Tricalysiosides A−G: Rearranged *ent*-Kauranoid Glycosides from the Leaves of *Tricalysia dubia*

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Seven rearranged *ent*-kaurane glycosides, named tricalysiosides A−G (**1**−**7**), were isolated from the leaves of *Tricalysia dubia* collected on Okinawa Island. Their C-18 and 19 methyls were found to have rearranged to form an α,*β*-unsaturated *γ*-lactone ring, with other functional groups remotely located only on C-15, -16, and -17 of the five-membered ring. Information available from various kinds of two-dimensional NMR spectra was limited and insufficient to allow total characterization of the structures. Finally, using X-ray crystallographic analysis, the structure of tricalysioside A (**1**) was determined to be 4-*nor*-18-*homo*-*ent*-kauran-4(18)-en-15*β*,16*β*,17-triol-3*β*,19-olide 16-*O*-*β*-D-glucopyranoside (**1**). On the basis of the crystal structure of **1**, the structures of the other tricalysiosides (**2**−**7**) were also established.

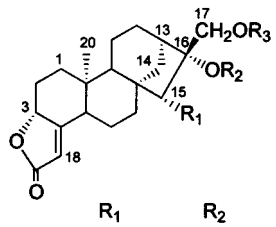
Tricalysia (Syn. *Canthium*), which comprises approximately 50 species, is distributed in subtropical and tropical areas of Asia and Africa.[1] Some species are used for medicinal purposes as folk medicines; for example, the roots, leaves, and stem bark of *Canthium subcordatum* are used for medicinal purposes in Africa.[2] Recently the chemical and biological active components of some of these species have been investigated and reported.[3] By means of bioassay-guided fractionation, several quinic acid derivatives exhibiting growth-retarding activity toward neonatal larvae of *Spodoptera littoralis* were isolated from *Canthium schimperianum*.

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.[1] In the course of our study of Okinawa's promising resource plants, constituents of *T. dubia* were investigated. Seven rearranged *ent*-kaurane glycosides were isolated, and this paper deals with their structural elucidation.

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. A *n*-BuOH-soluble fraction was separated by column chromatography on a highly porous synthetic resin (Diaion HP-20), normal and reversed-phase silica gels, and by liquid−liquid partition chromatography to afford seven diterpenoid glycosides, named tricalysiosides A−G (**1**−**7**). The structure of tricalysioside A (**1**) was first solved by X-ray crystallographic analysis. The structures of tricalysiosides B−G (**2**−**7**) were then elucidated from spectroscopic evidence.

Tricalysioside A (**1**) was isolated as colorless needles, whose elemental composition was determined to be



	R_1	R_2	R_3
1	OH	Glc	H
1a	OAc	Glc(OAc)$_4$	Ac
1b	OH	H	H
2	H	Glc	H
3	H	H	Glc
4	H	Glc(6')Api	H
5	H	Glc(6')Glc	H
6	OAc	Glc	H
7	OAc	H	Glc

Glc: β-D-glucopyranoside
Api: β-D-apiofuranoside

$C_{26}H_{38}O_{10}$ from the observation of a quasi-molecular ion peak ([M − H]$^-$) on negative-ion HRFAB mass spectrometry. The IR spectrum indicated that **1** was a glycosidic compound (3529−3311 cm^{-1}) and possessed a ketone function (1753 cm^{-1}). In the ^{13}C NMR spectrum, a total of 26 carbon signals were observed, with six assignable to the terminal *β*-glucopyranoside. From the remaining signals, **1** was expected to be a diterpenoid derivative with the following carbons: one methyl, eight methylenes, one of which was substituted by an oxygen function, five methines, two of which carried oxygen atoms, one trisubstituted double bond with highly shielded (δ_C 111.4, d) and deshielded (δ_C 175.1, s) signals, one carbonyl, and three quaternary carbons with one oxygen atom. On acetylation of **1**, hexaacetate (**1a**) was obtained. Thus, at least two hydroxyl groups were present other than those on glucose. Even with further extensive analyses involving two-dimensional NMR spectroscopy including ^{1}H−^{1}H COSY, HSQC, HMBC, and phase-sensitive NOESY, it was an arduous task to assemble the whole structure with the available spectroscopic information. From the HMBC spectrum, the position of the *β*-glucopyranose moiety was

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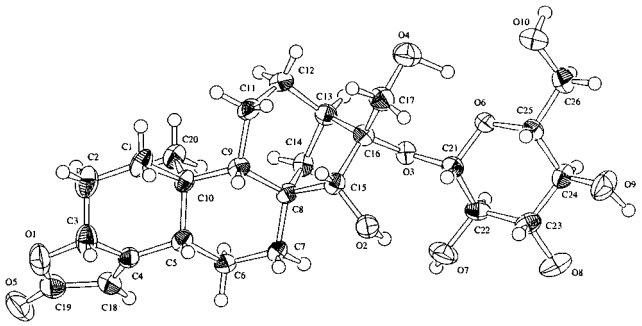

Figure 1. Perspective ORTEP drawing of tricalysioside A (**1**). The sugar carbons have crystallographic numbering.

deduced to be on the tertiary alcohol of the carbon atom which appeared at δ_C 90.0 (s). Therefore, X-ray crystallographic analysis was attempted using a suitable crystal. Figure 1 shows a perspective drawing of tricalysioside A (**1**). The crystallographic results showed the functional groups were located only on rings A and D. This explains the lack of information on NMR correlations between these rings. The structure obtained has a relatively rare rearranged kauranoid skeleton. Since acid hydrolysis gave D-glucose, the aglycone (**1b**) was found to be in an enantio form. Therefore, tricalysioside A (**1**) was elucidated to be 4-*nor*-18-*homo*-*ent*-kauran-4(18)-en-15β,16β,17-triol-19,3β-olide 16-*O*-β-D-glucopyranoside.

Tricalysioside B (**2**) was isolated as colorless crystals with an elemental composition of $C_{26}H_{38}O_9$, which is one oxygen atom less than **1**. Signals for an α,β-unsaturated γ-lactone were observed in the ^{1}H and ^{13}C NMR spectra, and one of the methine groups with an oxygen atom [C-15, δ_C 84.6 with δ_H 3.72 (s)] must be reduced to a methylene carbon [δ_C 52.1 with δ_H 1.73 (d, J = 14 Hz) and 2.08 (d, J = 14 Hz)]. The HMBC spectrum indicated that the sugar moiety was also at the tertiary alcohol on C-16, and the significant correlations of the methylene carbon (C-15, δ_C 52.1) with H-9 [δ_H 1.11 (d, J = 8 Hz)], H-14a [δ_H 1.80 (d, J = 14 Hz)], H-17a [δ_H 4.06 (d, J = 14 Hz)], and H-17b [δ_H 4.13 (d, J = 14 Hz)] supported the structure. Thus, the structure of **2** was elucidated to be as shown.

Tricalysioside C (**3**) was isolated as colorless crystals, and its elemental composition was the same as that of **2**. Signals for an α,β-unsaurated γ-lactone were observed in the ^{13}C NMR spectrum and the carbon signal with the tertiary alcohol shifted upfield from δ_C 90.1 to 80.7, whereas that with the primary alcohol shifted downfield from δ_C 63.1 to 75.5 (Table 1). Thus, **3** is a positional isomer of **2** with respect to the glucose moiety as shown.

Spectroscopic analyses of tricalysiosides D (**4**) and E (**5**) indicated that these compounds were analogous to tricalysioide B (**2**), except for the presence of 6-substituted β-glucopyranoses and additional sugars such as terminal β-apiofuranose and β-glucopyranose, respectively. Other NMR data for **4** and **5** were essentially superimposable to those of **2**. The linkages of these sugars to the hydroxyl groups at the C-6′ positions were confirmed by the HMBC spectra.

Tricalysioside F (**6**) was isolated as colorless crystals, and its elemental composition was $C_{28}H_{40}O_{11}$. In the NMR spectra, a singlet methyl proton signal at δ_H 2.21 (3H) and two additional carbon signals at δ_C 21.1 (q) and 171.1 (s) were observed. These were expected to arise from the acetyl group. The position of the acetyl group was expected to be the hydroxyl group at C-15, since in the ^{1}H NMR spectrum, a significant downfield shift of the H-15 signal (δ_H 5.24) was observed, compared with that of **1** (δ_H 3.72), and H-15 crossed δ_C 171.1 in the HMBC spectrum. The sugar position was the same as that in **1**. Therefore, the structure of **6** was elucidated to be as shown.

Table 1. ^{13}C NMR Data (δ) for Tricalysiosides A−G (**1**−**7**) and the Aglycone (**1b**) of **1**

	1	**1b**	**2**	**3**	**4**	**5**	**6**	**7**
1	35.9	36.0	35.9	35.9	35.9	35.9	35.8	35.8
2	29.9	29.9	29.9	29.9	29.9	29.9	29.8	29.8
3	81.4	81.4	81.4	81.4	81.4	81.4	81.3	81.3
4	175.1	175.2	175.0	175.0	175.1	175.0	174.7	174.7
5	48.7	48.9	48.7	48.7	48.7	48.7	48.4	48.4
6	21.3	21.4	22.0	22.0	22.0	22.0	21.0	21.1
7	34.7	34.7	39.9	40.0	39.9	39.9	33.5	33.7
8	47.8	47.8	44.5	44.4	44.6	44.7	47.4	47.3
9	52.5	52.6	53.4	53.4	53.5	53.4	51.8	52.0
10	43.0	43.1	42.9	42.9	43.0	42.9	42.9	42.9
11	19.4	19.7	19.4	19.3	19.5	19.3	19.6	19.5
12	25.4	25.8	25.6	26.3	25.6	25.7	25.0	25.4
13	42.5	43.6	42.9	46.2	43.1	43.6	41.7	44.7
14	36.1	36.5	37.5	37.8	37.5	37.7	36.6	37.0
15	84.6	82.6	52.1	53.2	51.9	51.0	84.4	83.7
16	90.0	81.3	90.1	80.7	90.2	90.3	88.8	81.1
17	62.2	66.2	63.2	75.5	63.0	62.9	63.3	75.2
18	111.4	111.5	111.4	111.4	111.4	111.4	111.5	111.6
19	173.7	173.7	173.6	173.7	173.7	173.6	173.6	173.6
20	14.8	14.9	14.7	14.7	14.7	14.7	14.8	14.8
1′	98.8		98.6	106.6	98.4	98.4	98.8	106.0
2′	75.7		75.4	75.5	75.3	75.5	75.8	75.5
3′	78.8		78.9	78.6	78.9	78.8	78.9	78.6
4′	71.9		71.8	71.8	72.1	72.0	71.8	71.7
5′	78.5		78.4	78.7	77.1	77.0	78.4	78.4
6′	62.7		62.9	62.9	69.2	69.9	62.9	62.8
1″					111.1	104.8		
2″					77.9	75.1		
3″					80.5	78.5		
4″					75.1	71.7		
5″					65.9	78.2		
6″						62.8		
*C*H$_3$CO−							21.1	21.1
CH$_3$CO−							171.1	171.2

Tricalysioside G (**7**) was a compound analogous to **3** and **6** (Table 1). Its sugar moiety was expected to be located on the hydroxyl group at C-17, since a significant downfield shift from δ_C 63.3 to 75.2 was observed in the ^{13}C NMR spectrum, and acetyl (δ_H 2.22, and δ_C 21.1 and 171.2) and H-15 (δ_H 5.08) signals similar to those of **6** were observed. Thus, the structure of tricalysioside G is a positional isomer of tricalysioside F with respect to the position of the sugar moiety. All other spectroscopic data also supported this assumption, and the structure is as shown.

This type of rearranged *ent*-kaurane has been found in some plant sources, such as cafestol[4,5] and kahweol[6] as nonglycosidic forms in coffee beans, and cafamarine,[7] mascaroside from *Caffea vianneyi*,[8] and mozambioside from *C. pseudozanguebariae*,[9,10] as glucosides. These rearranged kauranes possess a furan ring with C-3, -4, -18, and -19 and an oxygen atom at the 3-position. However, in *ent*-kauranoid glycosides isolated from *T. dubia* an α,β-unsaturated γ-lactone ring was formed from the same carbon and oxygen atoms with an extra carbonyl function on the C-19 carbon atom. We believe this is the first such reported case.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a Union Giken PM-1 automatic digital polarimeter. IR and UV spectra were recorded on a Horiba FT-710 Fourier transform infrared spectrophotometer and a JASCO V-520 UV/vis spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra, at 400

and 100 MHz, respectively, were obtained on a JEOL JNM α-400 spectrometer with tetramethylsilane as the internal standard. HRFAB mass spectra were acquired by a JEOL JMS SX-102 mass spectrometer. Cosmosil (ODS, 75C_{18}-OPN) was purchased from Nacalai Tesque Co., Ltd. (Kyoto, Japan) [Φ = 5.0 cm, *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 droplet counter-current chromatograph (DCCC) (Tokyo Rikakikai, Tokyo) was equipped with 500 glass columns (Φ = 2 mm, *L* = 40 cm), and the lower and upper layers of a solvent mixture, $CHCl_3$−MeOH−H_2O−*n*-PrOH (9:12:8:2), were used as the stationary and mobile phases, respectively. Five-gram fractions (5 g each) were collected and numbered according to their order of elution with the mobile phase.

Plant Material. The plant material was collected in Kunigami Village, Okinawa, in July 1998, and was identified by one of the authors (T.S.). A voucher specimen has been deposited in the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University Faculty of Medicine (98-TD-Okinawa-0709).

Extraction and Isolation. Air-dried leaves (6.04 kg) of *T. dubia* were extracted with MeOH (30 L × 3), and the MeOH extract was concentrated to 3 L. To the concentrate was added 150 mL of H_2O, followed by washing with an equivalent amount of *n*-hexane (56 g). The methanolic layer was concentrated to a viscous gum. The gummy material was suspended in H_2O (3 L) and then extracted successively with EtOAc (3 L) (EtOAc-soluble fraction, 360 g) and *n*-BuOH (3 L) (*n*-BuOH-soluble fraction, 290 g). Evaporation of the water layer left 325 g of a H_2O-soluble fraction.

The *n*-BuOH-soluble fraction was separated first by column chromatography on a highly porous synthetic resin, Diaion HP-20 (Φ = 5.0 cm, *L* = 60 cm) (Mitsubushi Chemical Co., Ltd., Tokyo, Japan), with MeOH−H_2O [(1:4, 8 L), (2:3, 8 L), (3:2, 8 L), and (4:1, 8 L)]; 500 mL fractions were collected. The residue (26.6 g in fractions 13−18) of the 40% MeOH eluate obtained on Diaion HP-20 column chromatography was subjected to silica gel (500 g) (70−230 mesh; Merck Co., Ltd., Darmstadt, Germany) column chromatography with $CHCl_3$ (2 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, 4.5 L), (7:1, 4.5 L), (17:3, 4.5 L), (33:7, 3 L), (4:1, 3 L), (4:1, 3 L), and (7:3, 3 L), fractions of 500 mL being collected. The residue (2.26 g in fractions 35−41) of the 6% MeOH eluate was separated by reversed-phase silica gel (Cosmosil) column chromatography to give 1.14 g of a residue, which was then purified by DCCC to give 563 mg of a residue in fractions 175−232 and 313 mg of **6** in fractions 233−261. The former was purified by DCCC again, followed by recrystallization from MeOH, to afford 11.0 mg of **7**.

The residue (6.00 g in fractions 42−49) of the 8−10% MeOH eluate was similarly purified by Cosmosil column chromatography to give a residue, 116 mg in fractions 155−170, which was then subjected to DCCC to furnish 33.2 mg of **2** in fractions 196−212. Recrystallization from MeOH and then filtration of the crystalline residue (2.84 g in fractions 50−55) of the 10% MeOH eluate gave 819 mg of **1** as colorless rods, and the filtrate was similarly worked up to the previous fractions on Cosmosil (304 mg in fractions 138−144) and DCCC gave 47.0 mg of **5** in fractions 107−130. The residue (2.75 g in fractions 64−72) of the 12.5−15% MeOH eluate was subjected to Cosmosil (524 mg in fractions 117−126), and then DCCC furnished 141 mg of **4** in fractions 71−85. The residue (41.3 g in fractions 19−22) of the 60% MeOH eluate obtained on Diaion HP-20 column chromatography was similarly separated by normal-phase (7.63 g from the 10% MeOH eluate) and reversed-phase (483 mg in fractions 160−175) silica gel column chromatographies, followed by DCCC, to give 43.8 mg (in fractions 117−135) of **3** in a crystalline state.

Tricalysioside A (1): colorless needles (MeOH); mp 285−288 °C; $[\alpha]_D^{21}$ −138° (*c* 1.22, pyridine); IR (KBr) ν_{max} 3529−3311, 2943, 1753, 1653, 1325, 1265, 1163, 1105, 1074 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 217 (4.14) nm; ^1H NMR (pyridine-d_5 + one drop of D_2O) δ 0.640 (3H, s, H_3-20), 0.940 (1H, td, *J* = 14, 4 Hz, H-1a), 1.24 (1H, d, *J* = 8 Hz, H-9), 1.35 (1H, m, H-6a), 1.40 (1H, m, H-12a), 1.41 (2H, m, H_2-11), 1.42 (1H, m, H-2a), 1.45 (1H, m, H-6b), 1.66 (1H, dt, *J* = 14, 3 Hz, H-1b), 1.75 (2H, m, H_2-7), 1.81 (1H, d, *J* = 12 Hz, H-14a), 1.88 (1H, m, H-12b), 1.92 (1H, br d, *J* = 12 Hz, H-5), 2.08 (1H, dd, *J* = 12, 4 Hz, H-14b), 2.21 (1H, tt, *J* = 10, 4 Hz, H-2b), 2.73 (1H, d, *J* = 3 Hz, H-13), 3.71 (1H, s, H-15), 4.97 (1H, ddd, *J* = 9, 6, 2 Hz, H-5′), 4.02 (1H, d, *J* = 13 Hz, H-17a), 4.04 (1H, br t, *J* = 9 Hz, H-2′), 4.06 (1H, t, *J* = 9 Hz, H-4′), 4.21 (1H, t, *J* = 9 Hz, H-3′), 4.25 (1H, *J* = 12, 6 Hz, H-6′a), 4.39 (1H, dd, *J* = 13 Hz, H-17b), 4.79 (1H, ddd, *J* = 10, 8, 1 Hz, H-3), 4.54 (1H, dd, *J* = 12, 2 Hz, H-6′b), 5.69 (1H, br s, H-18), 5.71 (1H, d, *J* = 8 Hz, H-1′); ^{13}C NMR (pyridine-d_5), Table 1; HRFABMS (negative-ion mode) *m*/*z* 509.2397 [M − H]$^-$ ($C_{26}H_{37}O_{10}$, 509.2387).

Tricalysioside B (2): colorless crystals (MeOH); mp 262−265 °C; $[\alpha]_D^{21}$ −121° (*c* 1.22, pyridine); IR (KBr) ν_{max} 3741−3435, 2931, 1749, 1649, 1160, 1136, 1076 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 219 (4.13) nm; ^1H NMR (pyridine-d_5 + one drop of D_2O) δ 0.606 (3H, s, H_3-20), 0.989 (1H, td, *J* = 14, 3 Hz, H-1a), 1.11 (1H, d, *J* = 8 Hz, H-9), 1.35−1.40 (4H, m, H-11a and -12a, and H_2-6), 1.46 (1H, m, H-2a), 1.57−1.68 (4H, m, H-1b, -7b, -11b, and -12b), 1.73 (1H, d, *J* = 14 Hz, H-15a), 1.80 (1H, d, *J* = 12 Hz, H-14a), 1.86 (1H, br t, *J* = 7 Hz, H-5), 2.10 (1H, m, H-14b), 2.10 (1H, d, *J* = 14 Hz, H-15b), 2.21 (1H, m, H-2b), 2.62 (1H, br s, H-13), 4.06 (1H, d, *J* = 14 Hz, H-17a), 3.96 (1H, ddd, *J* = 9, 6, 2 Hz, H-5′), 4.02 (1H, br t, *J* = 8 Hz, H-2′), 4.13 (1H, d, *J* = 14 Hz, H-17b), 4.16 (1H, t, *J* = 9 Hz, H-4′), 4.26 (1H, t, *J* = 9 Hz, H-3′), 4.29 (1H, dd, *J* = 12, 5 Hz, H-6′a), 4.51 (1H, dd, *J* = 12, 2 Hz, H-6′b), 4.80 (1H, td, *J* = 8, 1 Hz, H-3), 5.06 (1H, d, *J* = 8 Hz, H-1′), 5.70 (1H, s, H-18); ^{13}C NMR (pyridine-d_5), Table 1; HRFABMS (negative-ion mode) *m*/*z* 493.2457 [M − H]$^-$ (calcd for $C_{26}H_{37}O_9$, 493.2438).

Tricalysioside C (3): colorless needles (MeOH); mp 213−214 °C; $[\alpha]_D^{26}$ −106° (*c* 0.95, pyridine); IR (KBr) ν_{max} 3600−3120, 2929, 2868, 1750, 1644, 1456, 1349, 1164, 1077, 1019 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 205 (4.09) nm; ^1H NMR (pyridine-d_5 + one drop of D_2O) δ 0.613 (3H, s, H_3-20), 1.02 (1H, td, *J* = 14, 4 Hz, H-1a), 1.09 (1H, d, *J* = 11 Hz, H-9), 1.35 (2H, m, H-11a and -12a), 1.42 (2H, m, H_2-6), 1.46 (3H, m, H-1b, -2a, and -7a), 1.59 (1H, m, H-11b), 1.61 (1H, m, H-7b), 1.62 (1H, d, *J* = 14 Hz, H-15a), 1.80 (1H, d, *J* = 14 Hz, H-15b), 1.83 (1H, d, *J* = 11 Hz, H-14a), 1.87 (1H, m, H-12b), 1.94 (2H, m, H-5 and -14b), 2.28 (1H, v br s, H-2b), 2.45 (1H, d, *J* = 3 Hz, H-13), 3.96 (1H, d, *J* = 11 Hz, H-17a), 3.99 (1H, ddd, *J* = 9, 6, 2 Hz, H-5′), 4.08 (1H, dd, *J* = 9, 8 Hz, H-2′), 4.15 (1H, t, *J* = 9 Hz, H-4′), 4.25 (1H, t, *J* = 9 Hz, H-3′), 4.33 (1H, dd, *J* = 12, 6 Hz, H-6′a), 4.49 (1H, d, *J* = 11 Hz, H-17b), 4.56 (1H, dd, *J* = 12, 2 Hz, H-6′b), 4.83 (1H, t, *J* = 9 Hz, H-3), 4.98 (1H, d, *J* = 8 Hz, H-1′), 5.72 (1H, s, H-18); ^{13}C NMR (pyridine-d_5), Table 1; HRFABMS (negative-ion mode) *m*/*z* 493.2429 [M − H]$^-$ (calcd for $C_{26}H_{37}O_9$, 493.2438).

Tricalysioside D (4): colorless amorphous powder; $[\alpha]_D^{21}$ −109° (*c* 1.11, pyridine); IR (KBr) ν_{max} 3786−3410, 1731, 1641, 1074, 1043, 1020, 1105, 1074 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 216 (4.09) nm; ^1H NMR (pyridine-d_5 + one drop of D_2O) δ 0.652 (3H, s, H_3-20), 0.940 (1H, td, *J* = 14, 4 Hz, H-1a), 1.10 (1H, d, *J* = 8 Hz, H-9), 1.35−1.74 (11H, m, H-1b, -2a, and -15a, and H_2-6, -7, -11, and -12), 1.84 (1H, d, *J* = 13 Hz, H-14a), 1.98 (1H, d, *J* = 13 Hz, H-5), 2.09 (1H, d, *J* = 13 Hz, H-14b), 2.13 (1H, d, *J* = 14 Hz, H-15b), 2.17 (1H, v br s, H-2b), 2.69 (1H, d, *J* = 3 Hz, H-13), 3.92 (1H, t, *J* = 8 Hz, H-2′), 4.00 (1H, t, *J* = 8 Hz, H-4′), 4.07 (1H, dd, *J* = 15, 8 Hz, H-6′a), 4. 19 (1H, d, *J* = 8 Hz, H-4′), 4.19 (2H, s, H_2-5″), 4.35 (1H, d, *J* = 9 Hz, H-4″a), 4.68 (1H, d, *J* = 9 Hz, H-4″b), 4.66 (1H, dd, *J* = 15, 6 Hz, H-6′b), 4.71 (1H, t, *J* = 8 Hz, H-3), 4.72 (1H, d, *J* = 2 Hz, H-2″), 5.03 (1H, d, *J* = 8 Hz, H-1′), 5.62 (1H, s, H-18), 5.70 (1H, d, *J* = 2 Hz, H-1″); ^{13}C NMR (pyridine-d_5), Table 1; HRFABMS (negative-ion mode) *m*/*z* 625.2839 [M − H]$^-$ (calcd for $C_{31}H_{45}O_{13}$, 625.2860).

Tricalysioside E (5): colorless crystals (MeOH); mp 185−188 °C; $[\alpha]_D^{21}$ −113° (*c* 0.876, pyridine); IR (KBr) ν_{max} 3400, 2931, 1734, 1643, 1350, 1302, 1254, 1165, 1074, 1041 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 216 (4.09) nm; ^1H NMR (pyridine-d_5 + one drop of D_2O) δ 0.626 (3H, s, H_3-20), 0.942 (1H, td, *J* = 14, 4, H-1a), 1.10 (1H, d, *J* = 8 Hz, H-9), 1.35−1.74 (11H, m,

H-1b, -2a, and -15a, and H_2-6, -7, -11, and -12), 1.87 (1H, d, J = 13 Hz, H-14a), 1.90 (1H, d, J = 13 Hz, H-5), 2.08 (1H, d, J = 14 Hz, H-15b), 2.09 (1H, d, J = 13 Hz, H-14b), 2.17 (1H, v br s, H-2b), 2.65 (1H, d, J = 3 Hz, H-13), 3.93 (1H, ddd, J = 8, 5, 2 Hz, H-5″), 3.97 (1H, t, J = 8 Hz, H-2″), 4.02 (1H, t, J = 9 Hz, H-4′), 4.04 (3H, m, H-2′ and H_2-17), 4.11 (2H, m, H-5′ and -6′a), 4.17 (1H, t, J = 8 Hz, H-4″), 4.21 (1H, t, J = 8 Hz, H-3′), 4.22 (1H, t, J = 8 Hz, H-3″), 4.33 (1H, dd, J = 12, 5 Hz, H-6″a), 4.49 (1H, dd, J = 12, 2 Hz, H-6″b), 4.75 (1H, t, J = 8 Hz, H-3), 4.83 (1H, dd, J = 16, 6 Hz, H-6′b), 4.97 (1H, d, J = 8 Hz, H-1″), 5.01 (1H, d, J = 8 Hz, H-1′), 5.66 (1H, s, H-18); ^{13}C NMR (pyridine-d_5), Table 1; HRFABMS (negative-ion mode) m/z 655.2988 $[M - H]^-$ (calcd for $C_{32}H_{47}O_{14}$, 655.2966).

Tricalysioside F (6): colorless needles (MeOH), mp 262−266 °C; $[\alpha]_D^{21}$ −105° (c 1.30, pyridine); IR (KBr) ν_{max} 3741−3359, 2991, 2939, 1736, 1649, 1230, 1078 cm^{-1}; UV (MeOH) λ_{max} 216 (4.14) nm (log ϵ); ^{1}H NMR (pyridine-d_5 + one drop of D_2O) δ 0.606 (3H, s, H_3-20), 0.900 (1H, td, J = 14, 4 Hz, H-1a), 1.30−1.70 (11H, m, H-1b, -2a, and -9, and H_2-6, -7, -11, and -12), 1.81 (1H, d, J = 12 Hz, H-14a), 1.88 (1H, d, J = 10 Hz, H-5), 2.17−2.20 (1H, m, H-2b), 2.21 (3H, s, CH_3CO−), 2.31 (1H, dd, J = 12, 4 Hz, H-14b), 2.81 (1H, d, J = 3 Hz, H-13), 3.97 (1H, ddd, J = 9, 6, 2 Hz, H-5′), 4.08 (1H, br t, J = 8 Hz, H-2′), 4.16 (1H, t, J = 9 Hz, H-4′), 4.23 (1H, t, J = 9 Hz, H-3′), 4.25 (2H, s, H_2-17), 4.33 (1H, dd, J = 12, 6 Hz, H-6′a), 4.53 (1H, dd, J = 2 Hz, H-6′b), 4.75 (1H, td, J = 8, 1 Hz, H-3), 5.24 (1H, s, H-15), 5.34 (1H, d, J = 8 Hz, H-1′), 5.72 (1H, s, H-18); ^{13}C NMR (pyridine-d_5), Table 1; HRFABMS (negative-ion mode) m/z 551.2495 $[M - H]^-$ (calcd for $C_{28}H_{39}O_{11}$, 551.2492).

Tricalysioside G (7): colorless needles (MeOH); mp 268−271 °C; $[\alpha]_D^{21}$ −125° (c 0.71, pyridine); IR ν_{max} (KBr) 3529−3311, 2945, 1753, 1720, 1645, 1277, 1163, 1039 cm^{-1}; UV λ_{max} (MeOH) 218 (4.36) nm (log ϵ); ^{1}H NMR (pyridine-d_5 + one drop of D_2O) δ 0.626 (3H, s, H_3-20), 0.932 (1H, td, J = 14, 4 Hz, H-1a), 1.30−1.55 (8H, m, H-2a, -7a, -9, and -12a, and H_2-6 and -11), 1.63 (1H, td, J = 14, 5 Hz, H-1b), 1.73−1.81 (2H, m, H-7b and -12b), 1.84 (1H, d, J = 12 Hz, H-14a), 1.94 (1H, d, J = 10 Hz, H-5), 2.14 (1H, dd, J = 12, 4 Hz, H-14b), 2.22 (3H, s, CH_3-CO−), 2.25−2.30 (1H, m, H-2b), 2.50 (1H, d, J = 3 Hz, H-13), 3.93 (1H, ddd, J = 9, 6, 2 Hz, H-5′), 4.01 (1H, br t, J = 8 Hz, H-2′), 4.16 (1H, t, J = 9 Hz, H-4′), 4.20 (1H, t, J = 9 Hz, H-3′), 4.27 (1H, d, J = 10 Hz, H-17a), 4.33 (1H, dd, J = 12, 6 Hz, H-6′a), 4.43 (1H, d, J = 10 Hz, H-17b), 4.53 (1H, dd, J = 12, 2 Hz, H-6′b), 4.81 (1H, td, J = 8, 1 Hz, H-3), 4.91 (1H, d, J = 8 Hz, H-1′), 5.08 (1H, s, H-15), 5.75 (1H, s, H-18); ^{13}C NMR (pyridine-d_5), Table 1; HRFABMS (negative-ion mode) m/z 551.2507 $[M - H]^-$ (calcd for $C_{28}H_{39}O_{11}$, 551.2492).

Acetylation of Tricalysioside A (1) to 1a. Compound **1** (26.0 mg) was acetylated with 1 mL each of Ac_2O and pyridine at 60 °C for 18 h. The reaction mixture was poured into ice−water, and the precipitates formed were collected by suction. Recrystallization of the precipitates from MeOH gave the hexaacetate (**1a**) as colorless needles (30.5 mg, 79%). Hexaacetate (**1a**): colorless needles; mp 238−240 °C; $[\alpha]_D^{25}$ −110° (c 0.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.843 (3H, s, H_3-20), 1.13 (1H, td, J = 14, 3 Hz, H-1a), 1.998 (3H), 2.022 (6H), 2.065 (3H), 2.095 (3H), 2.114 (3H) (each s, CH_3CO− × 6), 2.37 (1H, m, H-2b), 2.48 (1H, s, H-13), 4.32 (1H, d, J = 13 Hz, H-17a), 4.67 (1H, dd, J = 12, 2 Hz, H-6′a), 4.20 (1H, dd, J = 12, 6 Hz, H-6′b), 4.63 (1H, d, J = 13 Hz, H-17b), 4.65 (1H, s, H-15), 4.67 (1H, m, H-3), 5.06 (1H, d, J = 8 Hz, H-1′), 5.63 (1H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 14.9 (C-20), 19.4 (C-11), 20.57 (×2), 20.62, 20.64, 20.67, 20.99 (CH_3CO− × 6), 20.9 (C-6), 25.6 (C-12), 29.3 (C-7), 33.4 (C-2), 36.0, 36.3 (C-1 and -14), 42.0 (C-13), 42.9 (C-10), 46.8 (C-8), 48.5 (C-5), 51.7 (C-9), 62.6(C-17), 67.8 (C-6′), 69.0, 71.2, 71.9, 73.0 (C-2′, -3′, -4′, and -5′), 81.1 (C-3), 83.4 (C-15), 86.5 (C-16), 97.2 (C-1′), 111.7 (C-18), 169.1, 169.4, 170.2, 170.3, 170.5, 170.8 (CH$_3$CO− × 6), 173.5, 173.6 (C-18 and -19); HRFABMS (positive-ion mode, m-nitrobenzyl alcohol as the matrix) m/z 763.3184 $[M + H]^+$ (calcd for $C_{38}H_{51}O_{16}$, 763.3177).

Acid Hydrolysis of Tricalysioside A (1) to 1b. Compound **1** (65 mg) was dissolved in 2 N H_2SO_4 and then refluxed for 2 h. The turbid hydrosylate was neutralized with $Ba(OH)_2$, and the precipitates were filtered off. The filtrate was concentrated and chromatographed on a silica gel column [Φ = 20 mm, L = 20 cm, $CHCl_3$, 50 mL, $CHCl_3$−MeOH (9:1), 50 mL, (8:2), 100 mL and $CHCl_3$−MeOH−H_2O (7:3:0.5), 200 mL; fractions of 15 mL being collected], to give 22.1 mg (50%) of aglycone (**1b**) in fractions 12−13 and 12.9 mg of glucose (56%) in fractions 28−37. Aglycone (**1b**): colorless needles (MeOH); mp 260−262 °C; $[\alpha]_D^{25}$ −151° (c 0.83, pyridine); ^{1}H NMR (pyridine-d_5) δ 0.694 (3H, s, H_3-20), 0.93 (1H, td, J = 14, 4 Hz, H-1a), 1.28 (1H, d, J = 9 Hz, H-9), 2.21 (1H, tt, J = 10, 4 Hz, H-2b), 2.50 (1H, br d, J = 4 Hz, H-13), 3.81 (1H, br d, J = 4 Hz, H-15), 4.11 (1H, d, J = 11 Hz, H-17a), 4.14 (1H, d, J = 11 Hz, H-17b), 4.72 (1H, ddd, J = 10, 8, 1 Hz, H-3), 5.74 (1H, s, H-18); ^{13}C NMR (pyridine-d_5), Table 1; HRFABMS (negative-ion mode) m/z 347.1861 $[M - H]^-$ (calcd for $C_{20}H_{27}O_5$, 347.1858). D-Glucose, $[\alpha]_D^{22}$ +31.4° (c 0.86, H_2O, 24 h after being dissolved in the solvent).

X-ray Analysis of Tricalysioside A (1).[11] Crystal data: $C_{26}H_{38}O_{10}$. M_r = 510.58, monoclinic, space group $P2_1$, a = 11.198(3) Å, b = 7.502(2) Å, c = 14.3031(9) Å, β = 96.57(1)°, V = 1193.8(3) Å^3, Z = 2, D_c = 1.420 Mg m^{-3}, F(000) = 548, μ(Mo Kα) = 1.080 cm^{-1}. The crystal used for data collection was a colorless prism with approximate dimensions of 0.5 × 0.4 × 0.4 mm. All data were obtained on a Rigaku AFC-5S automated four-circle diffractometer with graphite-monochromated Mo Kα radiation. Unit cell parameters were determined by least-squares refinement of the optimized setting of 22 reflections in the range 10.0−13.4°. The intensities were measured using $\omega/2\theta$ scans up to 55°. Three standard reflections were monitored every 150 measurements. The data were corrected for Lorentz and polarization factors. Absorption and decay corrections were not applied. Of the 2962 independent reflections that were collected, 1927 reflections with $I > 2.0\sigma(I)$ were used for structure determination and refinements. The structure was solved by a direct method using the teXsan crystallographic software package.[12] All non-H atoms were found on a Fourier map. The refinements of atomic parameters were carried out by full matrix least-squares refinement, using anisotropic temperature factors for all non-H atoms. All H atoms were located in difference Fourier maps and included in the refinement calculation at fixed positions. The final refinement converged with R = 0.044 and R_w = 0.048 for 324 parameters. Atomic scattering factors were taken from *International Tables for X-ray Crystallography*.

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(11) Crystallographic data for tricalysioside A (**1**) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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