Inhibitory Effects of Acylated Acyclic Sesquiterpene Oligoglycosides from the Pericarps of *Sapindus rarak* on Tumor Necrosis Factor-α-Induced Cytotoxicity

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Four new acylated acyclic sesquiterpene oligoglycosides (1—4) were isolated from the pericarps of *Sapindus rarak* Dc. together with four known acyclic sesquiterpene oligoglycosides, mukuroziosides Ia (5), Ib (6), IIa (7), and IIb (8). Their structures were elucidated on the basis of chemical and physicochemical evidence. These newly isolated compounds (1—4) were found to show inhibitory effects on tumor necrosis factor-α-induced cytotoxicity in L929 cells at concentrations of 30—100 μM.

Key words *Sapindus rarak*; acyclic sesquiterpene oligoglycoside; tumor necrosis factor-α; Sapindaceae; Thai natural medicine

During the course of our studies on bioactive constituents from natural medicines originating in Thailand,[1—9] we have reported that the methanolic extract from pericarps of *Sapindus rarak* Dc. (Sapindaceae) showed pancreatic lipase inhibitory activity (*in vitro*)[5] and inhibitory effect on plasma triglyceride elevation in olive oil-loaded mice (*in vivo*).[6] By bioassay-guided separation, several saponin constituents were isolated as their active compounds.[5,6] As a continuing study on this natural medicine, we further isolated four new acylated acyclic sesquiterpene oligoglycosides (**1**—**4**). This paper deals with isolation and structure elucidation of these new sesquiterpenes (**1**—**4**) as well as their inhibitory effects on tumor necrosis factor-α (TNF-α)-induced cytotoxicity in L929 cells.

The methanolic extract of pericarps of *S. rarak* collected in Thailand (67.5%) was subjected to Diaion HP-20 column chromatography ($H_2O \rightarrow$MeOH) to give H_2O- and MeOH-eluted fractions (26.8, 40.7%, respectively). From the MeOH-eluted fraction, the authors already isolated 20 saponins and four acyclic sesquiterpene oligoglycosides, mukuroziosides Ia (**5**, 0.60%), Ib (**6**, 0.02%), IIa (**7**, 3.71%), and IIb (**8**, 3.61%), as were reported.[5,6] By the intensive chromatographies on the MeOH-eluted fraction, four acylated acyclic sesquiterpene oligoglycosides, **1** (0.27%), **2** (0.22%), **3** (0.13%), and **4** (0.02%) have been newly isolated (Chart 1).

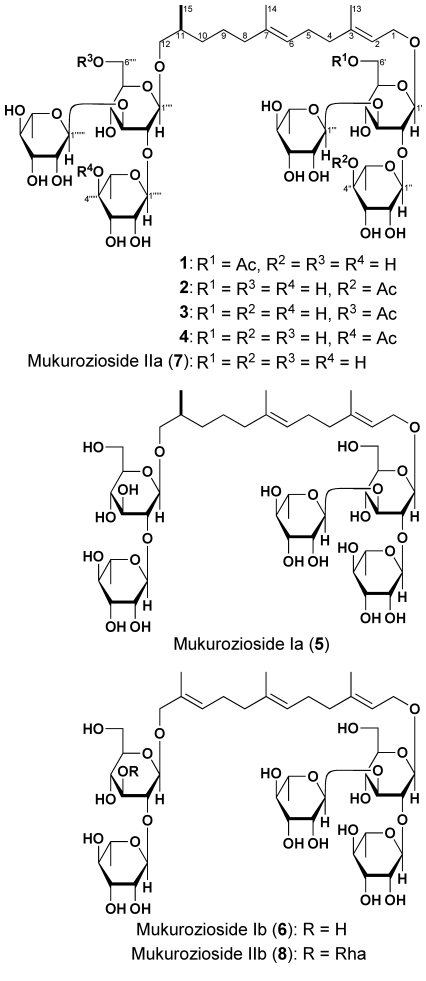

Chart 1.

Structures of Acylated Acyclic Sesquiterpene Oligoglycosides (1—4) Compound **1** was obtained as a white powder with negative optical rotation ($[\alpha]_D^{27}$ −51.6 in MeOH). The IR spectrum of **1** showed absorption bands at 3568, 1734, and 1044 cm^{-1} ascribable to hydroxyls, olefins, and ether functions, respectively. The positive- and negative-ion FAB-MS spectra of **1** showed quasimolecular ion peaks at *m/z* 1213 $(M+Na)^+$ and *m/z* 1189 $(M-H)^-$, respectively, and the molecular formula was determined as $C_{53}H_{90}O_{29}$ by high-resolution positive-ion FAB-MS measurement. The ^{1}H- and ^{13}C-NMR spectra of **1** (pyridine-d_5, Tables 1, 2), which were assigned by various NMR experiments,[10] showed signals assignable to three methyls [δ 0.99 (3H, d, J=6.7 Hz, 15-H_3), 1.61, 1.63 (3H each, both s, 14, 13-H_3)], two methylenes bearing an oxygen function [δ 3.41, 3.91 (1H each, both m, 12-H_2), 4.31, 4.58 (1H each, both m, 1-H_2)], two trisubstituted olefins [δ 5.22 (1H, dd, J=6.7, 6.7 Hz, 6-H), 5.68 (1H, dd, J=6.2, 6.2 Hz, 2-H)], two β-D-glucopyranosyl [δ 4.66 (1H, d, J=7.7 Hz, Glc-1‴-H), 4.68 (1H, d, J=7.7 Hz, Glc-1′-H)], and four α-L-rhamnopyranosyl moieties [δ 1.62 (3H, d,

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Table 1. ^{1}H-NMR Data (600 MHz, Pyridine-d_5) for **1**—**4** and **7**

Position	**1**	**2**	**3**	**4**	**7**
1	4.31 (m)	4.27 (m)	4.29 (m)	4.30 (m)	4.32 (m)
	4.58 (m)	4.56 (m)	4.57 (dd, 6.5, 11.5)	4.58 (dd, 6.6, 11.3)	4.57 (dd, 6.2, 11.2)
2	5.68 (dd, 6.2, 6.2)	5.71 (dd, 6.2, 6.2)	5.66 (dd, 6.0, 6.5)	5.67 (dd, 6.6, 6.6)	5.66 (dd, 6.1, 6.2)
4	2.10 (m)	2.09 (m)	2.08 (m)	2.10 (m)	2.08 (m)
	2.22 (m)	2.18 (m)	2.18 (m)	2.21 (dd, 7.2, 15.1)	2.20 (m)
5	1.98 (m)	1.97 (m)	1.97 (m)	2.00 (m)	1.99 (m)
	2.11 (m)	2.10 (m)	2.11 (m)	2.10 (m)	2.15 (m)
6	5.22 (dd, 6.7, 6.7)	5.22 (dd, 6.8, 6.8)	5.20 (dd, 6.8, 6.8)	5.22 (dd, 6.7, 6.7)	5.21 (dd, 6.8, 6.8)
8	1.91 (m)	1.91 (m)	1.98 (m)	1.90 (m)	1.92 (m)
	2.24 (m)	2.24 (m)	2.20 (m)	2.21 (m)	2.21 (m)
9	1.41 (m)	1.39 (m)	1.39 (m)	1.41 (m)	1.43 (m)
	1.57 (m)	1.61 (m)	1.57 (m)	1.53 (m)	1.59 (m)
10	1.13 (m)	1.12 (m)	1.14 (m)	1.14 (m)	1.11 (m)
	1.51 (m)	1.49 (m)	1.57 (m)	1.51 (m)	1.51 (m)
11	1.96 (m)	1.97 (m)	1.95 (m)	1.92 (m)	1.92 (m)
12	3.41 (m)	3.35 (m)	3.41 (dd, 6.7, 9.4)	3.39 (dd, 6.8, 9.5)	3.36 (dd, 6.9, 9.2)
	3.91 (m)	3.87 (m)	3.89 (dd, 6.7, 9.4)	3.94 (dd, 6.8, 9.5)	3.90 (dd, 6.9, 9.2)
13	1.63 (s)	1.65 (s)	1.63 (s)	1.65 (s)	1.64 (s)
14	1.61 (s)	1.62 (s)	1.61 (s)	1.62 (s)	1.62 (s)
15	0.99 (d, 6.7)	1.01 (d, 6.7)	1.01 (d, 6.7)	1.04 (d, 6.7)	0..99 (d, 6.8)
Glc-1′	4.68 (d, 7.7)	4.68 (d, 7.8)	4.75 (d, 7.7)	4.75 (d, 7.7)	4.74 (d, 7.8)
2′	4.02 (m)	4.01 (m)	4.03 (m)	4.02 (m)	4.01 (m)
3′	4.09 (dd, 8.8, 9.0)	4.08 (dd, 9.0, 9.0)	4.10 (dd, 8.8, 9.0)	4.10 (m)	4.10 (dd, 8.8, 9.0)
4′	4.04 (m)	4.01 (m)	4.02 (m)	4.01 (m)	4.01 (m)
5′	3.91 (m)	4.00 (m)	4.02 (m)	4.02 (m)	4.01 (m)
6′	4.76 (2H, m)	4.31 (m)	4.29 (m)	4.31 (m)	4.33 (m)
		4.44 (m)	4.42 (m)	4.44 (m)	4.44 (m)
6′-OAc	2.08 (s)				
Rha-1″	5.76 (br s)	5.89 (br s)	5.84 (br s)	5.76 (br s)	5.71 (br s)
2″	4.82 (m)	4.83 (m)	4.79 (m)	4.81 (m)	4.76 (m)
3″	4.49 (m)	4.64 (m)	4.56 (m)	4.58 (m)	4.51 (m)
4″	4.30 (m)	5.42 (dd, 9.2, 9.4)	4.44 (m)	4.29 (m)	4.41 (m)
5″	4.68 (m)	4.83 (m)	4.68 (m)	4.69 (m)	4.68 (m)
6″	1.69 (d, 6.2)	1.44 (d, 6.3)	1.68 (d, 6.2)	1.69 (d, 6.2)	1.69 (d, 6.2)
4″-OAc		1.99 (s)			
Rha-1‴	5.69 (br s)	5.67 (br s)	5.69 (br s)	5.67 (br s)	5.68 (br s)
2‴	4.85 (br s)	4.80 (m)	4.78 (m)	4.85 (m)	4.78 (m)
3‴	4.64 (m)	4.51 (m)	4.44 (m)	4.64 (m)	4.42 (m)
4‴	4.34 (m)	4.23 (m)	4.24 (m)	4.32 (m)	4.17 (m)
5‴	4.72 (m)	4.62 (m)	4.62 (m)	4.70 (m)	4.62 (m)
6‴	1.62 (d, 6.2)	1.62 (d, 6.1)	1.62 (d, 6.2)	1.62 (d, 6.3)	1.63 (d, 6.0)
Glc-1⁗	4.66 (d, 7.7)	4.67 (d, 7.8)	4.63 (d, 7.7)	4.65 (d, 7.7)	4.69 (d, 7.8)
2⁗	4.01 (m)	4.01 (m)	4.02 (m)	4.02 (m)	3.95 (m)
3⁗	4.06 (m)	4.06 (m)	4.08 (m)	4.08 (m)	4.09 (m)
4⁗	4.01 (m)	4.02 (m)	4.04 (m)	4.01 (m)	4.01 (m)
5⁗	4.01 (m)	4.02 (m)	3.84 (br dd, 5.0, 8.9)	4.02 (m)	3.95 (br dd, 5.0, 8.9)
6⁗	4.26 (m)	4.25 (m)	4.62 (dd, 5.0, 11.3)	4.27 (m)	4.27 (dd, 5.0, 11.8)
	4.41 (m)	4.41 (m)	4.85 (br d, 11.3)	4.44 (m)	4.41 (br d, 11.8)
6⁗-OAc			1.98 (s)		
Rha-1⁗	5.86 (br s)	5.85 (br s)	5.84 (br s)	5.86 (br s)	5.83 (br s)
2⁗	4.71 (m)	4.70 (m)	4.65 (m)	4.65 (m)	4.61 (m)
3⁗	4.44 (m)	4.50 (m)	4.46 (m)	4.70 (m)	4.44 (m)
4⁗	4.28 (m)	4.28 (m)	4.27 (m)	5.69 (dd, 9.2, 9.5)	4.40 (m)
5⁗	4.68 (br s)	4.69 (m)	4.68 (m)	4.81 (m)	4.68 (m)
6⁗	1.67 (d, 6.3)	1.67 (d, 6.2)	1.68 (d, 6.2)	1.44 (d, 6.2)	1.68 (d, 6.2)
4⁗-OAc				2.08 (s)	
Rha-1⁗	5.67 (br s)	5.67 (br s)	5.66 (br s)	5.66 (br s)	5.68 (br s)
2⁗	4.78 (m)	4.80 (m)	4.74 (m)	4.75 (m)	4.80 (m)
3⁗	4.58 (m)	4.59 (m)	4.56 (m)	4.57 (m)	4.62 (m)
4⁗	4.26 (m)	4.26 (m)	4.27 (m)	4.31 (m)	4.21 (m)
5⁗	4.75 (m)	4.71 (m)	4.68 (m)	4.63 (m)	4.71 (m)
6⁗	1.63 (d, 6.3)	1.63 (d, 6.3)	1.62 (d, 6.2)	1.63 (d, 6.2)	1.63 (d, 6.0)

Table 2. ^{13}C-NMR (150 MHz, Pyridine-d_5) Data for **1**—**4** and **7**

Position	**1**	**2**	**3**	**4**	**7**	Position	**1**	**2**	**3**	**4**	**7**
1	66.3	65.7	65.9	65.9	65.9	Rha-1‴	103.9	103.9	103.8	103.8	103.9
2	120.7	120.9	121.0	121.3	121.0	2‴	72.8	72.8	72.8	72.8	72.8
3	140.3	140.9	140.4	140.3	140.4	3‴	72.3	72.7	72.7	72.3	72.7
4	40.3	40.2	39.9	39.9	40.0	4‴	73.8	73.8	73.9	73.8	73.9
5	26.8	26.8	26.8	26.8	26.8	5‴	70.6	70.7	70.6	70.6	70.7
6	124.4	124.3	124.3	124.4	124.3	6‴	18.8	18.4	18.7	18.2	18.4
7	136.0	136.0	136.0	136.0	136.0						
8	40.3	40.3	40.3	40.3	40.3	Glc-1⁗	102.9	102.7	102.9	102.8	102.9
9	25.8	25.8	25.7	25.3	25.6	2⁗	78.1	77.6	78.1	77.7	78.0
10	33.8	33.8	33.8	33.7	33.8	3⁗	87.6	87.9	87.6	87.8	87.6
11	34.0	34.0	34.0	34.0	34.0	4⁗	70.3	70.3	70.7	70.2	70.2
12	75.6	75.7	75.6	75.6	75.7	5⁗	78.1	78.0	74.7	77.7	78.0
13	16.6	16.6	16.6	16.6	16.6	6⁗	62.4	62.4	64.3	62.4	62.4
14	16.1	16.1	16.1	16.0	16.0	6⁗-OAc			170.7		
15	17.3	17.4	17.3	17.4	17.4				20.7		
Glc-1′	101.3	101.5	101.5	101.4	101.4	Rha-1⁗	102.3	102.4	102.5	101.9	102.4
2′	78.1	78.1	78.2	78.2	78.1	2⁗	72.5	72.5	72.5	72.5	72.5
3′	87.8	88.0	87.6	87.6	87.8	3⁗	72.4	72.4	72.5	70.6	72.5
4′	70.2	70.2	70.0	70.2	70.1	4⁗	73.7	73.6	73.6	75.9	73.6
5′	75.8	78.1	78.0	78.0	78.1	5⁗	69.9	70.0	70.1	67.2	69.9
6′	65.8	62.4	62.4	62.4	62.4	6⁗	18.6	18.6	18.4	18.2	18.6
6′-OAc	170.7					4⁗-OAc				170.7	
	21.2									21.1	
Rha-1″	102.8	101.6	102.9	103.0	103.0	Rha-1⁗	103.8	103.8	103.9	103.9	103.9
2″	72.5	72.4	72.6	72.5	72.4	2⁗	72.6	72.6	72.6	72.6	72.6
3″	72.2	70.9	72.5	72.0	72.2	3⁗	72.6	72.5	72.5	72.6	72.6
4″	73.6	75.9	73.7	73.6	72.8	4⁗	73.7	73.6	73.6	73.6	73.9
5″	70.1	67.0	70.0	69.9	69.9	5⁗	70.3	70.2	70.1	70.2	70.6
6″	18.8	18.2	18.5	18.5	18.8	6⁗	18.4	18.4	18.4	18.4	18.4
4″-OAc		170.7									
		20.7									

J=6.2 Hz, Rha-6‴-H_3), 1.63 (3H, d, J=6.3 Hz, Rha-6⁗-H_3), 1.67 (3H, d, J=6.3 Hz, Rha-6⁗-H_3), 1.69 (3H, d, J=6.2 Hz, Rha-6″-H_3), 5.67 (1H, br s, Rha-1⁗-H), 5.69 (1H, br s, Rha-1‴-H), 5.76 (1H, br s, Rha-1″-H), 5.86 (1H, br s, Rha-1⁗-H)] together with an acetyl group [δ 2.08 (3H, s)]. The ^{1}H- and ^{13}C-NMR spectra were superimposable on those of mukurozioside IIa[11] (**7**), except for the signals due to the acetyl group. Treatment of **1** with 0.5% sodium methoxide (NaOMe)–MeOH provided **7**. The position of the acetyl group in **1** was clarified on the basis of the heteronuclear multiple bond connectivity (HMBC) experiment, which showed long-range correlation between 6′-position in the glucopyranosyl moiety [δ 4.76 (2H, m, Glc-6′-H_2)] and the acetyl carbonyl carbon (δ_C 170.7). Comparison of the ^{13}C-NMR data for **1** with those for **7** (pyridine-d_5, Table 2) revealed characteristic acetylation shifts at 5′- and 6′-positions in the glucopyranosyl moiety [**1**: δ_C 75.8 (Glc-5′), 65.8 (Glc-6′); **7**: δ_C 78.1 (Glc-5′), 62.4 (Glc-6′)]. On the basis of the above-mentioned evidence, the structure of **1** was determined to be 6′-*O*-acetylmukurozioside IIa.

Compound **2** was isolated as a white powder with negative optical rotation ($[\alpha]_D^{27}$ −49.7 in MeOH). By high-resolution positive-ion FAB-MS measurement, the molecular formula of **2** was found to be the same as that of **1**. The ^{1}H- and ^{13}C-NMR data of **2** (pyridine-d_5, Tables 1, 2) were very similar to those of **1** except for the signals due to the 1-*O*-carbohydrate moiety {a β-D-glucopyranosyl [δ 4.68 (1H, d, J=7.8 Hz, Glc-1′-H)] and two α-L-rhamnopyranosyl moieties [δ 1.44 (3H, d, J=6.2 Hz, Rha-6″-H_3), 1.62 (3H, d, J=6.3 Hz, Rha-6‴-H_3), 5.67 (1H, br s, Rha-1‴-H), 5.89 (1H, br s, Rha-1″-H)]} and an acetyl group [δ 1.99 (3H, s)]}. Treatment of **2** with 0.5% NaOMe–MeOH provided **7**. The position of the acetyl group in **2** was elucidated by the HMBC experiment and by consideration of the acetylation shift. Thus, a long-range correlation in the HMBC experiment of **2** was observed between 4″-position in the rhamnopyranosyl moiety [δ 5.42 (1H, dd, J=9.2, 9.4 Hz, Rha-4″-H)] and the acetyl carbonyl carbon (δ_C 170.7). The acetylation shift was detected around the 4″-position in the rhamnopyranosyl moiety [**2**: δ_C 70.9 (Rha-3″), 75.9 (Rha-4″), 67.0 (Rha-5″); **7**: δ_C 72.2 (Rha-3″), 72.8 (Rha-4″), 69.9 (Rha-5″)]. On the basis of the above-mentioned evidence, the structure of **2** was determined to be 4″-*O*-acetylmukurozioside IIa.

Compounds **3** and **4**, $C_{53}H_{90}O_{29}$, were also obtained as white powders with negative optical rotations (**3**: $[\alpha]_D^{26}$ −44.5; **4**: $[\alpha]_D^{25}$ −43.7 both in MeOH). By treatment of **3** and **4** with 0.5% NaO–MeOH, the desacyl derivative (**7**) was obtained. The ^{1}H- and ^{13}C-NMR spectra of **3** and **4** (pyridine-d_5, Tables 1, 2) resembled those of **7** except for the signals due to their 12-*O*-carbohydrate moieties {a β-D-glucopyranosyl [δ **3**: 4.63 (1H, d, J=7.7 Hz, Glc-1⁗-H); **4**: 4.65 (1H, d, J=7.7 Hz, Glc-1⁗-H)] and two α-L-rhamnopyranosyl moieties [δ **3**: 1.62 (3H, d, J=6.2 Hz, Rha-6⁗-H_3), 1.68 (3H, d, J=6.2 Hz, Rha-6⁗-H_3), 5.66 (1H, brs, Rha-1⁗-H), 5.84 (1H, br s, Rha-1⁗-H); **4**: 1.44 (3H, d, J=6.2 Hz, Rha-6⁗-H_3), 1.63 (3H, d, J=6.2 Hz, Rha-6⁗-H_3), 5.66 (1H, br s, Rha-1⁗-H), 5.86 (1H, brs, Rha-1⁗-H)], and an acetyl group [δ **3**: 1.98 (3H, s): **4**: 2.08 (3H, s)]}. The connectivities of the

Table 3. Inhibitory Effects of Acyclic Sesquiterpene Oligoglycosides (**1**—**8**) on TNF-α-Induced Cytotoxicity in L929 Cells

	Inhibition (%)				
	0 μM	3 μM	10 μM	30 μM	100 μM
1	0.0±1.6	3.3±2.2	−1.4±1.3	6.2±5.4	42.0±3.0**
2	0.0±1.6	1.2±1.3	−0.1±2.0	8.6±0.5**	24.6±1.2**
3	0.0±3.9	2.8±1.3	5.2±2.9	8.4±3.6	10.5±2.6*
4	0.0±1.7	0.4±3.3	3.1±3.3	10.3±0.3**	39.7±1.5**
Mukurozioside Ia (**5**)	0.0±1.6	3.8±2.7	2.7±1.2	0.0±3.0	−0.5±1.8
Mukurozioside Ib (**6**)	0.0±0.9	2.4±3.0	2.0±3.1	1.5±2.3	7.0±2.6
Mukurozioside IIa (**7**)	0.0±1.3	−2.5±0.9	−3.6±1.5	−4.7±0.6	−6.5±1.8
Mukurozioside IIb (**8**)	0.0±2.6	1.4±2.2	−9.6±3.3	−3.5±1.7	−11.4±1.6
Piperine[16,17,20)]	0.0±1.3	5.5±1.6*	5.3±1.4*	10.6±0.9**	41.8±1.4**
Silybin[a,16,17,20)]	0.0±2.6	5.3±2.8	22.0±3.8**	48.0±4.1**	50.8±3.9**

Each value represents the mean±S.E.M. (n=4). Significantly different from the control, $*p<0.05$, $**p<0.01$. *a*) Commercial silybin was purchased from Funakoshi Co., Ltd. (Tokyo, Japan).

acetyl group in **3** and **4** were clarified by the HMBC experiments, which showed long-range correlations between the following proton and carbon pairs {**3**: 6‴-position in the glucopyranosyl moiety [δ 4.62 (1H, dd, J=5.5, 11.3 Hz), 4.85 (1H, br d, J=11.3 Hz), Glc-6‴-H_2]} and the acetyl carbonyl carbon (δ_C 170.7); **4**: 4⁗-position in the rhamnopyranosyl moiety [δ 5.69 (1H, dd, J=9.2, 9.5 Hz, Rha-4⁗-H)] and the acetyl carbonyl carbon (δ_C 170.7)}, respectively. Consequently, the structures of **3** and **4** were determined to be 6‴-*O*-acetyl and 4⁗-*O*-acetylmukuroziosides IIa, respectively.

Effects of the Constituents on TNF-α-Induced Cytotoxicity in L929 Cells TNF-α is known to mediate a variety of organ injury through its induction of cellular apoptosis. In the case of liver, biological effects of TNF-α have been implicated in hepatic injury induced by hepatic toxins, ischemia/reperfusion, viral hepatitis, and alcohol.[12—14)] Therefore, TNF-α is considered to be an important marker to develop anti-inflammatory and hepatoprotective agents. On the basis of above-mentioned concept, we have been exploring such constituents as have reducing effect on TNF-α-induced cell death in L929 cells, a TNF-α-sensitive cell line.[15)] Previously, we have reported that several constituents from *Piper chaba*,[2,7—9)] *Boesenbergia rotunda*,[3,4)] *Punica granatum*,[16)] *Helichrysum arenarium*,[17—19)] and *Cistanche tubulosa*[20—22)] were found to show inhibitory effects on TNF-α-induced cytotoxicity in L929 cells. Inhibitory effects of the newly isolated compounds (**1**—**4**) on TNF-α-induced cell death in L929 cells were also examined. As shown in Table 3, acylated acyclic sesquiterpene oligoglycosides such as **1** (inhibition: 42.0±3.0% at 100 μM), **2** (24.6±1.2%), **3** (10.5±2.6%), and **4** (39.7±1.5%) were found to show significant activity. However, their desacyl compound, mukurozioside IIa (**7**), and related constituents [mukuroziosides Ia (**5**), Ib (**6**), and IIb (**8**)] showed no activity. Thus, the acetyl moiety was found essential for these acyclic sesquiterpene oligoglycosides to exhibit their activity. Although a large number of compounds, which inhibit cell death caused by production of TNF-α, have been known,[23,24)] not many compounds have been reported to reduce selectively the sensitivity of L929 cells to TNF-α. The compounds (**1**—**4**) are rare examples bearing such activity.

Experimental

The following instruments were used to obtain spectral and physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-ECA600 (600 MHz) and JNM-ECS400 (400 MHz) spectrometers; ^{13}C-NMR spectra, JEOL JNM-ECA600 (150 MHz) and JNM-ECS400 (100 MHz) spectrometer with tetramethylsilane as an internal standard; FAB-MS and HR-FAB-MS, JEOL JMS-SX 102A mass spectrometers; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV–VIS detectors; HPLC column, Cosmosil 5C_{18}-MS-II and HILIC (Nacalai Tesque, Inc.), Wakopak Navi C-30-5 (Wako Pure Chemical Industries Ltd.) (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography, silica gel 60N (Kanto Chemical Co., Ltd., 63—210 mesh, spherical, neutral); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, pre-coated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was carried out spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Plant Material This item was described in a previous report.[5,6)]

Extraction and Isolation The dried pericarps of *S. rarak* (452 g) were extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (305.0 g, 67.5%).[5,6)] The methanolic extract (232.0 g) was subjected to Diaion HP-20 column chromatography (2.0 kg, H_2O→MeOH, twice) to give H_2O-eluted fraction (92.0 g, 26.8%) and MeOH-eluted fraction (140.0 g, 40.7%). The MeOH-eluted fraction (123.0 g) was subjected to normal-phase silica gel column chromatography [3.7 kg, $CHCl_3$–MeOH–H_2O (20 : 3 : 1→10 : 3 : 1→7 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give 11 fractions [Fr. 1 (1.11 g), Fr. 2 (3.88 g), Fr. 3 (1.27 g), Fr. 4 (1.95 g), Fr. 5 (0.67 g), Fr. 6 (8.04 g), Fr. 7 (4.11 g), Fr. 8 (31.71 g), Fr. 9 (28.44 g), Fr. 10 (39.55 g), and Fr. 11 (2.24 g)] as reported previously.[5,6)] Fraction 9 (688.0 mg) was subjected to HPLC [Wakopak Navi C30-5, CH_3CN–H_2O (30 : 70, v/v)] to furnish 16 fractions {Fr. 9-1 (161.0 mg), Fr. 9-2 (5.0 mg), Fr. 9-3 (9.7 mg), Fr. 9-4 [=mukurozioside IIa (**7**, 23.7 mg, 0.50%)], Fr. 9-5 (20.3 mg), Fr. 9-6 (89.0 mg), Fr. 9-7 (9.6 mg), Fr. 9-8 [=**1** (18.7 mg, 0.39%)], Fr. 9-9 (15.3 mg), Fr. 9-10 (16.3 mg), Fr. 9-11 (18.6 mg), Fr. 9-12 [=**2** (14.9 mg, 0.31%)], Fr. 9-13 (9.2 mg), Fr. 9-14 (20.3 mg), Fr. 9-15 (7.9 mg), and Fr. 9-16 (98.7 mg)}. Fraction 9-9 (15.3 mg) was separated by HPLC [Cosmosil HILIC, CH_3CN–H_2O (85 : 15, v/v)] to give **4** (5.3 mg, 0.11%). Fraction 9-11 (18.6 mg) was purified by HPLC [Cosmosil HILIC, CH_3CN–H_2O (85 : 15, v/v)] to give **3** (9.1 mg, 0.17%).

1: A white powder, $[\alpha]_D^{27}$ −51.6 (c=0.67, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{90}O_{29}Na$ $(M+Na)^+$ 1213.5465; Found 1213.5471. IR (KBr, cm^{-1}): 3568, 1734, 1044. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1. ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS m/z: 1213 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1189 $(M-H)^-$, 1043 $(M-C_6H_{11}O_4)^-$, 1001 $(M-C_8H_{14}O_5)^-$, 735 $(M-C_{18}H_{31}O_{13})^-$, 693 $(M-C_{20}H_{33}O_{14})^-$.

2: A white powder, $[\alpha]_D^{27}$ −49.7 (c=0.91, MeOH). High-resolution posi-

tive-ion FAB-MS: Calcd for $C_{53}H_{90}O_{29}Na$ $(M+Na)^+$ 1213.5465; Found 1213.5458. IR (KBr, cm^{-1}): 3568, 1749, 1044. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1. ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 1213 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 1189 $(M-H)^-$, 1043 $(M-C_6H_{11}O_4)^-$, 693 $(M-C_{20}H_{33}O_{14})^-$.

3: A white powder, $[\alpha]_D^{27}$ −44.5 (c=0.61, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{90}O_{29}Na$ $(M+Na)^+$ 1213.5465; Found 1213.5460. IR (KBr, cm^{-1}): 3568, 1749, 1043. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1. ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 1213 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 1189 $(M-H)^-$, 1043 $(M-C_6H_{11}O_4)^-$, 735 $(M-C_{18}H_{31}O_{13})^-$, 693 $(M-C_{20}H_{33}O_{14})^-$.

4: A white powder, $[\alpha]_D^{25}$ −43.7 (c=0.16, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{90}O_{29}Na$ $(M+Na)^+$ 1213.5465; Found 1213.5471. IR (KBr, cm^{-1}): 3551, 1741, 1044. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1. ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 1213 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 1189 $(M-H)^-$, 1043 $(M-C_6H_{11}O_4)^-$, 1001 $(M-C_8H_{14}O_5)^-$, 735 $(M-C_{18}H_{31}O_{13})^-$, 693 $(M-C_{20}H_{33}O_{14})^-$.

Deacylation of 1—4 Solutions of **1**—**4** (5.0, 5.0, 3.0, 3.0 mg) in 0.5% sodium methoxide (NaOMe)–MeOH (1.0 ml) were stirred at room temperature for 3 h. Each solution was neutralized with Dowex HCR W2 (H^+ form), and the resins were removed by filtration. Condensation of the filtrates under reduced pressure yielded the corresponding deacylated products, which were purified by HPLC [column: Cosmosil HILIC, CH_3CN–H_2O (85 : 15, v/v)] to give mukurozioside IIa (**7**, 4.5 mg from **1**, 4.1 mg from **2**, 2.3 mg from **3**, and 2.0 mg from **4**).

Bioassay Method. Inhibitory Effect on TNF-α-Induced Cytotoxicity in L929 Cells Inhibitory effect on TNF-α-induced cell death in L929 cells was assayed by the method described in our previous report.[16,17] Briefly, a suspension of 1×10^4 cells [obtained from Dainippon Pharmaceutical (Osaka, Japan)] in 100 μl of minimum essential medium Eagle supplemented with 1% non-essential amino acid solution (Invitrogen), fetal bovine serum (FBS, 10%), penicillin G (100 units/ml), and streptomycin (100 μg/ml) was incubated in a 96-well microplate. After 44 h of incubation in the medium containing TNF-α (1 ng/ml) with or without a test sample, the viability of the cells was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Thus, the medium was exchanged with 100 μl of the fresh medium, and 10 μl of MTT [5 mg/ml in phosphate buffered saline (PBS)] solution was added to the medium. After 4 h of cultivation, the medium was removed, and 100 μl of isopropanol containing 0.04 M HCl was then added to dissolve the formazan produced in the cells. The optical density (OD) of the formazan solution was measured by microplate reader at 570 nm (reference: 655 nm). Inhibition (%) was obtained by following formula. Each test compound was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to the medium (final concentration in DMSO 0.5%).

$$\text{inhibition (\%)}=[(\text{OD (sample)}-\text{OD (control)})/(\text{OD (normal)}-\text{OD (control)})]\times100$$

normal: TNF-α (−), sample (−); control: TNF-α (+), sample (−); sample: TNF-α (+), sample (+).

Statistics Values were expressed as means±S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis. Probability (p) values less than 0.05 were considered significant.

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