5 [13][17], a (24*S*) configuration gives a resonance at δ(C) 77.0–77.2 [18]. The deshielded chemical shift of C(24) of **1** at δ(C) 86.1 indicated a glycosylation at this position, which is in good agreement with the literature data of eremophiloside A (=(3β,6α,16β,24*R*)-16-(acetyloxy)-3,6-bis[(6-deoxy-α-L-mannopyranosyl)oxy]-25-(β-D-xylopyranosyloxy)-9,19-cyclolanostan-24-yl 6-deoxy-β-D-galactopyranoside) [5] and led us to the conclusion that the absolute configuration at C(24) of **1** is (*R*) (*Fig.*). The four anomeric H-atom signals at δ(H) 5.37 (*d*, *J* = 8.1 Hz), 5.34 (br. *s*), 5.28 (br. *s*), and 4.98 (*d*, *J* = 7.3 Hz) which gave correlations in the HSQC spectrum with four signals of anomeric C-atoms at δ(C) 104.7, 103.7, 104.6, and 99.2, respectively, indicated the presence of four sugar units in **1** (*Table 2*). Complete assignment of the ^{1}H- and ^{13}C-NMR signals of the aglycone moiety of **1** revealed glycosylation shifts at δ(C) 88.2 (Agly C(3)), 79.4 (Agly C(6)), 86.1 (Agly C(24)), and 82.2 (Agly C(25)) (*Table 1*). These data, in combination with the absence of any ^{13}C glycosylation shifts for the C-atoms of the four sugar units led us to suggest that **1** has a tetradesmosidic structure. Complete assignments of the ^{1}H- and ^{13}C-NMR signals of the sugar moieties by ^{1}H,^{1}H-COSY, TOCSY, HSQC, and HMBC experiments (*Table 2*) allowed the identification of two α-rhamnopyranosyl (Rha I and Rha II), one β-glucopyranosyl (Glc), and one β-xylopyranosyl (Xyl) unit. The establishment of the linkage of the sugar units to the aglycone was shown by the HMBCs δ(H) 5.37 (*d*, *J* = 8.1 Hz, Glc H–C(1))/δ(C) 86.1 (Agly C(24)), δ(H) 5.34 (br. *s*, Rha II H–C(1))/δ(C) 79.4 (Agly C(6)), δ(H) 5.28 (br. *s*, Rha I H–C(1))/δ(C) 88.2 (Agly C(3)), and δ(H) 4.98 (*d*, *J* = 7.3 Hz, Xyl H–C(1))/δ(C) 82.2 (Agly C(25)). On the basis of the above results, the structure of compound **1** was elucidated as (3β,6α,16β,24*R*)-16-(acetyloxy)-3,6-bis(α-L-rhamnopyranosyloxy)-25-(β-D-xylopyranosyloxy)cycloartan-24-yl-β-D-glucopyranoside, named ciceroside A (*Fig.*).

Ciceroside B (**2**) was determined as $C_{47}H_{78}O_{18}$ by HR-ESI-MS (positive-ion mode) showing a quasimolecular-ion peak $[M+Na]^+$ at *m*/*z* 953.5080 (calc. 953.5086) and by the negative-ion-mode ESI-MS showing a quasimolecular-ion peak at *m*/*z* 929 ($[M-H]^-$) in accordance with an M_r of 930. Other fragment-ion peaks were observed at *m*/*z* 783 ($[M-H-146]^-$) and 639 ($[M-H-146-162+H_2O]^-$) suggesting the successive loss of one pentosyl and one hexosyl unit. ^{1}H-NMR Signals of three anomeric H-atoms at δ(H) 5.45 (*d*, *J* = 8.1 Hz), 5.35 (br. *s*), and 4.96 (*d*, *J* = 7.7 Hz) giving correlations with three signals of anomeric C-atoms at δ(C) 104.8, 104.7, and 99.1, respectively, indicated the presence of three sugar units (*Table 2*). Complete assignments of the ^{1}H- and ^{13}C-NMR signals of the sugar moieties, assigned by extensive 2D-NMR experiments, led to the identification of one α-rhamnopyranosyl (Rha), one β-glucopyranosyl (Glc), and one β-xylopyranosyl (Xyl) unit. Full

assignment of the ^{1}H- and ^{13}C-NMR signals of the aglycone moiety of **2** showed glycosylation shifts at δ(C) 83.8 (Agly C(3)), 87.2 (Agly C(24)), and 82.1 (Agly C(25)) (*Table 1*). These data, in combination with the absence of any ^{13}C glycosylation shifts for the C-atoms of the three sugar units, led us to deduce that **2** has a tridesmosidic structure. The establishment of the linkage of the sugar units to the aglycone was shown by the HMBCs δ(H) 5.45 (*d*, $J = 8.1$ Hz, Glc H–C(1))/δ(C) 87.2 (Agly C(24)), δ(H) 5.35 (br. *s*, Rha H–C(1))/δ(C) 83.8 (Agly C(3)), and δ(H) 4.96 (*d*, $J = 7.7$ Hz, Xyl H–C(1))/δ(C) 82.1 (Agly C(25)). The NMR data based on correlations observed in the COSY, ROESY, HSQC, and HMBC spectra of the aglycone moiety of **2** were in good agreement with those of huangqiyegenin II (=(3β,16β,24*S*)-3,16,24,25-tetrahydroxy-9,19-cyclolanostan-6-one) [19], exept for the configuration at position C(24). The comparison of the chemical shift of C(24) of **2** (δ(C) 87.2) with that of **1** (δ(C) 86.1) and that of eremophiloside A (δ(C) 86.8) [5] led us to deduce the absolute configuration of this asymmetric center to be (*R*). The presence of a keto function in **2** was shown by the signal in the ^{13}C-NMR spectrum at δ(C) 212.4. Its location at position C(6) was suggested by the presence of a *s* in the ^{1}H-NMR spectrum at δ(H) 3.31 (*s*, Agly H–C(5)) and confirmed by HMBC cross-peaks between δ(H) 3.31 (*s*, Agly H–C(5)), 2.33 (Agly CH_2(7)), and 2.65 (*dd*, $J = 7.3$, 4.06, Agly H–C(8)) and δ(C) 212.4 (Agly C(6)), respectively. Thus, **2** was established as (3β,16β,24*R*)-24-(β-D-glucopyranosyloxy)-16-hydroxy-3-(α-L-rhamnopyranosyloxy)-25-(β-D-xylopyranosyloxy)cycloartan-6-one, named ciceroside B.

The presence of tridesmosidic cycloartane-type saponins in the genus *Astragalus* was shown in some species [1][4][20–23], whereas the presence of tetradesmosidic structural analogs seems to be very rare in the genus *Astragalus* [5].

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*). Vacuum liquid chromatography (VLC): polyamide *SC 6* (50–160 μm, *Macherey-Nagel*). Flash chromatography (FC): *CombiFlash* (*Serlabo Technologies*); *RetiSep C-18* reversed-phase 130 g (40–63 μm; *Teledyne ISCO*); silica gel (SiO_2; 30–70 μm) glass column (180 × 50 mm). Medium-pressure liquid chromatography (MPLC): SiO_2 *60* (15–40 μm; *Merck*), HPLC pump *426* (*Alltech*), fraction collector *Biotech SuperFrac* (*Pharmacia*); SiO_2 *RP-18 Spherical C18* (300 Å, 75–200 μm; *Silicycle*), pump module *C-601* (*Büchi*), fraction collector *C-660* (*Büchi*), *Büchi* glass columns (460 × 25 mm and 460 × 15 mm), *Büchi* precolumn (110 × 15 mm). Gas chromatography (GC): *Termoquest* gas chromatograph with FID; *DB-1701* cap. column (30 m × 0.25 mm; *J & W Scientific*); detector temp.: 80° for 5 min, then increase to 270° at 15°/min, carrier gas He. TLC: SiO_2 plate *F-254* (60 Å; *Silicycle*) or SiO_2 *60* F_{254} (*Merck*) for high performance; eluent $CHCl_3$/MeOH/H_2O 70:23:4 (a); detection with *Komarowsky* reagent, *i.e.*, 2% 4-hydroxybenzaldehyde in MeOH/50% H_2SO_4 soln. 5:1 (1), followed by heating. Optical rotation: *AA-OR automatic* polarimeter. 1D- and 2D-NMR Spectra: *Varian Inova-600* (600 MHz) NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI- (neg.) and HR-ESI-MS (pos.): *Q-TOF-1* micromass spectrometer; in *m*/*z* (rel. %).

Plant Material. The aerial parts of *A. cicer* L. were collected at the Combe de Malaval, Les Freaux (Hautes-Alpes), France, in 2006. The roots of *A. sempervirens* LAM. were collected near the village of Saint-Disdier en Dy (Hautes-Alpes), France, in 2005. Two voucher specimens (N° 24032010, *A. cicer* L.; N° 6627, *A. sempervirens* LAM.) were deposited with the Herbarium of the Laboratory of Pharmacognosy, Burgundy University, Dijon, France. Both species were identified by *Gérard Ducerf*, Promonature, Briant, France. The roots of *A. ptilodes* BOISS. var. *cariensis* BOISS. were collected from the montain of Spil (1450 m), Manisa, Turkey, in July 1993 and identified by *Özcan Seçmen*, Department of

Biology, Faculty of Sciences, Ege University, Izmir, Turkey. A voucher specimen (EGE 19568) was deposited with the Herbarium of Ege University.

Extraction and Isolation. The air-dried and powdered aerial parts of *A. cicer* L. (390 g) were subjected to a maceration with 70% MeOH for 94 h, and then the residue of the maceration in MeOH was refluxed twice for 1 h at 70–80° (2 × 3 l). After filtration of the combined MeOH solns., the solvent was evaporated yielding 5.120 g of extract. This MeOH extract was dissolved in H_2O (300 ml), and successively partitioned with petroleum ether (3 × 300 ml), CH_2Cl_2 (4 × 300 ml), and BuOH sat. with H_2O (5 × 300 ml). The BuOH extract (4.320 g) was purified by CC (*Sephadex LH-20*, MeOH) yielding one main fraction (3.950 g) rich in saponins. The latter was subjected to FC (*RP-18*, MeOH/H_2O 60:40 → 100:0; 10.0 ml/min): *Fractions 1–6*. *Fr. 3* (117.7 mg) was subjected to MPLC (SiO_2, $CHCl_3$/MeOH/H_2O 80:20:2, 70:23:4, 64:40:8, and 0:100:0; 3 ml/min): *Frs. 3.1–3.9*. *Fr. 3.6* afforded **2** (6.7 mg). *Fr. 4* (770.3 mg) was divided in three equal parts (*Frs. 4.A*, *4.B*, and *4.C*), each of them was subjected to MPLC (SiO_2, $CHCl_3$/MeOH/H_2O 80:20:2, 70:23:4, 64:40:8, and 0:100:0; 5 ml/min) to yield a total of 31 fractions, *Frs. 4.A1–4.A11*, *Frs. 4.B1–4.B11*, and *Frs. 4.C1–4.C9*. *Fr. 4.C6* gave *eremophiloside B* (*= (3β,6α,16β,24*R*)-16-(acetyloxy)-24-hydroxy-25-(β-*D*-xylopyranosyloxy)-9,19-cyclolanostane-3,6-diyl bis[6-deoxy-α-*L*-mannopyranoside]*; 11.7 mg): *Fr. 4.A11* (22.8 mg), *Fr. 4.B9* (5.2 mg), *Fr. 4.B10* (4.1 mg), and *Fr. 4.C8* (12.4 mg) were combined, giving the new fraction *Fr. 4.X1* (44.5 mg), which was submitted to MPLC (*RP-18*, MeOH/H_2O 40:60 → 100:0; 3 ml/min): affording *Frs. 4.X1.1–4.X1.5*. *Fr. 4.X.1.5* gave **1** (7.1 mg).

The dried and powdered roots of *A. sempervirens* LAM. (320 g) were subjected to a maceration with 70% MeOH for 20 h, and then the residue of the maceration in MeOH was refluxed twice for 1 h at 70–80° (2 × 3 l). After filtration of the combined MeOH soln., the solvent was evaporated. The residue of the MeOH extract was dissolved in H_2O (200 ml) and partitioned with BuOH sat. with H_2O (5 × 200 ml) to yield 19.240 g. Part of the latter (12 g) was purified by FC (SiO_2, $CHCl_3$/MeOH/H_2O 90:10:0, 80:20:0, 75:20:0, 80:20:0, 80:20:2, 70:23:4, 64:40:8, 30:60:10, and 0:100:0; 50 ml/min): *Fractions A–M*. An aliquot of *Fr. I* (334 mg) and *J* (301 mg) were respectively subjected to several separations by MPLC (SiO_2, $CHCl_3$/MeOH/H_2O 80:20:2, 70:23:4, 64:40:8, and 0:100:0; and SiO_2 *RP-18*, MeOH/H_2O 40:60 → 100:0 and 75:25 → 100:0) giving together a total of 32 fractions. After combination of three of them, the new fraction (60 mg) was submitted to two successive purifications by MPLC (*RP-18*, MeOH/H_2O 75:25 → 90:10 and 55:45 → 80:20): *(3β,6α,16β,20*R*,24*S*)-20,24-epoxy-25-(β-*D*-glucopyranosyloxy)-6,16-dihydroxy-9,19-cyclolanostan-3-yl 2-*O*-α-*L*-arabinopyranosyl-β-*D*-xylopyranoside* (9.2 mg).

The dried and powdered roots of *A. ptilodes* BOISS. var. *cariensis* BOISS. (1.5 kg) were extracted with MeOH (3 × 5 l) at r.t. during 24 h for each extraction, and the solvent was evaporated, yielding 64 g. This residue was dissolved in H_2O (500 ml), and successively partitioned with hexane (3 × 300 ml), $CHCl_3$ (3 × 300 ml), and BuOH sat. with H_2O (3 × 300 ml). The latter gave, after removal of the solvent, 36 g of extract. Part of the BuOH extract (5 g) was purified by VLC (polyamide, 750 ml of H_2O, 750 ml of 50% MeOH, and 1250 ml of 100% MeOH). The 100% MeOH fraction (4.010 g) was submitted to FC (*RP-18*, MeOH/H_2O 60:40 → 100:0): *Fractions a–j*. An aliquot of *Fr. d* (390 mg) was separated by MPLC (SiO_2, $CHCl_3$/MeOH/H_2O 80:20:2, 70:23:4, 64:40:8, and 0:100:0): *astragaloside VII* (*= (3β,6α,16β,20*R*,24*S*)-20,24-epoxy-16-hydroxy-3-(β-*D*-xylopyranosyloxy)-9,19-cyclolanostane-6,25-diyl bis[β-*D*-glucopyranoside]*; 6.1 mg). A mixture of an aliquot of *Fr. e* and *f* was subjected to MPLC in the same way as *Fr. d*: *cyclosiversiosides E* (*= (3β,6α,16β,20*R*,24*S*)-20,24-epoxy-16,25-dihydroxy-9,19-cyclolanostane-3,6-diyl bis[β-*D*-xylopyranoside]*; 15.2 mg) and *F* (*= (3β,6α,16β,20*R*,24*S*)-20,24-epoxy-16,25-dihydroxy-3-(β-*D*-xylopyranosyloxy)-9,19-cyclolanostan-6-yl β-*D*-glucopyranoside*; 10.1 mg), and *astragaloside I* (*= (3β,6α,16β,20*R*,24*S*)-3-[(2,3-di-*O*-acetyl-β-*D*-xylopyranosyl)oxy]-20,24-epoxy-16,25-dihydroxy-9,19-cyclolanostan-6-yl β-*D*-glucopyranoside*; 11.8 mg) and *cyclosiversioside A* (*= (3β,6α,16β,20*R*,24*R*)-3-[(2,3-di-*O*-acetyl-β-*D*-xylopyranosyloxy]-20,24-epoxy-16,25-dihydroxy-9,19-cyclolanostan-6-yl β-*D*-xylopyranoside*; 7.3 mg).

Acid Hydrolysis and GC Analysis. Each compound (3 mg) was hydrolyzed with 2N aq. CF_3COOH (5 ml) for 3 h at 95°. After extraction with CH_2Cl_2 (3 × 5 ml), the aq. layer was repeatedly concentrated with MeOH until neutral and then analyzed by TLC (SiO_2, $CHCl_3$/MeOH/H_2O 8:5:1) by comparison with authentic samples. The (trimethylsilyl)thiazolidine derivatives of the sugar residue of each compound were prepared and analyzed by GC [24]. L-Rhamnose, D-glucose, and D-xylose were detected

in each case (**1** and **2**) by co-injection of the hydrolyzate with standard silylated samples, giving single peaks at t_R 13.16, 18.54, and 13.43 min, resp.

*(3β,6α,16β,24*R*)-16-(Acetyloxy)-3,6-bis[(6-deoxy-α-*L*-mannopyranosyl)oxy]-25-(β-*D*-xylopyranosyl-oxy)-9,19-cyclolanostan-24-yl β-*D*-Glucopyranoside* (= *Ciceroside A*; **1**): White amorphous powder. $[\alpha]_D^{25} = +3.2$ ($c = 0.18$, MeOH). TLC (a): R_f 0.27. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 1076 ($[M-H-43]^-$), 987 ($[M-H-132]^-$), 914 ($[M-H-43-162]^-$), 843 ($[M-H-162-132+18]^-$), 713 ($[M-H-146-146-132+18]^-$), 654 ($[M-H-43-162-146-132+18]^-$).

*(3β,16β,24*R*)-3-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-24-(β-*D*-glucopyranosyloxy)-16-hydroxy-25-(β-*D*-xylopyranosyloxy)-9,19-cyclolanostan-6-one* (= *Ciceroside B*; **2**). White amorphous powder. $[\alpha]_D^{25} = +9.5$ ($c = 0.18$, MeOH). TLC (a): R_f 0.52. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 783 ($[M-H-146]^-$), 638 ($[M-H-146-163+18]^-$).

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