  Bull. Chem. Soc. Jpn., 62, 469—473 (1989) 

# The Structure of Rotungenoside, a New Bitter Triterpene Glucoside from *Ilex Rotunda*

Munehiro NAKATANI,* Shuichi HATANAKA, Hajime KOMURA,† Takashi KUBOTA,†† and Tsunao HASE
Department of Chemistry, Faculty of Science, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890
†Suntory Institute for Bioorganic Research, Shimamoto-cho, Mishima-gun, Osaka 618
††Department of Chemistry, Faculty of Science, Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka 558
(Received July 27, 1988)

A new bitter triterpene ester glucoside have been isolated along with known bitter triterpene glucoside pedunclosside and some minor triterpenes ulsolic acid, rotundic acid, and rotungenic acid from the unripe fruits of *Ilex rotunda*. The structure was characterized as β-D-glucopyranosyl 3β,19α,24-trihydroxyurs-12-en-28-oate by spectral data and chemical means.

*Ilex rotunda* Thunb. (Aquifoliaceae) yields red fruits in winter. Birds pick up the ripe fruits in early spring but don't the unripe ones. Some triterpenoids have been isolated from this plant, i.e. a triterpene rotundic acid (**1**) from the seeds[1] and a triterpene glucoside pedunclosside (**2**) from the leaves.[2] But there is no detailed study on the constituents of the fruits. Then we were interested in the difference of their constituents and first investigated on the ripe fruits to get new triterpenes rotungenic acid (**3**) and rotundioic acid (**4**).[3] We now studied the constituents of the unripe fruits and isolated a new bitter triterpene ester glucoside rotungenoside (**5**) as minor glucoside along with major pedunclosside (**2**) and minor triterpenes ulsolic acid, rotundic acid (**1**), and rotungenic acid (**3**). These bitter glucosides **1** and **2** exhibited a weak insect antifeedant activity against the larvae of *Spodoptera litura* Fab. with the leaf disk choice test.

## Results and Discussion

The fresh unripe fruits of *Ilex rotunda* were extracted with methanol. After concentration to one-tenth of its original volume, the extract was suspended in water and then extracted with ethyl acetate. The extract was roughly divided into two groups by passing through LH-20 column, one of which was a mixture of triterpenes and the other contained some triterpene glycosides. Triterpenoids were detected by the red-violet colour given by the Liebermann-Burchard test. Crystallization of the glycoside fraction afforded a mixture of two compounds which were separated to a major known pedunclosside (**2**) (28-β-D-glucopyranosyl 3β,19α,23-trihydroxyurs-12-en-28-oate) and minor new rotungenoside (**5**) on a DCCC(droplet countercurrent chromatography) using chloroform–methanol–water solvent system in ascending mode. From the triterpene fraction, three known compounds ulsolic acid, rotundic acid (**1**) (3β,19α,23-trihydroxyurs-12-en-28-oic acid) and rotungenic acid (**3**) (3β,19α,24-trihydroxyurs-12-en-28-oic acid) were isolated.

Rotungenoside (**5**), an amorphous white powder, $[\alpha]_D^{25}$ +5° (MeOH), was assigned the molecular formula $C_{36}H_{58}O_{10}$ from SIMS (*m/z* 673[M+Na]$^+$). Its IR and UV spectra showed the presence of hydroxyl and ester groups (3450 and 1720 cm$^{-1}$) and double bond (209 nm: ε 3000). Its $^{13}$C NMR spectrum revealed thirty-six carbon signals ($CH_3$-×6, -$CH_2$-×9, >CH-×4, >C<-×5, -$CH_2$-O×2, >CH-O×5, >C<-O×1, -CH<O,O>×1, >C=CH-×1, -CO-O×1). The SI mass spectrum showed a characteristic peak at *m/z* 489 denoting the loss of 1 mol of glucose and two peaks of 471 and 453 indicated further successive loses of 1 and 2 mol of water. The $^{13}$C NMR experiment (Table 1) suggested that this glycoside **5** had the same sugar (β-D-glucopyranose) with pedunclosside (**2**) which was linked to the aglycone with ester linkage. The anomeric carbon of **5** was also found at a higher field (δ 95.7) owing to the esterification effect;[4] pedunclosside (**2**): δ 96.0. Careful examination of $^{13}$C NMR spectra of these aglycone part, clarified that these compounds had very similar aglycone; the only difference is the substitution around C-3 and C-4 in 3,18-dihydroxyurs-12-en-18-oic skeleton. In particular, the exchange of

Table 1. $^{13}$C NMR Data of Pedunclosside (**2**) and Rotungenoside (**5**) in Py-$d_5$ Solution

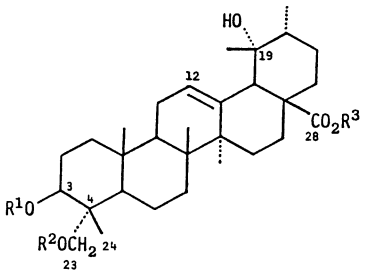
| Carbon No. | 2 | 5 | Carbon No. | 2 | 5 |
|---|---|---|---|---|---|
| 1 | 39.1t | 38.9t | 20 | 42.3d | 42.1d |
| 2 | 27.8t | 28.5t | 21 | 26.9t | 26.8t |
| 3 | 74.2d | 80.4d | 22 | 37.9t | 37.8t |
| 4 | 43.0s | 43.2s | 23 | 68.2t | 23.7q |
| 5 | 48.8d | 48.0d | 24 | 13.2q | 64.7t |
| 6 | 19.0t | 19.4t | 25 | 17.7q | 17.4q |
| 7 | 33.5t | 33.9t | 26 | 16.9q | 16.8q |
| 8 | 40.8s | 40.6s | 27 | 24.8q | 24.6q |
| 9 | 48.0d | 48.0d | 28 | 177.2s | 177.0s |
| 10 | 37.4s | 37.2s | 29 | 27.2q | 27.1q |
| 11 | 24.3t | 24.4t | 30 | 16.3q | 16.2q |
| 12 | 128.7d | 128.4d | | | |
| 13 | 139.5s | 139.3s | 1′ | 96.0d | 95.5d |
| 14 | 42.3s | 42.1s | 2′ | 73.9d | 74.1d |
| 15 | 29.5t | 29.3t | 3′ | 79.3d | 79.3d |
| 16 | 26.3t | 26.2t | 4′ | 71.4d | 71.3d |
| 17 | 48.8s | 48.7s | 5′ | 79.0d | 79.0d |
| 18 | 54.6d | 54.5d | 6′ | 62.6t | 62.4t |
| 19 | 72.9s | 72.7s | | | |

the signal at $\delta$ 13.2 to $\delta$ 23.7 led to the conclusion that the C-23 methyl was replaced with a hydroxymethyl group since the signal of the C-24 methyl in most ursene triterpenoids having 3$\beta$-OH group has been observed to about 10 ppm higher field than the C-23 methyl signal.[5,6]

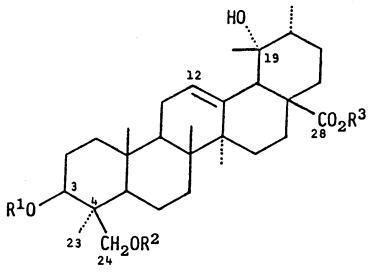
The glucoside **5** was acetylated to give the hexaacetate **6**. Its $^1$H NMR spectrum (Table 2) supported very well the ester linked $\beta$-anomeric configuration ($\delta$ 5.52, 1H, d, $J$= 8Hz; H-1′) including glucose moiety and showed the presence of an 3$\beta$-acetoxyl group ($\delta$ 4.58, 1H, dd, $J$=11 and 5.5 Hz; H-3$\alpha$). A signal at $\delta$ 2.53 (1H, br s; H-18) suggested the presence of the 19-*O*-substituted urs-12-ene skeleton,[7] while the 4-acetoxylmethylene signals at $\delta$ 4.14 and 4.36 (each d, $J$=12 Hz) showed the large down-field shift of 0.25 and 0.67 ppm compared to those of **7** (hexaacetate of **2**), which also suggested the 4$\beta$-$CH_2OH$ configuration in **5**.

The aglycone of **3** was obtained by alkaline hydrolysis and identified as **3** by means of $^{13}$C NMR and mass spectroscopies. It follows that **5** can be formulated as 28-$\beta$-D-glucopyranosyl 3$\beta$,19$\alpha$,24-trihydroxyurs-12-en-28-oate. In the $^1$H NMR spectrum of the diacetate (**8**), a W-type long-range coupling was observed between the H-3$\alpha$ signal at $\delta$ 4.58 (dd, $J$=10.5 and 5 Hz) and higher one of the axial 4$\beta$-acetoxymethyl signals at $\delta$ 4.38 and 4.12 (each d, $J$=11.5 Hz) but not observed in the hexaacetate **6**, which suggested a conformation change in the glucoside **6**.

Acid hydrolysis of **5** with hydrochloric acid in methanol afforded glucose (identified by TLC and paper chromatography) and a mixture of conjugated and nonconjugated dienes **9** and **10**. The dehydration product **9** showed a hindered conjugated diene system at 229 nm ($\varepsilon$ 3400) in its UV spectrum and the $^1$H NMR spectrum showed the presence of one olefinic methyl group at $\delta$ 1.75. On the other hand, compound **10** had a nonconjugated UV absorption at 207.5 nm ($\varepsilon$ 6500) and showed two olefinic methyl signals at $\delta$ 1.53 and 1.63 in its $^1$H NMR spectrum. These compounds were also obtained from rotungenic acid (**3**) by the acid treatment. A similar dehydration has been observed in the reaction of 19-hydroxyurs-12-ene or 19-hydroxyolean-12-enes[8,9] and so the compounds from **3** and **5**




| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 1: | H | H | H |
| 2: | H | H | glu |
| 4: | H | 4α-$CO_2$H | H |
| 7: | Ac | Ac | glu(Ac) |

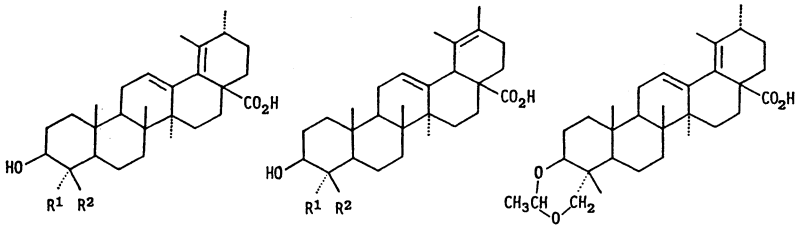



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 3: | H | H | H |
| 5: | H | H | glu |
| 6: | Ac | Ac | glu(Ac) |
| 8: | Ac | Ac | H |

Table 2. $^1$H NMR Data of Acetylglucosides **6** and **7**, and Acetylrotungenic Acid (**8**)

| H | Aglycone | | | | | | | | | H | Glucosyl | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **6** | | | **7** | | | **8** | | | | **6** | | **7** | |
| | $\delta$ | Mult | $J$/Hz | $\delta$ | Mult | $J$/Hz | $\delta$ | Mult | $J$/Hz | | $\delta$ | Mult $J$/Hz | $\delta$ | Mult $J$/Hz |
| 3 | 4.58 | dd | 11, 5.5 | 4.80 | dd | 11, 5 | 4.58 | dd | 10.5, 5 | 1′ | 5.52 | d 8 | 5.52 | d 8 |
| 12 | 5.38 | brt | 3.5 | 5.38 | brt | 3 | 5.35 | brt | 3.5 | 2′ | 5.17 | dd 9, 8 | 5.17 | dd 9, 8 |
| 18 | 2.53 | brs | | 2.53 | brs | | 2.54 | brs | | 3′ | 5.25 | t 9 | 5.25 | t 9 |
| 23 | 0.95 | s | | 3.69 | d | 11.5 | 0.95 | s | | 4′ | 5.12 | dd 10, 9 | 5.12 | dd 10, 9 |
| | | | | 3.89 | brd | 11.5 | | | | 5′ | 3.78 | ddd 10, 4.5, 2.3 | 3.78 | ddd 10, 4.5, 2.2 |
| 24 | 4.14 | d | 12 | 0.84 | s | | 4.12 | brd | 11.5 | 6′ | 4.05 | dd 12, 2.3 | 4.05 | dd 12, 2.2 |
| | 4.36 | d | 12 | | | | 4.38 | d | 11.5 | | 4.27 | dd 12, 4.5 | 4.27 | dd 12, 4.5 |
| 25 | 1.02 | s | | 0.98 | s | | 1.02 | s | | Ac | 2.01 | s | 2.01 | s |
| 26 | 0.71 | s | | 0.72 | s | | 0.73 | s | | | 2.02 | s | 2.02 | s |
| 27 | 1.22 | s | | 1.21 | s | | 1.22 | s | | | 2.02 | s | 2.03 | s |
| 29 | 1.25 | s | | 1.25 | s | | 1.26 | s | | | 2.07 | s | 2.07 | s |
| 30 | 0.94 | s | | 0.94 | s | | 0.96 | s | | | | | | |
| Ac | 2.03 | s | | 2.03 | s | | 2.03 | s | | | | | | |
| | 2.06 | s | | 2.06 | s | | 2.05 | s | | | | | | |

Measured in $CDCl_3$.



9: $R^1$= $CH_3$, $R^2$= $CH_2OH$ 10: $R^1$= $CH_3$, $R^2$= $CH_2OH$ 13

11: $R^1$= $CH_2OH$, $R^2$= $CH_3$ 12: $R^1$= $CH_2OH$, $R^2$= $CH_3$

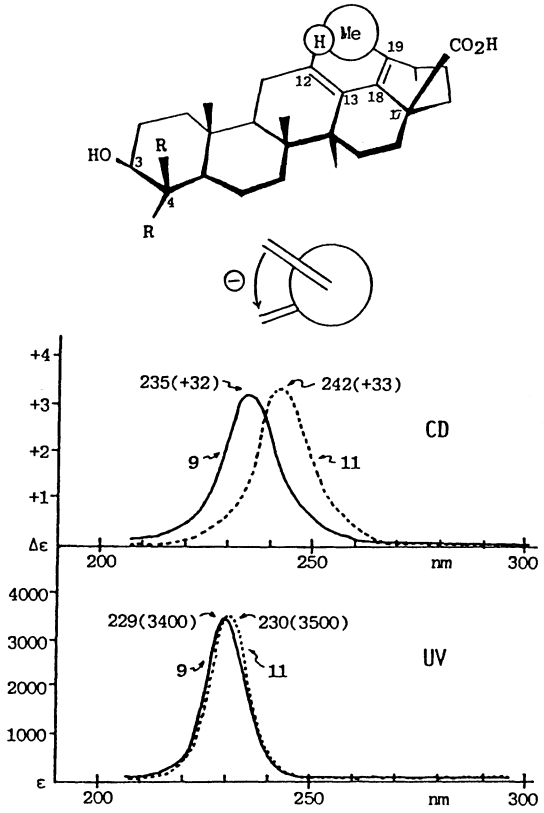



Fig. 1. UV and CD spectra of conjugated diene **9** and **11**.

were formulated as structures **9** and **10**. In the case of the reaction of **2** with dil sulfuric acid in ethanol, an acetal **13** was given in addition to dehydration products **11** and **12**.

The conjugated diene **9** exhibited a strong positive Cotton effect in the CD spectrum at 235 nm ($\Delta\varepsilon$ +32) like **11** (242 nm; $\Delta\varepsilon$ +33) due to $\pi$-$\pi^*$ transition of conjugated diene (Fig. 1). The positive Cotton effect was well correlated with the chirarlity of chromophore, which is positive when the planes of the two double bonds are skewed in a right-handed helix. In the heteroannular cisoid diene, the sign of $\pi$-$\pi^*$ cd is opposite to the chirality of the diene system.[10] Thus the positive effect expected for the negative chirality of the diene system in **9**, as predicted from its Dreiding model, was observed.

Among the compounds isolated in this work, peduncloside and rotungenoside were active as an insect antifeedant against the larvae of *Spodoptera litura* Fab.

## Experimental

Mps are uncorrected. Concentrations were performed under reduced pressure at bath temperatures not exceeding 50 °C. $^1$H NMR spectra were obtained at 360 MHz and $^{13}$C NMR spectra at 25.2 MHz. All the compounds were finally purified by HPLC on a $C_{18}$ semiprep column using $H_2O$/MeOH solvent system. UV spectra were measured in methanol (MeOH).

**Plant Material.** Unripe fruits of the plant were collected at Kagoshima University in December 1983.

**Extraction and Isolation.** The unripe fruits (2 kg) were extracted with MeOH (3×10 l). After concn to 500 ml, $H_2O$ (300 ml) was added and extracted with ethyl acetate (AcOEt) to give 18 g of an extract, which was passed through LH-20 column with MeOH to give two fractions. The first eluted fraction (7.2 g) was crystallized twice from AcOEt to give 2.1 g of a mixture of glucosides. The mixture (165 mg) was separated to a major component **2** (134 mg; 81.3%) and minor one **5** (14 mg; 8.4%) on a DCCC using $CHCl_3$-MeOH-$H_2O$ (7 : 13 : 8 v/v) in ascending mode. The major component was eluted out at fractions 70—100 and the minor one at fractions 104—136. The elution of the compounds was monitored by TLC. Chromatography of the second fraction (3.4 g) on silica gel afforded ulsolic acid (25 mg) and a mixture of two triterpenes (1.2 g), which was separated to **1** (840 mg) and **3** (43 mg) by recrystallization from MeOH followed by HPLC (solvent; 20% $H_2O$/MeOH).

**Rotungenoside (5).** A white amorphous powder; $[\alpha]_D^{25}$ +5° (*c* 0.04, MeOH); UV 209 nm ($\varepsilon$ 3000); IR (Nujol) 3500—3200, 1720 cm$^{-1}$; SIMS *m/z* 673 $(M+Na)^+$, 651 $(M+1)^+$, 581, 535, 489 $(651-C_6H_{11}O_5)^+$, 471 $(489-H_2O)^+$, 461, 453 $(471-H_2O)^+$, 435, 425, 407. Calcd for $C_{36}H_{59}O_{10}$: M+1, 651.

**Peduncloside (2).** Prisms from AcOEt mp 213—214°C; $[\alpha]_D^{25}$ +22° (*c* 0.05, MeOH); SIMS *m/z* 673 $(M+Na)^+$, 489 $(M+1-C_6H_{11}O_5)^+$, 471 $(489-H_2O)^+$, 425, 407; IR (Nujol) 3500—3200, 1720 cm$^{-1}$; UV 210 nm ($\varepsilon$ 4500). Found: C, 66.32; H, 8.83%. Calcd for $C_{36}H_{58}O_{10}$: C, 66.47; H, 8.92%.

**Ursolic Acid.** Mp 290 °C; EIMS *m/z* 456 $(M)^+$, 438, 410, 248, 207, 203, 189, which was identified by direct comparison (IR and MS) with an authentic sample.

**Rotundic Acid (1).** Prisms from MeOH, mp 272—274 °C (decomp); $[\alpha]_D^{25}$ +24° (*c* 0.1, MeOH); SIMS *m/z* 511 $(M+Na)^+$; EIMS *m/z* 488 $(M)^+$, 470, 452, 442, 264, 246, 223,

201, 175, 146; IR (Nujol) 3500—2600, 1690, 1640 $cm^{-1}$; UV 209 nm ($\varepsilon$4000); $^{13}C$ NMR (py-$d_5$) $\delta$=13.1 (C-24), 16.0 (C-30), 16.8 (C-26), 17.3 (C-25), 18.8 (C-6), 24.1 (C-11), 24.7 (C-27), 26.5 (C-16), 27.0 (C-21), 27.2 (C-29), 27.7 (C-2), 29.4 (C-15), 33.4 (C-7), 37.3 (C-10), 38.5 (C-22), 38.9 (C-1), 40.4 (C-8), 42.2 (C-14), 42.4 (C-20), 42.9 (C-4), 47.9 (C-9), 48.3 (C-17), 48.8 (C-5), 54.7 (C-18), 68.2 (C-23), 72.7 (C-19), 73.7 (C-3), 128.1 (C-12), 140.0 (C-13), 180.7 (C-28). HRMS Found: $m/z$ 488.3503. Calcd for $C_{30}H_{48}O_5$: M, 488.3502.

**Rotungenic Acid (3).** Column from $H_2O$-MeOH, mp 295—298 °C (decomp); $[\alpha]_D^{24}$ +50° ($c$ 0.07, MeOH); SIMS $m/z$ 489 $(M+1)^+$; EIMS $m/z$ 488 $(M)^+$, 470, 452, 442, 264, 246, 223, 219, 205, 201, 175, 146; IR (Nujol) 3500—2600, 1690, 1640 $cm^{-1}$; UV 209 nm ($\varepsilon$ 4000): $^{13}C$ NMR (py-$d_5$) $\delta$=16.1 (C-30), 16.8 (C-26), 17.2 (C-25), 19.3 (C-6), 23.7 (C-23), 24.3 (C-11), 24.7 (C-27), 26.5 (C-16), 27.0 (C-21), 27.2 (C-29), 28.5 (C-2), 29.4 (C-15), 34.0 (C-7), 37.2 (C-10), 38.6 (C-22), 38.8 (C-1), 40.4 (C-8), 42.1 (C-14), 42.4 (C-20), 43.2 (C-4), 47.9 (C-9), 48.4 (C-17), 54.7 (C-18), 56.6 (C-5), 64.6 (C-24), 72.8 (C-19), 80.3 (C-3), 127.9 (C-12), 140.0 (C-13), 180.8 (C-28). HRMS Found: $m/z$ 488.3504. Calcd for $C_{30}H_{48}O_5$: M, 488.3502.

**Hexaacetylrotungenoside (6).** Rotungenoside (**5**) (4 mg) was treated with acetic anhydride ($Ac_2O$) in py at 60 °C for 24 h to give the hexaacetate **6** (4 mg), mp 141—142.5 °C; $[\alpha]_D^{25}$ +15° ($c$ 0.1, MeOH); IR (Nujol) 3450, 1740, 1720 $cm^{-1}$; UV 208 nm ($\varepsilon$ 3700). Found: C, 63.77; H, 7.95%. Calcd for $C_{48}H_{70}O_{16}$: C, 63.84; H, 7.81%.

**Hexaacetylpeduncloside (7).** Peduncloside (**2**) (17 mg) was treated with $Ac_2O$ in py at 60 °C for 24 h to give the hexaacetate **7** (15 mg), mp 160—161 °C; $[\alpha]_D^{25}$ +47° ($c$ 0.1, MeOH); IR (Nujol) 3500, 1745, 1720 $cm^{-1}$; UV 209 nm ($\varepsilon$ 3800). Found: C, 63.89; H, 7.92%. Calcd for $C_{48}H_{70}O_{16}$: C, 63.84; H, 7.81%.

**3,24-Diacetylrotungenic Acid (8).** Compound **3** (4.5 mg) was acetylated with $Ac_2O$ in py to give the diacetate **8** (3 mg), mp 164—167 °C; EIMS $m/z$ 554 $(M-H_2O)^+$, 526, 512, 264, 246.

**Alkaline Hydrolysis of Rotungenoside (5).** Rotungenoside (**5**) (11 mg) was treated with 10% aq potassium carbonate ($K_2CO_3$) (400 mg) in MeOH (10 ml) under reflux for 3 h. The crude product gave **3** (7 mg) as plates from MeOH, mp 295—298 °C (decomp).

**Alkaline Hydrolysis of Peduncloside (2).** Compound **2** (11 mg) was treated with 10% aq $K_2CO_3$ in MeOH under reflux for 10 h. Work-up as usual gave **1** (6.5 mg) as plates from $H_2O$-MeOH, mp 272—275 °C (decomp).

**Acid Hydrolysis of Rotungenoside (5).** Compound **5** (10 mg) was treated with 2M hydrochloric acid (HCl; 1M=1 mol $dm^{-3}$) (2 ml) in MeOH (10 ml) under reflux for 4 h. The product was extracted with ether and the extract was purified by HPLC (17.5% $H_2O$/MeOH) to give **9** (3 mg) and **10** (1 mg). Compound **9**, mp 198—199 °C as plates from MeOH; IR (Nujol) 3500—2500, 1690, 1650 $cm^{-1}$; UV 229 nm ($\varepsilon$ 3400); CD (MeOH) 235 nm ($\Delta\varepsilon$ +32); EIMS $m/z$ 470 $(M)^+$, 424 $(M-HCO_2H)^+$, 246, 223, 205, 201, 69 (base peak); $^1H$ NMR ($CDCl_3$) $\delta$=0.88, 0.92, 0.97 (each 3H, s), 1.09 (3H, d, $J$=7.1 Hz), 1.25 (3H, s), 1.75 (3H, s) 3.34 (1H, br d, $J$=11.4 and 3.7 Hz), 3.45 (1H, br dd, $J$=11.5 and 5.0 Hz), 4.21 (1H, d, $J$=11.2 Hz), 5.44 (1H, dd, $J$=4.2 and 3.7 Hz). HRMS Found: $m/z$ 470.3387. Calcd for $C_{30}H_{46}O_4$: M, 470.3395. Compound **10**; UV 207.5 nm ($\varepsilon$ 6500); CD (MeOH) 210 nm ($\Delta\varepsilon$ −5.2); EIMS $m/z$ 470 $(M)^+$, 472 $(M-H_2O)^+$, 426 $(M-CO_2)^+$, 424, 313, 246, 223, 205, 201, 149, 75 (base peak); $^1H$ NMR ($CDCl_3$) $\delta$= 0.81, 0.90, 0.96, 1.24, 1.53, 1.63 (each 3H, s), 3.16 (1H, br s), 3.34 (1H, br d, $J$=11.4 Hz), 3.44 (1H, br dd, $J$=12 and 4 Hz), 4.21 (1H, d, $J$=11.2 Hz), 5.48 (1H, t, $J$=3.7 Hz). HRMS Found: $m/z$ 470.3335. Calcd for $C_{30}H_{46}O_4$: M, 470.3395.

**Methyl Ester of 9.** Compound **9** was methylated with diazomethane ($CH_2N_2$) in MeOH to give the methyl ester **14**, a white amorphous powder; EIMS $m/z$ 484 $(M)^+$, 260, 247, 201; $^1H$ NMR ($CDCl_3$) $\delta$=0.84 (3H, s; 9-Me), 0.92 (3H, s; 4$\alpha$-Me), 0.96 (3H, s; 11-Me), 1.07 (3H, d, $J$=7Hz; 20-Me), 1.25 (3H, s; 14-Me), 1.72 (3H, s; 19-Me), 3.34 (1H, m; 3-H), 3.45 (1H, br dd, $J$=11.5 and 4 Hz; 24-H), 4.21 (1H, d, $J$=11.5 Hz; 24-H), 5.35 (1H, dd, $J$=4 and 3.5 Hz; 12-H).

**Methyl Ester of 10.** Compound **10** was methylated with $CH_2N_2$ in MeOH to give the methyl ester **15**, mp 264—267 °C; EIMS $m/z$ 484 $(M)^+$, 260, 248, 247, 215, 201 187; $^1H$ NMR ($CDCl_3$) $\delta$=0.79 (3H, s; 9-Me), 0.90 (3H, s; 4$\alpha$-Me), 0.95 (3H, s; 11-Me), 1.25 (3H, s; 14-Me), 1.53 (3H, s; 20-Me), 1.63 (3H, s; 19-Me), 3.20 (1H, br, s; 18-H), 3.33 (1H, br d, $J$=11 Hz; 3-H), 3.44 (1H, br dd, $J$=11.5 and 4 Hz; 24-H), 4.20 (1H, d, $J$=11.5 Hz; 24-H), 5.47 (1H, t, $J$=3.5 Hz; 12-H).

**Acid Hydrolysis of Peduncloside (2).** (i) Compound **2** (95 mg) was refluxed with 2 M HCl in MeOH for 3 h. HPLC separation of the crude product gave **11** (42 mg) and **12** (8 mg). Compound **11**, mp 145—146 °C; IR (Nujol) 3500—2500, 1690, 1650 $cm^{-1}$; UV 230 nm ($\varepsilon$ 3500); CD (MeOH) 242 nm ($\Delta\varepsilon$ +33); SIMS $m/z$ 493 $(M+Na)^+$, 471 $(M+1)^+$; $^1H$ NMR ($CDCl_3$) $\delta$=0.87, 0.98, 1.03, 1.08 (each 3H, s), 1.09 (3H, d, $J$=7 Hz), 1.74 (1H, s), 3.45 (1H, d, $J$=10.5 Hz), 3.67 (1H, dd, $J$=9 and 7 Hz), 3.74 (1H, d, $J$=10.5 Hz), 5.46 (1H, t, $J$=4 Hz). HRMS Found: $m/z$ 470.3391. Calcd for $C_{30}H_{46}O_4$: M, 470.3395. Compound **12**, mp 165—167 °C; IR (Nujol) 3500—2500, 1680, 1650 $cm^{-1}$; UV 207 nm ($\varepsilon$ 4000); $^1H$ NMR ($CDCl_3$) $\delta$=0.86, 0.89, 0.97, 1.01, 1.54, 1.61 (each 3H, s), 3.17 (1H, br s), 3.43 (1H, d,$J$=10.5 Hz), 3.64 (1H, dd, $J$=9 and 7 Hz), 3.73 (1H, d, $J$=10.5 Hz), 5.49 (1H, t, $J$=3.8 Hz). HRMS Found: $m/z$ 470.3319. Calcd for $C_{30}H_{46}O_4$: M, 470.3395. (ii) Compound **2** (97 mg) was refluxed with 1 M sulfuric acid (15 ml) in ethanol (20 ml) for 5 h. Chromatography of the crude product on silica gel afforded an acetal **13** (8 mg) in addition to a mixture of **11** and **12**. Compound **13**, mp 215—216 °C; IR ($CHCl_3$) 3200—2500, 1690,1600 $cm^{-1}$; UV 247 nm ($\varepsilon$ 4500); SIMS $m/z$ 497 $(M+1)^+$, 451 $(497-HCO_2H)^+$, 404 $(451-CH_3CHO)^+$; $^1H$ NMR ($CDCl_3$) $\delta$=0.87, 0.98, 1.03, 1.08 (each 3H, s), 1.09 (3H, d, $J$=7 Hz), 1.35 (3H, d, $J$=5.3 Hz; $C\underline{H}_3CH\begin{matrix}O\\O\end{matrix}$), 3.24 (1H, d, $J$=10.4 Hz; 23-H), 3.55 (1H, dd, $J$=10 and 4.5 Hz; 3-H), 3.77 (1H, d, $J$=10.4 Hz; 23-H), 4.74 (1H, q, $J$=5.3 Hz; $CH_3C\underline{H}\begin{matrix}O\\O\end{matrix}$), 5.42 (1H, t ,$J$=3.5 Hz). SIMS Found: $m/z$ 497. Calcd for $C_{32}H_{49}O_4$: M+1, 497.

**Reaction of Rotungenic Acid (3) with Acid.** Compound **3** (7 mg) was refluxed with 2 M HCl in MeOH for 3 h. Work-up as usual gave **9** (3 mg) and **10** (1 mg).

**Reaction of Rotundic Acid (1) with Acid.** Compound **1** was treated with 2 M HCl in a manner similar to **3**. The crude product gave **11** and **12**.

**Insect Antifeedant Activity.** The triterpenoids **1**, **2**, **3**, and **5** were tested against the larvae of the pest insect *Spodoptera litura* Fab. with the leaf disk method.[11] Peduncloside (**2**) and rotungenoside (**5**) showed the antifeedant activity at 2000 ppm concn.

We are grateful to Dr. Hideo Naoki and Takashi Iwashita (Suntory Institute for Bioorganic Research) for MS and NMR spectra and to Mr. Mitsuo Tahara (Sankei Chemical Co., Ltd.) for the supply of the insect.

This work was partially supported by Grant-in-Aid for the Scientific Research (No. 60540355) from the Ministry of Education, Science and Culture.

**References**

1) T. Oyama, H. Aoyama, K. Yamada, T. Mitsuhashi, and N. Sugiyama, *Tetrahedron Lett.*, **1968**, 4639.

2) T. Hase, H. Hagii, M. Ishizu, M. Ochi, N. Ichikawa, and T. Kubota , *Nippon Kagaku Kaishi*, **1973**, 778.

3) M. Nakatani, Y. Miyazaki, T. Iwashita, H. Naoki, and T. Hase, *Phytochemistry*, in press.

4) K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai, and O. Tanabe, *Tetrahedron Lett.*, **1976**, 1005.

5) P. J. Houghton and L. M. Lian, *Phytochemistry*, **25**, 1939 (1986).

6) R. Chemli, A. Badadjamian, R. Faure, K. Boukef, G. Balansard, and E. Vidal, *Phytochemistry*, **26**, 1785 (1987).

7) I. Yoshioka, T. Sugawara, and I. Kitagawa, *Chem. Pharm. Bull.*, **19**, 1700 (1971).

8) C. H. Brieskorm and H. Wandere, *Chem. Ber.*, **100**, 1252 (1967).

9) J. Bermejo, J. L. Breton, G. de la Fuente, and A. G. Gonzalez, *Tetrahedron Lett.*, **1967**, 4649.

10) A. W. Burgstahler and R. C. Barkhurst, *J. Am. Chem. Soc.*, **92**, 7601 (1970).

11) I. Kubo, M. Taniguchi, A. Chapya, and K. Tsujimoto, *Planta Med.*, **1980**, 185.