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Sesquiterpene Glycosides and Saponins from *Cynara cardunculus* L.

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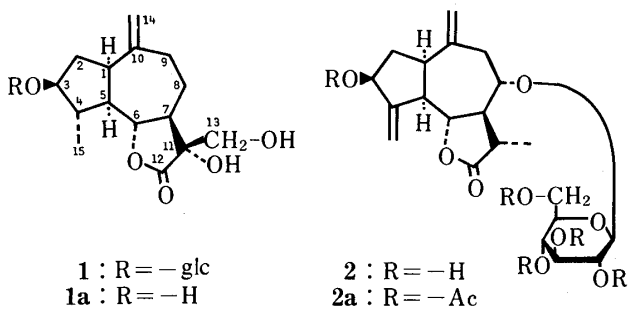
A new guaiane-type sesquiterpene glycoside, cynaroside A, seven new ursane-type saponins, cynarasaponins A—G, and three new oleanane-type saponins, cynarasaponins H—J, have been isolated from *Cynara cardunculus* L., together with a known guaiane-type sesquiterpene glycoside, 11β,13-dihydrodesacylcynaropicrin-8-β-D-glucoside, and a known oleanane-type saponin, chikusetsusaponin IVa. The structures of the new compounds were established on the basis of chemical and spectral data. This is the first isolation of saponin from Compositae plants.

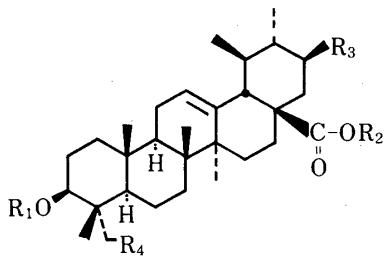
Keywords——*Cynara cardunculus*; Compositae; saponin; cynarasaponin; sesquiterpene glycoside

In connection with a study on the sesquiterpene glycosides of some plants in Compositae, we have also investigated *Cynara cardunculus* L. (cardon), which is used as a green vegetable and a tonic. The water extract of the aerial parts was passed through an Amberlite XAD-2 column and the adsorbed material was eluted with methanol. From the methanol eluate, a new guaiane-type sesquiterpene glycoside, cynaroside A (**1**), has been isolated together with a known guaiane-type sesquiterpene glycoside, 11β,13-dihydrodesacylcynaropicrin-8-β-D-glucoside (**2**). The water extract of the roots was passed through an Amberlite XAD-2 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 methylated with CH_2N_2. From the reaction mixture, seven new ursane-type saponins, cynarasaponins A (**3**), B (**4**), C (**5**), D (**6**), E (**7**), F (**8**) and G (**9**), and three new oleanane-type saponins, cynarasaponins H (**10**), I (**11**) and J (**12**), have been isolated as their methyl esters together with a known oleanane-type saponin, chikusetsusaponin IVa (**13**). The structures of the new compounds were determined on the basis of some chemical transformations and spectroscopic studies. A known sesquiterpene glycoside, 11β,13-dihydrodesacylcynaropicrin-8-β-D-glucoside (**2**), and a known saponin, chikusetsusaponin IVa (**13**), were identified by comparison of their proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra with reported data.[1,2]



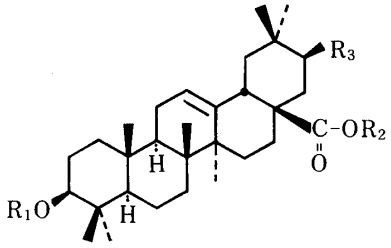
1 : R=−glc
1a : R=−H
2 : R=−H
2a : R=−Ac

Chart 1



	R_1	R_2	R_3	R_4
3	–glc·UA²–ara (COOCH₃)	–glc	–H	–H
3a	–glc·UA²–ara (COOCH₃)	–glc	–H	–H
3c	–glc·UA	$-CH_3$	–H	–H
3d	–H	$-CH_3$	–H	–H
3e	–glc²–ara	–glc	–H	–H
4	–glc·UA²–ara (COOCH₃)	–H	–H	–H
4a	–glc·UA²–ara (COOCH₃)	$-CH_3$	–H	–H
5	–glc·UA (COOCH₃)	–glc	–H	–H
5a	–glc·UA	–glc	–H	–H
6	–glc·UA²–ara (COOCH₃)	–glc	–H	–OH
6a	–glc·UA²–ara (COOCH₃)	–glc	–H	–OH
6g	–H	–H	–H	–OH
6f	–Ac	$-CH_3$	–H	–Ac
7	–glc·UA (COOCH₃)	–glc	–H	–OH
7a	–glc·UA	–glc	–H	–OH

	R_1	R_2	R_3	R_4
8	–glc·UA²–ara (COOCH₃)	–H	–OH	–H
8a	–glc·UA²–ara (COOCH₃)	$-CH_3$	–OH	–H
8d	–H	$-CH_3$	–OH	–H
9	–glc·UA²–ara (COOCH₃)	–glc	–OH	–H
9a	–glc·UA²–ara (COOCH₃)	–glc	–OH	–H



	R_1	R_2	R_3
10	–glc·UA²–ara (COOCH₃)	–glc	–H
10a	–glc·UA²–ara (COOCH₃)	–glc	–H
10d	–H	$-CH_3$	–H
11	–glc·UA²–ara (COOCH₃)	–H	–OH
11a	–glc·UA²–ara (COOCH₃)	$-CH_3$	–OH
11d	–H	$-CH_3$	–OH
12	–glc·UA²–ara (COOCH₃)	–glc	–OH
12a	–glc·UA²–ara (COOCH₃)	–glc	–OH
13	–glc·UA (COOCH₃)	–glc	–H
13a	–glc·UA	–glc	–H

UA = uronic acid

Chart 2

Cynaroside A (1)

The ^{1}H-NMR spectrum exhibited a doublet methyl signal at δ 1.44 (J=7 Hz), an exomethylene proton signal at δ 4.87 (br s) and an anomeric proton signal at δ 4.95 (d, J= 8 Hz). In the ^{13}C-NMR spectrum, twenty-one signals, including six signals due to a glucopyranosyl moiety, were observed. Acid hydrolysis afforded glucose as the sugar moiety and enzymatic hydrolysis afforded **1a** as an aglycone. Compound **1a** was assumed to be cynaratriol, which had been isolated from this plant,[3] and this was confirmed by direct comparison with an authentic sample (^{1}H- and ^{13}C-NMR). In the ^{13}C-NMR spectrum of **1**, the C-2 (δ 38.3) and C-4 (δ 45.6) signals were shifted upfield by 1.1 and 2.0 ppm, respectively, and the C-3 (δ 87.3) signal was shifted downfield by 9.2 ppm compared with those of **1a**. Thus, the structure of cynaroside A was decided to be **1**.

Cynarasaponin A Methyl Ester (3a)

In the ^{13}C-NMR spectrum, **3a** had three anomeric carbon signals and two ester carbonyl signals (Table I). Compound **3a** was methylated with CH_2N_2 followed by partial acid hydrolysis with AcCl–MeOH (1:20) to give **4a** and **3c** as prosapogenins and **3d** as a sapogenin. Compound **3d** was identified as ursolic acid methyl ester by direct comparison with an authentic sample [^{1}H- and ^{13}C-NMR, mass spectrum (MS), mixed melting point]. In the ^{13}C-NMR spectrum of **3a**, a carboxyl carbon signal (δ 176.2) is attributable to C-28 of the aglycone moiety, and consequently the carbomethoxyl group (δ 170.4) should be in the sugar

TABLE I. ^{13}C-NMR Chemical Shifts

	1[a]	**1a**[a]	**2**[a]	**3a**[a]	**3c**[a]	**3d**[b]	**3e**[a]	**4a**[a]	**5a**[a]	**6a**[a]	**7a**[a]	**8a**[a]	**8d**[b]	**9a**[a]	**10a**[a]	**11a**[a]	**11d**[b]	**12a**[a]	**13a**[a]
Aglycone																			
C-1	42.9	42.7	44.2	39.2	38.9	38.6	39.2	39.0	39.2	39.4	39.0	39.0	38.6	39.1	38.8	38.8	38.5	38.9	38.7
C-2	38.3	39.4	39.6	26.8	26.7	27.2	26.8	26.7	26.7	28.8	28.8	26.7	27.2	26.8	26.7	26.7	27.2	26.8	26.6
C-3	87.3	78.1	73.1	89.3	89.2	78.9	89.1	89.3	89.2	82.0	82.4	89.3	78.9	89.4	89.3	89.3	79.0	89.3	89.2
C-4	45.6	47.6	155.2	39.4	39.6	38.6	39.4	39.6	39.4	43.6	43.5	39.6	38.7	39.6	39.6	39.6	38.7	39.6	39.6
C-5	52.0	52.6	50.7	56.0	55.9	55.2	56.1	55.9	55.9	47.5	47.6	55.9	55.2	56.0	55.9	55.9	55.3	56.0	55.8
C-6	78.4	78.6	80.0	18.5	18.5	18.3	18.6	18.5	18.5	18.2	18.2	18.5	18.3	18.6	18.5	18.5	18.3	18.6	18.5
C-7	55.6	55.8	54.8	33.6	33.4	32.9	33.6	33.4	33.6	33.4	33.3	33.4	32.9	33.7	33.2	33.2	32.7	33.4	33.2
C-8	27.8	28.0	84.5	40.2	39.9	39.4	40.3	39.9	40.2	40.3	40.3	39.9	39.4	40.3	39.9	39.7	39.3	40.0	39.9
C-9	37.5	37.8	43.7	48.1	48.0	47.5	48.1	47.9	48.1	48.2	48.2	47.9[o]	47.5	48.1[r]	48.1	48.0	47.6	48.1	48.1
C-10	150.7	150.9	144.9	36.9	37.0	37.0	37.0	36.9	36.9	36.8	36.8	36.9	37.0	37.0	37.0	36.8	37.1	36.8	37.0
C-11	85.0	85.2	41.8	23.8	23.6	23.2	23.9	23.6	23.8	23.9	23.9	23.7	23.3	23.8	23.7	23.8	23.4	23.9	23.7
C-12	180.3	180.3	179.4	126.2	126.0	125.5	126.2	126.0	126.1	126.3	126.2	126.4	126.2	126.6	122.9	123.3	123.0	123.3	122.6
C-13	64.8	64.8	17.1	138.5	138.8	138.1	138.5	138.8	138.4	138.5	138.4	138.8	137.7	138.5	144.2	143.5	142.4	143.5	144.1
C-14	112.3	112.1	115.8	42.6	42.4	41.9	42.6	42.4	42.5	42.6	42.6	42.4	41.9	42.6	42.2	42.1	41.7	42.3	42.2
C-15	19.2	19.0	109.7	28.8	28.5	28.1	28.8	28.5	28.7	26.2	26.1	28.6	28.1	28.9	28.3	28.3	27.7	28.5	28.2
C-16				24.8	24.7	24.2	24.7	24.7	24.8	24.8	24.7	25.8	25.2	26.0	23.7	25.0	24.4	25.0	23.7
C-17				48.4	48.4	48.1	48.5	48.4	48.4	48.5	48.4	49.4	48.8	49.5	47.0	48.9	48.4	49.0	47.0
C-18				53.4	53.4	52.9	53.4	53.4	53.4	53.5	53.4	53.2	52.3	53.3	41.8	41.6	40.9	41.6	41.8
C-19				39.6	39.3	39.0	39.6	39.3	39.6	39.2	39.4	38.6	37.9	38.6	46.3	47.1	46.4	47.2	46.3
C-20				39.6	39.3	38.8	39.6	39.3	39.6	39.2	39.2	47.7[o]	46.6	47.7[r]	30.8	37.0	36.1	37.1	30.8
C-21				30.9	30.9	30.6	30.9	30.9	30.9	31.0	30.9	70.3	71.1	70.4	34.1	72.2	73.4	72.4	34.1
C-22				36.9	37.1	36.6	37.0	37.1	36.9	36.8	36.8	46.4	44.8	46.1	32.6	41.4	40.0	41.1	32.6
C-23				28.0	28.3	28.1	28.1	28.0	28.2	13.3	13.7	28.0	28.1	28.0	28.0	28.0	28.1	28.0	28.2

C-24			15.8[c]	15.7[e]	15.4[f]	15.8[g]	15.7[j]	15.8[k]	63.8	64.4	15.7[p]	15.4[q]	15.8[s]	15.6[u]	15.5[w]	15.3[x]	15.7[y]	15.5[α]
C-25			16.5[c]	17.0[e]	15.5[f]	16.6[g]	16.5[j]	17.0[k]	16.4	16.4	16.5[p]	15.6[q]	16.5[s]	16.4[u]	16.5[w]	15.5[x]	16.5[y]	17.0[α]
C-26			17.4	17.3	16.8	17.5	17.2	17.4	17.4	17.4	17.2	16.8	17.8	17.5	17.2	16.8	17.6	17.5
C-27			23.8	23.9	23.6	23.9	23.9	23.8	23.9	23.8	23.9	23.5	23.8	26.2	26.1	25.7	26.1	26.1
C-28			176.2	177.7	177.9	176.2	177.7	176.1	176.3	176.2	177.0	176.5	175.5	176.4	177.2	176.7	175.7	176.4
C-29			17.7	17.4	16.9	17.7	17.4	17.7	17.8	17.8	17.8	17.1	17.8	33.2	29.8	28.9	29.8	33.2
C-30			21.4	21.3	21.1	21.4	21.3	21.3	21.4	21.3	16.5	15.6	16.5	23.7	17.8	17.0	17.7	23.7
$COOCH_3$				51.5	51.3		51.5				51.6	51.5			51.7	51.6		
glc-1	105.9	105.5	95.7			95.8		95.7	95.8	95.7			95.9	95.7			96.0	95.7
glc-2	75.5	75.5	74.1			74.1		74.1	74.3	74.1			74.1	74.2			74.3	74.1
glc-3	78.6	79.0	79.2[d]			79.2[h]		79.0[l]	79.2[m]	79.2[n]			79.3[t]	79.2[v]			79.4[z]	79.3[β]
glc-4	71.9	71.8	71.4			71.4		71.3	71.4	71.3			71.4	71.2			71.2	71.2
glc-5	78.6	79.0	78.9[d]			78.9[h]		78.8[l]	78.9[m]	78.9[n]			78.9[t]	78.9[v]			78.9[z]	78.9[β]
glc-6	63.1	63.0	62.4			62.5		62.4	62.5	62.4			62.3	62.3			62.3	62.3
						(3-*O*-glc)												
glc·UA-1			105.4	107.3		105.2	105.4	107.3	104.5	106.4	105.4		105.5	105.3	105.4		105.4	107.3
glc·UA-2			83.4	75.4		83.9	83.4	75.4	83.3	75.5	83.4		83.4	83.3	83.4		83.4	75.4
glc·UA-3			76.8	78.0		78.2[i]	76.8	77.9	76.8	77.9	76.8		76.8	76.7	76.8		76.8	77.9
glc·UA-4			73.7	73.1		71.8	73.8	73.1	73.7	73.1	73.8		73.7	73.7	73.8		73.7	73.1
glc·UA-5			77.4	77.2		78.0[i]	77.4	77.2	77.4	77.3	77.4		77.4	77.3	77.4		77.4	77.2
glc·UA-6			170.4	170.8		63.0	170.4	170.7	170.4	170.8	170.5		170.5	170.4	170.5		170.4	170.8
$COOCH_3$			52.2	52.1			52.1	52.0	52.1	52.1	52.2		52.2	52.1	52.1		52.1	52.0
ara-1			106.7			106.7	106.8		106.6		106.8		106.7	106.6	106.8		106.7	
ara-2			72.9			73.7	72.9		72.9		72.9		72.9	72.8	72.9		72.9	
ara-3			74.2			74.3	74.3		74.1		74.3		74.3	74.2	74.3		74.3	
ara-4			69.1			69.1	69.2		69.2		69.2		69.2	69.1	69.2		69.2	
ara-5			67.1			67.0	67.1		67.1		67.1		67.1	67.0	67.1		67.1	

Run at 22.5 MHz *a*) in pyridine-d_5 and *b*) in $CDCl_3$ solution. *c*—*z*) α, β) Assignments may be interchanged in each column.

TABLE II. ^{1}H-NMR Chemical Shifts and Coupling Constants

	H-12	H-18	glc·UA^6—OMe	28-OMe	Anomeric H
3a	5.42 (br s)	2.50 (d, 10)	3.73 (s)		4.97 (d, 6) 5.14 (d, 7) 6.22 (d, 7)
4a	5.36 (br s)	2.40 (d, 10)	3.74 (s)	3.68 (s)	4.98 (d, 7) 5.16 (d, 7)
5a	5.42 (br s)	2.50 (d, 10)	3.74 (s)		4.96 (d, 7) 6.22 (d, 7)
6a	5.42 (br s)	2.48 (d, 10)	3.68 (s)		Overlapped 5.18 (d, 6) 6.22 (d, 7)
7a	5.42 (br s)	2.48 (d, 10)	3.71 (s)		Overlapped 6.22 (d, 8)
8a	5.43 (br s)	2.64 (d, 10)	3.72 (s)		Overlapped 5.18 (d, 6)
9a	5.40 (br s)	2.54 (d, 10)	3.76 (s)	3.68 (s)	4.96 (d, 7) 5.14 (d, 7) 6.24 (d, 6)
10a	5.44 (br s)		3.75 (s)		5.01 (d, 7) 5.20 (d, 7) 6.34 (d, 7)
11a	5.46 (br s)		3.72 (s)		Overlapped 5.18 (d, 7)
12a	5.42 (br s)		3.71 (s)	3.67 (s)	4.98 (d, 7) 5.18 (d, 7) 6.32 (d, 7)
13a	5.42 (br s)		3.74 (s)		4.98 (d, 7) 6.32 (d, 7)

Run at 89.55 MHz in pyridine-d_5 solution.

TABLE III. Molecular Rotation Value of the Saponins of *Cynara cardunculus* L.

Saponins	Found $[M]_D$ in MeOH	Δc
3b	+76.8°	+57.0°
3c	+19.8°	

The following $[M]_D$ values were used to determine the configurations of glycosidic linkages. Me α-L-arabinopyranoside, +17.3°; Me β-L-arabinopyranoside, +245°.[7]

moiety. Compound **3c** was concluded to be the 3-*O*-6′-*O*-methylglucuronopyranoside of ursolic acid methyl ester by comparing its ^{13}C-NMR spectrum with that of **3d**. In the ^{13}C-NMR spectra of **4a**, most of the carbon signals coincided with those of **3c**, except for C-1′, C-2′ and C-3′, which showed glucosylation shifts, and additional carbons of an arabinopyranose moiety. Therefore, **4a** was considered to be an arabinopyranosyl-(1→2)-6′-*O*-methyl-glucuronopyranoside of ursolic acid methyl ester. In the ^{13}C-NMR spectra of **3a**, most of the carbon signals coincided with those of **4a**, except for C-28 (δ 176.2) and additional carbons of a glucose moiety. Therefore, **3a** was considered to be ursolic acid 3-*O*-arabinopyranosyl-(1→2)-6′-*O*-methylglucuronopyranosido-28-*O*-glucopyranoside. Standard gas chromatographic (GC) sugar analysis on the alditol acetate of the acid-hydrolyzed product of **3a** showed L-arabinose and D-glucose in a molar ratio of 1 : 1 (uronic acid is not detected by this method). Compound **3a** was treated with $NaBH_4$, and the reduction product

was hydrolyzed; L-arabinose and D-glucose in a molar ratio of 1 : 2 were detected by alditol acetate analysis. From these results, it is apparent that D-glucose, L-arabinose and 6′-*O*-methyl-D-glucuronopyranose exist in a molar ratio of 1 : 1 : 1. The configurations of the sugar linkages were determined as β for the 6′-*O*-methyl-D-glucuronopyranosyl and glucopyranosyl from the ^{1}H-NMR coupling constants ($J=6$ Hz and $J=7$ Hz) of the anomeric protons of **3a**. As regards the L-arabinopyranosyl moiety, the configuration was determined as α from the ^{1}H-NMR signal pattern of the anomeric proton of **3a** ($J=7$ Hz) and the molecular rotation difference (+57°) between **3b** and **3c** (Table III). The coupling constant $J_{C_1-H_1}$ [glucose (161.8 Hz), methyl glucuronate (159.8 Hz), arabinose (157.6 Hz)] also supported the above configuration. On the basis of the above-mentioned results and spectral evidence, **3a** was concluded to be ursolic acid 3-*O*-α-L-arabinopyranosyl-(1→2)-6′-*O*-methyl-β-D-glucuronopyranosido-28-*O*-β-D-glucopyranoside.

Cynarasaponin B Methyl Ester (4a)

The ^{1}H- and ^{13}C-NMR spectra of **4a** were in good agreement with those of the partial acid hydrolysis product of **3a**, and therefore, the structure of this compound was decided to be **4a**.

Cynarasaponin C Methyl Ester (5a)

Based on a comparison of the ^{1}H- and ^{13}C-NMR spectra of **5a** with those of **3a** and **3c**, the structure of this compound was concluded to be ursolic acid 3-*O*-6′-*O*-methyl-β-D-glucuronopyranosido-28-*O*-β-D-glucopyranoside.

Cynarasaponin D Methyl Ester (6a)

A comparison of the ^{13}C-NMR spectrum of **6a** with that of **3a** indicated that the carbon signals due to the sugar moiety were in good agreement, except for δ 104.5, which was assigned to an anomeric carbon of 6′-*O*-methyl-β-D-glucuronopyranoside. Acid hydrolysis of **6a** gave a sapogenin, which was identified as 23-hydroxyursolic acid (**6g**) after conversion to the methyl ester acetate (**6f**), mp 180—183 °C, $[\alpha]_D$ +71.4°, by comparison of its ^{1}H-NMR, MS and melting point with reported data (mp 180—181.5 °C, $[\alpha]_D$ +64.1°).[4] On the basis of the above-mentioned results and spectral evidence, **6a** was concluded to be 23-hydroxyursolic acid 3-*O*-α-L-arabinopyranosyl-(1→2)-6′-*O*-methyl-β-D-glucuronopyranosido-28-*O*-β-D-glucopyranoside.

Cynarasaponin E Methyl Ester (7a)

Based on a comparison of the ^{1}H- and ^{13}C-NMR spectra of **7a** with those of **5a** and **6a**, the structure of this compound was concluded to be 23-hydroxyursolic acid 3-*O*-6′-*O*-methyl-β-D-glucuronopyranosido-28-*O*-β-D-glucopyranoside.

Cynarasaponin F Methyl Ester (8a)

In the ^{13}C-NMR spectrum of **8a**, the signals due to the sugar and aglycone moiety showed similar chemical shifts to those of **4a**, except for the carbons of the E ring (C-19, C-20, C-21 and C-22) and those attached to the E ring (C-17, C-29 and C-30). Compound **8a** was methylated with CH_2N_2 followed by acid hydrolysis to give a monomethyl ester of an aglycone (**8d**), mp 230—231.5 °C, $[\alpha]_D$ +75.0°. The MS of **8d** showed ion peaks at *m/z* 208 and 278, the characteristic retro-Diels–Alder cleavage peaks of an urs-12-ene-28-oic acid methyl ester derivative which possesses a hydroxyl group on either the D or E ring.[5] In the ^{13}C-NMR spectrum of **8d**, the C-20 (δ 46.6), C-21 (δ 71.1) and C-22 (δ 44.8) signals were shifted downfield by 7.8, 40.5 and 8.2 ppm, respectively, compared with those of **3d**. In the ^{1}H-NMR spectrum of **8d**, the signals of two carbinyl protons δ 3.25 (dd, $J=10$, 5 Hz) and 3.45 (dt, $J=5$, 10 Hz) were assigned to 3α-H and 21α-H, respectively. The above-mentioned results were indicative of two axial protons geminal to two equatorial hydroxyl groups. Finally, the

hydroxyl group at C-21 was β-oriented. Therefore, **8d** was considered to be 21β-hydroxyursolic acid methyl ester. On the basis of above-mentioned results and spectral evidence, **8a** was concluded to be the 3-*O*-α-L-arabinopyranosyl-(1→2)-6′-*O*-methyl-β-D-glucuronopyranoside of 21β-hydroxyursolic acid methyl ester.

Cynarasaponin G Methyl Ester (9a)

In the ^{13}C-NMR spectrum of **9a**, most of the carbon signals coincided with those of **8a**, except for C-28, and additional carbon signals of the glucose moiety. Therefore, **9a** was concluded to be 21β-hydroxyursolic acid 3-*O*-α-L-arabinopyranosyl-(1→2)-6′-*O*-methyl-β-D-glucuronopyranosido-28-*O*-β-D-glucopyranoside.

Cynarasaponin H Methyl Ester (10a)

In the ^{13}C-NMR spectrum of **10a**, the signals due to the sugar moiety showed similar chemical shifts to those of **3a**. Compound **10a** was methylated with CH_2N_2 followed by acid hydrolysis to give **10d**, mp 201—203 °C. Compound **10d** was identified as oleanolic acid methyl ester by direct comparison with an authentic sample (^{1}H-NMR, MS, mixed melting point). Therefore, **10a** was concluded to be oleanolic acid 3-*O*-α-L-arabinopyranosyl-(1→2)-6′-*O*-methyl-β-D-glucuronopyranosido-28-*O*-β-D-glucopyranoside.

Cynarasaponin I Methyl Ester (11a)

In the ^{13}C-NMR spectrum of **11a**, the signals due to the sugar moiety were in good agreement with those of **4a**, and the signals due to the aglycone moiety showed similar chemical shifts to those of **10a**, except for the carbons of the E ring (C-19, C-20, C-21 and C-22) and those attached to the E ring (C-17, C-29 and C-30). Compound **11a** was methylated with CH_2N_2 followed by acid hydrolysis to give a monomethyl ester of an aglycone (**11d**), mp 238—239 °C. Compound **11d** was identified as machaerinic acid methyl ester, by comparison of its ^{13}C-NMR, MS and melting point with reported data (mp 234—236 °C).[6] Therefore, **11a** was concluded to be the 3-*O*-α-L-arabinopyranosyl-(1→2)-6′-*O*-methyl-β-D-glucuronopyranoside of machaerinic acid methyl ester.

Cynarasaponin J Methyl Ester (12a)

The ^{13}C-NMR spectrum indicated that **12a** is a bisdesmoside of the same sapogenin, machaerinic acid (**11d**), as that of **11a**, and the signals of the sugar moiety are in good agreement with those of **3a**. Therefore, **12a** was concluded to be machaerinic acid 3-*O*-α-L-arabinopyranosyl-(1→2)-6′-*O*-methyl-β-D-glucuronopyranosido-28-*O*-β-D-glucopyranoside.

In this work, a new sesquiterpene glycoside, cynaroside A, and ten new saponins, cynarasaponins A—J, were isolated and their structures were established. It should be noted that this is the first isolation of saponin from Compositae plants, and this is also the first report on the glycosides of ursolic acid.

Experimental

Melting points were taken on a Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. MS were measured on a JEOL JMS-D100 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL FX90Q (89.55 and 22.5 MHz, respectively) and JEOL GX400 (399.65 MHz) NMR spectrometers. Chemical shifts are given on the δ scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). GC was done on a Hitachi K 53 gas chromatograph. Conditions: column, Spelco capillary column SPB 35, 0.75 mm × 30 m; column temperature, 200 °C; carrier gas, N_2. High-performance liquid chromatography (HPLC) was done on a Kyowa Seimitsu model K 880 instrument. Conditions: column, YMC-Pack ODS-7, 20 mm × 25 cm, CH_3CN–H_2O or MeOH–H_2O system.

Extraction and Isolation——Fresh aerial parts (flowering) (16.5 kg) of *Cynara cardunculus* L. cultivated in Shizuoka, Japan, were extracted twice with hot water. The extract was passed through an Amberlite XAD-2 column and the adsorbed material was eluted with methanol. The methanol eluate (87 g) was chromatographed on a

polyamide column and eluted with water. After repeated chromatography of the eluate (58 g) on silica gel with a chloroform–methanol system, two sesquiterpene glycosides were isolated. Fresh roots (3.5 kg) were extracted twice with hot water. The extract was passed through an Amberlite XAD-2 column and the adsorbed material was eluted with methanol. The methanol eluate (30 g) was passed through an Amberlite IR-120 column and concentrated to give a residue, which was methylated with CH_2N_2. After repeated chromatography on silica gel with a chloroform–methanol system, eleven saponins were isolated.

Cynaroside A (1)——Amorphous powder (26 mg), $[\alpha]_D^{23}$ +38.3° (c=1.37, MeOH). *Anal.* Calcd for $C_{21}H_{32}O_{10}\cdot H_2O$: C, 54.54; H, 7.41. Found: C, 54.74; H, 7.14. ^{1}H-NMR (pyridine-d_5) δ: 1.44 (3H, d, J=7 Hz, H_3-15), 4.22, 4.41 (each 1H, d, J=10 Hz, H-13), 4.87 (2H, br s, H_2-14), 4.95 (1H, d, J=8 Hz, H-1′). ^{13}C-NMR: Table I.

11β,13-Dihydrodesacylcynaropicrin-8-β-D-glucoside (2)——Amorphous powder (55 mg), $[\alpha]_D^{23}$ +24.5° (c=1.10, MeOH). *Anal.* Calcd for $C_{21}H_{30}O_9\cdot 1/2H_2O$: C, 57.92; H, 7.18. Found: C, 57.82; H, 7.13. ^{1}H-NMR (pyridine-d_5) δ: 1.83 (3H, d, J=10 Hz, H_3-13), 4.70 (1H, d, J=7 Hz, H-1′), 4.86 (2H, br s, H_2-14). ^{13}C-NMR: Table I.

Cynarasaponin A Methyl Ester (3a)——Amorphous powder (1.8 g), $[\alpha]_D^{25}$ +4.1° (c=1.33, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{18}\cdot 3/2H_2O$: C, 59.55; H, 8.22. Found: C, 59.32; H, 7.93. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin B Methyl Ester (4a)——Amorphous powder (100 mg), $[\alpha]_D^{21}$ +9.7° (c=1.81, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{13}\cdot 3/2H_2O$: C, 63.00; H, 8.67. Found: C, 62.98; H, 8.45. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin C Methyl Ester (5a)——Amorphous powder (26 mg), $[\alpha]_D^{21}$ −7.5° (c=0.93, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{14}\cdot 3/2H_2O$: C, 61.80; H, 8.50. Found: C, 61.86; H, 8.55. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin D Methyl Ester (6a)——Amorphous powder (220 mg), $[\alpha]_D^{25}$ +7.6° (c=1.16, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{19}\cdot 2H_2O$: C, 58.05; H, 8.12. Found: C, 58.05; H, 7.87. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin E Methyl Ester (7a)——Amorphous powder (290 mg), $[\alpha]_D^{25}$ +3.7° (c=0.95, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{15}\cdot 2H_2O$: C, 59.98; H, 8.43. Found: C, 60.10; H, 8.30. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin F Methyl Ester (8a)——Amorphous powder (100 mg), $[\alpha]_D^{25}$ +23.4° (c=0.47, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{14}\cdot 3/2H_2O$: C, 61.78; H, 8.56. Found: C, 61.69; H, 8.30. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin G Methyl Ester (9a)——Amorphous powder (70 mg), $[\alpha]_D^{25}$ +11.1° (c=0.85, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{19}\cdot 2H_2O$: C, 58.05; H, 8.12. Found: C, 57.88; H, 7.90. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin H Methyl Ester (10a)——Amorphous powder (250 mg), $[\alpha]_D^{21}$ +0.68° (c=1.48, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{18}\cdot 2H_2O$: C, 59.02; H, 8.20. Found: C, 58.75; H, 8.02. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin I Methyl Ester (11a)——Amorphous powder (80 mg), $[\alpha]_D^{25}$ +21.0° (c=0.90, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{14}\cdot 3/2H_2O$: C, 61.78; H, 8.56. Found: C, 61.51; H, 8.29. ^{1}H- and ^{13}C-NMR: Tables I and II.

Cynarasaponin J Methyl Ester (12a)——Amorphous powder (80 mg), $[\alpha]_D^{25}$ +15.6° (c=0.95, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{19}\cdot 2H_2O$: C, 58.05; H, 8.12. Found: C, 57.90; H, 7.89. ^{1}H- and ^{13}C-NMR: Tables I and II.

Chikusetsusaponin IVa Methyl Ester (13a)——Amorphous powder (18 mg), $[\alpha]_D^{21}$ +1.4° (c=0.74, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{14}\cdot 5/2H_2O$: C, 60.49; H, 8.56. Found: C, 60.33; H, 8.51. ^{1}H- and ^{13}C-NMR: Tables I and II.

Enzymatic Hydrolysis of Cynaroside A (1)——A solution of **1** (13 mg) in water (1 ml) was treated with cellulase (Sigma type II) (20 mg) at room temperature for 22 h, then the reaction mixture was extracted with ethyl acetate 4 times. The ethyl acetate extract was purified by HPLC to give **1a** (3.8 mg) as an amorphous powder. ^{1}H-NMR (pyridine-d_5) δ: 1.46 (3H, d, J=6 Hz, H_3-15), 3.90 (1H, m, H-3), 4.23, 4.44 (1H each, d, J=11 Hz, H-13), 4.62 (1H, t, J=10 Hz, H-6), 4.98 (2H, br s, H_2-14). MS m/z: 282 (M^+, trace), 264 (1), 252 (2), 234 (4), 223 (1), 205 (5), 189 (10), 179 (10), 161 (25), 149 (16), 131 (20), 42 (100). This product was shown to be identical with cynaratriol by comparison of spectral data.[3]

Acetylation of 11β,13-Dihydrodesacylcynaropicrin-8-β-D-glucoside (2)——**2** (2 mg) was dissolved in pyridine and acetic anhydride (each 2 drops), and the reaction mixture was left overnight at room temperature. The reagents were evaporated off *in vacuo* to give the pentaacetate (**2a**). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, d, J=7 Hz, H_3-13), 2.01, 2.03, 2.04, 2.07, 2.10 (3H each, s, OAc), 2.18 (1H, m, H-7), 2.86 (1H, m, H-1), 3.68 (1H, m, H-8), 4.72 (1H, d, J=8 Hz, H-1′), 5.05 (2H, br s, H_2-14), 5.28, 5.44 (1H, each, br s, H-15), 5.56 (1H, m, H-3).

Partial Acid Hydrolysis of Cynarasaponin A Methyl Ester (3a)——**1a** (140 mg) was dissolved in AcCl–MeOH (1 : 20) (20 ml) and the solution was refluxed for 1 h. The reagents were evaporated off, and the residue was methylated with CH_2N_2, then the reaction mixture was purified by HPLC to give **4a** (40 mg), **3c** (30 mg) and **3d** (9 mg). **3d**; colorless needles, mp 173—174.5 °C. MS m/z: 470 (M^+, 3), 411 (3), 410 (3), 262 (100), 203 (95). ^{1}H-NMR ($CDCl_3$) δ: 2.24 (1H, d, J=11 Hz, H-18), 3.26 (1H, t, J=9 Hz, H-3), 3.62 (3H, s, OMe), 5.26 (1H, br s, H-12). ^{13}C-NMR: Table I. **3c**; amorphous powder, $[\alpha]_D^{20}$ +3.0° (c=1.48, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 2.44 (1H, d, J=10 Hz, H-18), 3.40 (1H, dd, J=10, 5 Hz, H-3), 3.71, 3.78 (3H each, s, OMe), 5.02 (1H, d, J=7 Hz, anomeric H of methyl glucuronate), 5.17 (1H, br s, H-12). ^{13}C-NMR: Table I. **4a**; amorphous powder. ^{1}H-NMR (pyridine-d_5) δ: 2.42 (1H, d, J=10 Hz, H-18), 3.32 (1H, dd, J=10, 5 Hz, H-3), 3.71, 3.76 (3H each, s, OMe), 5.00 (1H, d, J=8 Hz, anomeric H of arabinose), 5.18 (1H, d, J=6 Hz, anomeric H of methyl glucuronate), 5.36 (1H, br s, H-12). ^{13}C-NMR: Table I.

Sodium Borohydride Reduction of Cynarasaponin A Methyl Ester (3a)——A solution of **3a** (100 mg) in methanol (5 ml) was treated with sodium borohydride (100 mg) at room temperature. The reaction mixture was stirred for 30 min, diluted with water, and passed through an Amberlite IR-120 column. The eluate was evaporated, and the product was purified by HPLC to give **3e** (34 mg) as an amorphous powder. ^{1}H-NMR (pyridine-d_5) δ: 2.56 (1H, d,

$J=10$ Hz, H-18), 3.63 (3H, s, OMe), 4.96 (1H, d, $J=7$ Hz, anomeric H of arabinose), 5.22 (1H, d, $J=7$ Hz, anomeric H of glucose), 5.47 (1H, br s, H-12), 6.29 (1H, d, $J=7$ Hz, anomeric H of glucose). ^{13}C-NMR: Table I.

Acid Hydrolysis of Cynarasaponin D Methyl Ester (6a)——A solution of **6a** (24 mg) in 10% H_2SO_4–MeOH (1 : 2) (10 ml) was refluxed for 6 h. The reaction mixture was extracted with ethyl acetate 4 times. The ethyl acetate extract was washed with H_2O, and purified by HPLC to give **6g**. **6g** was methylated with CH_2N_2, and then acetylated with pyridine–acetic anhydride to give **6f** as colorless needles, mp 180—183 °C, $[\alpha]_D^{25}$ +71.4° ($c=0.35$, $CHCl_3$) (lit.[4] mp 180—181.5 °C, $[\alpha]_D^{21}$ +64.1°). MS *m/z*: 570 (M^+, 1), 510 (5), 450 (3), 262 (90), 249 (15), 203 (100), 189 (30), 133 (75). ^{1}H-NMR ($CDCl_3$) δ: 0.76, 0.84, 0.98, 1.08 (3H each, s, Me), 0.87 (3H, d, $J=5$ Hz, H_3-29), 0.94 (3H, d, $J=6$ Hz, H_3-30), 2.03, 2.07 (3H each, s, OAc), 2.22 (1H, d, $J=12$ Hz, H-18), 3.60 (3H, s, OMe), 3.71, 3.87 (1H each, d, $J=12$ Hz, H-23), 4.78 (1H, dd, $J=10$, 5 Hz, H-3), 5.24 (1H, t, $J=3$ Hz, H-12).

Acid Hydrolysis of Cynarasaponin F Methyl Ester (8a)——A solution of **8a** (18 mg) in 10% H_2SO_4–MeOH (1 : 2) (10 ml) was refluxed for 6 h. The reaction mixture was extracted with ethyl acetate 4 times. The ethyl acetate extract was washed with H_2O, methylated with CH_2N_2, and then purified by HPLC to give **8d** as colorless needles, mp 230—231.5 °C, $[\alpha]_D^{25}$ +75.0° ($c=0.48$, $CHCl_3$). MS *m/z*: 486 (M^+, trace), 468 (6), 278 (9), 260 (18), 247 (19), 219 (12), 218 (11), 208 (10), 207 (35), 201 (100). ^{1}H-NMR ($CDCl_3$) δ: 0.78, 0.82, 0.96, 1.02, 1.09 (3H each, s, Me), 0.94, 1.11 (3H each, d, $J=7$ Hz, Me), 2.30 (1H, d, $J=11$ Hz, H-18), 3.25 (1H, dd, $J=10$, 5 Hz, H-3), 3.45 (1H, dt, $J=5$, 10 Hz, H-21), 5.30 (1H, t, $J=4$ Hz, H-12). ^{13}C-NMR: Table I.

Acid Hydrolysis of Cynarasaponin H Methyl Ester (10a)——**10a** (10 mg) was hydrolyzed in the same way as **8a** to give **10d** as colorless needles, mp 201—203 °C. MS *m/z*: 470 (M^+, trace), 411 (2), 410 (1), 262 (60), 203 (100). ^{1}H-NMR ($CDCl_3$) δ: 2.84 (1H, dd, $J=10$, 5 Hz, H-3), 3.20 (1H, t, $J=8$ Hz, H-18), 3.62 (3H, s, OMe), 5.28 (1H, t, $J=4$ Hz, H-12). ^{13}C-NMR: Table I.

Acid Hydrolysis of Cynarasaponin I Methyl Ester (11a)——**11a** (18 mg) was hydrolyzed in the same way as **8a** to give **11d** as colorless needles, mp 238—239 °C (lit.[6] 234—236 °C), MS *m/z*: 486 (M^+, 3), 468 (9), 426 (8), 409 (7), 278 (6), 260 (36), 247 (44), 208 (12), 207 (24), 201 (100), 190 (19). ^{1}H-NMR ($CDCl_3$) δ: 0.75, 0.82, 0.94, 0.95, 1.02, 1.16 (3H each, s, Me), 2.95 (1H, dd, $J=14$, 4 Hz, H-18), 3.25 (1H, dd, $J=11$, 5 Hz, H-3), 3.55 (1H, dd, $J=11$, 5 Hz, H-21), 5.35 (1H, t, $J=4$ Hz, H-12). ^{13}C-NMR: Table I.

Acid Hydrolysis of Sesquiterpene Glycosides 1 and 2, and Saponin Methyl Esters 3a, 3e, 4a, 5a, 6a, 7a, 8a, 9a, 10a, 11a, 12a and 13a—— A solution of a glycoside (*ca.* 0.1 mg) in 10% H_2SO_4 (2 drops) was heated in a boiling water bath for 30 min. The solution was passed through an Amberlite IR-45 column and concentrated to give a residue, which was reduced with $NaBH_4$ (*ca.* 1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and the eluate was concentrated to dryness. Boric acid was removed by co-distillation with methanol and the residue was acetylated with acetic anhydride and pyridine (1 drop each) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. From **1**, **2**, **5a**, **7a** and **13a**, glucitol acetate (t_R 12.0 min), from **4a**, **8a** and **11a**, arabinitol acetate (t_R 6.0 min), from **3a**, **6a**, **9a**, **10a** and **12a**, glucitol acetate and arabinitol acetate (1 : 1), and from **3e**, glucitol acetate and arabinitol acetate (2 : 1) were detected by GC.

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