Tricalysiamides A−D, Diterpenoid Alkaloids from *Tricalysia dubia*

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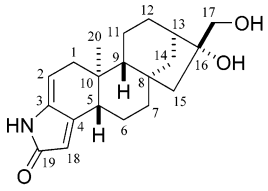
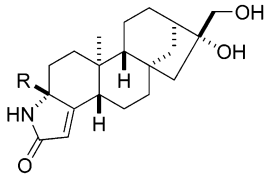
Four rearranged *ent*-kaurane diterpenoid alkaloids, tricalysiamides A−D (**1**−**4**), having a cafestol-type carbon framework were isolated from the wood of *Tricalysia dubia*. Their absolute structures were determined on the basis of 2D NMR spectroscopy, X-ray crystallographic analysis, and chemical methods.

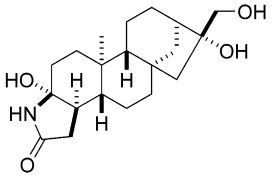
Tricalysia dubia (Lindl.) Ohwi (Rubiaceae) is an evergreen shrub or tree that is distributed in Taiwan and the southern parts of China and Japan. From the leaves of this plant the rearranged *ent*-kaurane glycosides tricalysiosides A−G[1] and the *ent*-kaurane glycosides tricalysiosides H−O[2] have been isolated. In a previous paper the structure elucidation of six rearranged *ent*-kaurane diterpenes, tricalysiolides A−F, from this plant was reported.[3] In the present study four new rearranged *ent*-kaurane diterpenoid alkaloids, tricalysiamides A−D (**1**−**4**), were isolated from the wood of this plant and their structures determined.



Tricalysiamide A (**1**)



Tricalysiamide B (**2**) R = H
Tricalysiamide C (**3**) R = OMe



Tricalysiamide D (**4**)

Results and Discussion

By a series of column chromatographies using highly porous synthetic resin (Diaion HP-20), silica gel, and aminopropyl-bonded silica gel, and ODS HPLC, a MeOH extract of air-dried wood of *T. dubia* afforded four diterpenoid alkaloids, tricalysiamides A−D (**1**−**4**).

Tricalysiamide A (**1**) was obtained as colorless prisms. Its molecular formula, $C_{20}H_{27}NO_3$, determined from the $[M + H]^+$ peak at *m*/*z* 330.2048 (calcd for $C_{20}H_{28}NO_3$, 330.2069) in the HRESIMS, indicated that **1** was an alkaloid. The IR spectrum indicated that **1** possessed hydroxyl (3355 cm^{-1}), amide NH (3220 cm^{-1}), and lactam carbonyl (1681 cm^{-1}) groups. The ^1H NMR spectrum showed the signals of one tertiary methyl group (δ 0.93), two olefinic protons (δ 5.98, 5.48), and one amide proton (δ 10.83) (Table 1). The ^{13}C NMR spectrum of **1** displayed signals due to one methyl, eight methylenes, three methines, two olefinic methines, three quaternary carbons, two olefinic quaternary carbons, and one carbonyl carbon (Table 2). The HMBC data revealed that **1** possessed a cafestol-type skeleton related to tricalysiolides A−F.[3] The correlations from H-18 to C-3, C-4, C-5, and C-19 and from NH to C-3, C-4, C-18, and C-19 indicated that C-3, C-4, C-18, C-19, and NH constitute an α,β-unsaturated-γ-lactam ring (Figure 1). The NOE correlations between H-14a/OH-16 and H-14b/H_3-20 indicated that the C-20 methyl, C-14 methylene, and the hydroxyl group at C-16 were α-oriented, and those between H-5/H-9, H-9/H-15b, and H-15b/H_2-17 indicated that H-5, H-9, H-15b, and the C-17 hydroxymethyl were β-oriented (Figure 2). From these observations, tricalysiamide A was proposed to have structure **1**, which was confirmed by a single-crystal X-ray analysis (Figure 3). Its absolute configuration was established by the preparation of **1** from tricalysiolide C (**5**),[3] whose absolute configuration was known (Scheme 1). Thus, treatment of **5** with aqueous ammonia gave hemiamidal **6**, which was subjected to acid-catalyzed dehydration to afford a product that was shown to be identical to natural **1** by comparison of their spectroscopic data and optical rotations. Thus, the absolute stereochemistry of tricalysiamide A was determined to be as shown in structure **1**.

Tricalysiamide B (**2**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{20}H_{29}NO_3$ from the $[M + H]^+$ peak at *m*/*z* 332.2189 (calcd for $C_{20}H_{30}NO_3$, 332.2226) in the HRESIMS. The NMR spectra of **2** were generally similar to those of **1**, except for the C-2 and C-3 signals. The C-2 olefinic methine (δ 106.3) and C-3 olefinic quaternary carbon (δ 139.5) signals in **1** were observed as aliphatic methylene (δ 30.7) and methine (δ 58.9) signals, respectively, in **2**. A NOESY correlation between H-3 and H-5 indicated that H-3 of **2** was β-oriented. The hydrogenation product of **1** was shown to be identical to natural **2** by comparison of their spectroscopic data and optical rotations. Thus, the absolute stereochemistry of tricalysiamide B was determined to be as shown in structure **2**.

Tricalysiamide C (**3**) was isolated as an amorphous powder. Its molecular formula was determined to be $C_{21}H_{31}NO_4$ from the $[M + H]^+$ peak at *m*/*z* 362.2349 (calcd for $C_{21}H_{32}NO_4$, 362.2331) in the HRESIMS. Similarity observed in the NMR spectra of **2** and **3** indicated that they had the same basic structure. The difference noted between them was that the NMR spectra of **3** displayed the presence of a methoxyl group (δ_H 3.09, δ_C 48.9). The methoxyl protons were correlated with the C-3 signal (δ_C 90.1) in the HMBC spectrum, demonstrating that it was located at position 3. A NOESY correlation between H-5 and OCH_3-3 indicated that the methoxyl group was β-oriented. Treatment of **6** with methanol in the presence of boron trifluoride diethyl etherate afforded a product that was shown to be identical to natural **3** by their spectroscopic data and optical rotations (Scheme 1). Thus, the absolute stereochemistry of tricalysiamide C was determined to be as shown in structure **3**.

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Table 1. ^{1}H NMR Data (δ) for Compounds **1**−**4** in C_5D_5N[a]

position	1	2	3	4
1a	2.35 (dd, 17.5, 6.7)	1.68 (m)	1.62 (m)	1.76 (m)
1b	1.84 (m)	0.97 (td, 13.7, 3.7)	1.38 (td, 13.6, 4.0)	1.08 (m)
2a	5.48 (d, 6.7)	2.14 (m)	2.33 (dt, 13.6, 2.4)	2.36 (m)
2b		1.47 (m)	1.82 (m)	2.33 (m)
3		3.90 (dd, 10.9, 7.2)		
4				2.24 (dd, 11.7, 6.6)
5	2.21 (dt, 11.8, 2.3)	1.88 (m)	2.08 (m)	0.97 (t, 12.1)
6a	1.71 (m)	1.51 (m)	1.56 (m)	1.42 (m)
6b	1.44 (m)	1.46 (m)	1.44 (m)	1.22 (td, 11.9, 3.1)
7a	1.66 (dt, 13.0, 3.0)	1.64 (m)	1.66 (m)	1.61 (m)
7b	1.56 (td, 13.0, 3.6)	1.53 (m)	1.57 (d, 9.1)	1.36 (m)
9	1.26 (d, 8.7)	1.15 (d, 8.8)	1.25 (d, 8.7)	0.93 (d, 7.2)
11a	1.71 (m)	1.71 (m)	1.72 (m)	1.70 (m)
11b	1.48 (m)	1.49 (m)	1.49 (m)	1.56 (m)
12a	1.89 (m)	1.87 (m)	1.89 (m)	1.90 (m)
12b	1.49 (m)	1.47 (m)	1.48 (m)	1.49 (m)
13	2.46 (d-like, 3.0)	2.45 (d-like, 2.9)	2.47 (d-like, 2.9)	2.47 (s-like)
14a	2.05 (dd, 11.6, 4.5)	2.05 (dd, 11.4, 4.1)	2.07 (m)	2.05 (dd, 10.9, 3.3)
14b	1.94 (d, 11.6)	1.96 (d, 11.4)	1.98 (d, 11.4)	2.00 (d, 10.9)
15a	1.85 (d, 13.6)	1.85 (d, 14.3)	1.87 (d, 14.2)	1.84 (d, 14.0)
15b	1.72 (d, 13.6)	1.73 (dd, 14.3, 1.3)	1.76 (d, 14.2)	1.72 (d, 14.0)
17a	4.15 (dd, 10.9, 5.3)	4.15 (dd, 10.9, 5.2)	4.15 (dd, 10.9, 5.3)	4.15 (dd, 10.7, 4.5)
17b	4.07 (dd, 10.9, 5.3)	4.06 (dd, 10.9, 5.2)	4.06 (dd, 10.9, 5.3)	4.06 (dd, 10.7, 4.5)
18a	5.98 (s)	5.83 (s)	5.93 (s)	3.21 (dd, 16.4, 6.5)
18b				2.35 (m)
20	0.93 (s, 3H)	0.71 (s, 3H)	0.77 (s, 3H)	1.04 (s, 3H)
NH	10.83 (s)	8.87 (s)	9.29 (s)	9.16 (s)
OMe			3.09 (s, 3H)	
OH-3				7.35 (s)
OH-16	5.22 (s)	5.22 (s)	5.19 (s)	5.17 (s)
OH-17	6.13 (t, 5.3)	6.12 (t, 5.2)	6.14 (t, 5.3)	6.15 (t, 4.5)

[a] Recorded at 500 MHz. Multiplicity and *J*-values in Hz are given in parentheses.

Table 2. ^{13}C NMR Data (δ) for Compounds **1**−**4** in C_5D_5N[a]

position	1	2	3	4
1	40.7	36.8	35.6	36.0
2	106.3	30.7	35.0	32.0
3	139.5	58.9	90.1	87.6
4	152.9	167.3	165.1	45.8
5	45.4	49.0	46.5	49.5
6	22.0	22.3	22.0	24.3
7	40.2	40.5	40.5	41.3
8	44.4	44.6	44.7	44.7
9	53.0	53.6	53.7	53.4
10	42.2	43.0	43.4	37.9
11	18.9	19.5	19.5	19.0
12	26.4	26.4	26.4	26.6
13	45.9	45.9	45.9	45.9
14	37.7	38.2	38.1	38.3
15	53.7	53.9	53.9	53.9
16	81.5	81.6	81.6	81.7
17	66.4	66.4	66.4	66.5
18	116.3	117.8	119.9	36.4
19	173.0	174.6	172.6	178.5
20	15.5	14.9	14.7	14.3
OMe			48.9	

[a] Recorded at 125 MHz.

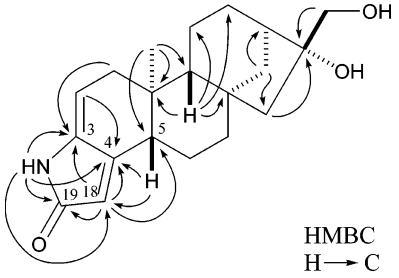


Figure 1. Selected HMBC correlations for **1**.

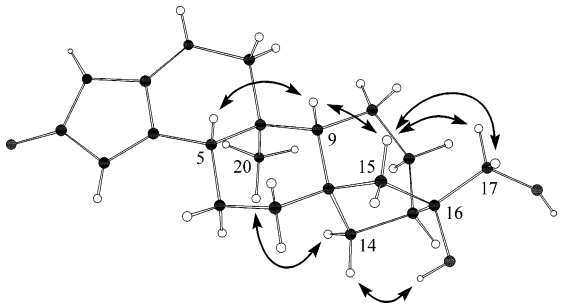


Figure 2. Selected NOE correlations for **1**.

Tricalysiamide D (**4**) was isolated as an amorphous powder. Its molecular formula was determined to be $C_{20}H_{31}NO_4$ from the $[M + H]^+$ peak at *m*/*z* 350.2330 (calcd for $C_{20}H_{32}NO_4$, 350.2331) in the HRESIMS. The NMR spectra indicated that **4** had aliphatic methine (δ_C 45.8) and methylene (δ_C 36.4) carbons at C-4 and C-18, respectively, and a hydroxyl group (δ_H 7.35), the location of which was assigned to position 3 by observation of HMBC correlations from the hydroxyl proton to C-3 (δ_C 87.6) and C-4. The NOESY correlations between H-4/H_3-20, H-4/OH-3, and H-5/NH indicated that H-4 and OH-3 were both α-oriented. Thus, compound **4** was considered to be the 4α,18-dihydro analogue of **6**. Catalytic hydrogenation of **6** afforded compound **7**, the OH-3*β*/H-4*β* isomer of **4**, whereas hydrogenation of methyl ester **9**, derived from tricalysiolide B (**8**) with known absolute configuration,[3] gave the H-4α product **10** and the H-4*β* product **11** in yields of 31% and 40%, respectively (Schemes 1 and 2). The stereochemistry of the hydrogenation products was determined on the basis of NOESY experiments. Subsequent treatment of **10** with methanolic aqueous ammonia produced a product that was shown to be identical to natural **4** by comparison of their spectroscopic data and optical rotations. Thus, tricalysiamide D was determined to have the absolute stereochemistry as shown in structure **4**.

Diterpenoid alkaloids having an *ent*-kaurane skeleton are of limited distribution among plants of the genera *Aconitum*, *Garrya*, and *Anopterus*.[4] They incorporate a nitrogen atom between C-19 and C-20. Tricalysiamides A−D (**1**−**4**) are the first examples of

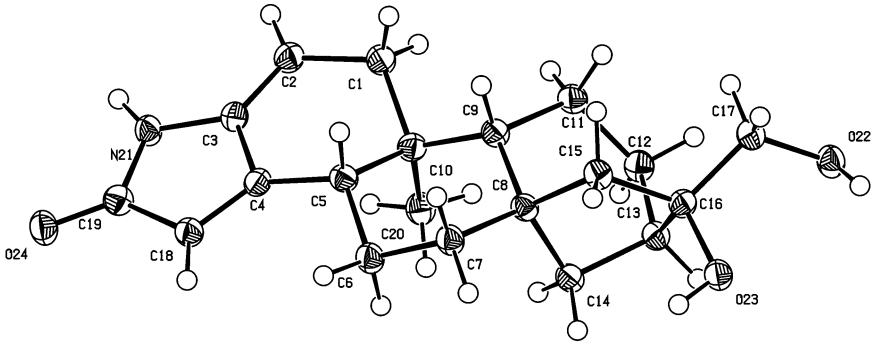
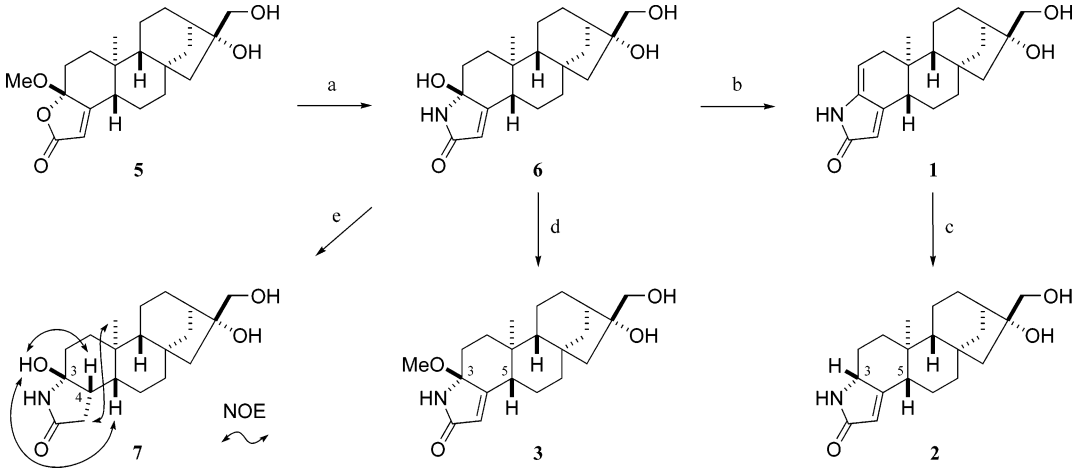


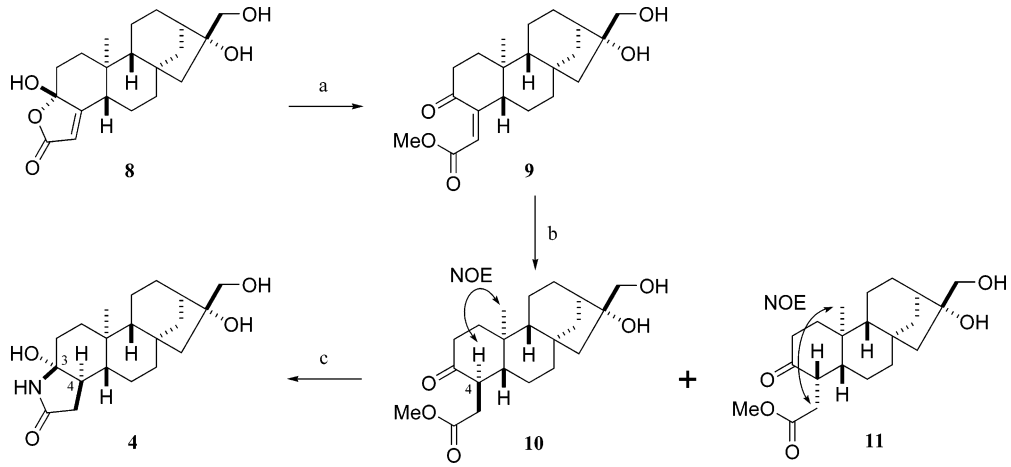
Figure 3. ORTEP representation of tricalysiamide A (**1**). Solvent has been omitted for clarity.

Scheme 1[a]



[a] Reagents and conditions: (a) aq NH_4OH, 48 h, 90%; (b) $BF_3{\cdot}OEt_2$, CH_2Cl_2-dioxane, 2 h, 75%; (c) H_2, Pd/C, EtOH, 2 h, 81%; (d) $BF_3{\cdot}OEt_2$, MeOH, 15 min, 84%; (e) H_2, Pd/C, EtOH, 2 h, 79%.

Scheme 2[a]



[a] Reagents and conditions: (a) MeI, K_2CO_3, acetone, 48 h, 65%; (b) H_2, Pd/C, EtOH, 3 h, 31% of **10** and 40% of **11**; (c) aq NH_4OH-MeOH, 48 h, 42%.

ent-kaurane diterpenoid alkaloids having a cafestol-type carbon framework with a nitrogen atom between C-3 and C-19.

Tricalysiamides A−C (**1**−**3**) showed no or weak cytotoxic activity against P-388 murine leukemia cells with IC_{50} values of >100, >100, and 73, respectively. Tricalysiamide D (**4**) was not evaluated for cytotoxicity due to the small quantity available.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P1030 digital polarimeter, IR spectra on a JASCO FT/IR 620 spectrophotometer, and UV spectra on a JASCO V-530 spectrophotometer. NMR spectra were measured in C_5D_5N on a Bruker DRX-500 spectrometer at 300 K. The ^1H chemical shifts were referenced to the resonance of residual C_5D_4HN in the solvent at 7.21 ppm, and the ^{13}C chemical shifts to the solvent resonance at 135.5 ppm. Mass spectra were obtained using a Micromass LCT spectrometer. Preparative HPLC was carried out on a JASCO PU-986 pump unit equipped with a UV-970 UV detector (λ = 220 nm) and an Inertsil PREP-ODS column (10 μm, 20 × 250 mm), using a MeOH−H_2O or a MeCN−H_2O solvent system at a flow rate of 10 mL/min. X-ray single-crystal analysis was taken on a Mac Science DIP diffractometer with Mo Kα radiation (λ = 0.71073 Å).

Plant Material. Wood of *T. dubia* was collected in Iriomote Island, Okinawa, in March 2005. The plant was identified by Dr. T. Kinoshita (Teikyo University, Japan). A voucher specimen (IRI-050323-06) has

been deposited at the Herbarium, Medicinal Plant Garden, Teikyo University (Sagamiko-machi, Kanagawa).

Extraction and Isolation. Cut and air-dried wood (17.1 kg) of *T. dubia* was extracted with MeOH (3 × 40 L). After removal of MeOH under reduced pressure, the residue (1.2 kg) was placed on a column of HP-20 (Diaion, 3.5 kg) and eluted with H_2O, H_2O−MeOH (1:1), H_2O−MeOH (1:4), MeOH, and acetone (each 15 L) sequentially to give five fractions. After removal of the solvent, the residue of the H_2O−MeOH (1:4) fraction (192.1 g) was subjected to silica gel (Merck Kieselgel 60, 230−400 mesh, 1.5 kg) column chromatography (CC) eluting sequentially with $CHCl_3$, $CHCl_3$−MeOH (20:1), $CHCl_3$−MeOH (10:1), $CHCl_3$−MeOH (3:1), and MeOH (each 6 L). After evaporation, the $CHCl_3$−MeOH (10:1) fraction (25.1 g) was subjected to amino-propyl-bonded silica gel (Chromatorex, 200−350 mesh, 200 g) CC eluting sequentially with $CHCl_3$−MeOH (30:1, 2 × 350 mL), $CHCl_3$−MeOH (20:1, 2 × 350 mL), $CHCl_3$−MeOH (10:1, 2 × 350 mL), and MeOH (800 mL) to give fractions 1−7. Fraction 1 (994 mg) was subjected to silica gel (30 g) CC eluting sequentially with $CHCl_3$−MeOH (30:1, 4 × 75 mL), $CHCl_3$−MeOH (15:1, 4 × 75 mL), and MeOH (300 mL) to give fractions A−I. Fraction F (149 mg) was further purified by repeated ODS HPLC using MeOH−H_2O (40:60) and then MeCN−H_2O (19:81) to afford **3** (6.1 mg). Fraction G (143 mg) was separated by repeated ODS HPLC using MeOH−H_2O (40:60) and then MeCN−H_2O (20:80) to afford **1** (8.4 mg) and **4** (1.4 mg). Separation of fraction H (294 mg) by ODS HPLC using MeOH−H_2O (35:65) and then MeCN−H_2O (19:81) afforded **2** (10.4 mg).

Tricalysiamide A (1): colorless prisms (MeOH), mp 293−295 °C; $[\alpha]^{25}_D$ −265 (*c* 0.07, pyridine); IR (film) ν_{max} 3355, 3220, 2921, 2861, 1681, 1656, 1449, 1053 1014 cm^{-1}; UV (MeOH) λ_{max} nm (log ϵ) 271 (3.37); ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 330.2048 $[M + H]^+$ (calcd for $C_{20}H_{28}NO_3$, 330.2069).

Tricalysiamide B (2): amorphous powder; $[\alpha]^{25}_D$ −210 (*c* 0.10, pyridine); IR (film) ν_{max} 3361, 2926, 2852, 1668, 1455, 1362, 1052, 1017 cm^{-1}; UV (MeOH) λ_{max} nm (log ϵ) 214 (4.24); ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 332.2189 $[M + H]^+$ (calcd for $C_{20}H_{30}NO_3$, 332.2226).

Tricalysiamide C (3): amorphous powder; $[\alpha]^{25}_D$ −212 (*c* 0.11, pyridine); IR (film) ν_{max} 3363, 3184, 2931, 2866, 1687, 1668, 1454, 1346, 1151, 1049, 1016 cm^{-1}; UV (MeOH) λ_{max} nm (log ϵ) 210 (4.09); ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 362.2349 $[M + H]^+$ (calcd for $C_{21}H_{32}NO_4$, 362.2331).

Tricalysiamide D (4): amorphous powder; $[\alpha]^{26}_D$ −77 (*c* 0.05, pyridine); IR (film) ν_{max} 3367, 3253, 2920, 2850, 1684, 1593, 1558, 1417, 1292, 1227, 1142 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 350.2330 $[M + H]^+$ (calcd for $C_{20}H_{32}NO_4$, 350.2331).

Preparation of Lactam 6. A solution of tricalysiolide C (**5**) (5.0 mg, 0.014 mmol) in 28% aqueous NH_4OH (2 mL) was stirred at room temperature for 48 h. Removal of the solvent and subsequent ODS HPLC of the residue with MeCN−H_2O (16:84) gave compound **6** (4.3 mg, 90%) as an amorphous powder: $[\alpha]^{25}_D$ −208 (*c* 0.11, pyridine); IR (film) ν_{max} 3379, 3174, 3099, 2929, 2864, 1657, 1450, 1356, 1161, 1128, 1070, 1024 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 9.48 (1H, s, NH), 7.79 (1H, s, OH-3), 6.13 (1H, s-like, OH-17), 5.77 (1H, s, H-18), 5.19 (1H, s, OH-16), 4.16 (1H, dd, J = 10.8, 2.6 Hz, H-17a), 4.06 (1H, dd, J = 10.8, 2.6 Hz, H-17b), 2.63 (1H, d, J = 10.6 Hz, H-5), 2.49 (1H, m, H-2), 2.47 (1H, s-like, H-13), 2.06 (1H, dd, J = 11.4, 3.9 Hz, H-14a), 2.00 (1H, d, J = 11.4 Hz, H-14b), 1.91 (1H, m, H-2b), 1.89 (1H, m, H-12a), 1.84 (1H, d, J = 14.5 Hz, H-15a), 1.75 (1H, d, J = 14.5 Hz, H-15b), 1.72 (1H, m, H-11a), 1.70 (1H, m, H-1a), 1.65 (1H, m, H-7a), 1.61 (1H, m, H-1b), 1.58 (1H, m, H-6a), 1.56 (1H, m, H-7b), 1.51 (1H, m, H-11b), 1.48 (1H, m, H-12b), 1.39 (1H, m, H-6b), 1.33 (1H, d, J = 8.6 Hz, H-9), 0.83 (3H, s, H_3-20); ^{13}C NMR (125 MHz, pyridine-d_5) δ 172.7 (C, C-19), 168.5 (C, C-4), 116.8 (CH, C-18), 86.4 (C, C-3), 81.6 (C, C-16), 66.4 (CH_2, C-17), 53.9 (CH, C-9), 53.9 (CH_2, C-15), 46.1 (CH, C-5), 45.9 (CH, C-13), 44.8 (C, C-8), 43.6 (C, C-10), 40.6 (CH_2, C-7), 38.1 (CH_2, C-14), 36.0 (CH_2, C-1), 35.9 (CH_2, C-2), 26.4 (CH_2, C-12), 22.2 (CH_2, C-6), 19.5 (CH_2, C-11), 14.6 (CH_3, C-20); HRESIMS *m*/*z* 348.2151 $[M + H]^+$ (calcd for $C_{20}H_{30}NO_4$, 348.2175).

Dehydration of 6. To a solution of **6** (11.0 mg, 0.032 mmol) in CH_2Cl_2−dioxane (10:1, 2 mL) was added BF_3·OEt_2 (11 μL, 0.089 mmol), and the mixture was stirred at room temperature for 2 h. After neutralization with sodium acetate (20 mg), the solvent was evaporated under reduced pressure. The residue was separated by ODS HPLC with MeOH−H_2O (35:65) to afford a product [7.8 mg, 75%, $[\alpha]^{26}_D$ −204 (*c* 0.11, pyridine)] that was shown to be identical to the natural product **1** by comparison of their ^1H NMR and mass spectra and optical rotations.

Hydrogenation of 1. Compound **1** (1.1 mg, 0.0033 mmol) was dissolved in EtOH (2 mL) and hydrogenated over 10% Pd/C (5 mg) for 2 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue was separated by ODS HPLC with MeOH−H_2O (40:60) to afford a product [0.9 mg, 81%, $[\alpha]^{27}_D$ −200 (*c* 0.10, pyridine)] that was shown to be identical to the natural product **2** by comparison of their ^1H NMR and mass spectra and optical rotations.

Treatment of 6 with Methanolic BF_3·OEt_2. BF_3·OEt_2 (1 μL, 0.008 mmol) was added to a solution of **6** (10.2 mg, 0.029 mmol) in MeOH (1 mL), and the mixture was stirred at room temperature for 15 min. After neutralization with sodium acetate (30 mg), the solvent was evaporated under reduced pressure. The residue was separated by ODS HPLC with MeOH−H_2O (35:65) to afford a product [8.9 mg, 84%, $[\alpha]^{25}_D$ −220 (*c* 0.10, pyridine)] that was shown to be identical to the natural product **3** by comparison of their ^1H NMR and mass spectra and optical rotations.

Hydrogenation of 6. Compound **6** (5.4 mg, 0.016 mmol) was dissolved in EtOH (2 mL) and hydrogenated over 10% Pd/C (16.5 mg) for 2 h. The catalyst was removed by filtration, and the filtrate was evaporated to give a residue, which was separated by ODS HPLC with MeCN−H_2O (12:88) to give compound **7** (4.3 mg, 79%) as an amorphous powder: $[\alpha]^{25}_D$ −69 (*c* 0.09, pyridine); IR (film) ν_{max} 3419, 3197, 2935, 2858, 1703, 1684, 1651, 1558, 1541, 1473, 1456, 1437, 1419, 1209, 1074, 1032 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 9.34 (1H, s, NH), 7.34 (1H, s, OH-3), 6.09 (1H, t, J = 5.0 Hz, OH-17), 5.15 (1H, s, OH-16), 4.13 (1H, dd, J = 10.8, 5.0 Hz, H-17a), 4.04 (1H, dd, J = 10.8, 5.0 Hz, H-17b), 2.65 (1H, m, H-4), 2.62 (1H, m, H-18a), 2.50 (1H, m, H-18b), 2.48 (1H, d-like, J = 2.2 Hz, H-13), 2.28 (1H, d, J = 14.3 Hz, H-2a), 2.05 (1H, m, H-2b), 2.04 (1H, m, H-14a), 2.00 (1H, d, J = 11.6 Hz, H-14b), 1.88 (1H, m, H-12a), 1.86 (1H, m, H-5), 1.82 (1H, d, J = 14.2 Hz, H-15a), 1.72 (1H, m, H-1a), 1.70 (1H, d, J = 14.2 Hz, H-15b), 1.69 (1H, m, H-11a), 1.65 (1H, m, H-7a), 1.57 (1H, m, H-7b), 1.54 (1H, m, H-11b), 1.51 (1H, m, H-12b), 1.49 (1H, m, H-6a), 1.40 (1H, td, J = 13.3, 3.4 Hz, H-1b), 1.14 (1H, d, J = 11.8 Hz, H-6b), 1.09 (1H, m, H-9), 1.07 (3H, s, H_3-20); ^{13}C NMR (125 MHz, pyridine-d_5) δ 174.5 (C, C-19), 87.2 (C, C-3), 81.5 (C, C-16), 66.4 (CH_2, C-17), 55.6 (CH, C-9), 54.1 (CH_2, C-15), 49.9 (CH, C-4), 46.1 (CH, C-13), 45.1 (C, C-8), 43.4 (CH, C-5), 41.9 (CH_2, C-7), 39.0 (C, C-10), 38.1 (CH_2, C-14), 36.1 (CH_2, C-1), 34.7 (CH_2, C-18), 33.9 (CH_2, C-2), 26.7 (CH_2, C-12), 25.7 (CH_2, C-6), 18.8 (CH_2, C-11), 16.9 (CH_3, C-20); HRESIMS *m*/*z* 350.2287 $[M + H]^+$ (calcd for $C_{20}H_{32}NO_4$, 350.2331).

Preparation of Methyl Ester 9. To a solution of tricalysiolide B (**8**) (10.3 mg, 0.030 mmol) in acetone (1 mL) were added iodomethane (1 mL) and potassium carbonate (51 mg, 0.37 mmol). After stirring at room temperature for 48 h, the mixture was filtered, and the filtrate was evaporated to dryness. By ODS HPLC with MeCN−H_2O (25:75), the residue gave compound **9** (7.0 mg, 65%) as an amorphous powder: $[\alpha]^{25}_D$ −139 (*c* 0.12, $CHCl_3$); IR (film) ν_{max} 3419, 2929, 2864, 1732, 1716, 1701, 1653, 1558, 1541, 1456, 1437, 1338, 1207, 1157, 1051, 1018 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 5.67 (1H, s, H-18), 4.13 (1H, d, J = 10.8 Hz, H-17a), 4.05 (1H, d, J = 10.8 Hz, H-17b), 3.68 (3H, s, H_3-21), 2.80 (1H, m, H-2a), 2.56 (1H, dd, J = 15.0, 3.8 Hz, H-2b), 2.46 (1H, s-like, H-13), 2.06 (1H, m, H-5), 2.04 (1H, m, H-14a), 2.00 (1H, m, H-1a), 1.91 (1H, d, J = 11.2 Hz, H-14b), 1.87 (1H, m, H-12a), 1.82 (1H, d, J = 14.5 Hz, H-15a), 1.71 (1H, m, H-11a), 1.68 (1H, d, J = 14.5 Hz, H-15b), 1.61 (1H, m, H-7a), 1.49 (1H, m, H-11b), 1.48 (1H, m, H-12b), 1.45 (1H, m, H-6a), 1.40 (1H, m, H-7b), 1.29 (1H, m, H-6b), 1.25 (1H, m, H-1b), 1.12 (1H, d, J = 8.6 Hz, H-9), 1.00 (3H, s, H_3-20); ^{13}C NMR (125 MHz, pyridine-d_5) δ 204.3 (C, C-3), 166.7 (C, C-19), 158.9 (C, C-4), 116.7 (CH, C-18), 81.5 (C, C-16), 66.4 (CH_2, C-17), 53.9 (CH, C-9), 53.5 (CH, C-5), 53.4 (CH_2, C-15), 51.6 (CH, C-21), 45.8 (CH, C-13), 44.4 (C, C-8), 42.3 (C, C-10), 39.9 (CH_2, C-7), 39.8 (CH_2, C-1), 39.1 (CH_2, C-2), 37.4 (CH_2, C-14), 26.4 (CH_2, C-12), 22.7 (CH_2, C-6), 19.6 (CH_2, C-11), 15.0 (CH_3, C-20); HRESIMS *m*/*z* 363.2180 $[M + H]^+$ (calcd for $C_{21}H_{31}O_5$, 363.2171).

Hydrogenation of 9. Compound **9** (4.2 mg, 0.012 mmol) was dissolved in EtOH (2 mL) and hydrogenated over 10% Pd/C (30 mg) for 3 h. The catalyst was removed by filtration, and the filtrate was evaporated to give a residue, which was separated by silica gel column

chromatography using $CHCl_3$−EtOAc (4:1) to give the H-4α product **10** (1.3 mg, 31%) and H-4*β* product **11** (1.7 mg, 40%). Compound **10**: amorphous powder; $[\alpha]^{26}_D$ −51 (*c* 0.08, $CHCl_3$); IR (film) ν_{max} 3444, 2924, 2850, 1732, 1707, 1651, 1635, 1456, 1261, 1165, 1095, 1020 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) *δ* 6.12 (1H, t, *J* = 5.4 Hz, OH-17), 5.18 (1H, s, OH-16), 4.13 (1H, dd, *J* = 10.8, 5.4 Hz, H-17a), 4.04 (1H, dd, *J* = 10.8, 5.4 Hz, H-17b), 3.66 (3H, s, H_3-21), 2.92 (1H, m, H-4), 2.80 (1H, dd, *J* = 16.6, 7.5 Hz, H-18a), 2.61 (1H, m), 2.52 (1H, m, H-18b), 2.47 (1H, m), 2.34 (1H, m), 2.00 (1H, m), 1.92−1.79 (4H, m), 1.71−1.60 (4H, m), 1.56−1.54 (2H, m), 1.52−1.36 (2H, m), 1.27 (1H, m), 1.10 (3H, s, H_3-20), 0.98 (1H, d, *J* = 11.4 Hz), 0.89 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (125 MHz, pyridine-d_5) *δ* 210.4 (C), 173.7 (C), 81.5 (C), 66.4 (CH_2), 54.0 (CH), 53.6 (CH_2), 51.4 (CH_3), 51.0 (CH), 47.3 (CH), 45.9 (CH), 44.2 (C), 41.0 (CH_2), 39.9 (CH_2), 38.8 (C), 37.6 (CH_2), 37.4 (CH_2), 31.7 (CH_2), 26.6 (CH_2), 24.3 (CH_2), 19.2 (CH_2), 14.8 (CH_3); HRESIMS *m*/*z* 365.2299 $[M + H]^+$ (calcd for $C_{21}H_{33}O_5$, 365.2328). Compound **11**: amorphous powder; $[\alpha]^{26}_D$ −54 (*c* 0.11, $CHCl_3$); IR (film) ν_{max} 3423, 2925, 2854, 1736, 1703, 1651, 1454, 1261, 1171, 1092, 1022 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) *δ* 6.14 (1H, s-like, OH-17), 5.20 (1H, s, OH-16), 4.11 (1H, d, *J* = 10.1 Hz, H-17a), 4.05 (1H, d, *J* = 10.1 Hz, H-17b), 3.67 (3H, s, H_3-21), 3.18 (1H, m, H-4), 2.77 (1H, dd, *J* = 15.8, 9.4 Hz, H-18a), 2.56 (1H, m, H-18b), 2.51 (1H, m), 2.46 (1H, m), 2.44 (1H, m), 2.02 (1H, dd, *J* = 11.2, 4.4 Hz), 1.90−1.83 (5H, m), 1.81 (1H, d, *J* = 13.8 Hz), 1.69 (1H, d, *J* = 13.8 Hz), 1.68−1.61 (3H, m), 1.52−1.47 (2H, m), 1.40 (1H, m), 1.27 (1H, m), 1.02 (1H, d, *J* = 8.6 Hz), 0.93 (3H, s, H_3-20); ^{13}C NMR (125 MHz, pyridine-d_5) *δ* 213.1 (C), 173.6 (C), 81.5 (C), 66.4 (CH_2), 55.1 (CH), 53.4 (CH_2), 51.7 (CH_3), 49.8 (CH), 47.1 (CH), 45.9 (CH), 44.6 (C), 40.9 (CH_2), 40.0 (CH_2), 38.3 (C), 37.1 (CH_2), 35.5 (CH_2), 33.0 (CH_2), 26.4 (CH_2), 24.7 (CH_2), 19.3 (CH_2), 18.1 (CH_3); HRESIMS *m*/*z* 365.2336 $[M + H]^+$ (calcd for $C_{21}H_{33}O_5$, 365.2328).

Preparation of Lactam 4. Compound **10** (3.2 mg, 0.0088 mmol) was dissolved in 28% aqueous NH_4OH−MeOH (1:1, 1 mL), and the mixture was stirred at room temperature for 48 h. After removal of the solvent under reduced pressure, the residue was separated by ODS HPLC with MeCN−H_2O (12:88) to give a compound [1.3 mg, 42%, $[\alpha]^{26}_D$ −67 (*c* 0.09, pyridine)] that was shown to be identical to the natural product **4** by comparison of their ^1H NMR and mass spectra and optical rotations.

Single-Crystal X-ray Crystallography of 1.[5] $C_{20}H_{27}NO_3 \cdot CH_3OH$, M = 361.47, 0.48 × 0.33 × 0.25 mm, orthorhombic, space group $P2_12_12_1$, a = 8.1460(2) Å, b = 9.2040(6) Å, c = 24.2500(16) Å, V = 1818.16(17) $Å^3$, Z = 4, D_X = 1.321 Mg m^{-3}, μ(Mo Kα) = 0.090 mm^{-1}, 2196 measured reflections, 2196 unique reflections, 1688 observed reflections [$I > 2\sigma(I)$], R1 = 0.0450, wR2 = 0.1021 (observed data), GOF = 0.944; R1 = 0.0567, wR2 = 0.1047 (all data).

The structure was solved by direct methods using the maXus crystallographic software package[6] and refined by full-matrix least-squares on F^2 using the program SHELXL-97.[7] The absolute structure could not be determined crystallographically.

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References and Notes

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(5) Crystallographic data for tricalysiamide A (**1**) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, under the reference number CCDC 622951. 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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