Two New Disulfated Triterpenoids from *Zygophyllum fabago*

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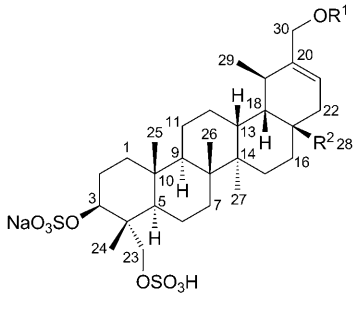
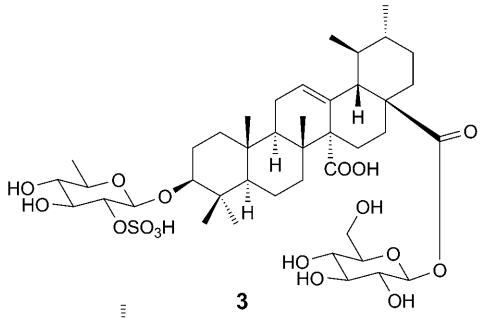
From the aerial parts of *Zygophyllum fabago*, two new monosodium salts of sulfated derivatives of ursolic acid, along with two known quinovic acid glycosides were isolated. The structures of the new compounds were determined as (3β,4α)-3,23,30-trihydroxyurs-20-en-28-al 3,23-di(sulfate) sodium salt (1:1) (**1**) and of (3β,4α)-3,23,28-trihydroxyurs-20-en-30-yl β-D-glucopyranoside 3,23-di(sulfate) sodium salt (1:1) (**2**) with the molecular formula $C_{30}H_{47}NaO_{10}S_2$ and $C_{36}H_{59}NaO_{15}S_2$, respectively. The structures of the known compounds were 3-*O*-(2-*O*-sulfo-β-D-quinovopyranosyl)quinovic acid 28-β-D-glucopyranosyl ester (**3**) and 3-*O*-(β-D-glucopyranosyl)quinovic acid 28-β-D-glucopyranosyl ester (**4**) (quinovic acid = (3β)-3-hydroxyurs-12-ene-27,28-dioic acid). The structures of all these compounds were determined by using 1D- and 2D-NMR spectroscopic techniques.

Introduction. – Chemical studies carried out on the family Zygophyllaceae have revealed the occurrence of important secondary metabolites such as sulfated triterpenoid saponins [1]. In an ongoing search for the bioactive compounds from Zygophyllaceae plants, the EtOH extract of *Zygophyllum fabago* was selected for investigation. From previous studies, several unusual disulfated ursane derivatives with a double bond at C(20)=C(21) have been reported which might have good potential in various biological activities. Compounds isolated in the present study include the two new disulfated triterpenoids **1** and **2**, as well as two known compounds 3-*O*-(2-*O*-sulfo-β-D-quinovopyranosyl)quinovic acid 28-β-D-glucopyranosyl ester (= zygophyloside E; **3**) [2] and 3-*O*-(β-D-glucopyranosyl)quinovic acid 28-β-D-glucopyranosyl ester (= guettarda saponin II; **4**) [3] (quinovic acid = (3β)-3-hydroxyurs-12-ene-27,28-dioic acid).

Results and Discussion. – Compound **1** was isolated as a white amorphous powder. Its molecular formula was determined as $C_{30}H_{47}NaO_{10}S_2$ on the basis of HR-FAB-MS which showed the quasi-molecular-ion peak at *m/z* 653.2435 ($[M-H]^-$). The IR spectra of **1** showed absorption bands for OH groups (3408 cm^{-1}), C–H stretch (2925 cm^{-1}), an aldehyde C=O (1710 cm^{-1}), an olefinic bond (1627 cm^{-1}), and a band



1 R^1 = H, R^2 = CHO
2 R^1 = Glc, R^2 = $HOCH_2$



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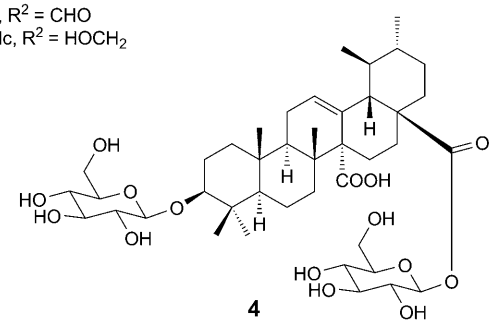

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typical for a sulfate stretch (1122 cm^{-1}). Acid hydrolysis of **1** followed by treatment with $BaCl_2$ yielded barium sulfate, hence established the presence of a sulfate residue in **1** [4]. The ^{1}H-NMR spectrum (*Table*) exhibited an olefinic H-atom resonance at δ(H) 5.56 (*d*, J = 6.5 Hz, H–C(21)), two CH groups at δ(H) 4.38 (*dd*, J = 4.5, 11.5 Hz) and 9.42 (*s*), two CH_2 groups at δ(H) 3.97 (*m*, H_a–C(23) overlapped with H_a–C(30)), 3.77 (*d*, J = 9.5 Hz, H_b–C(23)), and δ(H) 3.94 (*d*, J = 12.5 Hz, H_b–C(30)), four tertiary Me groups at δ(H) 0.76 (*s*, Me(24)), 0.93 (*s*, Me(25)), 0.94 (*s*, Me(26)), and 1.02 (*s*, Me(27)), and one secondary Me group at δ(H) 1.08 (*d*, J = 6.5 Hz, Me(29)). In addition, the ^{13}C-NMR spectra (*Table*) displayed one oxygenated CH group signal at δ(C) 80.8, two oxygenated CH_2 groups at δ(C) 64.8 and 70.0, and five Me groups at δ(C) 13.3, 14.8, 16.5, 17.2, and 23.8. The chemical shift of the CH(18) group, usually found at δ(C) 50.7–51.1 in ursane-type triterpenoids [5], was shifted upfield to δ(C) 41.3 in compound **1** and unambiguously assigned by a HMBC to H–C(19). Accordingly, **1** was suggested to contain an urs-20-ene skeleton and a disulfate group similar to zygofaboside A [6], except for the presence of an additional CH_2O group and the absence of a glycosidic ester linkage. The additional CH_2O group was placed at C(30) of **1** as a consequence of the low-field-shifted signals of the olefinic bond at δ(C) 148.1 (C(20)) and 118.3 (C(21)), and the HMBC (*Fig. 1*) H–C(21) (δ(H) 5.56)/C(30) (δ(C) 64.8). The ^{1}H-NMR signal at δ(H) 9.42 was consistent with the presence of an aldehyde H-atom in conjunction with the ^{13}C-NMR signal at δ(C) 209.0. This spectroscopic evidence suggested that a CHO group could be best accommodated at the C(28) position. On the basis of the above evidences, the structure of **1** was assigned as (3β,4β)-3,23,30-trihydroxyurs-20-en-28-al 3,23-di(sulfate) sodium salt (1:1), which is a new natural compound.

Table. *^{13}C- and ^{1}H-NMR Data* (125 and 500 MHz, resp., CD_3OD) *of* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.75 (br. *s*), 0.98 (br. *s*)	39.4	1.76 (br. *s*), 0.99 (br. *s*)	39.4
CH_2(2)	2.14–2.11 (*m*), 1.83–1.81 (*m*)	25.0	2.14–2.11 (*m*), 1.83–1.18 (*m*)	25.0
H–C(3)	4.38 (*dd*, *J* = 11.5, 4.5)	80.8	4.40 (*dd*, *J* = 11.5, 4.5)	81.0
C(4)		42.6		42.6
H–C(5)	1.32 (br. *s*)	masked	1.33 (br. *s*)	48.1
CH_2(6)	1.60 (br. *s*), 1.41–1.38 (*m*)	18.7	1.61 (br. *s*), 1.43–1.39 (*m*)	18.7
CH_2(7)	2.18–2.09 (*m*), 1.92–1.91 (*m*)	36.1	2.15–2.12 (*m*), 1.92–1.91 (*m*)	35.4
C(8)		42.0		42.2
H–C(9)	1.41–1.38 (*m*)	51.7	1.43–1.41 (*m*)	51.6
C(10)		37.8		37.8
CH_2(11)	1.72–1.67 (*m*), 1.23–1.22 (*m*)	22.7	1.50–1.47 (*m*), 1.29 (*s*)	22.7
CH_2(12)	1.41–1.38 (*m*), 1.15–1.12 (*m*)	29.3	1.41–1.38 (*m*), 1.15–1.12 (*m*)	28.9
H–C(13)	2.37 (*t*, *J* = 7.5)	33.6	1.42 (br. *s*)	39.8
C(14)		43.0		43.3
CH_2(15)	1.15–1.11 (*m*), 1.08–1.05 (*m*)	28.7	1.15–1.11 (*m*), 1.02–1.01 (*m*)	27.8
CH_2(16)	1.41–1.39 (*m*), 1.28 (br. *s*)	30.7	1.32–1.28 (*m*), 1.27 (br. *s*)	30.5
C(17)		51.9		39.5
H–C(18)	2.08–2.11 (*m*)	41.3	2.12–2.15 (*m*)	49.5 (masked)
H–C(19)	1.36 (br. *s*)	48.1	1.26 (br. *s*)	32.9
C(20)		148.1		142.4
H–C(21)	5.56 (*d*, *J* = 6.5)	118.3	5.63 (*d*, *J* = 6.5)	122.8
CH_2(22)	1.53 (br. *s*), 1.34 (br. *s*)	34.8	1.53 (br. *s*), 1.34 (br. *s*)	34.7
CH_2(23)	3.97 (*m*, ovlp., H_a), 3.77 (*d*, *J* = 9.5, H_b)	70.0	3.99 (*d*, *J* = 9.5, H_a), 3.76 (*d*, *J* = 9.0, H_b)	70.0
Me(24)	0.76 (*s*)	13.3	0.76 (*s*)	13.3
Me(25)	0.93 (*s*)	17.2	0.94 (*s*)	17.1
Me(26)	0.94 (*s*)	16.5	1.09 (*s*)	15.3
Me(27)	1.02 (*s*)	14.8	1.02 (*s*)	16.5
H–C(28) or CH_2(28)	9.42 (*s*)	209.0	3.64–3.67 (*m*, ovlp., H_a), 3.51 (*d*, *J* = 11.5, H_b)	59.2
Me(29)	1.08 (*d*, *J* = 6.5)	23.8	1.01 (*d*, *J* = 6.5)	23.2
CH_2(30)	3.97 (*m*, ovlp., H_a), 3.94 (*d*, *J* = 12.5, H_b)	64.8	4.21 (ovlp., 2 H)	73.5
Glucose:				
H–C(1′)			4.27 (*d*, *J* = 8.0)	104.6
H–C(2′)			3.19–3.17 (*m*)	75.3
H–C(3′)			3.33 (masked)	78.1
H–C(4′)			3.25 (masked)	71.6
H–C(5′)			3.24 (masked)	78.0
CH_2(6′)			3.87 (br. *d*, *J* = 12.5), 3.64–3.67 (*m*, ovlp.)	62.6

Compound **2** was also isolated as a white amorphous powder. The HR-FAB-MS of **2** gave a quasi-molecular-ion peak at *m*/*z* 817.3179 ($M-H]^-$) corresponding to a molecular formula $C_{36}H_{58}NaO_{15}S_2^-$. The ^{13}C-NMR signals (*Table*) of **2** were closely related to those of **1**, except for additional signals concerning rings *D* and *E*. Compound **2** differed from **1** by the presence of an additional hexose moiety, and a CH_2OH group

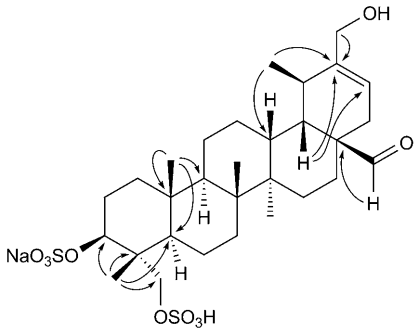

Fig. 1. *Important HMBC* (H → C) *interactions of* **1**

instead of a CHO group. The appearance of signals in the ^{1}H-NMR at δ(H) 3.64–3.67 (*m*, overlapped, H_a–C(28)) and 3.51 (*d*, $J = 11.5$ Hz, H_b–C(28)), and the absence of a CHO signal established the reduction of CHO of **1** to CH_2OH in **2**. This CH_2OH group was unambiguously assigned to the C(28) position. The downfield-shifted signal of CH_2(30)OH at δ(C) 73.5 with the corresponding ^{1}H-NMR signal at δ(H) 4.21 (overlapped, 2 H) and the HMBC (*Fig. 2*) of the anomeric H-atom of the sugar unit at δ(H) 4.27 (*d*, $J = 8.0$ Hz, H–C(1′)) with C(30) (δ(C) 73.5) showed the attachment of a hexose unit at C(30). The absolute configuration of the sugar unit was assigned as D-glucose after acid hydrolysis of **2** followed by the determination of the optical rotation of the isolated sugar. On the basis of all the above evidences, the structure of **2** was assigned as (3β,4α)-3,23,28-trihydroxyurs-20-en-30-yl β-D-glucopyranoside 3,23-di(sulfate) sodium salt (1:1), which is a new compound.

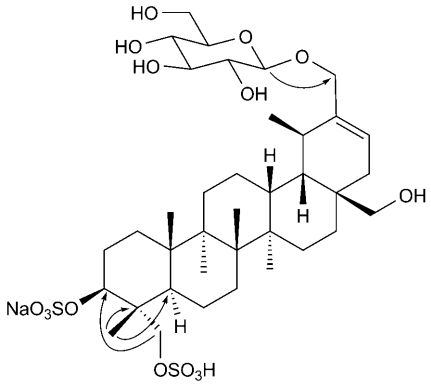

Fig. 2. *Important HMBC* (H → C) *interactions of* **2**

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Experimental Part

General. Column chromatography (CC): *Polygroprep C-18* (15–25 μm, 10 nm; *Macherey–Nagel*) and *Sephadex LH-20* (25–100 μm; *Sigma–Aldrich*). Flash chromatography (FC): *Eyela Flash Chromatography EF-10*, column (200 mm × 20 mm i.d.; *Eyela*) filled with *Polygroprep C-18* (25–

40 μm, 10 nm; *Macherey–Nagel*). MPLC: *Eyela-VSP-3050* instrument, column (200 mm × 25 mm i.d.; *Eyela*) filled with *Polygroprep C-18* (15–25 μm, 10 nm; *Macherey–Nagel*). Optical rotations: *Jasco-DIP-360* automatic digital polarimeter. IR Spectra: *Vector-22* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AC-500* and *-AV-600* spectrometers; δ in ppm rel. to Me_4Si as internal standard, J in Hz. FAB-MS: *Jeol-JMS-HX-110* mass spectrometer; in m/z.

Plant Material. The aerial parts of *Z. fabago* were collected from Ankara, Turkey, in June 2002. The plant was identified by one of us (*B. S.*). A voucher specimen (GUE # 2312) was deposited with the Herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Extraction and Isolation. The EtOH extract was prepared from aerial parts (12 kg) of *Z. fabago* by maceration. The dark-green residue (450 g) was dissolved in H_2O and partitioned between hexane, AcOEt, and MeOH. The MeOH extract (4 × 25 ml) was subjected to CC (*Sephadex LH-20*, pure H_2O (2.0 l), then polarity decrease by MeOH addition in steps of 25% (2.0 l each), up to 100% MeOH (2.0 l)). The fraction (250 mg) obtained with 25% MeOH/H_2O was subjected to reversed-phase MPLC (H_2O/MeOH 1:1): **1** and **2**. The purity of the compounds was checked by HP-TLC (visualization by spraying with $Ce(SO_4)_2$ reagent, followed by heating).

(3β,4β)-3,23,30-Trihydroxyurs-20-en-28-al 3,23-Di(sulfate) Sodium Salt (1:1) (**1**): White amorphous powder (12 mg). $[\alpha]_D^{25} = +13.3$ ($c = 0.042$, MeOH). IR (KBr): 3408, 2925, 1710, 1627, 1122. ^{1}H- and ^{13}C-NMR: *Table*. HR-FAB-MS: 653.2435 ($[M-H]^-$, $C_{30}H_{46}NaO_{10}S_2^-$; calc. 653.2418).

*(3β,4α)-3,23,28-Trihydroxyurs-20-en-30-yl β-*D*-Glucopyranoside 3,23-Di(sulfate) Sodium Salt (1:1)* (**2**): White amorphous powder (10 mg). $[\alpha]_D^{25} = +51.4$ ($c = 0.054$, MeOH). IR (KBr): 3418, 2924, 2854, 1625, 1443, 1385, 1200, 1119, 1073, 671, 648. ^{1}H- and ^{13}C-NMR: *Table*. HR-FAB-MS: 817.3179 ($[M-H]^-$, $C_{36}H_{58}NaO_{15}S_2^-$; calc. 817.3099). FAB-MS: 817 ($[M-H]^-$), 715, 653, 643.

Acid Hydrolysis. Compound **2** (5 mg) in MeOH (5 ml) was hydrolyzed with 10% aq. HCl soln. for 3 h at 100°. On cooling, the aglycone was extracted with AcOEt. The aq. hydrolyzate was neutralized with Ag_2CO_3 and concentrated. The sugar was found to be glucose by co-TLC (R_f 0.46; AcOEt/MeOH/AcOH/H_2O 11:2:2:2 and visualization with aniline phthalate reagent). The sugar was identified as D-glucose by the sign of its optical rotation ($[\alpha]_D^{25} = +52.2$).

Detection of Sulfate Group. A 1–2 mg aliquot of compounds **1** and **2** was heated under reflux with 10% HCl soln. (4 ml) for 4 h and then extracted with Et_2O. An aliquot of the aq. layer of each was treated with 70% $BaCl_2$ soln. to give a white precipitate of $BaSO_4$.

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