Tricalysionoside A, a Megastigmane Gentiobioside, Sulfatricalysines A—F, and Tricalysiosides X—Z, *ent*-Kaurane Glucosides, from the Leaves of *Tricalysia dubia*

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Further isolation work on the water-soluble fraction of a MeOH extract of *Tricalysia dubia* afforded one new megastigmane gentiobioside, named tricalysionoside A (1), and three sulfates, named sulfatricalysines A—C (2—4). Extensive isolation work on the 1-BuOH-soluble fraction of a MeOH extract of *T. dubia* yielded sulfatricalysines D—F (5—7) and three new *ent*-kaurane glucosides, named tricalysiosides X—Z (8—10). The structures of the new compounds were elucidated by analyses of one- and two-dimensional NMR spectroscopic data. The absolute stereochemistry of tricalysionoside A (1) was established by modified Mosher's method.

Key words *Tricalysia dubia*; Rubiaceae; megastigmane glucoside; sulfate; *ent*-kaurane glucoside

Tricalysia (Syn. *Canthium*), which comprises approximately 50 species, is distributed 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 (Syn. *Canthium dubium* Lindl.) (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 the southern part of Japan.[2] In the course of our study of Okinawa's promising resource plants, constituents of the title plant, *T. dubia*, were investigated. In previous studies, considerable numbers of rearranged *ent*-kaurane glucosides[3] and *ent*-kaurane glucosides[4,5] were isolated from the leaves of *T. dubia* and stems of the same plant also provided some *ent*-kaurane derivatives.[6] Sulfates are frequently found in sugar polymers, such as dermatane sulfate and keratan sulfate. Bile acids are conjugated with sulfate. Compounds are sometimes conjugated with sulfate in a metabolic system. Sulfuric acid esters are also found in plant products.[7—13] However, the occurrence of sulfates in plants is relatively rare. From the leaves of *T. dubia*, one megastigmane glucoside (**1**) and six glucoside sulfates (**2**—**7**) were isolated together with three new *ent*-karurane glucosides (**8**—**10**). This paper deals with their structural elucidation.

Results and Discussion

From the H_2O- and 1-BuOH-soluble fractions of a MeOH extract of the leaves of *T. dubia*, one megastigmane glucoside (**1**), six glucoside sulfates (**2**—**7**), and three *ent*-kaurane glucosides (**8**—**10**) were isolated by a combination of various kinds of chromatography. Their structures were elucidated by spectroscopic analyses and by modified Mosher's method.

Tricalysionoside A (**1**), $[\alpha]_D^{24}$ −16.9, was isolated as an amorphous powder and its elemental composition was determined to be $C_{25}H_{42}O_{13}$ by high-resolution (HR)-electrospray ionization (ESI)-mass spectrometry (MS). In the IR spectrum, absorption bands for hydroxyl groups (3388 cm^{-1}) and a conjugated ketone (1712 cm^{-1}) were observed. The UV spectrum also suggested the presence of a conjugated system (234 nm). In the ^{1}H-NMR spectrum, the signals for two singlet methyls, two doublet methyls (J=6 Hz and J=1 Hz), one olefinic proton, and two anomeric protons [δ_H 4.32 (d, J=8 Hz) and 4.39 (d, J=8 Hz)] were observed. The ^{13}C-NMR spectrum exhibited six signals assignable for a terminal β-glucopyranose and further six signals for the sugar moiety. The remaining 13 signals comprised those of four methyls, three methylenes, one trisubstituted double bond, one oxymethine, two quaternary carbons, and a carbonyl carbon (Table 1). One of the quaternary carbons was expected to possess an oxygen atom from its chemical shift (δ_C 79.4). From the above evidence, **1** was assumed to be a megastigmane derivative with two sugar units. A set of ^{13}C-NMR signals for the sugar moiety showed a close resemblance to that of gentiobiose,[14] and detailed inspection of the one- and two-dimensional NMR spectra established the structure of the aglycone to be 9-*epi*-blumenol B.[15,16] Thus the structure of **1** was expected to be similar to that of blumenol B gentiobioside, namely icariside B_5 6′-*O*-β-D-glucopyranoside (Fig. 1).[17,18] Absolute configuration of glucose was determined to

Table 1. ^{13}C-NMR Spectroscopic Data for Tricalysionoside A (**1**) (CD_3OD, 100 MHz)

C		C	
1	43.1	Glc 1′	104.3
2	51.1	2′	75.3
3	201.1	3′	78.1
4	126.7	4′	71.7
5	171.7	5′	77.1
6	79.4	6′	70.0
7	34.7	Glc 1″	104.9
8	33.1	2″	75.1
9	78.3	3″	78.1
10	22.3	4″	71.6
11	24.5	5″	78.2
12	24.1	6″	62.8
13	22.0		

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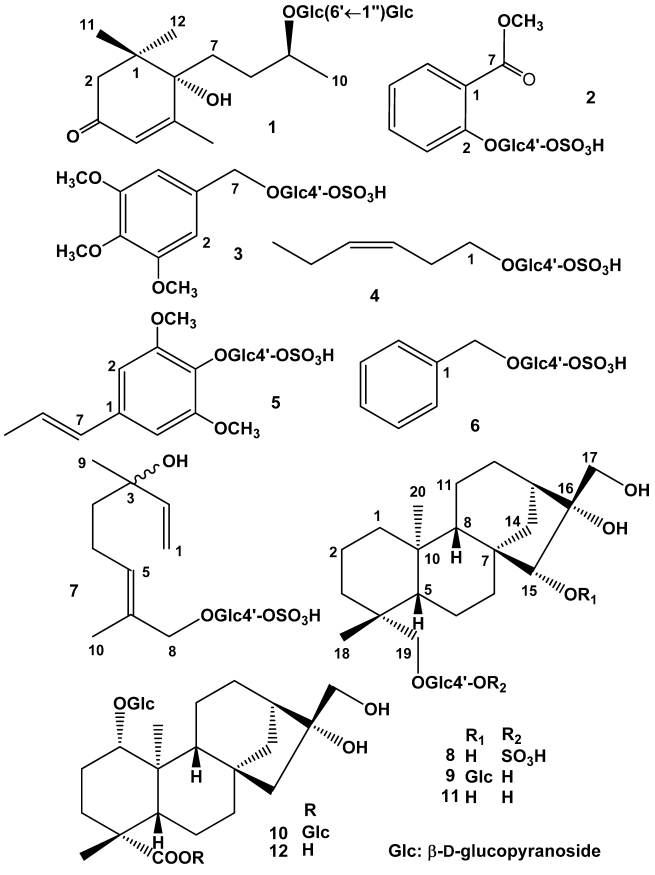

Fig. 1. Structures of Isolated Compounds

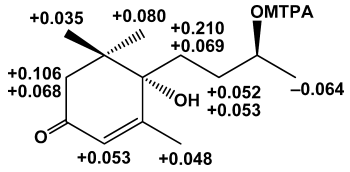

Fig. 2. Results with the Modified Mosher's Method (δ_{S-R})

Table 2. ^{13}C-NMR Spectroscopic Data for Sulfates (**2**—**7**) (CD_3OD, 100 MHz)

C	2	3	4	5	6	7
1	122.6	135.1	70.6	136.4	139.2	112.1
2	158.5	106.6	28.8	105.0	129.32	146.3
3	119.2	154.5	125.9	154.3	129.27	73.8
4	135.2	138.6	134.6	135.3	128.7	43.0
5	123.9	154.5	21.6	154.3	129.27	23.5
6	132.1	106.6	14.6	105.0	129.32	130.3
7	168.6	71.7		132.0	71.9	132.9
8				126.5		76.1
9				18.4		27.7
10						14.1
1′	103.6	102.8	104.1	105.1	103.1	102.9
2′	74.9	75.2	75.1	75.6	75.2	75.1
3′	76.2	76.8	76.8	76.5	76.9	76.9
4′	77.1	77.9	77.9	77.5	77.9	77.9
5′	76.7	76.5	76.3	76.7	76.4	76.3
6′	62.1	62.6	62.4	62.3	62.6	62.6
–OCH_3	52.9					
3,5-OCH_3		56.7		57.0		
4-OCH_3		61.2				

be of the D-series from its optical rotation value and that at the 6-position was confirmed to be *S* from the Cotton effects observed in the circular dichroism (CD) spectrum.[18] The absolute configuration at the 9-position was independently determined to be *S* from the results with the modified Mosher's method[19] applied to the aglycone, tricalysionol A (**1a**) (Fig. 2). This was opposite to that of blumenol B and dihydrovomifoliol β-D-glucopyranoside, and the same as that of icariside B_5.[20]

Sulfatricalysine A (**2**), $[\alpha]_D^{25}$ −23.3, was isolated as an amorphous powder and its elemental composition was determined to be $C_{14}H_{18}O_{11}S$ by HR-ESI-MS. The IR and UV spectra indicated that **2** was a glycosidic compound (3416 cm^{-1}) with an aromatic ring (1600, 1489 cm^{-1} and 283 nm, respectively) and a carbonyl group (1712 cm^{-1}). In the ^{1}H-NMR spectrum, signals for sequentially arranged four aromatic protons, methylene protons in a primary alcohol, an anomeric proton and methoxy protons were observed. The coupling pattern of other protons that might comprise a sugar moiety was exactly the same as that of β-glucopyranose. The ^{13}C-NMR spectrum exhibited six aromatic carbons, one carbonyl, one methoxy carbon, and six carbon signals for the sugar moiety (Table 2). From the above evidence, **2** was expected to be a methyl salicylate glucoside (**2a**) with a sulfate moiety. On acid hydrolysis of **2**, salicylic acid was taken up in the organic layer and the aqueous layer was determined by HPLC to contain D-glucose. Detailed analysis of ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) spectra indicated that the ^{1}H and ^{13}C signals for the 4-position of the glucopyranoside moiety were obviously shifted downfield due to esterification (δ_H of **2a**: 3.46—3.65 and that of **2**: 4.28, and δ_C of **2a**: 71.5 and that of **2**: 77.1).[21] Thus the structure of **2** was elucidated to be methyl salicylate β-D-glucopyranoside 4′-*O*-sulfate, as shown in Fig. 1. There is the possibility that the methyl ester was an artifact. Sulfate (*m*/*z*: 80.9646) and phosphate (*m*/*z*: 80.9742) are both present in living systems and the difference in mass between them on HR-MS is 10 millimass units. From the following observations, the presence of the sulfate was confirmed. At first, in the NMR spectra, no P–O–C or P–O–C–H coupling was observed. Second, when the aqueous layer of the acid hydrolyzate was neutralized with $Ba(OH)_2$, a white precipitate was obtained and this precipitate was not soluble in 2 M HCl.[7] Finally, on negative-ion MS, **2** was found to possess only three exchangeable protons on the addition of D_2O.

Sulfatricalysine B (**3**), $[\alpha]_D^{24}$ −24.9, was isolated as an amorphous powder and its elemental composition was determined to be $C_{16}H_{24}O_{12}S$ by HR-ESI-MS. In the NMR spectrum, the signals for the sugar moiety were essentially the same as those of **2**. The aglycone comprised a symmetrically substituted aromatic ring, three methoxy groups, and a primary alcohol. Since the aromatic protons (δ_H 6.76) showed a correlation cross peak with the methylene carbon (δ_C 71.7) in the heteronuclear multiple bond connectivity (HMBC) spectrum, the structure of the aglycone was elucidated to be a 4,5,6-trimethoxybenzyl alcohol. Therefore the structure of **3** is as shown in Fig. 1.

Sulfatricalysine C (**4**), $[\alpha]_D^{24}$ −4.6, was also isolated as an amorphous powder and its elemental composition was deter-

mined to be $C_{12}H_{22}O_9S$. From the NMR spectroscopic data, the sugar moiety was also expected to be a β-D-glucopyranose 4′-*O*-sulfate and the signals assignable to the aglycone were essentially the same as those reported for (*Z*)-hex-3-en-1-ol.[22] Therefore the structure of **4** was elucidated to be as shown in Fig. 1.

Sulfatricalysine D (**5**), $[\alpha]_D^{24}$ −10.9, was isolated as an amorphous powder and its elemental composition was determined to be $C_{17}H_{24}O_{11}S$. From the NMR spectroscopic data, the sugar moiety was expected to be the same as the preceding sulfates, and the aglycone part comprised a symmetrically substituted aromatic ring, two equivalent methoxy groups, a *trans* double bond, and a terminal methyl group. In the HMBC spectrum, correlation cross peaks between the aromatic protons (δ_H 6.67) and an olefinic carbon (δ_C 132.0) and anomeric proton (δ_H 4.88), and an aromatic carbon (δ_C 135.3) with an oxygen atom were observed. Thus the structure of **5** was elucidated to be 3,5-dimethoxy-1-(*E*)-propenylphenol 4-*O*-β-D-glucopyranoside-4′-*O*-sulfate, as shown in Fig. 1.

Sulfatricalysine E (**6**), $[\alpha]_D^{25}$ −11.8, was an amorphous powder with an elemental composition of $C_{13}H_{18}O_9S$. The NMR spectroscopic data showed that **6** possessed a monosubstituted benzene ring and a primary alcohol (Table 2). Signals assignable to 4′-*O*-sulfated β-D-glucopyranose were also observed and thus the structure of **6** was elucidated to be benzyl alcohol *O*-β-D-glucopyranoside-4′-*O*-sulfate, as shown in Fig. 1.

Sulfatricalysine F (**7**), $[\alpha]_D^{24}$ −3.3, was an amorphous compound with an elemental composition of $C_{16}H_{28}O_{10}S$. As shown by the spectroscopic data, **7** also possessed a *O*-β-D-glucopyranoside-4′-*O*-sulfate moiety in its molecule and the structure of the aglycone was expected to be an acyclic monoterpene. The NMR data for the aglycone section were essentially the same as those for betulabuside A isolated from *Erigeron breviscapus*.[23] Therefore the structure of **7** was betulabuside A 4′-*O*-sulfate, as shown in Fig. 1. The absolute stereochemistries at the 3-positions of betulabuside A and **7** remain to be determined.

Tricalysioside X (**8**), $[\alpha]_D^{24}$ −18.2, was isolated as an amorphous powder and its elemental composition was determined to be $C_{26}H_{44}O_{12}S$ by HR-ESI-MS. ^{13}C-NMR spectroscopic data were essentially the same as those reported for **11**,[5] except for the sugar moiety, whose ^{13}C-NMR data were indistinguishable from those of the preceding sulfates (Table 3). Therefore the structure of **8** was elucidated to be tricalysioside T (**11**) 4′-*O*-sulfate, as shown in Fig. 1.

Tricalysioside Y (**9**), $[\alpha]_D^{23}$ −36.8, was isolated as an amorphous powder and its elemental composition was determined to be $C_{32}H_{54}O_{14}$ by HR-ESI-MS. Of 32 signals observed in the ^{13}C-NMR spectrum, 12 were assignable to two sets of terminal β-glucopyranosides. The remaining 20 signals for the aglycone portion showed strong similarity to those of **11**, except for an obvious downfield shift of C-15 by 11.9 ppm and a slight downfield shift of C-8 by 1.3 ppm (Table 3). In the HMBC spectrum, one (δ_H 5.04) of the anomeric protons showed a significant correlation cross peak with the C-15 (δ_C 94.6) signal. Therefore the structure of **9** was elucidated to be 15-*O*-β-D-glycopyranoside of **11**, as shown in Fig. 1.

Tricalysioside Z (**10**), $[\alpha]_D^{23}$ −8.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{32}H_{52}O_{15}$ by HR-ESI-MS. In the ^{13}C-NMR spectrum, signal for a carbonyl carbon (δ_C 176.8), and similarly to in the case of **9**, 12 signals assignable to two sets of β-glucopyranoses were observed. One of the anomeric carbon signals, however, appeared at δ_C 96.0, which implied that one of the β-glucopyranosyl units was connected to the aglycone through an ester bond. The ^{13}C-NMR spectral signals for the aglycone showed a close resemblance to those of **12**,[4] except for a significant upfield shift of the carbonyl carbon by 3.1 ppm (Table 3). To this carbonyl carbon, the anomeric proton (δ_H 6.21) on δ_C 96.0 was correlated in the HMBC spectrum. Therefore the structure of **10** was elucidated to be tricalysioside H 19-*O*-β-D-glucopyranosyl ester, as shown in Fig. 1.

Table 3. ^{13}C-NMR Spectroscopic Data for Tricalysiosides X—Z (**8**—**10**), and Tricalysiosides T (**11**) and H (**12**) (C_5D_5N, 100 MHz)

C	**8**	**9**[b]	**10**	**11**[c]	**12**[d]
1	40.6 (41.8)[a]	40.5	92.5	40.7	92.6
2	18.7 (19.4)	18.7	28.2	18.7	28.3
3	36.1 (37.6)	36.5	36.5	36.2	36.7
4	38.3 (40.7)	38.4	43.9	38.4	43.6
5	57.1 (58.3)	56.8	56.5	57.1	56.0
6	20.4 (21.0)	20.3	22.5	20.4	22.8
7	37.2 (37.4)	37.4	43.7	37.3	43.7
8	48.0 (48.7)	49.3	45.9	48.0	46.0
9	56.4 (57.5)	56.4	55.6	56.4	55.7
10	39.8 (39.1)	40.0	45.8	39.8	45.8
11	18.8 (19.5)	19.1	21.4	18.9	21.2
12	26.2 (26.8)	25.8	27.3	26.3	27.4
13	43.6 (44.1)	44.2	45.7	43.6	45.7
14	36.8 (36.6)	36.8	38.8	36.8	39.0
15	82.8 (83.6)	94.6	53.6	82.7	53.6
16	81.7 (82.6)	81.7	82.2	81.3	82.1
17	66.1 (66.5)	66.8	66.4	66.2	66.4
18	28.3 (28.4)	28.2	28.7	28.3	29.3
19	73.5 (74.1)	73.1	176.8	73.2	179.9
20	18.4 (18.9)	18.5	12.7	18.4	13.0
1′	105.0 (104.8)	105.4	104.8	105.5	104.6
2′	75.0 (75.2)	75.4	75.5	75.4	75.8
3′	76.8 (77.0)	78.9	79.2	78.8	79.1
4′	77.3 (77.8)	72.0	71.8	71.9	71.8
5′	76.1 (76.0)	78.5	78.5	78.3	78.5
6′	62.0 (62.4)	63.0	62.9	63.0	62.9
1″		106.6	96.0		
2″		75.4	74.1		
3″		78.8	79.3		
4″		72.0	71.2		
5″		78.4	79.0		
6″		62.9	62.3		

a) Data for CD_3OD. *b*) At 150 MHz. *c*) Data from ref. 5. *d*) Data from ref. 4.

It is noteworthy to mention that from the leaves of *T. dubia*, seven sulfated glucosides, sulfatricalysines A—F (**2**—**7**) and tricalysioside X (**8**) were isolated. In these compounds, only the 4-position of glucopyranose was sulfated. Sulfated compounds were rarely found in some plants. Saponins with 2-*O*-sulfated glucopyranoside and 4-*O*-sulfated xylopyranoside have been isolated from *Eclipta alba*[24] and *Mollugo spergula*,[25] respectively. Phenolic compounds with 6-*O*-sulfated glucopyranoside were found in bark of *Bursera simaruba*.[26] In *T. dubia*, highly site-specific sulfotransferase may operate to form 3-*O*-sulfated glucopyranosides.

Experimental

General Optical rotations were measured on a JASCO P-1030 digital

polarimeter. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on JEOL JNM α-400 and ECA-600 spectrometers at 400 or 600 MHz and 100 or 150 MHz, respectively, with tetramethylsilane as internal standard. CD spectra were obtained with a JASCO J-720 spectropolarimeter. Positive-ion HR-ESI-MS was performed with an Applied Biosystems QSTAR XL NanoSpray™ System.

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and ODS open CC on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1.5 l)→(7 : 3, 1.5 l), fractions of 10 g being collected]. The 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 as 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=250 mm, 1.6 ml/min), and the eluate was monitored by UV detector at 254 nm, and a refractive index monitor. Crude hesperidinase was a generous gift from Tanabe Pharmaceutical Co., Ltd. (Tokyo, Japan). The (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-trifluoromethylpheylacetic acids (MTPA) were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

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 The H_2O-soluble fraction (324 g)[3] was subjected to Diaion HP-20 CC (Φ=60 mm, L=60 cm), using H_2O–MeOH (9 : 1, 8 l), (7 : 3, 3 l), (1 : 1, 8 l), and (3 : 7, 8 l), and MeOH (3 l), 21 fractions being collected. The residue (13.0 g in fractions 6—9) of the 30—50% MeOH eluate obtained on HP-20 CC was subjected to silica gel (250 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2 l), and $CHCl_3$–MeOH (99 : 1, 3 l), (97 : 3, 3 l), (19 : 1, 3 l), (37 : 3, 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)], and $CHCl_3$–MeOH–H_2O (70 : 30 : 4, 3 l), 500 ml fractions being collected. The residue (1.88 g) in fractions 35—42 was separated by octadecyl silica (ODS) open CC then the residue (364 mg) in fractions 29—42 was purified by DCCC. This residue (169 mg) was further separated by Sephadex LH-20 CC (Φ=25 mm, L=120 cm, MeOH, 10 g fraction being collected). Compounds **4** (22.5 mg), **3** (10.3 mg), and **2** (45.0 mg) were obtained as precipitates from fractions 51—53, 54—58, and 59—63, respectively. The residue (1.97 g) in fractions 43—52 obtained on silica gel CC was separated by ODS open CC, and the residue (218 mg) in fractions 68—78 was purified by DCCC. This residue (76.7 mg) was separated by Sephadex LH-20 CC to yield 20.0 mg of **1** in fractions 35—37.

Separation of the 1-BuOH-soluble fraction (324 g) with Diaion HP-20 and the residue (55.9 g) by silica gel CC have been described previously.[3] An aliquot (1.79 g) of the residue (5.16 g) in fractions 35—39 was separated by ODS open CC and 17.5 mg of **5** was obtained from fractions 76—81 as a precipitate. An aliquot (2.13 g) of the residue (3.70 g) in fractions 54—59 was separated by ODS open CC and the residue (186 mg) in fractions 31—41 was purified by DCCC. The residue (31.1 mg) in fractions 15–18 was finally purified by HPLC (MeOH–H_2O, 1 : 9) to give 3.9 mg of **6** from the peak at 9 min. The residue (105 mg) obtained on ODS open CC was separated by DCCC, then the residue (58.3 mg) in fractions 12—15 was separated by Sephadex LH-20 CC. The residue (44.8 mg) in fractions 48—53 was purified by HPLC (MeOH–H_2O, 3 : 17) to give 7.1 mg of **7** from the peak at 17 min. The residue (30.1 mg) in fractions 219—223 obtained on ODS open CC was purified by HPLC (MeOH–H_2O, 1 : 1) to afford 3.8 mg of **9** from the peak at 15 min.

The residue (2.24 g) in fractions 60—65 obtained on silica gel CC was separated by ODS open CC, then the residue (82.2 mg) in fractions 116—121 was purified by HPLC (MeOH–H_2O, 2 : 3) to give 7.1 mg of **10** from the peak at 11 min. The residue (116 mg) in fraction 142—155 was purified by DCCC to give 19.7 mg **8** in fractions 10—12.

Tricalysionoside A (**1**): Amorphous powder, $[\alpha]_D^{24}$ −16.9 (c=0.36, MeOH); IR ν_{max} (film) cm^{-1}: 3388, 2926, 2886, 1712, 1634, 1376, 1072, 1043; UV λ_{max} (MeOH) nm (log ε): 250sh (3.57), 234 (3.66); ^{1}H-NMR (CH_3OD, 400 MHz) δ: 5.83 (1H, qd, J=1, 1 Hz, H-4), 4.39 (1H, d, J=8 Hz, H-1″), 4.32 (1H, d, J=8 Hz, H-1′), 4.11 (1H, dd, J=12, 2 Hz, H-6′a), 3.86 (1H, dd, J=12, 2 Hz, H-6″a), 3.86 (1H, overlapped, H-9), 3.79 (1H, dd, J=12, 6 Hz, H-6′b), 3.67 (1H, dd, J=12, 6 Hz, H-6″b), 3.43 (1H, m, H-5′), 3.32 (2H, m, H-4′, 4″), 3.30 (2H, m, H-3′, 3″), 3.28 (1H, m, H-5″), 3.21 (1H, dd, J=9, 8 Hz, H-2″), 3.16 (1H, dd, J=9, 8 Hz, H-2′), 2.65 (1H, d, J=18 Hz, H-2a), 2.14 (1H, dd, J=8, 1 Hz, H-2b), 2.07 (1H, m, H-7a), 2.04 (3H, d, J=1 Hz, H_3-13), 1.85 (1H, m, H-7b), 1.80 (1H, m, H-8a), 1.50 (1H, m, H-8b), 1.24 (3H, d, J=6 Hz, H_3-10), 1.09 (3H, s, H_3-12), 1.02 (3H, s, H_3-11); ^{13}C-NMR (CD_3OD, 100 MHz): Table 1; CD $\Delta\varepsilon$ (nm): +0.65 (326), −2.57 (256), +4.52 (221) (c=4.58×10^{-5} M); HR-ESI-MS (positive-ion mode) m/z: 573.2513 $[M+Na]^+$ (Calcd for $C_{25}H_{42}O_{13}Na$: 573.2517).

Sulfatricalysine A (**2**): Amorphous powder, $[\alpha]_D^{25}$ −23.3 (c=0.49, MeOH); IR ν_{max} (film) cm^{-1}: 3416, 2949, 1712, 1600, 1489, 1451, 1242, 1079, 1046, 986; UV λ_{max} (MeOH) nm (log ε): 283 (3.35), 227 (3.81), 208 (3.94); ^{1}H-NMR (CH_3OD, 400 MHz) δ: 7.76 (1H, dd, J=8, 1 Hz, H-6), 7.54 (1H, ddd, J=8, 8, 1 Hz, H-4), 7.39 (1H, dd, J=8, 1 Hz, H-3), 7.14 (1H, ddd, J=8, 8, 1 Hz, H-5), 4.96 (1H, d, J=8 Hz, H-1′), 4.28 (1H, dd, J=10, 9 Hz, H-4′), 3.94 (1H, dd, J=12, 2 Hz, H-6′a), 3.89 (3H, s, –OCH_3), 3.81 (1H, dd, J=9, 9 Hz, H-3′), 3.82 (1H, dd, J=12, 5 Hz, H-6′b), 3.64 (1H, dd, J=9, 8 Hz, H-2′), 3.61 (1H, ddd, J=10, 5, 2 Hz, H-5′); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; HR-ESI-MS (negative-ion mode) m/z: 393.0494 $[M-H]^-$ (Calcd for $C_{14}H_{17}O_{11}S$: 393.0497); HR-ESI-MS (on addition of D_2O) (negative-ion mode) m/z: 396.0679 $[M-H]^-$ (Calcd for $C_{14}H_{14}D_3O_{13}S$: 396.0685).

Sulfatricalysine B (**3**): Amorphous powder, $[\alpha]_D^{24}$ −24.9 (c=0.37, MeOH); IR ν_{max} (film) cm^{-1}: 3432, 2939, 1592, 1507, 1460, 1422, 1237, 1124, 1079, 984; UV λ_{max} (MeOH) nm (log ε): 230sh (3.80), 211 (4.13); ^{1}H-NMR (CH_3OD, 400 MHz) δ: 6.76 (2H, s, H-2, 6), 4.85 (1H, d, J=12 Hz, H-7a), 4.65 (1H, d, J=12 Hz, H-7b), 4.37 (1H, d, J=8 Hz, H-1′), 4.14 (1H, dd, J=10, 9 Hz, H-4′), 3.93 (1H, dd, J=12, 2 Hz, H-6′a), 3.85 (6H, s, –OCH_3 at C-3, C-5), 3.77 (1H, dd, J=12, 6 Hz, H-6′b), 3.75 (3H, s, –OCH_3 at C-4), 3.66 (1H, dd, J=9, 9 Hz, H-3′), 3.40 (1H, ddd, J=10, 6, 2 Hz, H-5′), 3.37 (1H, dd, J=9, 8 Hz, H-2′); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; HR-ESI-MS (negative-ion mode) m/z: 439.0920 $[M-H]^-$ (Calcd for $C_{16}H_{23}O_{12}S$: 439.0915); HR-ESI-MS (on addition of D_2O) (negative-ion mode) m/z: 442.1105 $[M-H]^-$ (Calcd for $C_{16}H_{20}D_3O_{12}S$: 442.1104).

Sulfatricalysine C (**4**): Amorphous powder, $[\alpha]_D^{24}$ −4.6 (c=0.35, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2931, 16332, 1455, 1419, 1377, 1236, 1076, 1028, 982; ^{1}H-NMR (CH_3OD, 400 MHz) δ: 5.46 (1H, dtd, J=11, 7, 1 Hz, H-4), 5.38 (1H, ddt, J=11, 7, 1 Hz, H-3), 4.31 (1H, d, J=8 Hz, H-1′), 4.14 (1H, dd, J=10, 9 Hz, H-4′), 3.87 (1H, dd, J=12, 2 Hz, H-6′a), 3.86 (1H, dt, J=9, 7 Hz, H-1a), 3.76 (1H, dd, J=12, 5 Hz, H-6′b), 3.66 (1H, dd, J=9, 9 Hz, H-3′), 3.56 (1H, dt, J=9, 7 Hz, H-1b), 3.40 (1H, ddd, J=10, 5, 2 Hz, H-5′), 3.28 (1H, dd, J=9, 8 Hz, H-2′), 2.38 (2H, dddd, J=7, 7, 7, 1 Hz, H_2-2), 2.08 (2H, qdd, J=7, 7, 1 Hz, H_2-5), 0.97 (3H, t, J=7 Hz, H_3-6); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; HR-ESI-MS (negative-ion mode) m/z: 341.0916 $[M-H]^-$ (Calcd for $C_{12}H_{21}O_9S$: 341.0911); HR-ESI-MS (on addition of D_2O) (negative-ion mode) m/z: 344.1105 $[M-H]^-$ (Calcd for $C_{12}H_{18}D_3O_9S$: 442.1104).

Sulfatricalysine D (**5**): Amorphous powder, $[\alpha]_D^{24}$ −10.9 (c=0.19, MeOH); IR ν_{max} (film) cm^{-1}: 3289, 2961, 1580, 1506, 1462, 1414, 1240, 1132, 981; UV λ_{max} (MeOH) nm (log ε): 260 (4.05), 220 (4.25); ^{1}H-NMR (CH_3OD, 400 MHz) δ: 6.67 (2H, s, H-2, 6), 6.34 (1H, dd, J=16, 1 Hz, H-7), 6.22 (1H, dd, J=16, 6 Hz, H-8), 4.88 (1H, d, J=8 Hz, H-1′), 4.26 (1H, dd, J=10, 9 Hz, H-4′), 3.85 (6H, s, –OCH_3 at C-3, C-5), 3.81 (1H, dd, J=12, 2 Hz, H-6′a), 3.75 (1H, dd, J=12, 5 Hz, H-6′b), 3.73 (1H, dd, J=9, 9 Hz, H-3′), 3.59 (1H, dd, J=9, 8 Hz, H-2′), 3.37 (1H, ddd, J=10, 5, 2 Hz, H-5′), 1.86 (3H, dd, J=6, 1 Hz, H_3-9); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; HR-ESI-MS (negative-ion mode) m/z: 435.0969 $[M-H]^-$ (Calcd for $C_{17}H_{23}O_{11}S$: 435.0966); HR-ESI-MS (on addition of D_2O) (negative-ion mode) m/z: 438.1158 $[M-H]^-$ (Calcd for $C_{17}H_{20}D_3O_{11}S$: 438.1154).

Sulfatricalysine E (**6**): Amorphous powder, $[\alpha]_D^{25}$ −11.8 (c=0.22, MeOH); IR ν_{max} (film) cm^{-1}: 3407, 2926, 1617, 1454, 1416, 1236, 1077, 1026, 981; UV λ_{max} (MeOH) nm (log ε): 209 (3.59); ^{1}H-NMR (CH_3OD, 400 MHz) δ: 7.42 (2H, br d, J=7 Hz, H-2, 6), 7.32 (2H, br dd, J=7, 7 Hz, H-3, 5), 7.27 (1H, dr dd, J=7, 7 Hz, H-4), 4.93 (1H, d, J=12 Hz, H-7a), 4.67 (1H, d, J=12 Hz, H-7b), 4.39 (1H, d, J=9 Hz, H-1′), 4.14 (1H, dd, J=10, 9 Hz, H-4′), 3.93 (1H, dd, J=12, 2 Hz, H-6′a), 3.77 (1H, dd, J=12, 6 Hz, H-6′b), 3.65 (1H, dd, J=9, 9 Hz, H-3′), 3.40 (1H, ddd, J=10, 6, 2 Hz, H-5′), 3.36 (1H, dd, J=9, 8 Hz, H-2′); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; HR-ESI-MS (negative-ion mode) m/z: 349.0604 $[M-H]^-$ (Calcd for $C_{13}H_{17}O_9S$: 349.0598); HR-ESI-MS (on addition of D_2O) (negative-ion mode) m/z: 352.0791 $[M-H]^-$ (Calcd for $C_{13}H_{14}D_3O_9S$: 352.0787).

Sulfatricalysine F (**7**): Amorphous powder, $[\alpha]_D^{24}$ −3.3 (c=0.49); IR ν_{max} (film) cm^{-1}: 3398, 2927, 1639, 1410, 1373, 1239, 1077, 984; ^{1}H-NMR (CH_3OD, 400 MHz) δ: 5.91 (1H, dd, J=17, 11 Hz, H-2), 5.48 (1H, ddd, J=7, 7, 1 Hz, H-6), 5.20 (1H, dd, J=17, 2 Hz, H-1a), 5.03 (1H, dd, J=11,

2 Hz, H-1b), 4.27 (1H, d, J=8 Hz, H-1′), 4.21 (1H, dd, J=12, 1 Hz, H-8a), 4.12 (1H, dd, J=10, 9 Hz, H-4′), 4.04 (1H, dd, J=12, 1 Hz, H-8b), 3.89 (1H, dd, J=12, 2 Hz, H-6′a), 3.75 (1H, dd, J=12, 6 Hz, H-6′b), 3.64 (1H, dd, J=9, 9 Hz, H-3′), 3.36 (1H, ddd, J=10, 6, 2 Hz, H-5′), 3.30 (1H, dd, J=9, 8 Hz, H-2′), 2.09 (2H, m, H_2-5), 1.68 (3H, d, J=1 Hz, H_3-10), 1.55 (2H, m, H_2-4), 1.25 (3H, s, H_3-9); ^{13}C-NMR (CD_3OD, 100 MHz): Table 2; HR-ESI-MS (negative-ion mode) m/z: 411.1331 $[M-H]^-$ (Calcd for $C_{16}H_{27}O_{10}S$: 411.1330); HR-ESI-MS (negative-ion mode) m/z: 415.1578 $[M-H]^-$ (Calcd for $C_{16}H_{23}D_4O_{10}S$: 415.1581).

Tricalysioside X (**8**): Amorphous powder, $[\alpha]_D^{24}$ −18.2 (c=0.28, pyridine); IR ν_{max} (film) cm^{-1}: 3354, 2930, 2871, 1634, 1443, 1375, 1242, 1071, 1024, 983; ^{1}H-NMR (C_5D_5N, 400 MHz) δ: 4.72 (1H, d, J=8 Hz, H-1′), 4.52 (1H, dd, J=12, 3 Hz, H-6′a), 4.45 (1H, dd, J=8, 8 Hz, H-3′), 4.44 (1H, dd, J=8, 8 Hz, H-4′), 4.43 (1H, dd, J=12, 5 Hz, H-6′b), 4.22 (1H, d, J=10 Hz, H-19a), 4.12 (2H, s, H_2-17), 4.04 (1H, dd, J=8, 8 Hz, H-2′), 3.85 (1H, m, H-5′), 3.83 (1H, s, H-15), 3.53 (1H, d, J=10 Hz, H-19b), 2.50 (1H, br s, H-13), 2.00 (1H, m, H-14a), 1.99 (1H, m, H-7a), 1.95 (1H, m, H-6a), 1.90 (1H, m, H-3a), 1.79 (2H, m, H-6b and 14b), 1.76 (1H, m, H-12a), 1.70 (1H, m, H-7b), 1.69 (1H, d, J=12 Hz, H-1a), 1.52 (1H, m, H-12b), 1.51 (2H, m, H_2-11), 1.30 (2H, m, H_2-2), 1.14 (3H, s, H_3-18), 1.11 (1H, br d, J=6 Hz, H-9), 1.10 (1H, m, H-3b), 1.00 (3H, s, H_3-20), 0.91 (1H, d, J=12 Hz, H-5), 0.67 (1H, br dd, J=12, 12 Hz, H-1b); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 4.24 (1H, d, J=8 Hz, H-1′), 4.17 (1H, dd, J=10, 10 Hz, H-4′), 4.05 (1H, d, J=10 Hz, H-19a), 3.89 (1H, dd, J=12, 2 Hz, H-6′a), 3.79 (1H, dd, J=12, 5 Hz, H-6′b), 3.67 (1H, d, J=11 Hz, H-17a), 3.66 (1H, dd, J=10, 9 Hz, H-3′), 3.65 (1H, d, J=11 Hz, H-17b), 3.44 (1H, br s, H-15), 3.40 (1H, ddd, J=10, 5, 2 Hz, H-5′), 3.31 (1H, d, J=10 Hz, H-19b), 3.30 (1H, dd, J=9, 8 Hz, H-2′), 2.10 (1H, br s, H-13), 1.86 (1H, d, J=12 Hz, H-1a), 1.86 (1H, m, H-3a), 1.85 (1H, d, J=10 Hz, H-14a), 1.74 (1H, m, H-6a), 1.64 (2H, m, H-2a, 11a), 1.60 (3H, m, H-3b, 7a, 14b), 1.57 (2H, m, H_2-12), 1.38 (4H, m, H-2b, 6b, 7b, 11b), 1.10 (1H, br d, J=9 Hz, H-9), 1.06 (3H, s, H_3-20), 1.02 (3H, s, H_3-18), 0.95 (1H, br d, J=11 Hz, H-5), 0.81 (1H, br dd, J=12, 12 Hz, H-1b); ^{13}C-NMR (C_5D_5N and CD_3OD, 100 MHz): Table 3; HR-ESI-MS (negative-ion mode) m/z: 579.2484 $[M-H]^-$ (Calcd for $C_{26}H_{43}O_{12}S$: 579.2480); HR-ESI-MS (on addition of D_2O) (negative-ion mode) m/z: 585.2853 $[M-H]^-$ (Calcd for $C_{26}H_{37}D_6O_{12}S$: 585.2857).

Tricalysioside Y (**9**): Amorphous powder, $[\alpha]_D^{23}$ −36.8 (c=0.21, pyridine); IR ν_{max} (film) cm^{-1}: 3367, 2929, 2872, 1446, 1415, 1074, 1030; ^{1}H-NMR (C_5D_5N, 600 MHz) δ: 5.04 (1H, d, J=8 Hz, H-1″), 4.84 (1H, d, J=8 Hz, H-1′), 4.58 (1H, dd, J=11, 2 Hz, H-6″a), 4.55 (1H, dd, J=11, 2 Hz, H-6′a), 4.38 (1H, dd, J=11, 5 Hz, H-6′b), 4.33 (1H, d, J=10 Hz, H-19a), 4.25 (2H, br dd, J=8, 8 Hz, H-3′ and 3″), 4.23 (1H, d, J=12 Hz, H-17a), 4.22 (1H, dd, J=8, 8 Hz, H-4′), 4.20 (1H, dd, J=8, 8 Hz, H-4″), 4.16 (1H, dd, J=11, 6 Hz, H-6″b), 4.08 (1H, d, J=12 Hz, H-17b), 4.03 (2H, br dd, J=8, 8 Hz, H-2′, 2″), 4.02 (1H, m, H-5′), 3.98 (1H, s, H-15), 3.96 (1H, m, H-5″), 3.51 (1H, d, J =10 Hz, H-19b), 2.40 (1H, br s, H-13), 2.08 (1H, m, H-14a), 2.07 (1H, m, H-7a), 2.06 (1H, m, H-3a), 2.03 (1H, m, H-7b), 1.88 (1H, d, J=11 Hz, H-14b), 1.73 (1H, m, H-12a), 1.68 (1H, d, J=12 Hz, H-1a), 1.68 (1H, m, H-6a), 1.52 (2H, br s, H_2-11), 1.52 (1H, m, H-12b), 1.40 (1H, m, H-6b), 1.32 (2H, m, H_2-2), 1.26 (1H, br s, H-9), 1.10 (3H, s, H_3-18), 0.98 (3H, s, H_3-20), 0.97 (1H, br d, J=12 Hz, H-5), 0.91 (1H, m, H-3b), 0.71 (1H, br dd, J=12, 12 Hz, H-1b); HR-ESI-MS (positive-ion mode) m/z: 685.3397 $[M+Na]^+$ (Calcd for $C_{32}H_{54}O_{14}Na$: 685.3405).

Tricalysioside Z (**10**): Amorphous powder, $[\alpha]_D^{23}$ −8.0 (c=0.50, pyridine); IR ν_{max} (film) cm^{-1}: 3367, 2928, 1729, 1593, 1446, 1371, 1073, 1025; ^{1}H-NMR (C_5D_5N, 600 MHz) δ: 6.21 (1H, d, J=8 Hz, H-1″), 4.97 (1H, d, J=8 Hz, H-1′), 4.52 (1H, dd, J=12, 3 Hz, H-6′a), 4.42 (1H, dd, J=12, 3 Hz, H-6″a), 4.38 (1H, dd, J=12, 5 Hz, H-6′b), 4.34 (1H, dd, J=12, 5 Hz, H-6″b), 4.30 (1H, dd, J=9, 9 Hz, H-4″), 4.23 (1H, br dd, J=8, 8 Hz, H-3′, 3″), 4.21 (1H, dd, J=8, 8 Hz, H-4′), 4.17 (1H, dd, J=8, 8 Hz, H-2″), 4.10 (1H, d, J=11 Hz, H-17a), 4.00 (1H, dd, J=8, 8 Hz, H-2′), 4.00 (1H, m, H-5′, 5″), 3.98 (1H, d, J=11 Hz, H-17b), 3.72 (1H, dd, J=12, 4 Hz, H-1), 3.54 (1H, dd, J=16, 7 Hz, H-11a), 2.64 (1H, m, H-2a), 2.55 (1H, ddd, J=14, 14, 4 Hz, H-6a), 2.44 (1H, m, H-2b), 2.36 (1H, m, H-3a), 2.34 (1H, br s, H-13), 2.29 (1H, d, J=13 Hz, H-14a), 2.10 (1H, m, H-11b), 2.05 (1H, m, H-14b), 2.01 (1H, dd, J=14, 2 Hz, H-6b), 1.91 (1H, m, H-12a), 1.85 (2H, s, H_2-15), 1.79 (1H, br d, J=14 Hz, H-7a), 1.65 (3H, s, H_3-20), 1.62 (1H, br d, J=9 Hz, H-9), 1.61 (1H, m, H-12b), 1.52 (1H, m, H-7b), 1.25 (3H, s, H_3-18), 1.19 (1H, ddd, J=14, 14, 4 Hz, H-3b), 1.11 (1H, br d, J=14 Hz, H-5); HR-ESI-MS (positive-ion mode) m/z: 699.3194 $[M+Na]^+$ (Calcd for $C_{32}H_{52}O_{15}Na$: 699.3198).

Enzymatic Hydrolysis of Tricalysionoside A (1) Tricalysionoside A (**1**) (11.6 mg) in 2 ml of H_2O was hydrolyzed with emulsin (19.8 mg) and crude hesperidinase (6.0 mg) at 37 °C for 15 h. The reaction mixture was evaporated to dryness, then the methanolic solution was absorbed on silica gel and subjected to silica gel CC (20 g, Φ=18 mm, L=18 cm) with $CHCl_3$ (150 ml) and $CHCl_3$–MeOH (19 : 1, 150 ml, 9 : 1, 150 ml, 17 : 3, 150 ml and 7 : 3, 450 ml), 12-ml fractions being collected. An aglycone, tricalysionol A (**1a**) (1.4 mg), and D-glucose (6.0 mg) were recovered in fractions 23—28 and 54—62, respectively.

Tricalysionol A (**1a**): Amorphous powder, $[\alpha]_D^{24}$ +61.7 (c=0.12, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.83 (1H, dq, J=1, 1 Hz, H-4), 3.66 (1H, dqd, J=8, 6, 5 Hz, H-9), 2.59 (1H, d, J=18 Hz, H-2a), 2.16 (1H, d, J=18 Hz, H-2b), 2.04 (3H, d, J=1 Hz, H_3-13), 1.98 (1H, ddd, J=13, 13, 5 Hz, H-7a), 1.79 (1H, ddd, J=13, 13 4, Hz, H-7b), 1.68 (1H, dddd, J=13, 13, 4, 4 Hz, H-8a), 1.39 (1H, dddd, J=13, 13, 8, 5 Hz, H-8b), 1.16 (3H, d, J=6 Hz, H_3-10), 1.09 (3H, s, H_3-12), 1.02 (3H, s, H_3-11); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 201.0 (C-3), 171.8 (C5), 126.7 (C-4), 79.2 (C-6), 69.3 (C-9), 51.2 (C-2), 43.0 (C-1), 35.7 (C-7), 35.4 (C-8), 24.5 (C-11), 24.1 (C-12), 23.7 (C-10), 21.8 (C-13); HR-ESI-MS (positive-ion mode) m/z: 249.1458 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_3Na$: 249.1461). D-Glucose: $[\alpha]_D^{23}$ +34.1 (c=0.25, H_2O, 24 h after being dissolved in the solvent).

Preparation of (*R*)- and (*S*)-MTPA Esters (1b and 1c, Respectively) from 1a A solution of **1a** (0.7 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (48 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardodiimide hydrochloride (EDC) (13 mg) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP) (9 mg), then the mixture was occasionally stirred at 25 °C for 30 min. After the addition of 1 ml of CH_2Cl_2, the solution was washed with H_2O (1 ml), 5% HCl (1 ml), $NaHCO_3$–saturated H_2O, then brine (1 ml), successively. The organic layer was dried over Na_2SO_4 then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 18 cm, with development with $CHCl_3$–$(CH_3)_2CO$ (19 : 1) for 9 cm, then elution with $CHCl_3$–MeOH (9 : 1)] to furnish an ester, **1b** (0.5 mg). Through a similar procedure, **1c** (0.5 mg) was prepared from **1a** (0.7 mg) using (*S*)-MTPA (25 mg), EDC (14 mg), and 4-DMAP (11 mg).

Tricalysionol A 9-*O*-(*R*)-MTPA ester (**1b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.53—7.50 (2H, m, aromatic protons), 7.41—7.36 (3H, m, aromatic protons), 5.80 (1H, qd, J=1, 1 Hz, H-4), 5.13 (1H, m, H-9), 3.55 (3H, q, J=1 Hz, –OCH_3), 2.27 (1H, d, J=18 Hz, H-2a), 2.15 (1H, d, J=18 Hz, H-2b), 1.93 (3H, d, J=1 Hz, H_3-13), 1.84 (1H, m, H-7a), 1.82 (1H, m, H-8a), 1.59 (1H, m, H-8b), 1.56 (1H, m, H-7b), 1.35 (3H, d, J=6 Hz, H_3-10), 1.00 (3H, s, H_3-11), 0.95 (3H, s, H_3-12); HR-ESI-MS m/z: 465.1863 $[M+Na]^+$ (Calcd for $C_{23}H_{29}O_5F_3Na$: 465.1859). Tricalysionol A 9-*O*-(*S*)-MTPA ester (**1c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.51—7.49 (2H, m, aromatic protons), 7.42—7.36 (3H, m, aromatic protons), 5.85 (1H, qd, J=1, 1 Hz, H-4), 5.15 (1H, m, H-9), 3.49 (3H, q, J=1 Hz, –OCH_3), 2.38 (1H, d, J=18 Hz, H-2a), 2.21 (1H, d, J=18 Hz, H-2b), 1.98 (3H, d, J=1 Hz, H_3-13), 1.91 (1H, m, H-7a), 1.88 (1H, m, H-8a), 1.77 (1H, m, H-7b), 1.64 (1H, m, H-8b), 1.29 (3H, d, J=6 Hz, H_3-10), 1.034 (3H, s, H_3-11), 1.029 (3H, s, H_3-12); HR-ESI-MS m/z: 465.1850 $[M+Na]^+$ (Calcd for $C_{23}H_{29}O_5F_3Na$: 465.1859).

Acid Hydrolysis of Sulfatricalysine A (2) Sulfatricalysine A (**2**) (12.1 mg) was hydrolyzed in 1 ml of 2 M HCl at 60 °C for 3 h. After cooling, the reaction mixture was extracted with 1.5 ml of $CHCl_3$ three times to give 1.1 mg of salicylicacid (**2a**). The aqueous layer was neutralized by addition of $Ba(OH)_2$ solution to give 4.1 mg of $BaSO_4$ as a precipitate. The precipitate was not soluble in 2 M HCl.

Salicylic acid (**2a**): Amorphous powder; IR ν_{max} (film) cm^{-1}: 3192, 3065, 2926, 2854, 1663, 1610, 1485, 1464, 1241, 1217; UV λ_{max} (MeOH) nm (log ε): 299 (3.26), 232sh (3.52) 209 (3.77); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 10.5 (1H, br s, –OH), 7.91 (1H, dd, J=8, 1 Hz, H-6), 7.51 (1H, ddd, J=8, 8, 2 Hz, H-5), 7.01 (1H, dd, J=8, 1 Hz, H-3), 6.92 (1H, ddd, J=8, 8, 2 Hz, H-4); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.8 (C-7), 162.2 (C-2), 137.0 (C-4), 130.9 (C-6), 119.6 (C-5), 117.8 (C-3), 111.3 (C-1); HR-ESI-MS (negative-ion mode) m/z: 137.0237 $[M-H]^-$ (Calcd for $C_7H_5O_3$: 137.0233).

Sugar Analysis All the glucosides (**3**—**10**) (500 mg each) were hydrolyzed with 100 ml of 1 M HCl at 90 °C for 2 h. The reaction mixtures were washed with 100 ml of EtOAc then the aqueous layers were analyzed by HPLC with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak NH2P-50 (4.6 mm×250 mm), CH_3CN–H_2O (3 : 1), 1 ml/min] to give peaks at 9.0 min with positive optical rotation signs. The peaks were identified by co-chromatography with authentic D-glucose. Neutralized aqueous layer of hydrolysate of **2**, obtained in the above experiment, was treated with Amberlite MB-3 then sugar was also analyzed to be D-glucose.

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