Where W_{in} is the amount of lauroyl-indapamide in liposomes and W_{total} is the total amount of lauroyl-indapamide in the liposomal preparation.

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An unusual new sulfated triterpene saponin from *Arenaria juncea*

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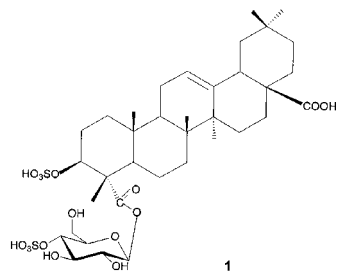
A novel triterpene saponin, 3-*O*-sulfate-gypsogenic acid-23-*O*-β-D-4-*O*-sulfate-glucopyranoside (junceoside D), has been isolated from the roots of *Arenaria juncea*. The structure was characterized mainly by a combination of 2D-NMR techniques, mass spectrometry and chemical methods.

Arenaria juncea Bieb. roots endemic to China (Family: Caryophyllaceae) is well-known in the Traditional Chinese Medicine in the Shanxi, Gansu Hubei province as a substitute of the Chinese drug Yin-Chai-Hu (root of *Stellaria dichotoma* var. *lanceolata* Bge) (Cui et al. 1992) and is used to treat fever due to Yin-deficiency and fever in infant malnutrition. Our previous phytochemical studies on the methanolic extract of *Arenaria juncea* roots led to the isolation of five triterpene-saponins (Gaidi et al. 2001). A detailed further investigation of the same extract has led to the isolation of an additional triterpene-glycoside **1**. The concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *Arenaria juncea* Bieb. (Caryophyllaceae) was purified by precipitation with diethyl ether. The crude saponin mixture was further dialysed and subjected to multiple chromatographic steps over Sephadex LH-20 and medium pressure liquid chromatography (MPLC) over reversed-phase Si RP-18 yielding a saponin, junceoside D (**1**). Its structure was elucidated mainly by FABMS, MALDITOFMS, HRESIMS and by 600 MHz NMR spectroscopic analysis including 1D and 2D NMR experiments ($^1H-^1H$ COSY, TOCSY, NOESY, HSQC, and HMBC).



1

Compound **1** was obtained as a yellow powder. The FABMS (negative-ion mode) exhibiting a quasi-molecu-

Table: ^{13}C NMR Data of the aglycon and sugar unit of saponin 1 (δ ppm, DMSO-d_6 as solvent)[a]

Position	Gypsogenic acid		
	Mult.[b]	δ ^{13}C	δ ^{1}H
1	CH_2	38.3	0.95, 1.52
2	CH_2	23.0	1.36, 1.72
3	CH	80.0	4.54, dd, J = 4.1 and J = 16.1
4	C	52.8	–
5	CH	51.6	1.36
6	CH_2	19.0	nd, nd
7	CH_2	33.7	1.30, 1.50
8	C	39.3	–
9	CH	48.0	1.46
10	C	36.3	–
11	CH_2	23.6	nd, nd
12	CH	121.5	5.05 (br s)
13	C	145.5	–
14	C	41.8	–
15	CH_2	28.0	1.18, nd
16	CH_2	23.7	nd, nd
17	C	46.7	–
18	CH	41.8	2.72
19	CH_2	46.4	0.98, 1.50
20	C	31.0	–
21	CH_2	34.6	0.90, nd
22	CH_2	28.0	0.80
23	C	174.6	–
24	CH_3	12.2	0.98 (s)
25	CH_3	15.7	0.83 (s)
26	CH_3	17.4	0.64 (s)
27	CH_3	26.0	1.01 (s)
28	C	180.0	–
29	CH_3	33.6	0.82 (s)
30	CH_3	24.1	0.80 (s)
		Glucose	
1		94.5	5.35 (d, J = 8.2)
2		72.8	3.23 (t, J = 8.5)
3		74.3	3.49 (t, J =9.1)
4		75.7	3.87 (br s)
5		75.8	3.02 (t, J = 7.0)
6		61.5	3.43, 3.59 (d, J = 11.4)

[a] The assignments were based on the COSY, TOCSY, NOESY, HSQC, and HMBC experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR), [b] Multiplicities were assigned from DEPT spectra. nd: not determined

lar ion peak at m/z 807 $[M-H]^-$ and the high-resolution ESI mass spectrum, HRESIMS (positive ion mode) showing a pseudomolecular ion peak at m/z 831.2568 $[M + Na]^+$ (Calcd for 831.2544, $C_{35}H_{52}O_{17}NaS_2$) indicated a molecular weight of 808, compatible with the molecular formula $C_{35}H_{52}O_{17}S_2$. Other significant ion peaks visible in the FABMS at m/z 727 $[(M-H)\text{-}80]^-$, 565 $[(M-H)\text{-}80\text{-}162]^-$, 485 $[(M-H)\text{-}80\text{-}162\text{-}80]^-$ corresponded to the successive losses of two sulfate groups $[SO_3]^-$ and one hexosyl moiety. The fragment ions at m/z 485 corresponded to the pseudomolecular ion of the aglycone. The presence of a sulfate group was concluded by the presence of a peak at m/z 97 $[OSO_3H]^-$ in the negative FABMS. The MALDITOFMS of **1** showing a $[M-H]^-$ ion peak at m/z 807 and $[(M + K-H)-H]^-$ ion peak at m/z 845 followed by a fragment ion peak at m/z 727 $[M-H-80]^-$ confirmed the proposed molecular weight. Mineral acid hydrolysis of **1** with 2 N TFA afforded an artifactual aglycone and glucose (co-TLC). The aglycone was identified as gypsogenic acid from 2D NMR spectra of **1** (Table) (Koike et al. 1999). The downfield shift observed in the HSQC spectrum for the Agly H-3/Agly C-3 resonance at δ_H 4.54 (dd, J = 4.1 and J = 16.1)/δ_C 80.5 suggested that the aglycone residue was esterified by the SO_3H group at the position C-3, that was in good agreement with literature data for similarly sulfated saponins (Acebes et al. 1998). Compound **1** was shown to contain one sugar residue from the HSQC spectrum which showed the anomeric ^{1}H NMR signal at δ 5.35 (d, J = 8.2 Hz) giving a correlation with the ^{13}C NMR signal at δ 94.5. The ring protons of the monosaccharide residue were assigned starting from the readily identifiable anomeric proton by means of the COSY, TOCSY, HSQC and HMBC spectra. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one β-glucopyranosyl moiety (Glc). The common D-configuration was assumed, according to those most often encountered among the plant glycosides. In the HMBC spectrum, the carbonyl C-28 observed at δ 180.0 was typical of a unsubstituted group but the upfield shift of C-23 at δ_C 174.6 indicated that C-23 was substituted by the glucosidic ester chain. Unequivocal demonstration of the linkage at this position was obtained from the HMBC experiment, giving a cross peak between $\delta_{H\text{-}24}$ 0.98 (s) and $\delta_{C\text{-}23}$ 174.6 and between the anomeric ^{1}H NMR signal at $\delta_{H\text{-}1}$ 5.35 (d, J = 8.2 Hz, Glc H-1) and the ^{13}C NMR signal at δ_C 174.6 (Agly C-23). The downfield shift observed in the HSQC spectrum for the Glc H-4/Glc C-4 resonance at δ_H 3.87 (br s)/δ_C 75.7 suggested that the Glc residue was substituted at the position C-4 but the substituent does not contain carbon atoms. It was suggested that the substituent is a second SO_3H group. This was confirmed by FABMS which presented a fragment ion at m/z 565 $[(M-H)\text{-}80\text{-}162]^-$.

Based on the above results, the structure of compound **1** was determined as 3-*O*-sulfate-gypsogenic acid-23-*O*-β-D-4-*O*-sulfate-glucopyranoside, a new natural compound.

Sulfated triterpene saponins have been already found in Caryophyllaceae (Acebes et al. 1998) but according to a an updated literature search and to previous reviews on sulfated-saponins (Amimoto et al. 1993; Higuchi et al. 1984; Kostova et al. 2002; Sánchez-Contreras et al. 1998, 2000; Sasmakov et al. 2003; Shaker et al. 2000; Sung et al. 1991), **1** is a first triterpene-saponin containing two sulfate groups.

Experimental

1. General procedures

All physical data of the isolated compound were obtained on the same instruments as those used in a previous paper (Gaidi et al. 2001). All NMR chemical shifts (δ) are given in ppm and the samples were solubilized in DMSO-d_6 (δ 39.5). Furthermore, HRESIMS spectra were carried out on a Q-TOF1 micromass spectrometer. MALDITOFMS was conducted using a PerSeptive Biosystems Voyager DE-STR mass spectrometer. TLC and HPTLC employed precoated Si gel plates 60 F_{254} (Merck). The following TLC solvent systems were used: for saponins (a) $CHCl_3$–MeOH–AcOH–H_2O (15 : 8 : 3 : 2); for sapogenins (b) toluene-Me_2CO (4 : 1); for monosaccharides (c) $CHCl_3$–MeOH–H_2O (8 : 5 : 1). Spray reagents for the saponin was: Komarowsky reagent, a mixture (5 : 1) of *p*-hydroxybenzaldehyde (2% in MeOH) and H_2SO_4 50%; for the sugars: diphenylaminephosphoric acid reagent. Isolations were carried out using a Gilson medium-pressure liquid chromatography (MPLC) system (Gaidi et al. 2001).

2. Plant material

The roots of *Arenaria juncea* were collected in July 1990 in Hubei Province, People Republic of China. A voucher specimen (No 48-11) is deposited in the Herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Burgundy (Gaidi et al. 2001).

3. Extraction

Dried powdered roots of *Arenaria juncea* (639 g) were defatted with 5 l *n*-hexane and extracted successively with 5 l $CHCl_3$ and 5 l MeOH. After removal of the solvent by evaporation under reduced pressure, the MeOH extract (30 g) was suspended in H_2O (400 ml) and submitted to partition between H_2O saturated *n*-BuOH (3×200 ml). After evaporation of the solvent under reduced pressure, 23 g of the *n*-BuOH fraction was obtained. The *n*-BuOH extract was solubilized in MeOH (10 ml) and precipitated in Et_2O (3×250 ml) yielding 7 g of a crude saponin fraction of which 4 g was dialysed for 3 days and submitted to column chromatography on Sephadex LH-20 eluted by MeOH yielding four fractions (F1–F4).

4. Isolation and characterization of 1

Five saponins were obtained from F2 (810 mg) (Gaidi et al. 2001). F3 (226 mg) was first fractionated by column chromatography on Sephadex LH-20 eluted by MeOH, then by MPLC on reversed phase material, Lichroprep RP-18, Merck (40–63 μm) eluted with MeOH–H_2O (linear gradient 50–60%) to give compound **1**. Final purification was carried out on Sephadex LH-20 eluted by MeOH to obtain compound **1** (10 mg).
Junceoside D (**1**): Yellow powder. $[\alpha]_{20}^{D} + 16°$ (c 0.10, MeOH); IR ν_{max} KBr cm^{-1} 3398, 2928, 1735, 1718, 1615, 1386, 1220; negative FABMS glycerol matrix: m/z 807 $[M-H]^-$, 727 $[(M-H)-80]^-$, 565 $[(M-H)-80-162]^-$, 485 [(M−H)-80-162-80]; positive HRESIMS: m/z 831.2568 $[M + Na]^+$ (Calcd for 831.2544, $C_{35}H_{52}O_{17}NaS_2$). MALDITOFMS: m/z 807 $[M-H]^-$, 845 $[(M + K-H)-H]^-$, 727 $[M-H-80]^-$. ^1H and ^{13}C NMR: Table.

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Clausine Z, a new carbazole alkaloid from *Clausena excavata* with inhibitory activity on CDK5

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A new carbazole alkaloid, named clausine Z, has been isolated from the stems and leaves of *Clausena excavata* Burm. (Rutaceae). Its structure was established by spectroscopic methods. The compound exhibits inhibitory activity against cyclin-dependent kinase 5 (CDK5) and shows protective effects on cerebellar granule neurons *in vitro*.

CDK5 is a member of the family of cyclin dependent kinases (CDKs). Contrarily to the other members of this family, CDK5 is not involved in the cell cycle control but plays a crucial role in the development of the central nervous system and the regulation of neuronal signal transduction. Experimental studies have shown that over-activation of CDK5 might contribute to the pathology of neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis and cerebral ischemia (Dhavan and Tsai 2001; Shelton and Johnson 2004).
In the course of our search for new CDK5 inhibitors, we noticed that an EtOAc extract of the stems and leaves of *Clausena excavata* Burm. (Rutaceae) showed *in vitro* CDK5 inhibitory activity. *C. excavata* is a shrub growing in Southeast Asia known to contain, among others, coumarins and carbazole alkaloids (Ito et al. 1996, 1997, 2000; Wu et al. 1999). Semipreparative fractionation of the bioactive extract by HPLC-UV and subsequent testing of the fractions enabled the activity to be attributed to a main peak (Rt 8.9 min) with a UV spectrum corresponding to a 9H-carbazole ring system. The compound (**1**) was isolated from an ethanolic extract of the stems and leaves by a combination of liquid/liquid partition and CC on silicagel and Sephadex LH20. The ESI TOF MS (positive ion mode, $[M + H]^+$ m/z 228.0630) allowed the molecular formula to be established as $C_{13}H_9NO_3$. 1D and 2D NMR data revealed the presence of a formyl group (δ^1H 9.91, $\delta^{13}C$ 191.6) and two hydroxy groups (δ^1H 10.24, δ^1H 9.07). The ^1H and ^{13}C resonances of the formyl group, derived from a HC-HSQC experiment, were used as starting point for the assignment of the regiochemistry of compound **1**. The close neighbourhood of the formyl proton with two aromatic protons (H-2 d, $J = 1.3$ Hz and H-4 d, $J = 1.3$ Hz) was confirmed in the HH-ROESY spectrum by the two corresponding intense cross peaks, as well as in the HC-HMBC spectrum, which showed HC