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TRITERPENOIDS FROM PLANTS OF THE GENUS *Tamarix*

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*The taraxeran-14-ene-type triterpenoids methyl 3-β-al-D-fridoolean-14-en-28-oate (**1**), 3-α-[3″,4′-dihydroxy-trans-cinnamoyl]-oxy-D-fridoolean-14-en-28-oic acid (**2**), and β-sitosterol (**3**) were isolated from the aerial part of plants from the genus* Tamarix *(*T. laxa, T. elongata*) (Tamaricaceae). The structures of the triterpenoids were proved using spectral data (IR, UV, PMR, ^{13}C NMR, 2D NMR $^{1}H—^{1}H$ COSY, HMQC, HMBC, mass). Compound **1** is new and not previously described in the literature.*

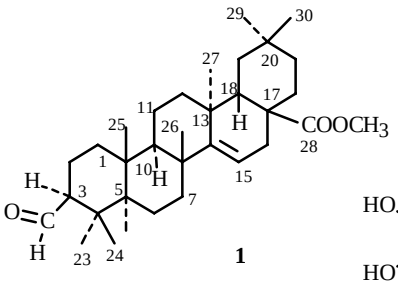
Key words: *Tamarix, T. laxa* Willd, *T. elongata* Ledeb., Tamaricaceae, methyl 3-β-al-D-fridoolean-14-en-28-oate, 3-α-[3″,4″-dihydroxy-*trans*-cinnamoyl]-oxy-D-fridoolean-14-en-28-oic acid, β-sitosterol.

The triterpene content of plants of the genus *Tamarix* (Tamaricaceae) has been little studied. The isolation of D-fridoolean-14-en-3-α-28-diol, D-fridoolean-14-en-3-β-28-diol, and 28-hydroxy-D-fridoolean-14-en-3-β-hydroxy from *T. aphylla* [1], β-amyrin, ursulic acid, and lupeol from *T. troupii* [2], 28-hydroxy-3-oxo-D-fridours-14-ene and 3-β-28-dihydroxy-D-fridours-14-ene from *T. chinensis* [3], and steroidal triterpenes from *T. gallica* [4] has been reported.

Kazakhstan species of *Tamarix* (*T. hispida*) have yielded 3-α-[3″,4″-dihydroxy-*trans*-cinnamoyl]-oxy-D-fridoolean-14-en-28-oic acid (isotamarixene), 3-α-hydroxytaraxeran-14-en-28-oic acid, and β-sitosterol [5, 6].

In the present work we investigated triterpenoids from the two species *T. laxa* Willd and *T. elongata* Ledeb. Phenolic acids and flavonoids were isolated previously from these species [7, 8].

The ethanol extract of the aerial part of *T. laxa* Willd and *T. elongata* Ledeb. collected in the Aral region and Almaty district contained according to TLC three triterpenes. Successive extraction of the alcohol extract by $CHCl_3$ and ethylacetate isolated from the $CHCl_3$ extract by adsorption chromatography over silica gel the known 3-α-[3″,4″-dihydroxy-*trans*-cinnamoyl]-oxy-D-fridoolean-14-en-28-oic acid (**2**), β-sitosterol (**3**), and the new, previously undescribed methyl 3-β-al-D-fridoolean-14-en-28-oate (**1**).



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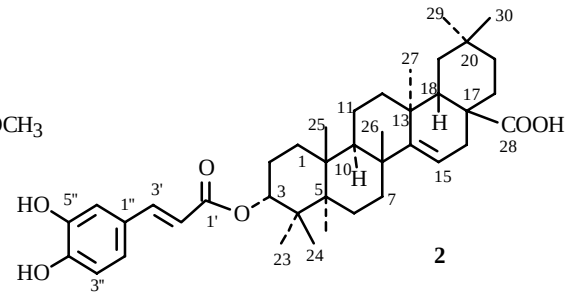

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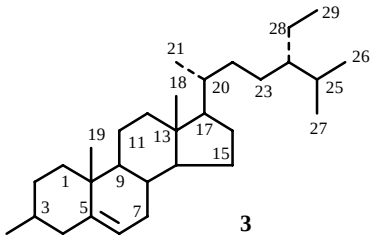

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The structures of the compounds were determined by physical chemical analyses.

Compound **1** was white needlelike crystals, mp 292-294°C. The IR spectrum contained absorption bands characteristic of methyl, methine, methylene, and ester groups at 2864-2933 cm^{-1} and 1689 cm^{-1}, respectively.

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TABLE 1. ^{13}C NMR and PMR Spectra of **1** and **2** (CD_3COCD_3, δ, ppm)

C atom	**1**		**2**		**4***	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	36.1	1.30 m	36.0	1.30	37.8	-
2	23.2	1.98 m	23.4	1.98 m	27.1	-
3	76.0	3.30 dd (9.0, 4.7)	78.2	4.69 t (2.5)	79.0	
4	38.1	-	38.4	-	38.7	3.18 dd (11.0, 5.0)
5	51.3	1.35	51.5	1.35	55.5	-
6	19.3	1.46; 1.65 m	19.2	1.40; 1.60 m	18.7	-
7	42.2	1.38; 1.91 m	41.9	1.40; 1.95 m	41.1	-
8	39.9	-	39.9	-	39.0	-
9	49.8	1.53	50.0	1.55	49.1	-
10	36.1	-	36.1	-	38.0	-
11	18.0	1.54; 1.66 m	18.0	1.55; 1.65 m	17.3	-
12	33.8	1.65; 1.70 m	33.8	1.60; 1.76 m	33.4	-
13	38.0	-	38.1	-	37.4	-
14	161.0	-	161.0	-	160.6	-
15	117.0	5.54 dd (3.4, 11.0)	117.1	5.54 dd (3.4, 11.0)	116.5	-
16	32.6	1.98; 2.37 m	32.5	1.96; 2.37 m	31.7	5.50 dd (4.0, 8.0)
17	52.0	-	51.5	-	51.4	-
18	42.5	2.37 m	42.5	2.37 m	41.8	-
19	34.5	1.10; 1.27 m	34.5	1.10; 1.27 m	35.5	-
20	32.4	-	32.4	-	29.3	-
21	34.3	1.08; 1.65	34.2	1.05; 1.60 m	33.8	-
22	31.3	1.48; 1.65	31.6	1.50; 1.70 m	31.1	-
23	27.0	0.98 s	28.3	0.99 s	28.0	-
24	22.5	0.82 s	22.2	0.87 s	15.4	0.97 s
25	15.7	0.89 s	15.6	0.92 s	15.5	0.79 s
26	26.4	0.90 s	26.5	0.94 s	26.3	0.90 s
27	22.6	0.92 s	22.6	0.94 s	22.4	0.92 s
28	179.0	-	179.3	-	178.5	0.93 s
29	31.6	0.93 s	30.0	0.95 s	32.1	-
30	30.0	0.94 s	30.1	0.95 s	28.7	0.93 s
$COOCH_3$	56.0	3.80 s	-	-	51.7	0.92 s
CHO	238.0	-	-	-	-	3.56 s
1′	-	-	167.0		-	-
2′	-	-	116.3	6.30 d (15.0)	-	-
3′	-	-	146.3	7.50 d (15.0)	-	-
1″	-	-	127.7	-	-	-
2″	-	-	115.0	7.13 d (2.0)	-	-
3″	-	-	145.3	-	-	-
4″	-	-	148.6	-	-	-
5″	-	-	117.1	6.84 d (8.1)	-	-
6″	-	-	122.3	6.98 d (1.9, 8.1)	-	-

*Data from ref. [10].

The PMR spectrum of **1** exhibited at strong field signals for seven methyls at δ 0.82-0.98 and multiplets for 20 methylene protons at δ 1.08-1.98. Olefinic proton H-15 resonated as a 1H doublet of doublets at δ 5.54 with SSCC J_1 = 10.0 and J_2 = 3.5 Hz. This indicated that **1** was a taraxeran-14-ene-type or a D-fridooleane derivative [9].

The C-3 proton resonated as a 1H doublet of doublets with SSCC J_1 = 10.0 and J_2 = 3.0 Hz at δ 3.30, corresponding to the β-form. A 3H singlet at δ 3.80 was assigned to the C-28 acetyl methyl.

The ^{13}C NMR spectrum of **1** exhibited 32 signals. Seven methyls (C-23-27, C-29-30) and four methines (C-5, C-9, C-15, C-18) were recorded in normal phase by DEPT as a function of rotation angle of β-radiofrequency pulses (β = 45°, 90°,

135°); ten methylene C atoms (C-1, C-2, C-6, C-7, C-11, C-12, C-16, C-19, C-21, C-22), in reverse phase. The C atoms of the double bond (C-14 and C-15) resonated at δ 161.0 and 117.0; C-28 and C-3, at δ 179.0 and 76.0, respectively. The C-28 acetyl methyl was observed at δ 56.0.

According to the literature, this assignment of signals in the PMR and ^{13}C NMR spectra corresponds to 3-β-hydroxy-28-acetoxy-D-fridoolean-14-ene (**4**) [10]. However, the ^{13}C NMR spectrum of **1** contained an additional signal at δ 238.0, typical of an aldehyde C atom.

The mass spectrum of **1** (EI, negative-ion FAB) exhibited a peak for the molecular ion with *m*/*z* 482, corresponding to the molecular formula $C_{32}H_{50}O_3$. The presence of the aldehyde and acetyl groups was confirmed by formation of a fragment with *m*/*z* 438 ($C_{30}H_{46}O_2$; $[M - CHO_3 - CH_3]^+$). Characteristic fragments with *m*/*z* 248 ($C_{16}H_{23}O_2$), 204 ($C_{16}H_{23}O_2$ - COO), 189 ($C_{14}H_{21}$), and 133 ($C_{15}H_{23}$ - C_5H_{11}) corresponded to Diels—Alder retro-diene decomposition of **1**.

Two dimensional homo-COSY-45° and heteronuclear HMBC and HMQC spectroscopies were used to prove the positions of the double bond, $COOCH_3$, CHO, and CH_3 groups.

The position of the double bond was proved by the HMQC spectrum. Proton H-15 (δ 5.54 ppm) correlated with the C-15 C atom (δ 117.0 ppm); in the HMBC spectrum, with C-14 (δ 161.0 ppm) and C-16 (δ 32.6 ppm). The COSY-45° spectrum also confirmed that vicinal H-15 and H-16 were correlated. Thus, according to 2D NMR spectra, the double bond was located between C-14 and C-15.

The position of the $COOCH_3$ group was confirmed by the HMBC spectrum. Signals of H-18, H-16, and H-22 correlated with the C-28 C atom (δ 179.0 ppm); the 3H singlet for the acetyl methyl at δ 3.80 ppm, with C atoms at δ 179.0 ppm (C-28).

The site of attachment of the aldehyde was also confirmed by the HMBC spectrum. The aldehyde C at δ 238 ppm correlated with the C-23 methyl proton (δ 0.98 ppm) and the C-2 methylene proton (δ 1.98 ppm). This confirmed that the aldehyde was in the C-3 position. A doublet of doublets in the PMR spectrum with SSCC J_1 = 9.0 and J_2 = 4.7 Hz indicated that the methine proton was located on C-3 in the axial position; the aldehyde, in the equatorial position, which corresponded with their β-placement.

The positions of the methyls were confirmed completely by HMBC and HMQC spectra.

Physical chemical analyses of **1** established its structure as methyl 3-β-al-D-fridoolean-14-en-28-oic acid. Compound **1** is new and has not been reported.

EXPERIMENTAL

TLC used silica gel plates (Merck, 60F, 254) and solvent systems hexane:acetone (9:1, 8:2, 7:3, 6:4, 5:5) and cerium sulfate developer with heating.

Column chromatography used silica gel 60 (70-230 mesh, Merck).

IR spectra were recorded on Jasco IR A-1 and Shimadzu IR-460 instruments in KBr disks; UV spectra, on Shimadzu UV-240; PMR spectra, on Bruker AM 500 FT NMR (500 MHz); ^{13}C NMR (125 MHz); and ^1H—^1H COSY-45°, ^1H—^{13}C HMBC, and HMQC, on Bruker AM 500 FT NMR (125 MHz); mass spectra, in Varian-MAT 112S and Finnigan MAT 112; EI and FAB mass spectra, in MAT 312 JEOL-JM S HX-110.

Melting points were measured on a Kofler block; optical rotation, on a JASCO DIP-360 instrument.

Isolation and Separation of Triterpenoids. Air-dried aerial part of the two species *T. laxa* Willd and *T. elongata* Ledeb. (5 kg) was ground to particle size 0.5-1 cm and extracted twice with aqueous ethanol (80%) at a 1:4 raw material:solvent ratio for 72 h. The combined extracts were concentrated in vacuo to an aqueous residual (1.5 L) that was treated successively with $CHCl_3$ and ethylacetate. The $CHCl_3$ and ethylacetate extracts were concentrated in a rotary evaporator to dryness to afford 55.6 g and 30.4 g of dry extracts, respectively. According to TLC, the main components of the $CHCl_3$ extract were triterpenoids **1-3** and a trace of flavonoids. The $CHCl_3$ extract was separated by adsorption chromatography over silica gel with elution by hexane and then hexane:acetone mixtures with increasing concentrations of acetone. Rechromatography of the resulting fractions containing the total triterpenoids over silica gel afforded pure **1** (0.64 g), **2** (0.56 g), and **3** (0.45 g).

Methyl 3β-al-D-Fridoolean-14-en-28-oate (1). $C_{32}H_{50}O_3$, white needlelike crystals, mp 292-294°C (CH_3OH).

IR spectrum (KBr, ν, cm^{-1}): 2933, 2864, 1689, 1632, 1601.

Mass spectrum (EI, 70, *m*/*z*): 482 (20.4) $[M]^+$, 438 (4.2), 248 (25.7), 204 (23.1), 189 (100.0), 133 (29.7). FAB-MS: 483 $[M + H]^+$, 481 $[M - H]^+$.

For the PMR and ^{13}C NMR, see Table 1.

3-α-[3″,4″-Dihydroxy-*trans*-cinnamoyl]-oxy-D-fridoolean-14-en-28-oic acid (2). $C_{39}H_{54}O_6$, white amorphous powder, mp 198-200°C (CH_3OH), $[\alpha]_D$ -22° (*c* 0.04, CH_3OH).

UV spectrum (MeOH, λ_{max}, nm): 216, 245, 329.

IR spectrum (KBr, ν, cm^{-1}): 3417, 1685, 1603, 1273, 813.

Mass spectrum (EI, 70, *m*/*z*): 617 (20.0) $[M]^+$, 438 (37.2), 248 (17.2), 204 (33.8), 189 (23.3), 133 (21.2). FAB-MS: 618 $[M + H]^+$, 616 $[M - H]^+$.

For the PMR and ^{13}C NMR, see Table 1.

β-Sitosterol (3). $C_{29}H_{50}O$, white crystalline powder, mp 136-139°C (CH_3OH).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.66 (3H, s, H-18), 5.33 (1H, br. s, H-6), 0.89 (3H, d, J = 6.5, H-21), 0.83 (3H, t, H-29), 0.82 (3H, d, J = 6.2, H-26), 0.84 (1H, t), 0.78 (3H, d, J = 6.2, H-27).

Mass spectrum (EI, 70, *m*/*z*): 414 (24.5) $[M]^+$, 396 (30.0), 329 (22.2), 303 (25.8), 255 (17.0), 144 (26.3), 95.0 (45.9).

REFERENCES

1. J. Merfort, J. Buddrus, and M. A. Nawwar, *Phytochemistry*, **11**, 403 (1992).
2. V. S. Parmar, S. S. Rathar, and S. A. Singh, *Phytochemistry*, **4**, 871 (1985).
3. S. K. Sharma and V. S. Parmar, *J. Sci. Ind. Res.*, **57**, 873 (1998).
4. C. K. Andhiwal and K. Kishore, *J. Nat. Prod.*, **45**, 222 (1982).
5. N. Sultanova, T. Makhmoor, A. Uasin, Z. A. Abilov, V. B. Omurkamzinova, Atta-ur-Rahman, and M. I. Choudhary, *Planta Med.*, **70**, 65 (2004).
6. N. A. Sultanova, Zh. A. Abilov, E. E. Shul′ts, and V. B. Omurkamzinova, *Khim. Prir. Soedin.*, 163 (2004).
7. A. K. Umbetova, N. A. Sultanova, V. B. Omurkamzinova, and Zh. A. Abilov, *Vestn. Kaz. Gos. Univ., Ser. Khim.*, **2**, 163 (2004).
8. A. K. Umbetova, Sh. Zh. Esirkegenova, I. M. Chaudry, V. B. Omurkamzinova, and Zh. A. Abilov, *Khim. Prir. Soedin.*, 250 (2004).
9. B. M. Shasi and P. A. Kundu, *Phytochemistry*, **37**, 1517 (1994).
10. V. U. Ahmad and Atta-ur-Rahman, *Handbook of Natural Products*, Amsterdam (1994).