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TRAGOPOGONOSIDES A–I, OLEANANE SAPONINS FROM *TRAGOPOGON PRATENSIS*

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Key Word Index—*Tragopogon pratensis*; Compositae; triterpene saponin; tragopogonoside; echinocystic acid; acacic acid lactone; tormentic acid.

Abstract—Nine new triterpenic saponins, named tragopogonosides A–I, were isolated from the whole plants of *Tragopogon pratensis*, together with five known triterpenic glycosides. The structures of these saponins were determined on the basis of spectral and chemical evidence.

INTRODUCTION

Tragopogon pratensis L. is widely distributed in Europe and eastern Asia and its root is edible [1]. In an earlier communication the isolation and structures of 18 oleanane-type triterpenic saponins from the roots of *T. porrifolius* L. were described [2]. In continuation of our investigations on the terpenic glycosides of Compositae plants, we now report the isolation of 14 triterpenic glycosides from *T. pratensis* L. Nine of these glycosides are new compounds.

RESULTS AND DISCUSSION

The water extract of the whole plants was passed through a Mitsubishi Diaion HP-20 column and the adsorbed material was eluted with methanol. The methanol eluate was passed through an Amberlite IR-120 column and concentrated to give a residue which was then treated with diazomethane. From the methylated fraction, 14 triterpenic glycosides were isolated after repeated chromatography. Compounds **2**–**5** were identified as tragopogonsapogins B, D, F and H [2], respectively, and compound **14** was tormentic acid ester glucoside [3], by comparison with reported data.

The ^{1}H NMR spectrum of tragopogonoside A methyl ester (**1a**) indicated that compound **1a** was the diglycoside of an oleanane-type triterpenoid showing two anomeric proton signals [δ4.98 (1H, *d*, J=8 Hz), 6.22 (1H, *d*, J=8 Hz)], seven singlet methyl signals [δ0.87, 0.98, 1.01, 1.08, 1.11, 1.28, 1.82 (each 3H, *s*)] and a trisubstituted olefinic proton signal [δ5.61 (1H, *t*-like)]. Methanolysis gave echinocystic acid (**1b**) as an aglycone, while methanolysis followed by $NaBH_4$ reduction gave glucose and xylose as the sugar moieties. In the ^{1}H NMR spectrum of compound **1a**, a carbomethoxyl signal [δ3.73 (3H, *s*)] and a methine proton signal [δ4.57 (1H, *d*, J=9 Hz)], which were characteristic of glucuronic acid methyl ester, were observed [2]. On irradiation of an anomeric proton signal at δ4.98 (1H, *d*, J=8 Hz), nuclear Overhauser effects (NOEs) were observed on the proton signals at δ3.39 (1H, *dd*, J=12, 4 Hz), at δ4.57 (1H, *d*, J=9 Hz) and at δ4.24 (1H, *t*, J=8.5 Hz) which were assigned to H-3 of the aglycone, and H-5 and H-3 of glucuronic acid methyl ester, respectively. An anomeric signal of xylose was shifted downfield at δ6.22 (1H, *d*, J=8 Hz) in the ^{1}H NMR and upfield at δ96.3 in the ^{13}C NMR spectrum of compound **1a**. These data indicated that compound **1** had one glucuronic acid and one xylose at C-3 and C-28, respectively.

The ^{1}H NMR spectrum of tragopogonoside B methyl ester (**6a**) showed two carbomethoxyl signals [δ3.68, 3.72 (each 3H, *s*)] and two anomeric proton signals [δ4.96 (1H, *d*, J=8 Hz), 5.18 (1H, *d*, J=8 Hz)]. Methanolysis followed by $NaBH_4$ reduction gave methyl echinocystate (**6b**) as an aglycone and glucose and galactose as the sugar moieties. On irradiation of an anomeric proton signal at δ5.18, an NOE was observed on the proton signal at δ4.21 (1H, *t*, J=8.5 Hz) which was assigned to the H-2 of the glucuronic acid moiety by detailed spin decoupling experiments. Irradiation of another anomeric proton signal at δ4.96 gave an NOE on the methine proton signal at δ3.30 (1H, *dd*, J=12, 4 Hz) which was assigned to H-3 of the aglycone moiety from its coupling pattern. In the ^{13}C NMR spectrum of compound **6a**, glycosylation shifts were observed at C-1 (−2 ppm) and C-2 (+8.3 ppm) of the glucuronic acid moiety, compared with those of compound **1a**. From these data, the structure of tragopogonoside B was determined to be **6**.

The ^{1}H NMR spectrum of tragopogonoside C methyl ester (**7a**) showed three anomeric proton signals at δ4.96 (1H, *d*, J=8 Hz), 5.19 (1H, *d*, J=7 Hz) and 6.21 (1H, *d*, J=7 Hz). The last one (shifted downfield) indicated the presence of an ester-type glycoside linkage. NOE, spin decoupling experiments and the ^{13}C NMR data indicated the presence of the same sugar chain at C-3 as in compound **6a**. Methanolysis of compound **7a** gave echinocystic acid (**1b**), glucose, galactose and xylose. Thus, xylose was attached to C-28.

The ^{1}H NMR spectrum of tragopogonoside D methyl ester (**8a**) indicated the presence of three anomeric proton signals at δ4.98 (1H, *d*, J=8 Hz), 5.22 (1H, *d*, J=8 Hz)

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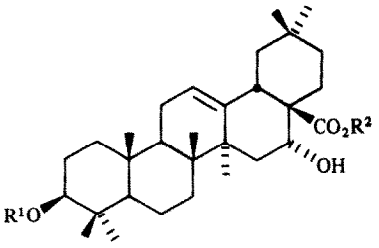
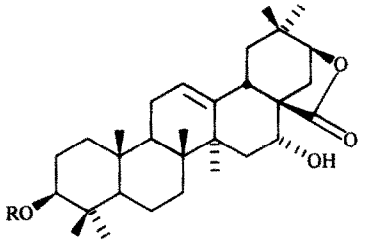
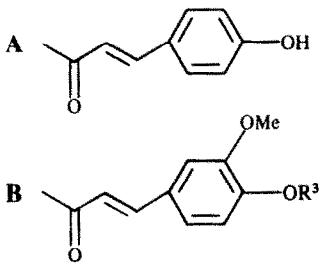
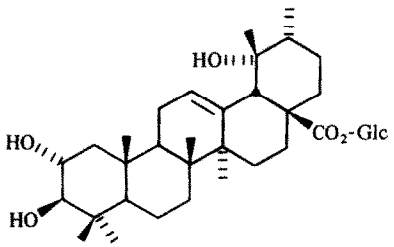


	R^1	R^2	R^3
1	Glc-UA	Xyl	
1a	Glc-UA-Me	Xyl	
1b	H	H	
2	Glc-UA	Xyl—^{2}—**A**	
2a	Glc-UA-Me	Xyl—^{2}—**A**	
3	Glc-UA	Xyl—^{2}—**B**	Glc
3a	Glc-UA-Me	Xyl—^{2}—**B**	Glc
4	Glc-UA	Xyl—^{2}—**A**	
4a	Glc-UA-Me	Xyl—^{2},^{3}—**A**(Glc)	
5	Glc-UA	Ara—^{2},^{3}—**A**(Glc)	
5a	Glc-UA-Me	Ara—^{2},^{3}—**A**(Glc)	
6	Glc-UA—^{2}—Gal	H—^{3}—Glc	
6a	Glc-UA-Me—^{2}—Gal	Me	
6b	H	Me	
7	Glc-UA—^{2}—Gal	Xyl	
7a	Glc-UA-Me—^{2}—Gal	Xyl	
8	Glc-UA	Xyl—^{3}—Glc	
8a	Glc-UA-Me	Xyl—^{3}—Glc	
9	Glc-UA—^{2}—Gal	Xyl—^{3}—Glc	
9a	Glc-UA-Me—^{2}—Gal	Xyl—^{3}—Glc	
10	Glc-UA—^{2}—Gal	Xyl—^{2}—**A**	
10a	Glc-UA-Me—^{2}—Gal	Xyl—^{2},^{3}—**A**(Glc)	
11	Glc-UA—^{2}—Gal	Xyl—^{2},^{3}—**A**(Glc)	
11a	Glc-UA-Me—^{2}—Gal	Xyl—^{2}—**A**	
12	Glc-UA—^{2}—Gal	Xyl—^{2}—**B**	H
12a	Glc-UA-Me—^{2}—Gal	Xyl—^{2}—**B**	H



	R
13	Glc-UA—^{2}—Gal
13a	Glc-UA-Me—^{2}—Gal
13b	H





14

and 6.12 (1H, *d*, $J = 7$ Hz). Methanolysis gave echinocystic acid (**1d**) as an aglycone and glucose–xylose (2:1) as the sugar moiety. On irradiation of the anomeric proton signal at δ4.98, an NOE was observed on the proton signal at δ3.39 (1H, *d*, $J = 11.5, 4$ Hz). The proton signal at δ3.39 was assigned to H-3 of the aglycone due to its coupling pattern. C-3 of xylose was shifted downfield by 10.0 ppm and C-2 and C-4 were shifted upfield by 1.1 ppm and 1.6 ppm, respectively, compared with those of compound **1a**. From these NMR data, glucuronic acid and glucose-(1→3)-xylose were attached to C-3 and C-28 of echinocystic acid, respectively.

In the ^{13}C NMR spectrum of tragopogonoside E methyl ester (**9a**), six more carbon signals due to a galactopyranosyl residue were observed and the carbon signal due to C-2 of glucuronic acid methyl ester was shifted downfield by 8.2 ppm and C-1 and C-3 of glucuronic acid methyl ester were shifted upfield by 1.9 and 0.4 ppm, respectively, compared with those of compound **8a**.

Methanolysis of tragopogonoside F methyl ester (**10a**) followed by $NaBH_4$ reduction gave echinocystic acid (**1b**), methyl *p*-coumarate and glucose–galactose–xylose (2:1:1). The ^{13}C NMR spectrum was similar to that of compound **9a** except for the presence of the *p*-coumaroyl residue. C-1 and C-3 of xylose were shifted upfield by 2.3 and 2.6 ppm, respectively, comparing with those of compound **9a**. Thus, *p*-coumaric acid was attached to C-2 of xylose.

Tragopogonoside G methyl ester (**11a**) and tragopogonoside H methyl ester (**12a**) were obtained as a mixture (*ca* 2:1). The ^{13}C NMR spectrum of the mixture was similar to that of compound **7a** except for the presence of cinnamic acid derivatives. Methanolysis of the mixture followed by $NaBH_4$ reduction gave methyl *p*-coumarate and methyl ferulate as an ester moiety. H-2 of xylose was shifted downfield by 1.67 ppm compared with that of compound **7a**, while C-1 and C-3 of xylose were shifted upfield by 2.6 and 1.6 ppm, respectively, compared with those of compound **7a**. Thus, *p*-coumaric acid was attached to C-2 of xylose in compound **11a** and ferulic acid in compound **12a**.

Tragopogonoside I methyl ester (**13a**) showed carbinyl proton signals at δ3.28 (1H, *dd*, $J = 12, 4$ Hz), 4.25 (1H, *d*,

Table 1. ^{13}C NMR spectral data for the glycosidic and ester part of saponin methyl esters, **1a**, **6a**–**13a**, in pyridine-d_5

	1a	**6a**	**7a**	**8a**	**9a**	**10a**	**11a**	**12a**	**13a**
Glucuronic acid									
1	107.3	105.3	105.2	107.7	105.3	105.2	105.4	105.4	105.3
2	75.4	83.7	83.7	75.5[a]	83.7	83.6	83.6	83.6	83.8
3	77.3	77.5	77.5	77.9	77.5	77.3	77.3	77.3	77.6
4	73.2	72.8	72.8	73.2	72.8	72.9	72.9	72.9	72.8
5	77.9	76.7	76.7	77.2	76.8	76.7	76.6	76.6	76.8
6	170.9	170.4	170.4	170.8	170.5	170.5	170.4	170.4	170.5
Me	52.1	52.0	52.0	52.0	52.0	52.1	52.0	52.0	52.1
Galactose									
1		107.1	107.1		107.1	107.0	107.0	107.0	107.1
2		74.6	74.6		74.7[b]	74.8[c]	74.7[d]	74.7[e]	74.7[f]
3		74.9	74.9		75.0[b]	74.8[c]	74.8[d]	74.8[e]	75.0[f]
4		69.5	69.5		69.6	69.6	69.6	69.6	69.6
5		76.9	76.9		77.0	77.0	76.9	76.9	77.0
6		61.3	61.3		61.4	61.4	61.3	61.3	61.4
Xylose									
1	96.3		96.3	95.8	95.9	93.6	93.7	93.7	
2	73.6		73.5	72.5	72.5	71.2	73.5	73.5	
3	78.1		77.8	88.1	88.2	85.6	76.2	76.2	
4	70.8		70.8	69.2	69.2	69.4	70.9	70.9	
5	67.6		67.5	67.2	67.2	67.1	67.5	67.5	
Glucose									
1				105.6	105.6	105.4			
2				75.4[a]	75.5	74.7[c]			
3				78.6	78.7	78.7			
4				71.8	71.8	71.7			
5				78.3	78.4	78.4			
6				62.6	62.6	62.8			
Ester									
α						166.6	166.4	166.4	
β						115.1	114.8	114.8	
γ						146.0	146.0	146.0	
1						126.2	125.9	126.3	
2						131.0	130.9	111.6	
3						116.7	116.8	151.3	
4						161.6	161.6	149.0	
5						116.7	116.8	116.8	
6						131.0	130.9	123.6	
Me								56.0	

[a–f] Assignments may be interchanged in each column.

J = 5.5 Hz) and 4.53 (1H, *dd*, J = 11, 4.5 Hz) in the ^{1}H NMR spectrum as an aglycone moiety and gave acacic acid lactone (**13b**) [4] as an aglycone on acid hydrolysis. The ^{13}C NMR chemical shifts of the sugar carbon were almost superimposable on those of compound **6a** and C-3 was shifted downfield by 11.2 ppm compared with that of compound **13b**. Therefore, the structure of tragopogonoside I was assigned as **13**.

The triterpenic saponins of *T. pratensis* L. are similar to those of *T. porrifolius* L., but the former has galactose as the sugar component.

EXPERIMENTAL

General. Instrumental analyses were carried out as described in ref. [2].

Plant material, extraction and isolation. Tragopogon pratensis was cultivated in Shizuoka, Japan, 1989. A voucher specimen is deposited in the Herbarium, School of Pharmaceutical Sciences, University of Shizuoka. Dried whole plants (4.7 kg) were extracted twice with hot H_2O. The extract was chromatographed on a Mitsubishi Diaion HP-20 column and the MeOH eluate (22 g) was passed through an Amberlite IR-120 column and treated with CH_2N_2. The methylated fraction was chromatographed on a silica gel column with $CHCl_3$–MeOH (9:1–4:1) and semi-prep. HPLC [ODS and PhA: MeCN–H_2O system] to give **1a** (162 mg), **2a** (187 mg), **3a** (20 mg), **4a** (122 mg), **5a** (26 mg), **6a** (141 mg), **7a** (34 mg), **8a** (20 mg), **9a** (6 mg), **10a** (92 mg), **11a** + **12a** (80 mg), **13a** (13 mg) and **14** (8 mg).

Tragopogonoside A methyl ester (**1a**). Amorphous powder, $[\alpha]_D^{24}$ −21.9° (MeOH; *c* 0.48); FABMS *m/z*: 817 $[M+Na]^+$, 795 $[M+H]^+$. (Found: C, 61.57; H, 8.43. $C_{42}H_{66}O_{14}\cdot 3/2H_2O$

requires: C, 61.37; H, 8.46%.) ^{1}H NMR (pyridine-d_5): δ0.87 (3H, *s*, H_3-25), 0.98 (3H, *s*, H_3-24), 1.01 (3H, *s*, H_3-29), 1.08 (3H, *s*, H_3-26), 1.11 (3H, *s*, H_3-30), 1.28 (3H, *s*, H_3-23), 1.82 (3H, *s*, H_3-27), 3.39 (1H, *dd*, J = 12, 4 Hz, H-3), 3.55 (1H, *dd*, J = 14, 4 Hz, H-18), 3.73 (3H, *s*, CO_2Me), 3.81 (1H, *br t*, J = 10 Hz, Xyl H-5), 4.06 (1H, *t*, J = 8 Hz, Glc·UA H-2), 4.13 (1H, *t*, J = 8 Hz, Xyl H-2), 4.18 (overlapped, Xyl H-4), 4.19 (overlapped, Xyl H-3), 4.24 (1H, *t*, J = 8.5 Hz, Glc·UA H-3), 4.36 (1H, *dd*, J = 11, 3 Hz, Xyl H-5), 4.45 (1H, *t*, J = 9 Hz, Glc·UA H-4), 4.57 (1H, *d*, J = 9 Hz, Glc·UA H-5), 4.98 (1H, *d*, J = 8 Hz, Glc·UA H-1), 5.25 (1H, *br s*, H-16), 5.61 (1H, *t*-like, H-12), 6.22 (1H, *d*, J = 8 Hz, Xyl H-1). ^{13}C NMR (pyridine-d_5): δ (aglycone C_1–C_{30}) 38.8, 26.7, 89.2, 39.6, 55.9, 18.5, 33.5, 40.1, 47.2, 37.0, 23.9, 122.8, 144.6, 42.1, 36.2, 74.3, 49.3, 41.3, 47.2, 30.9, 36.0, 32.4, 28.2, 15.7, 17.0, 17.6, 27.2, 176.2, 33.3, 24.7. Glycosidic part: see Table 1.

Tragopogonoside B methyl ester (**6a**). Amorphous powder, $[\alpha]_D^{24}$ −9.4° (MeOH; *c* 2.07); FABMS *m/z*: 861 $[M+Na]^+$, 839 $[M+H]^+$. (Found: C, 60.18; H, 8.63. $C_{44}H_{70}O_{15}\cdot 2H_2O$ requires: C, 60.39; H, 8.52%.) ^{1}H NMR (pyridine-d_5): δ0.87 (3H, *s*, H_3-25), 0.89 (3H, *s*, H_3-24), 1.02 (3H, *s*, H_3-29), 1.09 (3H, *s*, H_3-26), 1.10 (3H, *s*, H_3-30), 1.29 (3H, *s*, H_3-23), 1.77 (3H, *s*, H_3-27), 3.30 (1H, *dd*, J = 12, 4 Hz, H-3), 3.38 (1H, *dd*, J = 13, 4 Hz, H-18), 3.68, 3.72 (each 3H, *s*, CO_2Me), 4.02 (1H, *br dd*, J = 8, 5 Hz, Gal H-5), 4.14 (1H, *dd*, J = 9, 3 Hz, Gal H-3), 4.21 (1H, *t*, J = 8.5 Hz, Glc·UA H-2), 4.27 (1H, *t*, J = 8.5 Hz, Glc·UA H-3), 4.36 (1H, *dd*, J = 11, 5 Hz, Gal H-6), 4.39 (1H, *t*, J = 9 Hz, Glc·UA H-4), 4.47 (1H, *d* J = 9.5 Hz, Glc·UA H-5), 4.52 (1H, *dd*, J = 9, 8 Hz, Gal H-2), 4.55 (1H, *dd*, J = 11, 8 Hz, Gal H-6), 4.65 (1H, *d*, J = 3 Hz, Gal H-4), 4.96 (1H, *d*, J = 8 Hz, Glc·UA H-1), 5.01 (1H, *br s*, H-16), 5.18 (1H, *d*, J = 8 Hz, Gal H-1), 5.52 (1H, *t*-like, H-12). ^{13}C NMR: see Table 1.

Tragopogonoside C methyl ester (**7a**). Amorphous powder, $[\alpha]_D^{24}$ −16.3° (MeOH; *c* 1.63); FABMS *m/z*: 979 $[M+Na]^+$. (Found: C, 56.59; H, 8.35. $C_{48}H_{76}O_{19}\cdot 7/2H_2O$ requires: C, 56.51; H, 8.20%.) ^{1}H NMR (pyridine-d_5): δ0.87 (3H, *s*, H_3-25), 1.01 (3H, *s*, H_3-29), 1.08 (6H, *s*, H_3-24, H_3-26), 1.11 (3H, *s*, H_3-30), 1.27 (3H, *s*, H_3-23), 1.80 (3H, *s* H_3-27), 3.29 (1H, *dd*, J = 12, 4 Hz, H-3), 3.55 (3H, *dd*, J = 14.5, 4 Hz, H-18), 3.72 (3H, *s*, CO_2Me), 3.80 (1H, *t*, J = 11 Hz, Xyl H-5), 4.02 (1H, *br t*, J = 7.5 Hz, Gal H-5), 4.12 (1H, *t*, J = 8 Hz, Xyl H-2), 4.14 (1H, *dd*, J = 9, 3 Hz, Gal H-3), 4.18 (overlapped, Xyl H-3, Xyl H-4), 4.21 (1H, *t*, J = 8 Hz, Glc·UA H-2), 4.27 (1H, *t*, J = 9 Hz, Glc·UA H-3), 4.36 (overlapped, Gal H-6, Xyl H-5), 4.39 (1H, *t*, J = 9 Hz, Glc·UA H-4), 4.47 (1H, *d*, J = 9.5 Hz, Glc·UA H-5), 4.53 (1H, *t*, J = 8 Hz, Gal H-2), 4.55 (1H, *dd*, J = 11, 8 Hz, Gal H-6), 4.65 (1H, *d*, J = 3 Hz, Gal H-4), 4.96 (1H, *d*, J = 8 Hz, Glc·UA H-1), 5.19 (1H, *d*, J = 7 Hz, Gal H-1), 5.24 (1H, *br s*, H-16), 5.60 (1H, *t*-like, H-12), 6.21 (1H, *d*, J = 7 Hz, Xyl H-1). ^{13}C NMR: see Table 1.

Tragopogonoside D methyl ester (**8a**). Amorphous powder, $[\alpha]_D^{23}$ −27.8° (MeOH; *c* 0.45); FABMS *m/z*: 979 $[M+Na]^+$, 957 $[M+H]^+$. (Found: C, 58.14; H, 8.30. $C_{48}H_{76}O_{19}\cdot 2H_2O$ requires: C, 58.05; H 8.12%.) ^{1}H NMR (pyridine-d_5): δ0.88 (3H, *s*, H_3-25), 0.98 (3H, *s*, H_3-24), 1.00 (3H, *s*, H_3-29), 1.06 (3H, *s*, H_3-30), 1.09 (3H, *s*, H_3-26), 1.28 (3H, *s*, H_3-23), 1.81 (3H, *s*, H_3-27), 3.39 (1H, *dd*, J = 11.5, 4 Hz, H-3), 3.52 (1H, *dd*, J = 14.5, 4.5 Hz, H-18), 3.71 (1H, *t*, J = 12 Hz, Xyl H-5), 3.74 (3H, *s*, CO_2Me), 3.97 (1H, *m*, Glc H-5), 4.05 (1H, *t*, J = 8.5 Hz, Glc H-2), 4.07 (1H, *t*, J = 8.5 Hz, Glc·UA H-2), 4.13 (overlapped, Xyl H-2, Xyl H-3), 4.14 (overlapped, Xyl H-4), 4.15 (overlapped, Glc H-4), 4.16 (overlapped, Glc H-3), 4.24 (1H, *t*, J = 9 Hz, Glc·UA H-3), 4.25 (1H, *dd*, J = 12, 6 Hz, Glc H-6), 4.28 (1H, *dd*, J = 12, 5 Hz, Xyl H-5), 4.45 (1H, *t*, J = 9 Hz, Glc·UA H-4), 4.49 (1H, *dd*, J = 12, 2 Hz, Glc H-6), 4.57 (1H, *d*, J = 9.5 Hz, Glc·UA H-5), 4.98 (1H, *d*, J = 8 Hz, Glc·UA H-1), 5.21 (1H, *br*, *s*, H-16), 5.22 (1H, *d*, J = 8 Hz, Glc H-1), 5.60 (1H, *t*-like, H-12), 6.12 (1H, *d*, J = 7 Hz, Xyl H-1). ^{13}C NMR: see Table 1.

Tragopogonoside E methyl ester (**9a**). Amorphous powder, $[\alpha]_D^{24}$ −22.9° (MeOH; *c* 0.35); FABMS *m/z*: 1141 $[M+Na]^+$, 1119 $[M+H]^+$. (Found: C, 53.32; H, 7.82. $C_{54}H_{86}O_{24}\cdot 11/2H_2O$ requires. C, 53.23, H, 8.03%.) ^{1}H NMR (pyridine-d_5): δ0.88 (3H, *s*, H_3-25), 1.00 (3H, *s*, H_3-29), 1.07 (3H, *s*, H_3-30), 1.09 (6H, *s*, H_3-24, H_3-26), 1.28 (3H, *s*, H_3-23), 1.80 (3H, *s*, H_3-27), 3.30 (1H, *dd*, J = 12, 4 Hz, H-3), 3.52 (1H, *dd*, J = 14, 4 Hz, H-18), 3.72 (3H, *s*, CO_2Me), 3.72 (1H, *t*, J = 12 Hz, Xyl H-5), 3.98 (1H, *m*, Glc H-5), 4.04 (1H, *br t*, J = 7 Hz, Gal H-5), 4.06 (1H, *t*, J = 8 Hz, Glc H-2), 4.13 (overlapped, Xyl H-2), 4.14 (overlapped, Xyl H-4, Glc H-4), 4.15 (overlapped, Xyl H-3), 4.16 (overlapped, Glc H-3, Gal H-3), 4.23 (1H, *t*, J = 8 Hz, Glc·UA H-2), 4.25 (1H, *dd*, J = 12, 5 Hz, Glc H-6), 4.28 (1H, *dd*, J = 12, 5 Hz, Xyl H-5), 4.29 (1H, *t*, J = 9 Hz, Glc·UA H-3), 4.38 (1h, *dd*, J = 11, 5 Hz, Gal H-6), 4.41 (1H, *t*, J = 9 Hz, Glc·UA H-4), 4.48 (1H, *d*, J = 9.5 Hz, Glc·UA H-5), 4.50 (1H, *dd*, J = 12, 2 Hz, Glc H-6), 4.55 (1H, *t*, J = 8 Hz, Gal H-2), 4.56 (1H, *dd*, J = 11, 8 Hz, Gal H-6), 4.66 (1H, *d*, J = 3 Hz, Gal H-4), 4.97 (1H, *d*, J = 8 Hz, Glc·UA H-1), 5.20 (1H, *d*, J = 8 Hz, Gal H-1), 5.22 (1H, *br s*, H-16), 5.23 (1H, *d*, J = 8 Hz, Glc H-1), 5.60 (1H, *t*-like, H-12), 6.13 (1H, *d*, J = 7 Hz, Xyl H-1). ^{13}C NMR: see Table 1.

Tragopogonoside F methyl ester (**10a**). Amorphous powder, $[\alpha]_D^{24}$ +1.3° (MeOH; *c* 1.16); UV λ_{max}^{MeOH} nm (log ε): 230 (3.98), 301 (sh 4.20), 317 (4.29): FABMS *m/z*: 1288 $[M+Na]^+$. ^{1}H NMR (pyridine-d_5): δ0.91 (3H, *s*, H_3-25), 0.99 (6H, *s*, H_3-24, H_3-29), 1.06 (3H, *s*, H_3-26), 1.10 (3H, *s*, H_3-30), 1.24 (3H, *s*, H_3-23), 1.72 (3H, *s*, H_3-27), 3.25 (1H, *dd*, J = 12, 4 Hz, H-3), 3.43 (1H, *dd*, J = 14.5, 4 Hz, H-18), 3.71 (3H, *s*, CO_2Me), 3.74 (1H, *t*, J = 11 Hz, Xyl H-5), 3.93 (1H, *t*, J = 8 Hz, Glc H-2), 4.03 (overlapped, Glc H-4, Glc H-5), 4.05 (overlapped, Gal H-5), 4.10 (overlapped, Xyl H-4, Glc H-6), 4.11 (overlapped, Glc H-3), 4.13 (1H, *dd*, J = 9, 3 Hz, Gal H-3), 4.21 (1H, *dd*, J = 11, 6 Hz, Glc H-6), 4.25 (1H, *t*, J = 8 Hz, Glc·UA H-2), 4.26 (1H, *t*, J = 8 Hz, Glc·UA H-3), 4.27 (overlapped, Xyl H-5), 4.28 (1H, *t*, J = 9 Hz, Xyl H-3), 4.38 (1H, *t*, J = 9 Hz, Glc·UA H-4), 4.41 (1H, *dd*, J = 11, 5 Hz, Gal H-6), 4.45 (1H, *d*, J = 9.5 Hz, Glc·UA H-5), 4.53 (1H, *dd*, J = 9, 8 Hz, Gal H-2), 4.57 (1H, *dd*, J = 11, 8 Hz, Gal H-6), 4.59 (1H, *d*, J = 3 Hz, Gal H-4), 4.94 (1H, *d*, J = 7.5 Hz, Glc·UA H-1), 5.05 (1H, *d*, J = 8 Hz, Glc H-1), 5.15 (1H, *d*, J = 8 Hz, Gal H-1), 5.19 (1H, *br s*, H-16), 5.56 (1H, *t*-like, H-12), 5.80 (1H, *t*, J = 9 Hz, Xyl H-2), 6.13 (1H, *d*, J = 8 Hz, Xyl H-1), 6.72 (1H, *d*, J = 16 Hz, ester H-β), 7.12 (2H, *d*, J = 8 Hz, ester H-3, H-5), 7.49 (2H, *d*, J = 8 Hz, ester H-2, H-6), 7.94 (1H, *d*, J = 16 Hz, ester H-γ). ^{13}C NMR: see Table 1.

Tragopogonoside G methyl ester (**11a**) *and tragopogonoside H methyl ester* (**12a**) (*ca* 2:1). Amorphous powder; FABMS *m/z*: 1125 $[M+Na]^+$, 1103 $[M+H]^+$, 1153 $[M+Na]^+$, 1133 $[M+H]^+$. ^{1}H NMR (pyridine-d_5): δ0.88 (3H, *s*, H_3-25), 0.97 (3H, *s*, H_3-24), 1.00 (3H, *s*, H_3-29), 1.07 (3H, *s*, H_3-26), 1.10 (3H, *s*, H_3-30), 1.25 (3H, *s*, H_3-23), 1.73 (3H, *s*, H_3-27), 3.26 (1H, *dd*, J = 12, 4 Hz, H-3), 3.45 (1H, *dd*, J = 13, 4 Hz, H-18), 3.71 (3H, *s*, CO_2Me), 3.82 (1H, *t*, J = 11 Hz, Xyl H-5), 3.86 (3/3H, *s*, OMe), 4.13 (1H, *dd*, J = 9.5, 3 Hz, Gal H-3), 4.23 (overlapped, Xyl H-4), 4.24 (1H, *t*, J = 8 Hz, Glc·UA H-2), 4.27 (1H, *t*, J = 8.5 Hz, Glc·UA H-3), 4.32 (1H, *dd*, J = 11, 4.5 Hz, Xyl H-5), 4.39 (1H, *t*, J = 8 Hz, Glc·UA H-4), 4.42 (1H, *dd*, J = 11, 5 Hz, Gal H-6), 4.46 (1H, *d*, J = 9.5 Hz, Glc·UA H-5), 4.54 (1H, *t*, J = 8.5 Hz, Gal H-2), 4.60 (1H, *d*, J = 3 Hz, Gal H-4), 4.65 (1H, *m*, Gal H-5), 4.95 (1H, *d*, J = 7 Hz, Glc·UA H-1), 5.17 (1H, *d*, J = 8 Hz, Gal H-1), 5.23 (1H, *br s*, H-16), 5.29 (1H, *t*, J = 8 Hz, Xyl H-3), 5.56 (1H, *t*-like, H-12), 5.79 (1H, *t*, J = 8.5 Hz, Xyl H-1), 6.19 (1H, *d*, J = 8.5 Hz, Xyl H-1), 6.60 (2/3H, *d*, J = 16 Hz, *p*-coumarate H-β), 6.62 (1/3H, *d*, J = 16 Hz, ferulate H-β), 7.17 (4/3H, *d*, J = 8 Hz, *p*-coumarate H-3, H-5), 7.17 (1/3H, *br d*, J = 8 Hz, ferulate H-6), 7.22 (1/3H, *d*, J = 8 Hz, ferulate H-5), 7.25 (1/3H, *br s*, ferulate H-2), 7.53 (4/3H, *d*, J = 8 Hz, *p*-coumarate H-2, H-6), 7.90 (3/3H, *d*, J = 16 Hz, *p*-coumarate H-γ, ferulate H-γ). ^{13}C NMR· see Table 1.

Tragopogonoside I methyl ester (**13a**). Amorphous powder, $[\alpha]_D^{23}$ −24.2° (MeOH; *c* 0.66); FABMS *m/z*: 845 $[M+Na]^+$, 823 $[M+H]^+$. (Found: C, 58.74; H, 8.17. $C_{43}H_{66}O_{15}\cdot 3H_2O$ requires: C, 58.87; H, 8.28%.) ^{1}H NMR (pyridine-d_5): δ0.81 (6H, *s*, Me × 2), 0.95, 1.07, 1.10, 1.30, 1.36 (each 3H, *s*, Me), 2.79 (1H, *m*, H-18), 3.28 (1H, *dd*, *J* = 12, 4 Hz, H-3), 3.73 (3H, *s*, CO_2Me), 4.05 (1H, *m*, Gal H-5), 4.17 (1H, *dd*, *J* = 9.5, 3 Hz, Gal H-3), 4.24 (1H, *t*, *J* = 8.5 Hz, Glc·UA H-2), 4.25 (1H, *d*, *J* = 5.5 Hz, H-21), 4.30 (1H, *t*, *J* = 8.5 Hz, Glc·UA H-3), 4.40 (1H, *dd*, *J* = 11, 5 Hz, Gal H-6), 4.42 (1H, *t*, *J* = 8.5 Hz, Glc·UA H-4), 4.49 (1H, *d*, *J* = 9.5 Hz, Glc·UA H-5), 4.53 (1H, *dd*, *J* = 11, 4.5 Hz, H-16), 4.57 (1H, *dd*, *J* = 9, 8 Hz, Gal H-2), 4.59 (1H, *dd*, *J* = 11, 8 Hz, Gal H-6), 4.68 (1H, *dd*, *J* = 3, 1 Hz, Gal H-4), 4.97 (1H, *d*, *J* = 8 Hz, Glc·UA H-1), 5.35 (1H, *t*-like, H-12), 5.52 (1H, *d*, *J* = 8 Hz, Gal H-1). ^{13}C NMR: see Table 1.

Hydrolysis of compound **1a**. Compound **1a** (39 mg) was refluxed with AcCl–MeOH (1:d20; 5 ml) for 3 hr. The reaction mixture was concd and partitioned between EtOAc and H_2O. After purification of the EtOAc layer by HPLC [YMC D-ODS-7, 20 mm × 25 cm, MeOH–H_2O (4:1)], an aglycone (**1b**; 6 mg) was obtained as colourless needles, mp 305–308°, $[\alpha]_D^{23}$ + 50.0° (MeOH; *c* 0.25). ^{1}H and ^{13}C NMR spectral data were identical to those of echinocystic acid [2].

Hydrolysis of compound **13a**. Compound **13a** (1 mg) was refluxed with 4 M HCl–dioxane (1:3; 0.8 ml) for 50 min. The reaction mixture was concd and partitioned between EtOAc and H_2O. From the EtOAc layer, acacic acid lactone (**13b**) [4] was detected by HPLC [Develosil ODS, 4.6 mm × 25 cm, MeOH–H_2O (4:1); flow rate, 1.3 ml min^{-1}; UV 205 nm; R_t 7.3 min].

Degradation of compounds **1a, 6a–12a** *with* AcCl–MeOH. Saponins (**1a, 6a–12a**) (*ca* 1 mg) were reduced with $NaBH_4$ (*ca* 1 mg) in MeOH (0.2 ml) overnight at room temperature. The reaction mixture was neutralized with dil. HCl and passed through a Mitsubishi Diaion HP-20 column and the MeOH eluate was concd under reduced pressure. The residue was refluxed in AcCl–MeOH (1:20; 1 ml) for 3 hr. After drying, the reaction mixture was partitioned between EtOAc and H_2O. The EtOAc layer was concd to dryness, and the residue was analyzed by HPLC with authentic samples [conditions: column YMC R-ODS-7, 4.6 mm × 25 cm, MeOH–H_2O (4:1); flow rate, 1.3 ml min^{-1}; UV 205 nm; R_t echinocystic acid, 9.4 min from **1a** and **7a–12a**]; [conditions: column, Develosil ODS, 4.6 mm × 25 cm, MeOH–H_2O (4:1); flow rate, 1.3 ml min^{-1}; UV 205 nm; R_t methyl echinocystate, 24.0 min from **6a**]; [conditions: column, Develosil ODS, 4.6 mm × 25 cm, MeCN–H_2O (3:7); flow rate, 1.3 ml min^{-1}; UV 330 nm; R_t methyl *p*-coumarate, 12.0 min from **10a** and **11a + 12a**; methyl ferulate, 13.0 min from **11a + 12a**]. The H_2O layer was also concentrated and the residue was heated in 5%H_2SO_4 (two drops) in a boiling water bath for 1 hr. The soln was passed through an Amberlite IR-45 column and the eluate was concd to give a residue which was reduced with $NaBH_4$ (*ca* 1 mg) for 1 hr at room temp. The reaction mixture was passed through an Amberlite IR-120 column and the eluate was concd to dryness. Boric acid was removed by co-distillation with MeOH and the residue was acetylated with Ac_2O and pyridine (one drop each) at 100° for 1 hr. The reagents were evapd off *in vacuo*. From each saponin glucitol acetate, xylitol acetate and galactitol acetate were detected by GC [conditions: column, Supelco SP-2380 capillary column (0.25 mm × 30 m); column temp., 250°; carrier gas, N_2; R_t glucitol acetate, 11.1 min; xylitol acetate, 7.2 min; galactitol acetate, 10.3 min].

Degradation of crude saponin fraction. The MeOH eluate (20 mg) of the Mitsubishi Diaion HP-20 column was refluxed in AcCl–MeOH (1:20; 3 ml) for 4 hr. The reaction mixture was concd and chromatographed on a silica gel column. From the C_6H_6–Me_2CO (17:3) eluate, echinocystic acid (**1b**), methyl *p*-coumarate and methyl ferulate were detected by HPLC, but methyl caffeate was not detected. HPLC conditions were the same as described previously.

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