Tricalysiosides P—U: *Ent*-kaurane Glucosides and a Labdane Glucoside from Leaves of *Tricalysia dubia* Ohwi

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Further extensive isolation work on the 1-BuOH-soluble fraction of a MeOH extract of *Tricalysia dubia* afforded five new *ent*-kaurane glucosides (4—8) and one new labdane glucoside (9), together with a known megastigmane glucoside, sammangaoside B (1), and monoterpene glucosides (2, 3). The structures of the new compounds were elucidated by analyses of one- and two-dimensional NMR spectroscopic data. The absolute configuration of the 9-position of sammangaoside B was revised to *S* and its total stereochemistry was established by the modified Mosher's method.

Key words *Tricalysia dubia*; Rubiaceae; diterpenoid; tricalysioside; *ent*-kaurane glucoside; labdane glucoside

The approximately 50 species of *Tricalysia* (Syn. *Canthium*) are found in subtropical and tropical areas of Asia and Africa. Some species are used for medicinal purposes, *e.g.* the roots, leaves, and stem bark of *Canthium subcordatum* are used as folk medicines in Africa.[1)]

Tricalysia dubia (Lindl.) Ohwi (Rubiaceae) is an evergreen shrub or tree that grows to a height of about 2—4 m. It is distributed in the south of China, Taiwan, and Okinawa.[2)] In the course of our study on Okinawa's promising resource plants, the constituents of the title plant, *T. dubia*, were investigated.

In previous papers, the structural elucidation of seven rearranged *ent*-kaurane glucosides, tricalysiosides A—G, and eight *ent*-kaurane glucosides, named tricalysiosides H—O, from leaves of *T. dubia* was reported.[3,4)] Non-glycosidic rearranged *ent*-kauranes were also isolated from the same plant species.[5,6)] Further extensive investigation of the same plant material afforded five new *ent*-kaurane glucosides (**4**—**8**) and one new labdane glucoside (**9**), together with one megastigmane glucoside, two monoterpene glucosides (**1**, **2**),[7—9)] and sammangaoside B (**3**).[10)] This paper deals with their structural elucidation and revision of the stereostructure of sammangaoside B.

Results and Discussion

Air-dried leaves of *T. dubia* were extracted with MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The 1-BuOH-soluble fraction was separated by various chromatographic procedures including column chromatography (CC) on a highly porous synthetic resin (Diaion HP-20), normal silica gel and reversed-phase octadecyl silica gel (ODS) CC, then droplet counter-current chromatography (DCCC) to afford six diterpenoid glucosides, named tricalysiosides P—U (**4**—**9**) (Fig. 1), together with one megastigamne glucoside, two monoterpene glucosides (**1**, **2**),[7—9)] and summangaoside B (**3**).[10)] The details and yields are given in the Experimental section. The structures of tricalysiosides P—U (**4**—**9**) were elucidated by spectroscopic analyses and comparison with tricalysiosides previously isolated based on spectroscopic evidence.

Sammangaoside B (**3**), $[\alpha]_D$ −35.0°, was first isolated from *Clerodendrum inerme* but the stereochemistry of its six-membered ring portion remained to be determined. Phase-sensitive NOE experiments supported the previously reported relative structure of the ring system, as depicted in the literature.[10)] Application of modified Mosher's method[11)] to the aglycone led to revision of the absolute configuration of the 9-position to *S* (Fig. 2) then established the total stereostructure of **3** to have the 3*S*,5*R*,6*S*,9*S* configuration, as

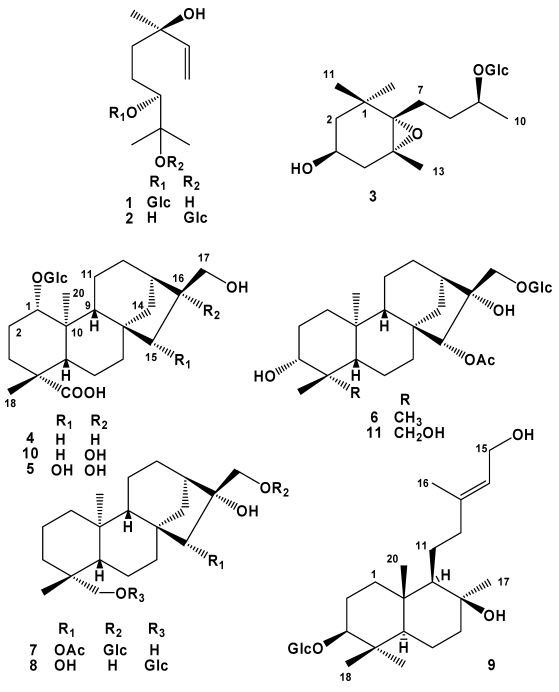

Fig. 1. Structures of Compounds **1**—**9**

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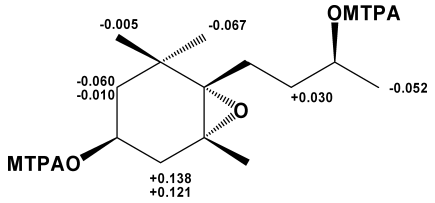

Fig. 2. Results of Modified Mosher's Method ($\Delta\delta_{S-R}$)

shown in Fig. 1. The assignment of the ^{13}C-NMR chemical shift of C-2 (47.9) in ref. 10 must be corrected to 49.3.

Tricalysioside P (**4**), $[\alpha]_D^{22}$ −22.8°, was isolated as colorless crystals and its elemental composition was determined to be $C_{26}H_{42}O_9$ by HR-ESI-TOF-MS. The ^{13}C-NMR spectrum showed signals for β-glucopyranose, the remaining 20 signals indicating that **4** was an *ent*-kaurane diterpenoid glucoside. The functional groups comprised one each of primary and secondary alcohols, and a carboxyl group. A closely resembling compound has already been isolated as tricalysioside H (**10**) from the same plant material, whose structure was established by X-ray crystallographic analysis.[4] The ^{13}C-NMR chemical shifts of rings A and B of **4** were essentially superimposable on those of **10** (Table 1). When the elemental compositions of **4** and **10** were compared, one of the oxygen functional groups in rings C and D was found to be obviously replaced by a hydrogen atom. In **10**, C-16 was observed as a singlet carbon and the chemical shift was 82.1 ppm, the singlet being replaced by a doublet carbon with a chemical shift of 38.7 ppm in **4**. Therefore tricalysioside P (**4**) was elucidated to be a deoxygenated derivative of tricalysioside H (**10**). The phase-sensitive NOESY correlation peak between H_2-17 (δ_H 3.67) and the H-11 axial proton at δ_H 3.52 confirmed that the C-17 carbinol was in the β-orientation. The position of the sugar moiety was also confirmed by the HMBC experiment, the anomeric proton (δ_H 4.96) showing a cross peak with the C-1 signal (δ_C 92.6). Since a series of compounds was isolated from the same plant material, kaurane must have an *entio*-skeleton and glucose of the D-series.[3—6] These suppositions were confirmed by evidence that acid hydrolysis of **4** gave D-glucose and then in the ^{13}C-NMR spectra, chemical shifts of the A-rings, to which the D-glucose was attached, of tricalysiolides P (**4**) and H (**10**) showed essentially the same chemical shifts. Therefore the structure of tricalysioside P (**4**) was elucidated to be *ent*-1β,17-dihydroxykauran-19-oic acid 1-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Tricalysioside Q (**5**), $[\alpha]_D^{22}$ −8.5°, was isolated as colorless crystals and mass spectroscopic analysis revealed its elemental composition to be $C_{26}H_{42}O_{11}$, which corresponds to two oxygens more than in that of tricalysioside P (**4**) and one more than in that of **10**. The NMR spectral analyses indicated that **5** was an analogous compound to **4**, and the ^{13}C-NMR chemical shifts of rings A and B were diagnostically the same as those of **4** and **10**, except at the C-7 and 8 positions, whose chemical shifts, however, were suggested that there was no oxygen function on them. This implied that extra oxygen functions must have been introduced to some carbon atoms of rings C and D, which influenced the chemical shifts of C-7 and 8. The most plausible positions are C-15 and 16, because the new alcoholic groups were secondary and tertiary alcohols and the same substitution of functional groups was found in the aglycones of tricalysioside A [C-15: δ_C 82.6 (d), 16: δ_C 81.3 (s) and 17: δ_C 66.2 (t)],[3] tricalysioside M [C-15: δ_C 82.7 (d), 16: δ_C 81.3 (s) and 17: δ_C 66.3 (t)],[4] and tricalysiolide G [C-15: δ_C 82.6 (d), 16: δ_C 81.3 (s) and 17: δ_C 66.2 (t)][6] (Table 1). This assumption was confirmed by the HMBC correlation peak between H_2-17 (δ_H 4.10) and C-15 (δ_C 82.3). From the NOESY experiment, the orientation of the hydroxyl group was concluded to be the same as that of the aforementioned compounds. Therefore the structure of tricalysioside Q (**5**) was elucidated to be *ent*-1β,15β,16β,17-tetrahydroxykauran-19-oic acid 1-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Tricalysioside R (**6**), $[\alpha]_D^{22}$ −13.9°, was isolated as an amorphous powder and its elemental composition was established to be $C_{28}H_{46}O_{10}$ by HR-ESI-TOF-MS. The ^{13}C- and ^{1}H-NMR spectroscopic data indicated that **6** was also an *ent*-kaurane-type diterpenoid glucoside with an acetyl group (δ_C 171.3, 21.1 and δ_H 2.18). When the ^{13}C-NMR spectroscopic data were compared with those of tricalysioside N (**11**), rings C and D of **6** were judged to have the same functional groups as those of **11** (Table 1). The position of the sugar linkage was confirmed by the HMBC spectroscopic data, the anomeric proton signal (δ_H 4.92) showing a cross peak with the carbon signal of the primary alcohol (C-17, δ_C 75.5). The acetyl group was similarly placed on the secondary alcohol at C-15 to that of **11**, as judged from a cross peak between H-15 (δ_H 5.13) and a carbonyl carbon of the acetyl group in the HMBC spectrum, and an observed significant downfield shift of H-15 (δ_H 5.13) from that (δ_H 4.00) in **5** on acetylation. The remaining secondary alcoholic functional group was placed at C-3 by following HMBC correlations; cross peaks from H_3-19 (δ_H 1.01) and H_3-18 (δ_H 1.19) to δ_C 78.0. Although the H-3 proton signal (δ_H 3.42, dd, J=8, 8 Hz) did not show a typical axial nor equatorial coupling pattern, judging from the NOESY cross peaks between H-3 and the equatorial methyl, δ_H 1.19, and H-5 (δ_H 0.80), it must occupy a pseudo axial space. On acetylation, the pentaacetate was obtained. In the ^{1}H-NMR spectrum, the H-3 signal was shifted downfield (δ_H 3.42→4.44) and its coupling pattern showed a typical axial feature, a doublet of a double (J=12, 5 Hz). Therefore, the structure of tricalysioside R (**6**) was elucidated to be 15-*O*-acetyl-*ent*-3β,15β,16β,17-tetrahydroxykaurane 17-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Tricalysioside S (**7**), $[\alpha]_D^{22}$ −20.0°, was isolated as an amorphous powder and its elemental composition was established to be $C_{28}H_{46}O_{10}$ by HR-ESI-TOF-MS. The NMR spectroscopic data of rings C and D were essentially the same as those of **6** and showed that the secondary alcohol of **6** was replaced by a primary alcohol. The NOESY correlation peak between H_3-20 (δ_H 0.97) and carbinol protons (δ_H 3.64, 3.96) indicated that the axial methyl on C-4 must carry an oxygen functional group. Therefore the structure of tricalysioside S (**7**) was elucidated to be 15-*O*-acetyl-*ent*-15β,16β,17,19-tetrahydroxykaurane 17-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Tricalysioside T (**8**), $[\alpha]_D^{22}$ −35.1°, was isolated as an amorphous powder and its elemental composition was established to be $C_{26}H_{46}O_9$ by HR-ESI-TOF-MS. Although the NMR spectroscopic data were similar to those of **6** and **7**, resonances assignable to an acetyl group were not observed.

Table 1. ^{13}C-NMR Data for Tricalysioside P—U (**4**—**9**) (100 MHz, Pyridine-d_5) and Reference Compounds (**10**[4] and **11**[4])

Carbon No.	**4**	**10**	**5**	**6**	**11**	**7**	**8**	**9**	**9a**
1	92.6	92.6	92.5	36.1	36.4	40.5	40.7	38.3	38.6
2	28.3	28.3	28.4	28.1	28.4	18.7	18.7	26.6	28.1 (−1.5)[b]
3	36.7	36.7	36.6	78.0	80.0	36.6	36.2	89.0	78.1 (+10.5)
4	43.6	43.6	43.6	39.4	43.3	39.8	38.4	39.7	39.5 (+0.2)
5	56.2	56.0	56.0	55.1	55.6	56.7	57.1	55.8	55.8
6	23.2	22.8	21.9	19.7	20.1	20.1	20.4	20.5	20.8
7	43.6	43.7	37.6[a]	39.6	38.7	36.2	37.3	45.1	45.2
8	46.1	46.0	49.1	47.5	47.5	47.6	48.0	73.0	73.1
9	55.2	55.7	54.8	55.6	55.6	55.8	56.4	61.9	62.0
10	45.8	45.8	45.9	38.9	39.3	39.2	39.8	38.9	39.3
11	21.7	21.2	21.4	18.8	18.9	18.9	18.9	24.6	24.7
12	32.5	27.4	26.9	26.0	25.8	25.9	26.3	43.7	43.8
13	44.9	43.7	43.3	44.8	44.8	44.8	43.6	138.6	138.6
14	38.5	39.0	37.4[a]	36.7	36.5	36.5	36.8	125.4	125.5
15	45.7	53.7	82.3	84.0	83.7	84.0	82.7	59.0	59.1
16	38.7	82.1	81.9	81.2	81.2	81.2	81.3	16.7	16.7
17	67.2	66.4	66.1	75.5	75.5	75.2	66.2	24.5	24.6
18	29.4	29.3	29.3	28.8	23.7	27.9	28.3	16.8	16.3
19	180.8	179.9	180.1	16.4	64.4	64.3	73.2	28.3	28.8
20	12.9	13.0	13.0	18.0	18.4	18.9	18.4	15.9	16.0
1′	104.6	104.6	104.5	105.9	105.9	105.8	105.5	106.9	
2′	75.8	75.8	75.7	75.2	75.6	75.4	75.4	75.8	
3′	79.1	79.1	78.9	78.6	78.6	78.5	78.8	78.8	
4′	71.7	71.8	71.7	71.7	71.7	71.7	71.9	71.9	
5′	78.4	78.5	78.3	78.3	78.3	78.3	78.3	78.3	
6′	62.9	62.9	62.8	62.7	62.7	62.7	63.0	63.0	
CH_3CO				21.1	21.1	21.0			
$CH_3\underline{C}O$				171.3	171.2	173.0			

a) Signals may be interchangeable. *b*) $\Delta\delta_9-\delta_{9a}$.

When the ^{13}C-NMR spectrum was compared with that of **7**, the carbinol carbon signal of C-17 was found to be shifted up (δ_C 75.2→66.2), while the other primary alcohol carbon signal at C-19 was shifted down (δ_C 64.3→73.2) due to glucosylation. This was confirmed by the HMBC correlation peak between the anomeric proton (δ_H 4.85) and C-19 (δ_C 73.2) and by the NOESY correlation peaks between H_2-19 (δ_H 3.57, 4.34) and H_3-20 (δ_H 1.02). Therefore, the structure of tricalysioside T (**8**) was elucidated to be *ent*-15β,16β,17,19-tetrahydroxykaurane 19-*O*-β-D-glucopyranoside, as shown Fig. 1.

On acid hydrolyses of tricalysiosides Q—T (**5**—**8**), D-glucoside was detected in each hydrolytic medium and used as a chiral probe. Comparisons of ^{13}C-NMR spectra with those of resembling compounds confirmed that the aglycones were in the *ent*-series (**5** with **4**, **6** with **11**, **7** with tricalysioside N,[4] and **8** with 16α,17,19-trihydroxy-*ent*-kaurane 19-*O*-β-D-glucopyranoside[12]).

Tricalysioside U (**9**), $[\alpha]_D^{22}$ −17.5°, was isolated as colorless crystals and its elemental composition was determined to be $C_{26}H_{46}O_8$ by HR-ESI-TOF-MS. The ^{13}C-NMR spectrum together with the ^{1}H-NMR spectrum indicated the presence of six signals assignable to β-glucopyranose, the remaining 20 signals comprising those of five methyls, six methylenes, two methines, two quarternary carbons, one each of primary, secondary, and tertiary alcohols, and one trisubstituted double bond. Four degrees of unsaturation, and the presence of one hexopyranose and one double bond suggested that the aglycone moiety must have a bicyclic system. In the H–H COSY spectrum, the primary alcohol protons (δ_H 4.49) showed correlation to the olefinic proton (δ_H 5.84), then correlated to methylene protons (δ_H 2.56, 2.23) on C-12 and methyl protons (δ_H 1.73). These methylene protons were further correlated to the methylene protons (δ_H 1.96, 1.40) on C-11, and then correlated to the methine proton (δ_H 1.34) on C-9. This evidence led us to the conclusion that the side chain consisted of six carbons and thus the aglycone had a labdane skeleton. The location of the tertiary alcohol was determined to be C-8 from the diagnostic HMBC correlations illustrated in Fig. 3a, H_3-17 [δ_H 1.31 (s)] showing a cross peak with C-8 [δ_C 73.0 (s)]. That of the secondary alcohol was also determined to C-3 from the HMBC correlations of H_3-19 to C-3 and H-3 to C-4, 18, and 19. In the phase-sensitive NOESY spectrum, the axial proton at C-3 showed NOE correlations with the axial protons at C-1 and C-5, and the axial methyl protons at C-4 with the axial methyl protons at C-10 and the axial proton at C-6 [Fig. 3b]. Since the axial proton (δ_H 0.70) at C-1 was correlated with the proton at C-9 (δ_H 1.34), the side chain must be in the equatorial orientation. The NOE correlation between the H_3-17 and H-11 protons enabled placing of the C-17 methyl in the equatorial position. The position of the sugar linkage was determined to be on the hydroxyl group at C-3 from the HMBC correlations between the anomeric proton (δ_H 4.93) and C-3 (δ_C 89.0), and H-3 (δ_H 3.42) and the anomeric carbon (δ_C 106.9). Therefore the relative structure of tricalysiosde U (**9**) was determined to be as shown in Fig. 1. All the kuarane derivatives isolated from *T. dubia* were expected to be in an *ent*-series due to the co-occurrence of *ent*-congeners. Whereas a labdane is a biosynthetic precursor of kauranes and only one labdane-type diterpenoid has been isolated from the same plant so far. When this labdane is in an *ent*-series, it must be a precursor of many kaurane derivatives such as tricalysiosides A—U and tricalysiolides. If it is not, it should be a

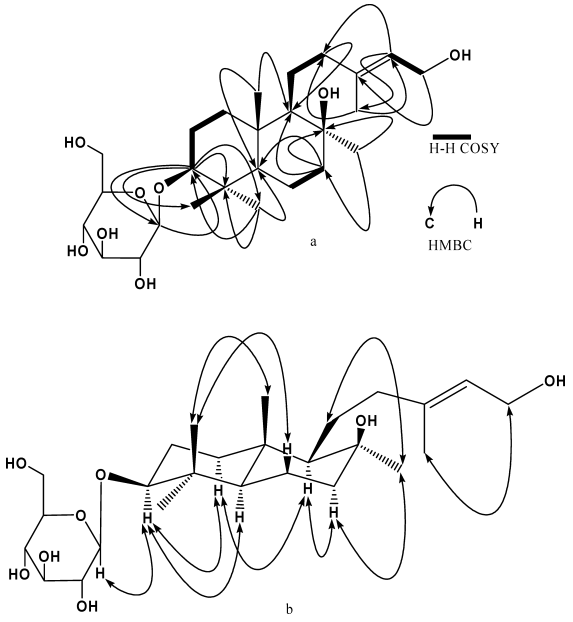

Fig. 3. H–H COSY and HMBC (a), and Phase-Sensitive NOE (b) Correlations of **9**

dead-end product on which no further biosynthetic enzymes operate. For the application of a glucosylation-induced shift-trend[13] to determine the absolute configuration of the 3-position, tricalysioside U was hydrolyzed to give an aglycone, tricalysiol U (**9a**), and D-glucose. Kasai *et al.*[13] have reported a glucosylation-induced shift-trend for dammarenediol-I with D-glucose at C-4: −1.2 ppm and C-2: +0.3 ppm, and one for dammaranediol-I with L-glucose, which corresponds to *ent*-dammaranediol-I with D-glucose, at C-4: −4.2 ppm and C-2: −0.8 ppm. From the observation that C-2 of **9a** shifted upfield by −1.5 ppm and C-4 shifted down by +0.2 ppm on β-D-glucopyranosylation (Table 1), the absolute stereochemistry of C-3 was expected to have the *S*-configuration and, in turn, the labdane was in a normal series, not in an *enantio* one. To confirm the stereochemistry, the modified Mosher's method was further applied.[11] The H-2a and 2b protons of the 3*R*-MTPA diester resonated at 1.87 ppm and 1.75 ppm, whereas on analyses of the H–H COSY spectrum, those of the 3*S*-MTPA diester werefound to be at between 1.58 and 1.68, and overlapped by many signals. Although the exact $\Delta_{\delta S-\delta R}$ values could not be calculated, they obviously had minus signs, whereas those of H_3-18 and H_3-19 showed plus values, +3 Hz and +31 Hz, respectively. Therefore, the structure of the labdane derivative, tricalysioside U (**9**) was elucidated to be 3*S*,8*S*,11*E*,15-trihydroxylabdene 3-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Experimental

General Experimental Procedures Melting points were determined by a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1030 polarimeter. IR spectra were measured on a Horiba FT-710 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane (TMS) as internal standard. Positive-ion HR-MS were taken on an Applied Biosystem QSTAR XL system ESI (Nano Spray)-TOF-MS.

Highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase [octadecyl silica gel (ODS)] open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1 l)→(1 : 1, 1 l), fractions of 10 g being collected], respectively. Droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=2 mm, L=40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-PrOH (9 : 12 : 8 : 2) were used for the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ=6 mm, L=25 cm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor.

Emulsin was purchased from Tokyo Chemical Industries Co., Ltd. (Tokyo, Japan), and crude hesperidinase was a gift from Tokyo Tanabe Pharmaceutical Co., Ltd. (Tokyo, Japan). *R*- and *S*-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA) were the products of Wako Pure Chemical Industry Ltd.

Plant Material Leaves of *T. dubia* (LINDL.) OHWI (Rubiaceae) were collected in Okinawa, Japan, in August 1990, and a voucher specimen was deposited in the Herbarium of the Department of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (90-TD-Okinawa-0822).

Extraction and Isolation of 1—9 Dried leaves of *T. dubia* (6.04 kg) were extracted three times with MeOH (45 l) at room temperature for 1 week then concentrated to 6 l *in vacuo*. The extract was washed with *n*-hexane (6 l) then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (6 l) and extracted with EtOAc (6 l) to give 361 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (6 l) to give a 1-BuOH-soluble fraction (290 g), and the remaining water-layer was concentrated to furnish 325 g of a water-soluble fraction.

The 1-BuOH-soluble fraction was separated first by CC on Diaion HP-20 (Φ=5.0 cm, L=60 cm), with MeOH–H_2O [20% (8 l), 40% (8 l), 60% (8 l), and 80% (8 l) in water, successively], 500-ml factions being collected. The fraction eluted with 20—40% MeOH (55.9 g in fractions 13—18) was subjected to a column of silica gel (1.0 kg) using $CHCl_3$ (3 l) and $CHCl_3$–MeOH [99 : 1 (3 l), 97 : 3 (3 l), 19 : 1 (3 l), 37 : 3 (3 l), 9 : 1 (6 l), 7 : 1 (6 l), 17 : 3 (6 l), 33 : 7 (6 l), 4 : 1 (6 l), 3 : 1 (6 l), and 7 : 3 (6 l); fractions of 1 l being collected] as a solvent system. The residue of fraction 27—34 (1.94 g out of 6.31 g, 10—12.5% MeOH eluate) was subjected to ODS CC, fractions of 10 g being collected, and the residue (70.5 mg) of fractions 69—71 was purified by DCCC to afford 33.4 mg of compound **2** in fractions 29—36. The residues (206 mg in fractions 72—84 and 99.8 mg in fractions 91—97) were similarly subjected to DCCC to give 109 mg of **1** in fractions 35—50 and 36.5 mg of **3** in fractions 46—54, respectively. Compound **3** was finally purified by HPLC to afford 8.5 mg as a pure state. A portion (2.13 g) of the residue (3.70 g in fractions 54—59, 25% MeOH eluate) was similarly subjected to ODS CC. The residue of fractions 106—113 (117 mg) was purified by DCCC to give 30.1 mg of crystalline **5** in fractions 28—36.

The 40% eluate (26.6 g in fractions 13—18) on Diaion HP-20 CC was subjected to a column of silica gel (500 g) using $CHCl_3$ (3 l) and $CHCl_3$–MeOH [99 : 1 (3 l), 49 : 1 (3 l), 97 : 3 (3 l), 24 : 1 (3 l), 19 : 1 (2 l), 47 : 3 (3 l), 23 : 2 (3 l), 9 : 1 (4.5 l), 7 : 3 (4.5 l), 17 : 3 (4.5 l), 33 : 7 (4.5 l), 4 : 1 (3 l), 3 : 1 (3 l), and 7 : 3 (3 l); fractions of 500 ml being collected] as a solvent system. A portion (1.97 g) of the residue (6.00 g in fractions 42—49, 8—10% MeOH eluate) was similarly subjected to ODS CC. From fractions 185—195, compound **9** (40.0 mg) was obtained in a crystalline state. The residue of fractions 60—63 (1.65 g) was subjected ODS CC, fractions of 10 g being collected, and the residue (42.7 mg) of fractions 176—187 was purified by DCCC to afford 11.1 mg of compound **8** in fractions 110—128. The residue (42.3 mg) of fractions 201—205 was subjected to DCCC to give 35.1 mg of crystalline compound **4** in fractions 16—26.

The fraction eluted with 80% MeOH (13.6 g in fractions 23—26) on Diaion HP-20 CC was subjected to a column of silica gel (300 g) using $CHCl_3$ (3 l) and $CHCl_3$–MeOH [99 : 1 (3 l), 49 : 1 (3 l), 97 : 3 (3 l), 24 : 1 (3 l), 19 : 1 (3 l), 47 : 3 (3 l), 23 : 2 (3 l), 9 : 1 (3 l), 7 : 1 (3 l), 17 : 3 (3 l), 33 : 7 (3 l), 4 : 1 (3 l), 3 : 1 (3 l), and 7 : 3 (3 l); fractions of 500 ml being collected] as the solvent system. The residue (2.25 g) of fractions 40—48 (6—8% MeOH eluate) was subjected to ODS CC, fractions of 10 g being collected, and the residue (133 mg) in fractions 189—204 was purified by DCCC. The residue (38.9 mg) in fractions 87—114 was finally purified by HPLC to give 5.4 mg of **6** and 7.5 mg of **7**.

Known Compounds Isolated 3,7-Dimethyloct-1-ene-3,6,7-triol 6-*O*-β-D-glucopyranoside (**1**): $[\alpha]_D^{22}$ −7.1° (*c*=1.14, MeOH).[7,8] 3,7-Dimethyloct-1-ene-3,6,7-triol 7-*O*-β-D-glucopyranoside (**2**), $[\alpha]_D^{22}$ −13.5° (*c*=2.23, MeOH).[9]

Sammangaoside B (**3**): $[\alpha]_D^{25}$ −35.0° (c=0.53, MeOH).[10)]

Tricalysioside P (**4**): Colorless crystals, mp 192—194 °C. $[\alpha]_D^{22}$ −22.8° (c=1.23, pyridine). IR ν_{max} (film) cm^{-1}: 3367, 2917, 2854, 1699, 1651, 1472, 1456, 1077, 1035. ^{1}H-NMR (pyridine-d_5) δ: 4.96 (1H, d, J=8 Hz, H-1′), 4.52 (1H, dd, J=12, 3 Hz, H-6′a), 4.37 (1H, dd, J=12, 5 Hz, H-6′b), 4.22 (1H, dd, J=9, 9 Hz, H-3′), 4.20 (1H, dd, J=9, 9 Hz, H-4′), 4.01 (1H, dd, J=9, 8 Hz, H-2′), 3.99 (1H, m, H-5′), 3.72 (1H, dd, J=11, 4 Hz, H-1), 3.67 (2H, d, J=8 Hz, H_2-17), 3.52 (1H, dd, J=15, 6 Hz, H-11a), 2.61 (1H, m, H-2a), 2.47 (2H, m, H-2b, 3a), 2.34 (1H, br s, H-14a), 2.23 (2H, m, H-6a, 13), 2.05 (1H, br d, J=14 Hz, H-6b), 2.02 (1H, m, H-11b), 1.97 (1H, br d, J=11 Hz, H-14b), 1.78 (1H, dd, J=13, 10 Hz, H-15a), 1.68 (1H, m, H-12a), 1.60 (1H, br d, J=8 Hz, H-9), 1.53 (1H, m, H-12b), 1.52 (2H, m, H_2-7), 1.51 (3H, s, H_3-20), 1.32 (3H, s, H_3-18), 1.21 (1H, m, H-3b), 1.20 (1H, m, H-16), 1.18 (1H, dd, J=13, 5 Hz, H-15b), 1.06 (1H, br d, J=14 Hz, H-5). ^{13}C-NMR (pyridine-d_5): Table 1. HR-ESI-TOF-MS (positive-ion mode) m/z: 521.2727 $[M+Na]^+$ (Calcd for $C_{26}H_{42}O_9Na$: 521.2721).

Tricalysioside Q (**5**): Colorless crystals, mp 150—152 °C. $[\alpha]_D^{22}$ −8.5° (c=0.94, pyridine). IR ν_{max} (film) cm^{-1}: 3393, 2934, 2872, 1700, 1651, 1513, 1456, 1246, 1160, 1075, 1034. ^{1}H-NMR (pyridine-d_5) δ: 4.92 (1H, d, J=8 Hz, H-1′), 4.51 (1H, dd, J=12, 2 Hz, H-6′a), 4.34 (1H, dd, J=12, 5 Hz, H-6′b), 4.23 (1H, dd, J=9, 9 Hz, H-3′), 4.20 (1H, dd, J=9, 9 Hz, H-4′), 4.20 (2H, s, H_2-17), 4.00 (1H, dd, J=9, 8 Hz, H-2′), 4.00 (1H, s, H-15), 3.95 (1H, m, H-5′), 3.72 (1H, dd, J=11, 4 Hz, H-1), 3.55 (1H, dd, J=15, 6 Hz, H-11a), 2.62 (1H, m, H-2a), 2.49 (1H, br s, H-13), 2.45 (1H, br dd, J=12, 12 Hz, H-2b), 2.41 (1H, br d, J=12 Hz, H-3a), 2.26 (1H, br dd, J=13, 13 Hz, H-6a), 2.12 (1H, br d, J=13 Hz, H-6b), 2.00 (4H, m, H-7a, 7b, 11b, 14a), 1.88 (1H, m, H-12a), 1.75 (1H, d, J=8 Hz, H-9), 1.71 (1H, dd, J=13, 2 Hz, H-14b), 1.65 (1H, m, H-12b), 1.51 (3H, s, H_3-20), 1.29 (3H, s, H_3-18), 1.23 (1H, br s, H-3b), 1.12 (1H, br d, J=13 Hz, H-5). ^{13}C-NMR (pyridine-d_5): Table 1. HR-ESI-TOF-MS (positive-ion mode) m/z: 553.2611 $[M+Na]^+$ (Calcd for $C_{26}H_{42}O_{11}Na$: 553.2619).

Tricalysioside R (**6**): Amorphous powder, $[\alpha]_D^{22}$ −13.9° (c=0.36, pyridine). IR ν_{max} (film) cm^{-1}: 3395, 2933, 2871, 1720, 1450, 1372, 1254, 1166, 1075, 1036. ^{1}H-NMR (pyridine-d_5) δ: 5.13 (1H, s, H-15), 4.92 (1H, d, J=8 Hz, H-1′), 4.53 (1H, dd, J=12, 2 Hz, H-6′a), 4.44 (1H, d, J=11 Hz, H-17a), 4.36 (1H, dd, J=12, 5 Hz, H-6′b), 4.26 (1H, d, J=11 Hz, H-17b), 4.20 (1H, dd, J=9, 9 Hz, H-3′), 4.18 (1H, dd, J=9, 9 Hz, H-4′), 4.00 (1H, dd, J=9, 8 Hz, H-2′), 3.91 (1H, m, H-5′), 3.42 (1H, dd, J=8, 8 Hz, H-3), 2.49 (1H, br s, H-13), 2.18 (3H, s, C<u>H</u>$_3$CO–), 2.15 (1H, dd, J=12, 2 Hz, H-14a), 1.91 (1H, d, J=12 Hz, H-14b), 1.84 (3H, m, H-1a, H_2-2), 1.78 (1H, m, H-12a), 1.71 (2H, m, H_2-7), 1.62 (1H, br d, J=13 Hz, H-6a), 1.51 (2H, br s, H_2-11), 1.48 (1H, m, H-12b), 1.39 (1H, dd, J=13, 4 Hz, H-1b), 1.30 (1H, m, H-6b), 1.23 (1H, br d, J=5 Hz, H-9), 1.19 (3H, s, H_3-18), 1.01 (3H, s, H_3-19), 0.98 (3H, s, H_3-20), 0.80 (1H, d, J=12 Hz, H-5). ^{13}C-NMR (pyridine-d_5): Table 1. HR-ESI-TOF-MS (positive-ion mode) m/z: 565.2992 $[M+Na]^+$ (Calcd for $C_{28}H_{46}O_{10}Na$: 565.2983).

Tricalysioside S (**7**): Amorphous powder, $[\alpha]_D^{22}$ −20.0° (c=0.50, pyridine). IR ν_{max} (film) cm^{-1}: 3384, 2932, 2871, 1730, 1451, 1376, 1253, 1166, 1077, 1032. ^{1}H-NMR (pyridine-d_5) δ: 5.11 (1H, s, H-15), 4.91 (1H, d, J=8 Hz, H-1′), 4.52 (1H, dd, J=12, 2 Hz, H-6′a), 4.43 (1H, d, J=11 Hz, H-17a), 4.34 (1H, dd, J=12, 6 Hz, H-6′b), 4.27 (1H, br dd, J=8, 8 Hz, H-3′), 4.25 (1H, d, J=11 Hz, H-17b), 4.24 (1H, br dd, J=8, 8 Hz, H-4′), 4.00 (1H, br dd, J=8, 8 Hz, H-2′), 3.96 (1H, d, J=11 Hz, H-19a), 3.91 (1H, m, H-5′), 3.64 (1H, d, J=11 Hz, H-19b), 2.48 (1H, br s, H-13), 2.18 (3H, s, C<u>H</u>$_3$CO–), 2.10 (1H, m, H-14a), 1.89 (1H, br d, J=12 Hz, H-14b), 1.80 (4H, m, H_2-3, 7), 1.78 (1H, m, H-12a), 1.76 (2H, m, H_2-6), 1.65 (1H, m, H-1a), 1.51 (1H, m, H-12b), 1.49 (2H, m, H_2-11), 1.38 (2H, m, H_2-2), 1.27 (1H, br d, J=6 Hz, H-9), 1.18 (3H, s, H_3-18), 0.97 (3H, s, H_3-20), 0.97 (1H, overlapped by methyl signal, H-5), 0.72 (1H, ddd, J=13, 13, 3 Hz, H-1b). ^{13}C-NMR (pyridine-d_5): Table 1. HR-ESI-TOF-MS (positive-ion mode) m/z: 565.2977 $[M+Na]^+$ (Calcd for $C_{28}H_{46}O_{10}Na$: 565.2983).

Tricalysioside T (**8**): Amorphous powder, $[\alpha]_D^{22}$ −35.1° (c=0.74, pyridine). IR ν_{max} (film) cm^{-1}: 3360, 2929, 2869, 1650, 1541, 1512, 1457, 1073, 1028. ^{1}H-NMR (pyridine-d_5) δ: 4.85 (1H, d, J=8 Hz, H-1′), 4.56 (1H, dd, J=12, 2 Hz, H-6′a), 4.38 (1H, dd, J=12, 5 Hz, H-6′b), 4.34 (1H, d, J=10 Hz, H-19a), 4.25 (1H, br dd, J=8, 8 Hz, H-3′), 4.22 (1H, br dd, J=8, 8 Hz, H-4′), 4.12 (1H, d, J=12 Hz, H-17a), 4.11 (1H, d, J=12 Hz, H-17b), 4.03 (1H, br dd, J=8, 8 Hz, H-2′), 3.99 (1H, m, H-5′), 3.79 (1H, s, H-15), 3.57 (1H, d, J=10 Hz, H-19b), 2.50 (1H, br s, H-13), 2.05 (1H, d, J=12, 2 Hz, H-14a), 1.95 (1H, m, H-14b), 1.90 (4H, m, H_2-3, 7), 1.80 (3H, m, H_2-7, 12a), 1.72 (1H, m, H-1a), 1.55 (2H, m, H_2-11), 1.51 (1H, m, H-12b), 1.30 (2H, m, H_2-2), 1.19 (3H, s, H_3-18), 1.13 (1H, d, J=5 Hz, H-9), 1.02 (3H, s, H_3-20), 0.93 (1H, d, J=12 Hz, H-5), 0.70 (1H, br dd, J=12, 12 Hz, H-1b). ^{13}C-NMR (pyridine-d_5): Table 1. HR-ESI-TOF-MS (positive-ion mode) m/z: 523.2882 $[M+Na]^+$ (Calcd for $C_{26}H_{44}O_9Na$: 523.2877).

Tricalysioside U (**9**): Colorless crystals, mp 145—148 °C. $[\alpha]_D^{22}$ −17.5° (c=0.80, pyridine). IR ν_{max} (film) cm^{-1}: 3360, 2940, 2872, 1650, 1457, 1390, 1165, 1077, 1026. ^{1}H-NMR (pyridine-d_5) δ: 5.84 (1H, br t, J=6 Hz, H-14), 4.93 (1H, d, J=8 Hz, H-1′), 4.57 (1H, dd, J=12, 3 Hz, H-6′a), 4.41 (1H, dd, J=12, 5 Hz, H-6′b), 4.49 (2H, d, J=6 Hz, H_2-15), 4.27 (1H, dd, J=8, 8 Hz, H-3′), 4.20 (1H, dd, J=8, 8 Hz, H-4′), 4.04 (1H, dd, J=8, 8 Hz, H-2′), 4.00 (1H, m, H-5′), 3.42 (1H, dd, J=13, 4 Hz, H-3), 2.56 (1H, ddd, J=13, 13, 5 Hz, H-12a), 2.25 (1H, dddd, J=13, 13, 13, 4 Hz, H-2a), 2.23 (1H, ddd, J=13, 13, 4 Hz, H-12b), 2.05 (1H, br d, J=12 Hz, H-7a), 1.96 (1H, m, H-11a), 1.87 (1H, m, H-2b), 1.75 (1H, m, H-7b), 1.73 (3H, s, H_3-16), 1.60 (1H, m, H-6a), 1.59 (1H, m, H-1a), 1.40 (1H, m, H-11b), 1.34 (1H, m, H-9), 1.31 (3H, s, H_3-17), 1.30 (3H, s, H_3-19), 1.30 (1H, m, H-6b), 1.11 (1H, ddd, J=13, 13, 3 Hz, H-1b), 1.01 (3H, s, H_3-18), 0.98 (1H, br d, J=11 Hz, H-5), 0.81 (3H, s, H_3-20). ^{13}C-NMR (pyridine-d_5): Table 1. HR-ESI-TOF-MS (positive-ion mode) m/z: 509.3075 $[M+Na]^+$ (Calcd for $C_{26}H_{46}O_8Na$: 509.3084).

Acetylation of Tricalysioside R (6) Tricalysioside R (1.5 mg) was acetylated with five drops each of acetic anhydride and pyridine at 20 °C for 4 h. The reagents were removed with a stream of N_2 to give the pentaacetate (**6b**) (2.1 mg).

Pentaacetate (**6b**): ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.18 (1H, dd, J=9, 9 Hz, H-3′), 5.06 (1H, dd, J=9, 9 Hz, H-4′), 4.96 (1H, dd, J=9, 8 Hz, H-2′), 4.81 (1H, s, H-15), 4.55 (1H, d, J=8 Hz, H-1), 4.44 (1H, dd, J=12, 5 Hz, H-3), 4.25 (1H, dd, J=12, 5 Hz, H-6′a), 4.13 (1H, dd, J=12, 2 Hz, H-6′b), 3.98 (1H, d, J=10 Hz, H-17a), 3.70 (1H, d, J=10 Hz, H-17b), 3.68 (1H, ddd, J=9, 5, 2 Hz, H-5′), 2.87 (1H, br s. H-13), 2.09, 2.08, 2.05, 2.03, 2.01, 1.99 (each 3H, each s, C<u>H</u>$_3$CO– ×6), 1.03 (3H, s, H_3-18), 0.84, 0.83 (each 3H, each s, H_3-19, 20); HR-ESI-TOF-MS (positive-ion mode) m/z: 775.3516 $[M+Na]^+$ (Calcd for $C_{38}H_{56}O_{15}Na$: 775.3511).

Enzymatic Hydrolysis of Sammangaoside B (3) Sammangaoside B (**3**) (8.5 mg) was hydrolyzed with emulsin (10 mg) at 37 °C for 18 h. The concentrated reaction mixture was subjected to silica gel CC (Φ=20 mm, L=15 cm) with elution with $CHCl_3$ (200 ml) and $CHCl_3$–MeOH (19 : 1, 150 ml, 9 : 1, 160 ml, 7 : 3, 300 ml), 12 ml fractions being collected. Sammangaol B (**3a**) was recovered in fractions 27—36 (3.4 mg, 68%) and glucose in fractions 52—60 (2.7 mg, 70%).

Sammangaol B (**3a**): Colorless syrup, $[\alpha]_D^{26}$ −6.5° (c=0.23, MeOH), ^{1}H-NMR (CD_3OD): δ 4.03 (1H, sextet, J=6 Hz, H-9), 3.67 (1H, m, H-3), 2.22 (1H, ddd, J=14, 5, 2 Hz, H-4eq), 1.95 (1H, m, H-7a), 1.71 (1H, ddd, J=12, 5, 2 Hz, H-8a), 1.60 (1H, dd, J=14, 9 Hz, H-4aq), 1.60 (2H, m, H-7b, 8b), 1.48 (1H, ddd, J=11, 4, 2 Hz, H-2eq), 1.34 (3H, s, H_3-13), 1.19 (3H, d, J=6 Hz, H_3-10), 1.17 (1H, overlapped, H-2ax), 1.09 (3H, s, H_3-11), 0.91 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD): δ 70.7 (C-6), 69.1 (C-9), 67.2 (C-5), 64.4 (C-3), 49.1 (C-2), 42.6 (C-4), 36.9 (C-8), 36.7 (C-1), 29.5 (C-11), 28.3 (C-7), 25.9 (C-12), 21.3 (C-10), 21.1 (C-13); HR-ESI-TOF-MS (positive-ion mode) m/z: 251.1623 $[M+Na]^+$ (Calcd for $C_{13}H_{24}O_3Na$: 251.1617). D-glucose, $[\alpha]_D^{25}$ +38.2° (c=0.18, H_2O).

Preparation of (*R*)- and (*S*)-MTPA Esters 3b and 3c from 3a A solution of **3a** (1.6 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (22 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (15 mg) and *N*,*N*-dimethylaminopyridine (DMAP) (11 mg), the mixture being occasionally stirred at 25 °C for 1 h. After the addition of 1 ml each of H_2O and CH_2Cl_2, the solution was successively washed with 5% HCl (1 ml), $NaHCO_3$-saturated H_2O (1 ml), and brine (1 ml). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), and developed with $CHCl_3$–$(CH_3)_2CO$ (19 : 1) and eluted with $CHCl_3$–MeOH (9 : 1)] to furnish the ester, **3b** (0.5 mg, 50%). Through a similar procedure, **3c** (1.0 mg, 28%) was prepared from **3a** (1.6 mg) by use of (*S*)-MTPA (27 mg), EDC (15 mg), and DMAP (11 mg).

Sammangaol B 3,9-di-(*R*)-MTPA Ester (**3b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 7.48—7.53 (4H, m, aromatic protons), 7.36—7.42 (6H, m, aromatic protons), 5.10 (1H, m, H-3), 5.06 (1H, qt, J=6, 6 Hz, H-9), 3.56 (3H, q, J=1 Hz, –OCH_3), 3.52 (3H, q, J=1 Hz, –OCH_3), 2.29 (1H, dd, J=16, 6 Hz, H-4a), 1.71 (1H, dd, J=15, 6 Hz, H-4b), 1.68 (1H, m, H-8a), 1.66 (1H, dd, J=14, 3 Hz, H-2eq), 1.53 (1H, m, H-8b), 1.32 (1H, overlapped, H-2ax), 1.32 (3H, d, J=6 Hz, H_3-10), 1.26 (3H, s, H_3-13), 1.08 (3H, s, H_3-12), 0.97 (3H, s, H_3-11), the locations of H_2-7 signals were obscure due to many overlapping signals; HR-ESI-MS (positive-ion mode) m/z: 683.2408 $[M+Na]^+$ (Calcd for $C_{33}H_{38}O_7F_6Na$: 683.2413).

Sammangaol B 3,9-di-(*S*)-MTPA Ester (**3c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 7.53—7.49 (4H, m, aromatic protons), 7.38—7.42 (6H, m, aromatic protons), 5.12 (1H, m, H-3), 5.06 (1H, qt, J=6, 6 Hz, H-9), 3.52

(3H, q, J=1 Hz, –OCH_3), 3.50 (3H, q, J=1 Hz, –OCH_3), 2.41 (1H, dd, J=15, 6 Hz, H-4a), 1.82 (1H, dd, J=15, 7 Hz, H-4b), 1.71 (1H, m, H-8a), 1.65 (1H, dd, J=13, 4 Hz, H-2eq), 1.53 (1H, m, H-8b), 1.27 (3H, d, J=6 Hz, H_3-10), 1.26 (1H, overlapped, H-2ax), 1.26 (3H, s, H_3-13), 1.02 (3H, s, H_3-12), 0.97 (3H, s, H_3-11), the locations of H_2-7 signals were obscure due to many overlapping signals; HR-ESI-MS (positive-ion mode) *m*/*z*: 683.2427 $[M+Na]^+$ (Calcd for $C_{33}H_{38}O_7F_6Na$: 683.2413).

Analyses of the Sugar Moiety About 2 mg of each tricalysioside P—T (**4**—**8**) was hydrolyzed with 1 N HCl (0.1 ml) at 100 °C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc and the water layers were analyzed by a chiral detector (JASCO OR-2090 plus) with an amino column [Asahipak NH2P-50 4E, CH_3CN–H_2O (80 : 20), 1 ml/min] to give peaks at retention times of *ca*. 13.6 min (positive optical rotation sign). The peaks were identified by co-chromatography with the authentic D-glucose.

Enzymatic Hydrolysis of Tricalysioside U (9) Tricalysioside U (**9**) (21.8 mg) was dissolved in 10% DMSO in H_2O (5 ml), and hydrolyzed with emulsin (20 mg) and crude hesperidinase (20 mg) at 37 °C for 18 h. The concentrated reaction mixture was subjected to silica gel CC (Φ=20 mm, L=20 cm) with elution with $CHCl_3$ (200 ml) and $CHCl_3$–MeOH (19 : 1, 150 ml, 9 : 1, 160 ml, 7 : 3, 300 ml), 12-ml fractions being collected. Tricalysiol U (**9a**) was recovered in fractions 27—36 (7.6 mg, 50%) and glucose in fractions 52—60 (6.2 mg, 90%).

Tricalysiol U (**9a**): Amorphous powder, $[\alpha]_D^{26}$ −14.4° (c=0.51, pyridine), ^{1}H-NMR (pyridine-d_5): δ 5.86 (1H, t, J=7 Hz, H-14), 4.49 (2H, d, J=7 Hz, H_2-15), 3.46 (3H, dd, J=11, 4 Hz, H-3), 2.59 (1H, ddd, J=13, 13, 6 Hz, H-12a), 2.26 (1H, ddd, J=13, 11, 5 Hz, H-2a), 2.07 (1H, br d, J=13 Hz, H-7a), 1.76 (3H, s, H_3-16), 1.37 (3H, s, H_3-17), 1.22 (3H, s, H_3-19), 1.04 (3H, s, H_3-18), 0.89 (3H, s, H_3-20); ^{13}C-NMR (pyridine-d_5): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m*/*z*: 347.2554 $[M+Na]^+$ (Calcd for $C_{20}H_{36}O_3Na$: 347.2562). D-glucose, $[\alpha]_D^{25}$ +44.1° (c=0.41, H_2O).

Preparation of (*R*)- and (*S*)-MTPA Esters 9b and 9c from 9a A solution of **9a** (3.6 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (37 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hyrochloride (EDC) (30 mg) and *N*,*N*-dimethylaminopyridine (DMAP) (25 mg), the mixture being occasionally stirred at 25 °C for 1 h. After the addition of 1 ml each of H_2O and CH_2Cl_2, the solution was successively washed with 5% HCl (1 ml), $NaHCO_3$-saturated H_2O (1 ml), and brine (1 ml). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), and developed with $CHCl_3$–$(CH_3)_2CO$ (19 : 1) and eluted with $CHCl_3$–MeOH (9 : 1)] to furnish the ester, **9b** (3.5 mg, 38%). Through a similar procedure, **9c** (1.0 mg, 11%) was prepared from **9a** (3.6 mg) by use of (*S*)-MTPA (37 mg), EDC (14 mg), and DMAP (29 mg).

Tricalysiol U 3,15-di-(*R*)-MTPA Ester (**9b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 7.55—7.51 (4H, m, aromatic protons), 7.40—7.38 (6H, m, aromatic protons), 5.36 (1H, br d, J=7 Hz, H-14), 4.84 (1H, dd, J=12, 7 Hz, H-15a) , 4.79 (1H, dd, J=12, 7 Hz, H-15b), 4.72 (1H, dd, J=12, 5 Hz, H-3), 3.56 (3H, s, –OCH_3), 3.54 (3H, s, –OCH_3); 1.87 (1H, br dd, J=13. 4 Hz, H-2a), 1.75 (1H, dddd, J=13, 13, 13, 4 Hz, H-2b), 1.72 (3H, s, H_3-16), 1.13 (3H, s, H_3-17), 0.84 (3H, s, H_3-20), 0.82 (3H, s, H_3-19), 0.78 (3H, s, H_3-18); HR-ESI-MS (positive-ion mode) *m*/*z*: 779.3351 $[M+Na]^+$ (Calcd for $C_{40}H_{50}O_7F_6Na$: 779.3352).

Tricalysiol U 3,15-di-(*S*)-MTPA Ester (**9c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ: 7.53—7.51 (4H, m, aromatic protons), 7.42—7.38 (6H, m, aromatic protons), 5.36 (1H, br d, J=7 Hz, H-14), 4.84 (1H, dd, J=12, 7 Hz, H-15a) , 4.78 (1H, dd, J=12, 7 Hz, H-15b), 4.68 (1H, dd, J=12, 5 Hz, H-3), 3.56 (3H, s, –OCH_3), 3.52 (3H, s, –OCH_3), 1.58—1.68 (2H, m, H_2-2), 1.72 (3H, s, H_3-16), 1.13 (3H, s, H_3-17), 0.90 (3H, s, H_3-19), 0.81 (3H, s, H_3-20), 0.79 (3H, s, H_3-18); HR-ESI-MS (positive-ion mode) *m*/*z*: 779.3355 $[M+Na]^+$ (Calcd for $C_{40}H_{50}O_7F_6Na$: 779.3352).

Acknowledgements The authors are grateful for access to the superconducting NMR instrument at the Analytical Center of Molecular Medicine of the Graduate School of Biomedical Sciences, Hiroshima University. This work was supported in part by Grants-in-Aids from the Ministry of Education, Science, Sports, Culture and Technology of Japan and the Ministry of Health, Labour and Welfare of Japan, and Japan Society for the Promotion of Science. Thanks are also due to the Astellas Foundation for Research on Medicinal Resources and the Takeda Science Foundation for financial support.

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