# Structures of Acetylated Oleanane-Type Triterpene Saponins, Rarasaponins IV, V, and VI, and Anti-hyperlipidemic Constituents from the Pericarps of *Sapindus rarak*

Yasunobu Asao,[a] Toshio Morikawa,[b] Yuanyuan Xie,[b,c] Masaki Okamoto,[b] Makoto Hamao,[a] Hisashi Matsuda,[a] Osamu Muraoka,[b] Dan Yuan,[c] and Masayuki Yoshikawa*[,a,b]

[a] *Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan:* [b] *Pharmaceutical Research and Technology Institute, Kinki University; 3–4–1 Kowakae, Higashi-Osaka, Osaka 577–8502, Japan: and* [c] *School of Traditional Medicine, Shenyang Pharmaceutical University; Wenhua Rd., Shenyang 110016, People's Republic of China.*
Received August 28, 2008; accepted November 25, 2008; published online November 26, 2008

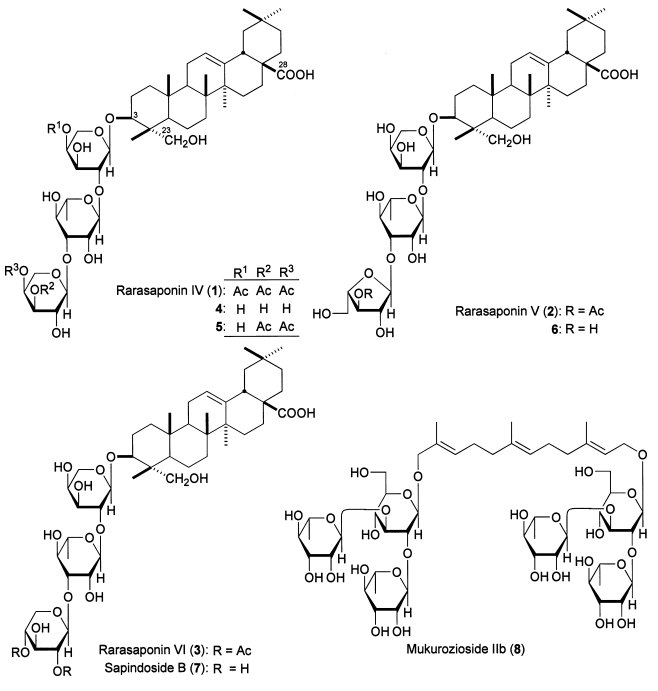
**The methanolic extract and its saponin fraction (methanol-eluted fraction) of the pericarps of *Sapindus rarak* DC. were found to suppress plasma triglyceride elevation in olive oil-treated mice. From the active fraction, three new acylated oleanane-type triterpene saponins, rarasaponins IV (1), V (2), and VI (3), were isolated. The structures of 1—3 were elucidated on the basis of chemical and spectroscopic evidence. The principle saponin constituents, hederagenin 3-*O*-α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (4) and hederagenin 3-*O*-(3,4-di-*O*-acetyl-α-L-arabinopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (5), showed inhibitory effects on plasma triglyceride elevation at a dose of 200 mg/kg, *per os*.**

**Key words** *Sapindus rarak*; anti-hyperlipidemic activity; triterpene oligoglycoside; rarasaponin; Sapindaceae

During the course of characterization studies on Thai natural medicines,[1—19] we found that a methanol extract from the pericarps of *Sapindus rarak* DC. (Sapindaceae) showed an inhibitory effect on pancreatic lipase activity.[1] Through bioassay-guided separation, four oleanane-type triterpene saponins named rarasaponins I—III and raraoside A were isolated from the active fraction together with 13 saponins and four acyclic sesquiterpene glycosides.[1] As a continuing study on the biological active constituents from *S. rarak*, the methanolic extract and its saponin fraction were found to inhibit plasma triglyceride (TG) elevation in olive-oil treated mice. From the saponin fraction, we isolated three new acetylated oleanane-type triterpene oligoglycosides called rarasaponins IV (**1**), V (**2**), and VI (**3**). This paper deals with the structure elucidation of these three new saponins (**1**—**3**) as well as the anti-hyperlipidemic activities of the major saponin constituents of this Thai medicine, hederagenin 3-*O*-α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**4**)[1,20] and hederagenin 3-*O*-(3,4-di-*O*-acetyl-α-L-arabinopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**5**).[1,20,21]

The pericarps of *S. rarak* collected in Thailand were extracted with methanol to give a methanolic extract (67.5% from the dried pericarps).[1] As shown in Table 1, the methanolic extract significantly suppressed plasma TG elevation 2 h after administration of olive oil at a dose of 250 mg/kg, *per os* (*p.o.*). The methanolic extract was subjected to Diaion HP-20 column chromatography ($H_2O$→MeOH) to give $H_2O$- and MeOH-eluted fractions (25.6% and 40.7%), respectively. The MeOH-eluted fraction significantly suppressed plasma TG elevation 2 h after administration of olive oil at a dose of 125 mg/kg, *p.o.* This fraction was subjected to normal- and reversed-phase column chromatographies, and finally HPLC to give rarasaponins IV (**1**, 0.02%), V (**2**, 0.20%), and VI (**3**, 0.12%).

**Structures of Rarasaponins IV (1), V (2), and VI (3)** Rarasaponin IV (**1**) was obtained as an amorphous powder with positive optical rotation ($[\alpha]_D^{27}$ +25.6° in MeOH). The IR spectrum of **1** showed absorption bands at 1736 and 1655 $cm^{-1}$ ascribable to ester carbonyl and olefin functions, and broad bands at 3488, 1086, and 1053 $cm^{-1}$, suggestive of an oligoglycoside structure. In the positive- and negative-ion fast atom bombardment (FAB)-MS of **1**, quasimolecular ion peaks were observed at *m/z* 1031 $(M+Na)^+$ and 1007 $(M-H)^-$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **1** to be $C_{52}H_{80}O_{19}$. The $^1$H- (Table 2) and $^{13}$C-NMR (Table 3) spectra (pyridine-$d_5$) of **1**, which were assigned by various NMR experiments,[22] showed signals assignable to six methyls [$\delta$ 0.93, 0.95, 1.01, 1.04, 1.15, 1.26 (3H each, all s, 29, 25, 30, 26, 24, 27-$H_3$)], a methylene and a methine bearing an oxygen function [$\delta$ 3.92, 4.27 (1H each, both d, $J$=10.8 Hz, 23-$H_2$), 4.27 (1H,




* To whom correspondence should be addressed. e-mail: myoshika@mb.kyoto-phu.ac.jp

Table 1. Inhibitory Effects of the MeOH Extract and its MeOH- and $H_2O$-Eluted Fractions from the Pericarps of *Sapindus rarak* on Plasma Triglyceride Elevation in Olive Oil-Treated Mice

| Treatment | Dose (mg/kg, *p.o.*) | *n* | Plasma triglyceride (mg/dl)[a] | | |
|---|---|---|---|---|---|
| | | | 2.0 h | 4.0 h | 6.0 h |
| Normal | — | 7 | 163.7±10.0** | 153.8±12.3** | 123.5±10.5** |
| Control | — | 7 | 588.2±53.5 | 705.8±89.6 | 445.5±106.0 |
| MeOH ext. | 250 | 7 | 316.7±64.9** | 449.3±88.1** | 375.0±62.4 |
| | 500 | 6 | 194.1±14.9** | 208.9±50.6** | 252.0±46.7 |
| Normal | — | 7 | 174.9±12.6** | 130.2±14.8** | 126.8±10.3** |
| Control | — | 9 | 680.3±77.5 | 580.3±56.5 | 480.8±51.4 |
| MeOH-eluted fraction | 125 | 7 | 345.9±50.8** | 360.5±68.0* | 513.2±92.3 |
| | 250 | 6 | 279.9±40.5** | 410.4±84.3 | 456.0±70.4 |
| | 500 | 7 | 207.0±25.7** | 203.8±20.4** | 260.7±111.6 |
| $H_2O$-eluted fraction | 500 | 7 | 871.4±75.3 | 786.3±56.3 | 484.7±53.2 |

*a*) Values represent the means±S.E.M. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

dd, $J$=4.8, 12.1 Hz, 3-H)], an olefin [$\delta$ 5.47 (1H, dd, $J$=3.6, 4.4 Hz, 12-H)], two arabinopyranosyl moieties [$\delta$ 5.02 (1H, d, $J$=7.0 Hz, *inner*-Ara(p)-1-H), 5.42 (1H, d, $J$=7.4 Hz, *terminal*-Ara(p)-1-H)], and a rhamnopyranosyl moiety [$\delta$ 1.64 (3H, d, $J$=6.2 Hz, Rha-6-$H_3$), 6.31 (1H, br s, Rha-1-H)] together with three acetyl groups [$\delta$ 1.87, 1.96, 2.05 (3H each, all s, Ac-$H_3$)]. Treatment of **1** with 0.5% sodium methoxide (NaOMe)–MeOH provided hederagenin 3-*O*-$\alpha$-L-arabinopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**4**).[1,20] The positions of the acetyl groups in **1** were clarified on the basis of a heteronuclear multiple bond connectivity (HMBC) experiment, which showed long-range correlations between the 4-proton in the *inner*-arabinopyranosyl moiety [$\delta$ 5.36 (1H, br s)] and the acetyl carbonyl carbon ($\delta_C$ 170.5), between the 3-proton in the *terminal*-arabinopyranosyl moiety [$\delta$ 5.49 (1H, dd, $J$=3.8, 9.6 Hz)] and the acetyl carbonyl carbon ($\delta_C$ 170.8), and between the 4-proton in the *terminal*-arabinopyranosyl moiety [$\delta$ 5.58 (1H, br s)] and the acetyl carbonyl carbon ($\delta_C$ 170.4). Comparison of the $^{13}$C-NMR data for **1** with those for hederagenin 3-*O*-(3,4-di-*O*-acetyl-$\alpha$-L-arabinopyranosyl)-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**5**),[1,20,21] which is one of the major saponin constituent from the pericarps of *S. rarak*, revealed acetylation shifts around the 4-position in the *inner*-arabinopyranosyl moiety [**1**: $\delta_C$ 73.3 (*inner*-Ara(p)-C-3), 72.9 (*inner*-Ara(p)-C-4), 63.8 (*inner*-Ara(p)-C-5); **5**: $\delta_C$ 74.9 (*inner*-Ara(p)-C-3), 69.6 (*inner*-Ara(p)-C-4), 66.0 (*inner*-Ara(p)-C-5)].[20] On the basis of the above-mentioned evidence, the structure of rarasaponin IV was determined to be hederagenin 3-*O*-(3,4-di-*O*-acetyl-$\alpha$-L-arabinopyranosyl)-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-4-*O*-acetyl-$\alpha$-L-arabinopyranoside (**1**).

Rarasaponin V (**2**) was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{29}$ −8.3° in MeOH). The IR spectrum of **2** showed absorption bands at 3569, 1734, 1717, 1656, and 1053 cm$^{-1}$, ascribable to hydroxyl, ester carbonyl, carboxyl, olefin, and ether functions. The molecular formula of **2**, $C_{48}H_{76}O_{17}$, was determined from the positive- and negative-ion FAB-MS [*m/z* 947 $(M+Na)^+$ and 923 $(M-H)^-$] and by high-resolution positive-ion FAB-MS measurement. The $^1$H- (Table 2) and $^{13}$C-NMR (Table 3) spectra[22] (pyridine-$d_5$) of **2** indicated the presence of six methyls [$\delta$ 0.93, 0.95, 1.00, 1.02, 1.07, 1.24 (3H each, all s, 29, 25, 30, 26, 24, 27-$H_3$)], a methylene and a methine bearing an oxygen function [$\delta$ 3.86, 4.18 (1H each, both d, $J$=10.9 Hz, 23-$H_2$), 4.21 (1H, dd, $J$=4.9, 12.1 Hz, 3-H)], an olefin [$\delta$ 5.46 (1H, br s, 12-H)], and an arabinopyranosyl [$\delta$ 5.03 (1H, d, $J$=7.2 Hz, Ara(p)-1-H)], an arabinofuranosyl [$\delta$ 6.13 (1H, br s, Ara(f)-1-H)], and a rhamnopyranosyl moieties [$\delta$ 1.57 (3H, d, $J$=6.3 Hz, Rha-6-$H_3$), 6.18 (1H, br s, Rha-1-H)] together with an acetyl group [$\delta$ 1.93 (3H, s, Ac-$H_3$)]. In addition, treatment of **2** with 0.5% NaOMe–MeOH gave hederagenin 3-*O*-$\alpha$-L-arabinofuranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**6**).[20] The $^1$H- and $^{13}$C-NMR data of **2** were superimposable on those of **6**, except for the signals due to the $\alpha$-L-arabinopyranosyl moiety. In the HMBC experiment on **2**, a long-range correlation was observed between the 3-position of the $\alpha$-L-arabinopyranosyl moiety [$\delta$ 5.57 (1H, br d, $J$=*ca.* 4 Hz)] and the acetyl carbonyl carbon ($\delta_C$ 170.6), so that the position of acetyl group was elucidated. On the basis of above-mentioned evidence, the structure of rarasaponin V was determined to be hederagenin 3-*O*-(3-*O*-acetyl-$\alpha$-L-arabinofuranosyl)-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**2**).[23]

Rarasaponin VI (**3**), $[\alpha]_D^{26}$ +1.9° (MeOH), was also obtained as an amorphous powder. The positive- and negative-ion FAB-MS of **3** showed quasimolecular ion peaks at *m/z* 989 $(M+Na)^+$ and *m/z* 965 $(M-H)^-$, respectively. The high-resolution positive-ion FAB-MS of **3** revealed the molecular formula to be $C_{50}H_{78}O_{18}$. The proton and carbon signals in the $^1$H- (Table 2) and $^{13}$C-NMR (Table 3) spectra[22] (pyridine-$d_5$) of **3** indicated the presence of an aglycon part {six methyls [$\delta$ 0.93, 0.96, 1.01, 1.03, 1.08, 1.24 (3H each, all s, 29, 25, 30, 26, 24, 27-$H_3$)], a methylene and a methine bearing an oxygen function [$\delta$ 3.88, 4.20 (1H each, both d, $J$=10.7 Hz, 23-$H_2$), 4.23 (1H, m, 3-H)], and an olefin [$\delta$ 5.47 (1H, dd, $J$=3.1, 3.4 Hz, 12-H)]}, an arabinopyranosyl [$\delta$ 5.05 (1H, d, $J$=6.5 Hz, Ara(p)-1-H)], a xylopyranosyl [$\delta$ 5.37 (1H, d, $J$=7.4 Hz, Xyl-1)], and a rhamnopyranosyl moieties [$\delta$ 1.53 (3H, d, $J$=6.2 Hz, Rha-6-$H_3$), 6.18 (1H, d, $J$=1.4 Hz, Rha-1-H)] together with two ester carbonyl carbons ($\delta_C$ 170.3, 170.3) suggesting the presence of two acetyl groups [$\delta$ 1.91, 1.99 (3H each, both s, Ac-$H_3$)]. Treatment of **3** with 0.5% NaOMe–MeOH yielded sapindoside B (**7**).[20] In the HMBC experiments on **3**, long-range correlations were observed between the 2-proton in the xylopyranosyl part [$\delta$ 5.52 (1H, dd, $J$=7.4, 8.9 Hz)] and the acetyl carbonyl carbon ($\delta_C$ 170.3) and between the 4-proton in the xylopyranosyl

Table 2. $^{1}$H-NMR Data (600 MHz, Pyridine-$d_5$) of Rarasaponins IV—VI (**1**—**3**)

| Position | **1** | **2** | **3** |
|---|---|---|---|
| 1 | 1.08 br dd, *ca.* 4, 14 | 1.08 m | 1.08 m |
| | 1.58 m | 1.58 m | 1.57 m |
| 2 | 2.02 m | 1.98 m | 2.00 m |
| | 2.24 m | 2.20 m | 2.22 m |
| 3 | 4.27 dd, 4.8, 12.1 | 4.21 dd, 4.9, 12.1 | 4.23 m |
| 5 | 1.76 br d, *ca.* 8 | 1.70 m | 1.78 m |
| 6 | 1.38 dd, 2.2, 12.4 | 1.35 m | 1.38 m |
| | 1.78 m | 1.74 m | 1.78 m |
| 7 | 1.33 m | 1.30 m | 1.28 m |
| | 1.67 m | 1.64 m | 1.63 ddd, 3.8, 12.9, 16.3 |
| 9 | 1.81 m | 1.70 m | 1.78 m |
| 11 | 1.94 m (2H) | 1.80 m | 1.78 m |
| | | 1.95 m | 1.98 m |
| 12 | 5.47 dd, 3.6, 4.4 | 5.46 br s | 5.47 dd, 3.1, 3.4 |
| 15 | 1.13 m | 1.14 m | 1.13 br d, *ca.* 14 |
| | 2.15 m | 2.14 m | 2.15 ddd, 3.1, 13.2, 13.7 |
| 16 | 2.06 m (2H) | 2.06 m | 2.08 m |
| | | 2.14 m | 2.12 m |
| 18 | 3.28 dd, 4.3, 13.9 | 3.27 dd, 4.1, 13.2 | 3.28 dd, 4.1, 13.9 |
| 19 | 1.30 m | 1.28 m | 1.28 m |
| | 1.78 m | 1.80 m | 1.80 m |
| 21 | 1.20 br d, *ca.* 12 | 1.20 m | 1.20 m |
| | 1.43 m | 1.46 m | 1.43 m |
| 22 | 1.81 m | 1.80 m | 1.80 m |
| | 2.04 m | 2.06 m | 2.04 m |
| 23 | 3.92 d, 10.8 | 3.86 d, 10.9 | 3.88 d, 10.7 |
| | 4.27 d, 10.8 | 4.18 d, 10.9 | 4.20 d, 10.7 |
| 24 | 1.15 s | 1.07 s | 1.08 s |
| 25 | 0.95 s | 0.95 s | 0.96 s |
| 26 | 1.04 s | 1.02 s | 1.03 s |
| 27 | 1.26 s | 1.24 s | 1.24 s |
| 29 | 0.93 s | 0.93 s | 0.93 s |
| 30 | 1.01 s | 1.00 s | 1.01 s |
| C-3-sugar | | | |
| *inner*-Ara(p)-1′ | 5.02 d, 7.0 | 5.03 d, 7.2 | 5.05 d, 6.5 |
| 2′ | 4.51 dd, 7.0, 8.8 | 4.50 dd, 7.2, 7.2 | 4.51 dd, 6.5, 7.7 |
| 3′ | 4.10 dd, 3.8, 8.8 | 4.61 dd, 3.8, 8.1 | 4.05 dd, 3.6, 8.1 |
| 4′ | 5.36 br s | 4.11 m | 4.15 m |
| 5′ | 3.66 dd, 1.6, 11.9 | 3.65 dd, 1.7, 11.7 | 3.67 dd, 2.0, 12.0 |
| | 4.18 dd, 2.7, 11.9 | 4.22 dd, 8.6, 11.7 | 4.25 m |
| Rha-1″ | 6.31 br s | 6.18 br s | 6.18 d, 1.4 |
| 2″ | 4.88 m | 4.84 br s | 4.81 dd, 1.4, 3.2 |
| 3″ | 4.78 m | 4.68 dd, 2.9, 9.8 | 4.62 dd, 3.2, 9.5 |
| 4″ | 4.44 dd, 9.3, 9.8 | 4.30 dd, 9.5, 9.8 | 4.30 dd, 9.5, 9.6 |
| 5″ | 4.75 m | 4.66 m | 4.60 m |
| 6″ | 1.64 d, 6.2 | 1.57 d, 6.3 | 1.53 d, 6.2 |
| *terminal*-Ara(p)-1‴ | 5.42 d, 7.4 | | |
| 2‴ | 4.44 dd, 7.4, 9.6 | | |
| 3‴ | 5.49 dd, 3.8, 9.6 | | |
| 4‴ | 5.58 br s | | |
| 5‴ | 3.81 dd, 1.1, 13.1 | | |
| | 4.07 dd, 2.4, 13.1 | | |
| *terminal*-Ara(f)-1‴ | | 6.13 br s | |
| 2‴ | | 4.78 br s | |
| 3‴ | | 5.57 br d, *ca.* 4 | |
| 4‴ | | 4.75 dd, 4.0, 8.3 | |
| 5‴ | | 4.14 m (2H) | |
| Xyl-1‴ | | | 5.37 d, 7.4 |
| 2‴ | | | 5.52 dd, 7.4, 8.9 |
| 3‴ | | | 4.23 m |
| 4‴ | | | 5.26 ddd, 5.1, 8.9, 9.2 |
| 5‴ | | | 3.47 dd, 9.2, 11.5 |
| | | | 4.17 dd, 5.1, 11.5 |
| Acetyl | | | |
| 4′ | 1.96 s | | |
| 2‴ | | | 1.99 s |
| 3‴ | 1.87 s | 1.93 s | |
| 4‴ | 2.05 s | | 1.91 s |

Table 3. $^{13}$C-NMR Data (150 MHz, Pyridine-$d_5$) of Rarasaponins IV—VI (**1**—**3**)

| Position | **1** | **2** | **3** |
|---|---|---|---|
| 1 | 39.1 | 39.1 | 39.0 |
| 2 | 26.5 | 26.3 | 26.2 |
| 3 | 81.6 | 81.2 | 81.4 |
| 4 | 43.7 | 43.6 | 43.6 |
| 5 | 47.9 | 48.0 | 47.9 |
| 6 | 18.2 | 18.2 | 18.3 |
| 7 | 33.0 | 33.0 | 33.0 |
| 8 | 39.9 | 39.8 | 39.8 |
| 9 | 48.3 | 48.2 | 48.2 |
| 10 | 37.0 | 37.0 | 37.0 |
| 11 | 23.8 | 23.7 | 23.8 |
| 12 | 122.6 | 122.6 | 122.6 |
| 13 | 144.9 | 144.8 | 144.8 |
| 14 | 42.1 | 42.0 | 42.2 |
| 15 | 28.4 | 28.4 | 28.4 |
| 16 | 23.9 | 23.9 | 23.9 |
| 17 | 46.7 | 46.7 | 46.5 |
| 18 | 42.3 | 42.2 | 42.0 |
| 19 | 46.5 | 46.5 | 46.7 |
| 20 | 31.0 | 31.0 | 31.0 |
| 21 | 34.3 | 34.3 | 34.3 |
| 22 | 33.3 | 33.3 | 33.2 |
| 23 | 64.1 | 64.3 | 64.3 |
| 24 | 14.1 | 14.0 | 14.0 |
| 25 | 16.1 | 16.1 | 16.1 |
| 26 | 17.5 | 17.5 | 17.5 |
| 27 | 26.2 | 26.2 | 26.2 |
| 28 | 180.2 | 180.1 | 180.1 |
| 29 | 33.3 | 33.3 | 33.2 |
| 30 | 23.8 | 23.8 | 23.8 |
| C-3-sugar | | | |
| *inner*-Ara(p)-1′ | 104.8 | 104.5 | 104.4 |
| 2′ | 75.4 | 75.7 | 75.6 |
| 3′ | 73.3 | 74.8 | 74.7 |
| 4′ | 72.9 | 69.4 | 69.4 |
| 5′ | 63.8 | 65.8 | 65.8 |
| Rha-1″ | 101.7 | 101.1 | 101.2 |
| 2″ | 71.9 | 72.0 | 71.6 |
| 3″ | 82.2 | 79.3 | 81.9 |
| 4″ | 73.0 | 72.6 | 72.2 |
| 5″ | 69.8 | 69.7 | 70.1 |
| 6″ | 18.4 | 18.5 | 18.4 |
| *terminal*-Ara(p)-1‴ | 106.9 | | |
| 2‴ | 70.2 | | |
| 3‴ | 74.0 | | |
| 4‴ | 69.3 | | |
| 5‴ | 64.2 | | |
| *terminal*-Ara(f)-1‴ | | 111.0 | |
| 2‴ | | 80.3 | |
| 3‴ | | 81.4 | |
| 4‴ | | 84.8 | |
| 5‴ | | 62.7 | |
| Xyl-1‴ | | | 103.9 |
| 2‴ | | | 75.0 |
| 3‴ | | | 72.2 |
| 4‴ | | | 72.6 |
| 5‴ | | | 62.8 |
| Acetyl | | | |
| 4′ | 170.5 | | |
| | 20.8 | | |
| 2‴ | | | 170.3 |
| | | | 21.0 |
| 3‴ | 170.8 | 170.6 | |
| | 20.9 | 20.8 | |
| 4‴ | 170.4 | | 170.3 |
| | 20.8 | | 20.7 |

part [$\delta$ 5.26 (1H, ddd, $J$=5.1, 8.9, 9.2 Hz)] and the acetyl carbonyl carbon ($\delta_C$ 170.3), so that the connectivities of acetyl carbonyl groups were clarified. Consequently, the structure of rarasaponin VI was determined to be hederagenin 3-*O*-(2,4-di-*O*-acetyl-$\beta$-D-xylopyranosyl)-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**3**).

**Inhibitory Effect of Principal Constituents (4, 5, 8) on Plasma TG Elevation in Olive Oil-Treated Mice** The effects of the principal constituents (**4**, **5**, **8**) of *S. rarak* on plasma TG elevation in olive oil-treated mice were examined. In contrast, a standard lipase inhibitor, orlistat,[24] showed a potent effect in this assay model (Table 4). Among the principal known saponin constituents of *S. rarak*, hederagenin 3-*O*-$\alpha$-L-arabinopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**4**)[20] and hederagenin 3-*O*-(3,4-di-*O*-acetyl-$\alpha$-L-arabinopyranosyl)-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**5**)[20,21] significantly suppressed the increase in plasma TG levels 2 h after administration of olive oil at doses of 200 mg/kg, *p.o.* However, the major acyclic sesquiterpene glycoside, mukurozioside IIb (**8**)[25] did not show such effect. Consequently, the saponin constituents from *S. rarak* may be useful for the prevention of obesity. Furthermore, other anti-obese experiments are considered to be studied in the near future.

## Experimental

The following instruments were used to obtain 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) spectrometer; $^{13}$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 spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV–VIS detectors; HPLC column, Cosmosil 5$C_{18}$-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 60$F_{254}$ (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** The pericarps of *S. rarak* collected in Thailand at September 2006. The plant material was identified by Dr. Yutana Pongpiriyadacha, a Lecturer of Rajamangala University of Technology Srivijaya (Thailand). A voucher specimen (2006.09. Raj-01) of this plant is on file in our laboratory.

**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%).[1] 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.[1] Fraction 1 (1.11 g) was subjected to reversed-phase silica gel column chromatography [30 g, MeOH–$H_2O$ (70 : 30→80 : 20→90 : 10, v/v)→MeOH] to afford seven fractions [Fr. 1-1 (30.0 mg), Fr. 1-2 (137.7 mg), Fr. 1-3 (104.3 mg), Fr. 1-4 (137.9 mg), Fr. 1-5 (130.2 mg), Fr. 1-6 (34.3 mg), and Fr. 1-7 (356.8 mg)]. Fraction 1-2 (137.7 mg) purified by HPLC [Cosmosil 5$C_{18}$-MS-II, MeOH–1% aqueous AcOH (80 : 20, v/v)] to

Table 4. Inhibitory Effects of **4**, **5**, and **8** on Plasma Triglyceride Elevation in Olive Oil-Treated Mice

| Treatment | Dose (mg/kg, *p.o.*) | *n* | Plasma triglyceride (mg/dl)[a)] | | |
|---|---|---|---|---|---|
| | | | 2.0 h | 4.0 h | 6.0 h |
| Normal | — | 7 | 119.3±11.8** | 132.5±10.6** | 101.1±5.2** |
| Control | — | 9 | 565.8±74.0 | 635.3±84.4 | 458.3±91.8 |
| **4** | 50 | 7 | 623.8±71.2 | 650.5±50.4 | 325.8±37.7 |
| | 100 | 7 | 401.1±81.1 | 563.0±93.2 | 557.6±88.4 |
| | 200 | 7 | 288.2±73.4* | 344.9±36.3* | 384.5±52.8 |
| Normal | — | 7 | 142.8±20.7** | 121.8±12.7** | 123.7±73.1** |
| Control | — | 9 | 513.0±83.0 | 397.2±63.4 | 259.3±48.8 |
| **5** | 50 | 7 | 377.5±75.9 | 367.5±43.0 | 287.2±30.1 |
| | 100 | 7 | 354.3±87.7 | 321.1±46.7 | 450.5±44.2 |
| | 200 | 7 | 177.7±23.6** | 178.4±21.5** | 169.9±28.9 |
| Mukurozioside IIb (**8**) | 200 | 7 | 595.0±82.0 | 480.6±61.0 | 350.0±24.1 |
| Normal | | 7 | 91.9±9.4** | 97.3±7.4** | 90.6±9.4** |
| Control | — | 9 | 440.3±60.2 | 393.2±60.1 | 263.3±45.0 |
| Orlistat | 5 | 7 | 371.3±41.5 | 297.0±67.4 | 171.9±24.9 |
| | 10 | 7 | 203.8±52.1** | 160.4±47.7** | 129.1±16.6** |
| | 20 | 7 | 198.6±24.1** | 131.0±16.8** | 114.5±7.6** |

*a*) Values represent the means±S.E.M. Significantly different from the control group, $*p<0.05$, $**p<0.01$.

give rarasaponin IV (**1**, 8.6 mg, 0.02%) and hederagenin 3-*O*-(3,4-di-*O*-acetyl-$\alpha$-L-arabinopyranosyl)-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**5**, 22.3 mg, 0.06%). Fraction 4-3 (113.6 mg), which was described previously,[1)] was further separated by HPLC [Wakopak Navi C30-5, $CH_3CN$–$H_2O$ (50 : 50, v/v)] to furnish rarasaponin VI (**3**, 6.2 mg, 0.12%). Fraction 5 (670.0 mg) was further purified by HPLC [Cosmosil HILIC, $CH_3CN$–$H_2O$ (92 : 8, v/v)] to afford rarasaponin V (**2**, 42.2 mg, 0.01%). Fraction 6 (250.0 mg) was separated by HPLC [Wakopak Navi C30-5, $CH_3CN$–$H_2O$ (50 : 50, v/v)] to give **2** (18.0 mg, 0.19%).

Rarasaponin IV (**1**): An amorphous powder, $[\alpha]_D^{27}$ +25.6° ($c$=0.43, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{80}O_{19}Na$ $(M+Na)^+$: 1031.5191. Found: 1031.5183. IR (KBr): 3488, 1736, 1655, 1254, 1086, 1053 $cm^{-1}$. $^1$H-NMR (600 MHz, pyridine-$d_5$) $\delta$: given in Table 2. $^{13}$C-NMR data (150 MHz, pyridine-$d_5$) $\delta_C$: given in Table 3. Positive-ion FAB-MS $m/z$: 1031 $(M+Na)^+$. Negative-ion FAB-MS $m/z$: 1007 $(M-H)^-$, 965 $(M-C_2H_3O)^-$, 791 $(M-C_9H_{13}O_6)^-$, 749 $(M-C_{11}H_{15}O_7)^-$, 603 $(M-C_{17}H_{25}O_{11})^-$, 471 $(M-C_{22}H_{33}O_{15})^-$.

Rarasaponin V (**2**): An amorphous powder, $[\alpha]_D^{29}$ −8.3° ($c$=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{48}H_{76}O_{17}Na$ $(M+Na)^+$: 947.4981. Found: 947.4987. IR (KBr): 3569, 1734, 1717, 1656, 1250, 1053 $cm^{-1}$. $^1$H-NMR (600 MHz, pyridine-$d_5$) $\delta$: given in Table 1. $^{13}$C-NMR data (150 MHz, pyridine-$d_5$) $\delta_C$: given in Table 2. Positive-ion FAB-MS $m/z$: 947 $(M+Na)^+$. Negative-ion FAB-MS $m/z$: 923 $(M-H)^-$, 881 $(M-C_2H_3O)^-$, 749 $(M-C_7H_{11}O_5)^-$, 603 $(M-C_{13}H_{21}O_9)^-$, 471 $(M-C_{18}H_{29}O_{13})^-$.

Rarasaponin VI (**3**): An amorphous powder, $[\alpha]_D^{26}$ +1.9° ($c$=0.41, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{50}H_{78}O_{18}Na$ $(M+Na)^+$: 989.5086. Found: 989.5082. IR (KBr): 3568, 1736, 1719, 1655, 1240, 1055 $cm^{-1}$. $^1$H-NMR (600 MHz, pyridine-$d_5$) $\delta$: given in Table 1. $^{13}$C-NMR data (150 MHz, pyridine-$d_5$) $\delta_C$: given in Table 2. Positive-ion FAB-MS $m/z$: 989 $(M+Na)^+$. Negative-ion FAB-MS $m/z$: 965 $(M-H)^-$, 749 $(M-C_9H_{13}O_6)^-$, 603 $(M-C_{15}H_{23}O_{10})^-$, 471 $(M-C_{20}H_{31}O_{14})^-$.

**Deacylation of Rarasaponins IV (1), V (2), and VI (3)** A solution of rarasaponins IV (**1**, 3.1 mg), V (**2**, 8.0 mg), and VI (**6**, 3.0 mg) in 0.5% sodium methoxide (NaOMe)–MeOH (1.0 ml) was stirred at room temperature for 3 h, respectively. The reaction mixture was neutralized with Dowex HCR-W2 ($H^+$ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave a residue, which was purified by HPLC [Cosmosil HILIC, $CH_3CN$–$H_2O$ (85 : 15, v/v)] to furnish **4** (from **1**, 1.9 mg, 71.0%), **6** (from **2**, 5.3 mg, 73.3%), and sapindoside B (**7**, 2.1 mg from **3**, 75.8%).

**Bioassay Method. Animals** Male ddY mice weighing about 25—30 g were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were fasted for 20—24 h prior to the beginning of the experiment, but were allowed free access to tap water. All of the experiments were performed with conscious mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

**Inhibitory Effect on Plasma TG Elevation in Olive Oil-Treated Mice** The experiments were performed as described in our previous method with a slight modification.[26—28)] Each test sample was administered orally to fasted mice and olive oil (5 ml/kg) was administered *p.o.* 30 min thereafter. Blood sample (*ca.* 0.25 ml) was collected from the infraorbital venosus plexus under ether anesthesia at 2, 4, and 6 h after oral administration of olive oil. The collected blood was immediately mixed with heparin sodium (5 units/tube). After centrifugation of blood sample, plasma TG was determined by enzymatic method using Triglyceride E test Wako (Wako Pure Chemical Ind., Ltd., Osaka, Japan).

**Statistics** Values were expressed as means±S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used.

**Acknowledgements** M. Yoshikawa, and H. Matsuda were supported by the 21st COE Program, Academic Frontier Project, and a Grant-in Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT). O. Muraoka and T. Morikawa were supported by High-tech Research Center Project (2007—2011) and a Grant-in Aid for Scientific Research from MEXT.

**References and Notes**

1) Morikawa T., Xie Y., Asao Y., Okamoto M., Yamashita C., Muraoka O., Matsuda H., Pongpiriyadacha Y., Yuan D., Yoshikawa M., *Phytochemistry*, in press.
2) Yoshikawa M., Morikawa T., Nakano K., Pongpiriyadacha Y., Murakami T., Matsuda H., *J. Nat. Prod.*, **65**, 1638—1642 (2002).
3) Yoshikawa M., Pongpiriyadacha Y., Kishi A., Kageura T., Wang T., Morikawa T., Matsuda H., *Yakugaku Zasshi*, **123**, 871—880 (2003).
4) Morikawa T., Kishi A., Pongpiriyadacha Y., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **66**, 1191—1196 (2003).
5) Kishi A., Morikawa T., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 1051—1055 (2003).
6) Matsuda H., Pongpiriyadacha Y., Morikawa T., Ochi M., Yoshikawa M., *Eur. J. Pharmacol.*, **471**, 59—67 (2003).
7) Matsuda H., Morikawa T., Managi H., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **13**, 3197—3202 (2003).
8) Matsuda H., Ando S., Morikawa T., Kataoka S., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **15**, 1949—1953 (2005).
9) Ando S., Matsuda H., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem.*, **13**, 3289—3294 (2005).
10) Morikawa T., Ando S., Matsuda H., Kataoka S., Muraoka O., Yoshikawa M., *Chem. Pharm. Bull.*, **53**, 625—630 (2005).
11) Morikawa T., Matsuda H., Yamaguchi I., Pongpiriyadacha Y., Yoshikawa M., *Planta Med.*, **70**, 152—159 (2004).
12) Matsuda H., Ninomiya K., Morikawa T., Yasuda D., Yamaguchi I., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **18**, 2038—2042 (2008).
13) Matsuda H., Tewtrakul S., Morikawa T., Nakamura A., Yoshikawa M.,

*Bioorg. Med. Chem.*, **12**, 5891—5898 (2004).
14) Matsuda H., Morikawa T., Xu F., Ninomiya K., Yoshikawa M., *Planta Med.*, **70**, 1201—1209 (2004).
15) Morikawa T., Xu F., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **54**, 1530—1534 (2006).
16) Matsuda H., Yoshida K., Miyagawa K., Asao Y., Takayama S., Nakashima S., Xu F., Yoshikawa M., *Bioorg. Med. Chem.*, **15**, 1539—1546 (2007).
17) Yoshikawa M., Xu F., Morikawa T., Pongpiriyadacha Y., Nakamura S., Asao Y., Kumahara A., Matsuda H., *Chem. Pharm. Bull.*, **55**, 308—316 (2007).
18) Yoshikawa M., Morikawa T., Funakoshi K., Ochi M., Pongpiriyadacha Y., Matsuda H., *Heterocycles*, **75**, 1639—1650 (2008).
19) Morikawa T., Funakoshi K., Ninomiya K., Yasuda D., Miyagawa K., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **56**, 956—962 (2008).
20) Kimata H., Nakashima T., Kokubun S., Nakayama K., Mitoma Y., Kitahara T., Yata N., Tanaka O., *Chem. Pharm. Bull.*, **31**, 1998—2005 (1983).
21) Kojima K., Zhu X.-B., Ogihara Y., *Phytochemistry*, **48**, 885—888 (1998).
22) The $^{1}$H- and $^{13}$C-NMR spectra of **1**—**3** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments.
23) Inhibitory effect of rarasaponin V (**2**) on pancreatic lipase activity was examined [inhibition (%): 0.0±1.6, 5.4±1.5, 42.2±5.1**, 91.8±2.2**, and 99.3±0.5** at 0, 50, 100, 200, and 400 $\mu$M, respectively ($IC_{50}$=118 $\mu$M)]. Values were expressed as means±S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Significantly different from the control group, $^{**}p<0.01$. The bioassay method of pancreatic lipase inhibitory activity was described previously.[1)]
24) McNeely W., Benfield P., *Drugs*, **56**, 241—249 (1998).
25) Kasai R., Fujino H., Kuzuki T., Wong W.-H., Goto C., Yata N., Tanaka O., Yasuhara F., Yamaguchi S., *Phytochemistry*, **25**, 871—876 (1986).
26) Yoshikawa M., Morikawa T., Yamamoto K., Kato Y., Nagatomo A., Matsuda H., *J. Nat. Prod.*, **68**, 1360—1365 (2005).
27) Ninomiya K., Matsuda H., Shimoda H., Nishida N., Kasajima N., Yoshino T., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **14**, 1943—1946 (2004).
28) Morikawa T., Li X., Nishida E., Ito Y., Matsuda H., Nakamura S., Muraoka O., Yoshikawa M., *J. Nat. Prod.*, **71**, 828—835 (2008).