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 μ M.

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 (H₂O→MeOH) to give H₂O- 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).

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 C₅₃H₉₀O₂₉ by high-resolution positive-ion FAB-MS measurement. The ¹H- and ¹³C-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-H₃), 1.61, 1.63 (3H each, both s, 14, 13-H₃)], two methylenes

- 1: R¹ = Ac, R² = R³ = R⁴ = H
 2: R¹ = R³ = R⁴ = H, R² = Ac
 3: R¹ = R² = R⁴ = H, R³ = Ac
 4: R¹ = R² = R³ = H, R⁴ = Ac

Mukurozioside IIa (**7**): R¹ = R² = R³ = R⁴ = H

Mukurozioside Ia (**5**)

Mukurozioside Ib (**6**): R = H
 Mukurozioside IIb (**8**): R = Rha

Chart 1.

bearing an oxygen function [δ 3.41, 3.91 (1H each, both m, 12-H₂), 4.31, 4.58 (1H each, both m, 1-H₂)], 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. ^1H -NMR Data (600 MHz, Pyridine- d_5) for **1**–**4** and **7**

Position	1	2	3	4	7
1	4.31 (m) 4.58 (m)	4.27 (m) 4.56 (m)	4.29 (m) 4.57 (dd, 6.5, 11.5)	4.30 (m) 4.58 (dd, 6.6, 11.3)	4.32 (m) 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) 2.22 (m)	2.09 (m) 2.18 (m)	2.08 (m) 2.18 (m)	2.10 (m) 2.21 (dd, 7.2, 15.1)	2.08 (m) 2.20 (m)
5	1.98 (m) 2.11 (m)	1.97 (m) 2.10 (m)	1.97 (m) 2.11 (m)	2.00 (m) 2.10 (m)	1.99 (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) 2.24 (m)	1.91 (m) 2.24 (m)	1.98 (m) 2.20 (m)	1.90 (m) 2.21 (m)	1.92 (m) 2.21 (m)
9	1.41 (m) 1.57 (m)	1.39 (m) 1.61 (m)	1.39 (m) 1.57 (m)	1.41 (m) 1.53 (m)	1.43 (m) 1.59 (m)
10	1.13 (m) 1.51 (m)	1.12 (m) 1.49 (m)	1.14 (m) 1.57 (m)	1.14 (m) 1.51 (m)	1.11 (m) 1.51 (m)
11	1.96 (m)	1.97 (m)	1.95 (m)	1.92 (m)	1.92 (m)
12	3.41 (m) 3.91 (m)	3.35 (m) 3.87 (m)	3.41 (dd, 6.7, 9.4) 3.89 (dd, 6.7, 9.4)	3.39 (dd, 6.8, 9.5) 3.94 (dd, 6.8, 9.5)	3.36 (dd, 6.9, 9.2) 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) 4.44 (m)	4.29 (m) 4.42 (m)	4.31 (m) 4.44 (m)	4.33 (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) 4.41 (m)	4.25 (m) 4.41 (m)	4.62 (dd, 5.0, 11.3) 4.85 (br d, 11.3)	4.27 (m) 4.44 (m)	4.27 (dd, 5.0, 11.8) 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'''-H₃), 1.63 (3H, d, $J=6.3$ Hz, Rha-6''''-H₃), 1.67 (3H, d, $J=6.3$ Hz, Rha-6''''-H₃), 1.69 (3H, d, $J=6.2$ Hz, Rha-6''-H₃), 5.67 (1H, brs, Rha-1''''-H), 5.69 (1H, brs, Rha-1'''-H), 5.76 (1H, brs, Rha-1''-H), 5.86 (1H, brs, Rha-1'-H)] together with an acetyl group [δ 2.08 (3H, s)]. The ^1H - and ^{13}C -NMR spectra were superimposable on those of mukurozioside IIa¹¹⁾ (**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'-H₂)] 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 ($[\alpha]_{\text{D}}^{27}$ –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 ^1H - 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''-H₃), 1.62 (3H, d, $J=6.3$ Hz, Rha-

6'''-H₃), 5.67 (1H, brs, Rha-1'''-H), 5.89 (1H, brs, 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**, C₅₃H₉₀O₂₉, were also obtained as white powders with negative optical rotations (**3**: $[\alpha]_{\text{D}}^{26}$ –44.5; **4**: $[\alpha]_{\text{D}}^{25}$ –43.7 both in MeOH). By treatment of **3** and **4** with 0.5% NaO–MeOH, the desacyl derivative (**7**) was obtained. The ^1H - 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''''-H₃), 1.68 (3H, d, $J=6.2$ Hz, Rha-6''''-H₃), 5.66 (1H, brs, Rha-1''''-H), 5.84 (1H, brs, Rha-1''''-H); **4**: 1.44 (3H, d, $J=6.2$ Hz, Rha-6''''-H₃), 1.63 (3H, d, $J=6.2$ Hz, Rha-6''''-H₃), 5.66 (1H, brs, 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 μ M	3 μ M	10 μ M	30 μ M	100 μ M
1	0.0 $\pm$ 1.6	3.3 $\pm$ 2.2	-1.4 $\pm$ 1.3	6.2 $\pm$ 5.4	42.0 $\pm$ 3.0**
2	0.0 $\pm$ 1.6	1.2 $\pm$ 1.3	-0.1 $\pm$ 2.0	8.6 $\pm$ 0.5**	24.6 $\pm$ 1.2**
3	0.0 $\pm$ 3.9	2.8 $\pm$ 1.3	5.2 $\pm$ 2.9	8.4 $\pm$ 3.6	10.5 $\pm$ 2.6*
4	0.0 $\pm$ 1.7	0.4 $\pm$ 3.3	3.1 $\pm$ 3.3	10.3 $\pm$ 0.3**	39.7 $\pm$ 1.5**
Mukurozioside Ia (5)	0.0 $\pm$ 1.6	3.8 $\pm$ 2.7	2.7 $\pm$ 1.2	0.0 $\pm$ 3.0	-0.5 $\pm$ 1.8
Mukurozioside Ib (6)	0.0 $\pm$ 0.9	2.4 $\pm$ 3.0	2.0 $\pm$ 3.1	1.5 $\pm$ 2.3	7.0 $\pm$ 2.6
Mukurozioside IIa (7)	0.0 $\pm$ 1.3	-2.5 $\pm$ 0.9	-3.6 $\pm$ 1.5	-4.7 $\pm$ 0.6	-6.5 $\pm$ 1.8
Mukurozioside IIb (8)	0.0 $\pm$ 2.6	1.4 $\pm$ 2.2	-9.6 $\pm$ 3.3	-3.5 $\pm$ 1.7	-11.4 $\pm$ 1.6
Piperine ^{16,17,20}	0.0 $\pm$ 1.3	5.5 $\pm$ 1.6*	5.3 $\pm$ 1.4*	10.6 $\pm$ 0.9**	41.8 $\pm$ 1.4**
Silybin ^{a,16,17,20}	0.0 $\pm$ 2.6	5.3 $\pm$ 2.8	22.0 $\pm$ 3.8**	48.0 $\pm$ 4.1**	50.8 $\pm$ 3.9**

Each value represents the mean $\pm$ 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'''-H₂]} 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 $\pm$ 3.0% at 100 μ M), **2** (24.6 $\pm$ 1.2%), **3** (10.5 $\pm$ 2.6%), and **4** (39.7 $\pm$ 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; ¹H-NMR spectra, JEOL JNM-ECA600 (600 MHz) and JNM-ECS400 (400 MHz) spectrometers; ¹³C-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 5C₁₈-MS-II and HILIC (Nacalai Tesque, Inc.), Wakopak Navi C-30-5 (Wako Pure Chemical Industries Ltd.) (250 $\times$ 4.6 mm i.d.) and (250 $\times$ 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 60F₂₅₄ (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(SO₄)₂–10% aqueous H₂SO₄, 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, H₂O $\rightarrow$ MeOH, twice) to give H₂O-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, CHCl₃–MeOH–H₂O (20:3:1 $\rightarrow$ 10:3:1 $\rightarrow$ 7:3:1, lower layer $\rightarrow$ 6:4:1, v/v/v) $\rightarrow$ 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, CH₃CN–H₂O (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, CH₃CN–H₂O (85:15, v/v)] to give **4** (5.3 mg, 0.11%). Fraction 9-11 (18.6 mg) was purified by HPLC [Cosmosil HILIC, CH₃CN–H₂O (85:15, v/v)] to give **3** (9.1 mg, 0.17%).

1: A white powder, [α]_D²⁷ -51.6 ($c=0.67$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₃₃H₅₀O₂₉Na (M+Na)⁺ 1213.5465; Found 1213.5471. IR (KBr, cm⁻¹): 3568, 1734, 1044. ¹H-NMR (600 MHz, pyridine-*d*₅) δ : given in Table 1. ¹³C-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-C₆H₁₁O₄)⁻, 1001 (M-C₈H₁₄O₅)⁻, 735 (M-C₁₈H₃₁O₁₃)⁻, 693 (M-C₂₀H₃₃O₁₄)⁻.

2: A white powder, [α]_D²⁷ -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. ¹H-NMR (600 MHz, pyridine-*d*₅) δ : given in Table 1. ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 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, $[\alpha]_D^{25} -44.5$ ($c=0.61$, 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. ¹H-NMR (600 MHz, pyridine-*d*₅) δ : given in Table 1. ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 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. ¹H-NMR (600 MHz, pyridine-*d*₅) δ : given in Table 1. ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 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 ($M-C_8H_{14}O_3$)⁻, 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, CH₃CN–H₂O (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 μ l 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 μ g/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 μ l of the fresh medium, and 10 μ l 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 μ l 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%).

$$\text{inhibition (\%)} = \frac{[(OD(\text{sample}) - OD(\text{control})) / (OD(\text{normal}) - OD(\text{control}))] \times 100}$$

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

Statistics Values were expressed as means $\pm$ 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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