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Tetrahedron 63 (2007) 4558–4562
Tetrahedron

# Three new diterpenoids, tricalysiolide H and tricalysiones A and B, from *Tricalysia dubia*

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Received 10 January 2007; accepted 19 February 2007
Available online 2 March 2007

**Abstract**—Three new diterpenoids, tricalysiolide H (**1**) and tricalysiones A (**2**) and B (**3**), with novel structural features were isolated from the wood of *Tricalysia dubia*, together with a known compound, cafestol (**4**). The structures of **1–3** were elucidated on the basis of 2D NMR spectroscopy and X-ray crystallographic analysis.


## 1. Introduction

*Tricalysia dubia* (Lindl.) Ohwi (Rubiaceae), an evergreen shrub or tree, 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 our previous paper, six rearranged *ent*-kaurane diterpenes, tricalysiolides A–F, were isolated from the wood of this plant and their structures were elucidated.[3] In the present study, from the wood of this plant, we isolated three novel kaurane diterpenoids, tricalysiolide H (**1**) and tricalysiones A (**2**) and B (**3**), and a known diterpene, cafestol (**4**),[4] and determined the structures of **1–3** (Fig. 1).

## 2. Results and discussion

By a series of column chromatography on highly porous synthetic resin (Diaion HP-20), silica gel, aminopropyl-bonded silica gel, and ODS HPLC, a MeOH extract of air-dried wood of *T. dubia* afforded new diterpenoids named tricalysiolide H (**1**), tricalysiones A (**2**) and B (**3**) along with a known compound, cafestol (**4**).[4] The identification of cafestol (**4**) was made by the spectroscopic data.

Tricalysiolide H (**1**) was isolated as colorless prisms. Its molecular formula, $C_{21}H_{32}O_5$, was determined from the $[M+H]^+$ peak at *m/z* 365.2286 (calcd for $C_{21}H_{33}O_5$ 365.2328) in the HRESIMS. The IR spectrum indicated that **1** possessed hydroxyl (3420 $cm^{-1}$) and γ-lactone (1765 $cm^{-1}$) groups. The $^1H$ NMR spectrum gave signals due to one tertiary methyl (δ 0.85, s, 3H) and one methoxyl group (δ 3.44, s, 3H) (Table 1), and the $^{13}C$ NMR spectrum gave the signals due to one methyl, 10 methylenes, four methines, five quaternary carbons, one of which being a carbonyl carbon, and one methoxyl carbon (Table 2). Detailed analysis of the $^1H$–$^1H$ COSY, HMQC, and HMBC spectra showed that **1** had the same B, C, and D ring structures as cafestol (**4**). By the

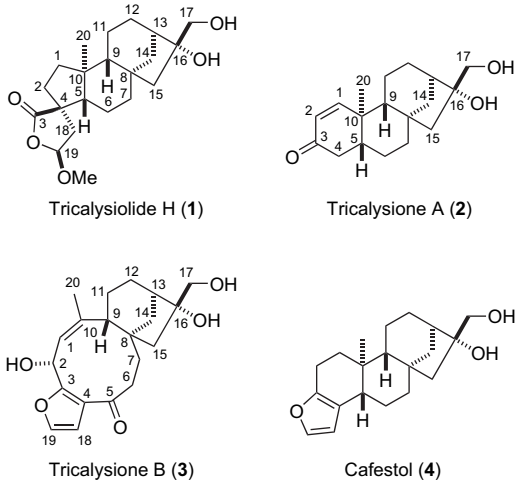


**Figure 1**. Structures of tricalysiolide H (**1**), tricalysiones A (**2**), B (**3**), and cafestol (**4**) from *Tricalysia dubia*.

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doi:10.1016/j.tet.2007.02.113

**Table 1**. $^1$H NMR data (500 MHz, $\delta$) for tricalysiolide H (**1**), tricalysiones A (**2**) and B (**3**) in $C_5D_5N$[a]

| Proton | Tricalysiolide H (**1**) | Tricalysione A (**2**) | Tricalysione B (**3**) |
|---|---|---|---|
| 1a | 1.67 (m) | 7.03 (d, 10.0) | 5.95 (d, 10.0) |
| 1b | 1.21 (m) | | |
| 2a | 2.32 (td, 13.9, 3.7) | 5.93 (d, 10.0) | 6.61 (dd, 10.0, 4.3) |
| 2b | 1.75 (m) | | |
| 4a | | 2.39 (dd, 17.6, 14.2) | |
| 4b | | 2.25 (dd, 17.6, 3.8) | |
| 5 | 1.97 (dd, 12.3, 2.2) | 1.71 (m) | |
| 6a | 1.49 (m) | 1.39 (dddd, 12.7, 12.7, 12.7, 3.4) | 3.19 (t, 12.3) |
| 6b | 1.45 (m) | 1.15 (m) | 2.67 (ddd, 12.0, 12.0, 8.9) |
| 7a | 1.60 (dt, 13.0, 3.1) | 1.57 (dt, 12.7, 3.1) | 1.92 (m) |
| 7b | 1.48 (td, 13.0, 3.9) | 1.50 (td, 12.7, 3.8) | 1.55 (ddd, 12.8, 12.8, 8.9) |
| 9 | 1.22 (d, 8.0) | 1.14 (d, 9.0) | 3.19 (d, 7.5) |
| 11a | 1.40 (dd, 11.3, 5.5) | 1.76 (m) | 1.85 (m) |
| 11b | 1.37 (m) | 1.64 (dd, 15.4, 7.2) | 1.46 (dd, 14.2, 5.9) |
| 12a | 1.91 (m) | 1.94 (m) | 1.90 (m) |
| 12b | 1.57 (m) | 1.49 (m) | 1.70 (m) |
| 13 | 2.45 (d-like, 3.0) | 2.47 (d-like, 3.4) | 2.46 (s-like) |
| 14a | 1.99 (dd, 11.5, 4.1) | 2.04 (dd, 11.3, 4.3) | 2.06 (m) |
| 14b | 1.88 (d, 11.5) | 1.85 (d, 11.3) | 1.69 (d, 11.7) |
| 15a | 1.83 (d, 14.0) | 1.82 (d, 14.0) | 2.05 (d, 14.0) |
| 15b | 1.66 (d, 14.0) | 1.70 (dd, 14.0, 1.6) | 1.71 (d, 14.0) |
| 17a | 4.12 (dd, 10.8, 4.7) | 4.13 (dd, 10.8, 4.4) | 4.11 (dd, 11.0, 4.5) |
| 17b | 4.03 (dd, 10.8, 4.7) | 4.06 (dd, 10.8, 4.4) | 4.05 (dd, 11.0, 4.5) |
| 18a | 2.31 (dd, 14.0, 3.8) | | 7.06 (d, 1.8) |
| 18b | 2.17 (dd, 14.0, 6.3) | | |
| 19 | 5.42 (dd, 6.3, 3.8) | | 7.69 (d, 1.8) |
| 20 | 0.85 (s, 3H) | 1.07 (s, 3H) | 1.89 (s, 3H) |
| OMe | 3.44 (s, 3H) | | |
| OH-2 | | | 7.66 (d, 4.3) |
| OH-16 | 5.12 (s) | 5.24 (s) | 5.29 (s) |
| OH-17 | 6.10 (t, 4.7) | 6.16 (t, 4.4) | 6.19 (t, 4.5) |

[a] Assignments based on $^1$H–$^1$H COSY, HMQC, and HMBC experiments. Multiplicity and *J*-values in Hz are given in parentheses.

$^1$H–$^1$H COSY correlations between $H_2$-1 and $H_2$-2, and the HMBC correlations from H-1a, $H_2$-2, and H-5 to C-4 and from $H_3$-20 to C-1, C-5, and C-10, the A ring of **1** was indicated to be a cyclopentane ring, and by the correlations from $H_2$-2, H-5, $H_2$-18, and H-19 to C-3, from $H_2$-18 to C-2 and C-4, from H-18b to C-5, and from $H_2$-2 and H-5 to C-18, the presence of a γ-lactone spiro-linked at C-4 of the A ring was implied. By an HMBC correlation from the methoxyl protons to C-19, the methoxyl group was indicated to be at C-19 (Fig. 2). NOE correlations 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, and those between H-14a/OH-16, H-14b/$H_3$-20, and $H_2$-18/$H_3$-20 indicated that the C-20 methyl, C-14 and C-18 methylenes, and the hydroxyl group at C-16 were α-oriented (Fig. 3). By an NOE correlation detected between H-6a and $OCH_3$-19, the relative stereochemistry on the lactone ring was assigned to be 4*R** and 19*S**. From these observations, tricalysiolide H (**1**) was considered to have the structure shown in Figure 1, which was confirmed by a single-crystal X-ray analysis (Fig. 4). Tricalysiolide H (**1**) is a diterpenoid having an unusual structure in which a γ-lactone is spiro-linked to the cyclopentane A ring of the rearranged kaurane skeleton. A possible biosynthetic route of **1** from cafestol (**4**), a known diterpenoid also isolated from this plant source in the present study, is depicted in Scheme 1. Oxidation of the furan ring of cafestol (**4**) produced keto-aldehyde **5**, which, via subsequent 1,2-migration of C-2 to C-4 through benzilic acid rearrangement-like reaction, produced acid **6** having the contracted A ring, which then converted to **1**.

**Table 2**. $^{13}$C NMR data (125 MHz, $\delta$) for tricalysiolide H (**1**), tricalysiones A (**2**) and B (**3**) in $C_5D_5N$

| Carbon | Tricalysiolide H (**1**) | Tricalysione A (**2**) | Tricalysione B (**3**) |
|---|---|---|---|
| 1 | 41.6 | 161.1 | 129.8 |
| 2 | 38.9 | 126.6 | 64.2 |
| 3 | 183.0 | 199.3 | 160.6 |
| 4 | 50.6 | 41.3 | 121.1 |
| 5 | 57.9 | 45.8 | 195.4 |
| 6 | 21.5 | 26.5 | 39.3 |
| 7 | 41.4 | 40.9 | 35.8 |
| 8 | 44.3 | 45.1 | 48.3 |
| 9 | 54.9 | 48.8 | 42.2 |
| 10 | 47.8 | 41.2 | 140.3 |
| 11 | 21.3 | 19.0 | 23.2 |
| 12 | 26.5 | 26.3 | 25.9 |
| 13 | 46.2 | 45.8 | 45.9 |
| 14 | 37.9 | 38.3 | 40.1 |
| 15 | 53.2 | 53.6 | 47.4 |
| 16 | 81.5 | 81.6 | 82.0 |
| 17 | 66.4 | 66.4 | 66.1 |
| 18 | 40.9 | | 111.2 |
| 19 | 103.7 | | 142.2 |
| 20 | 17.4 | 17.3 | 24.7 |
| OMe | 56.8 | | |

Tricalysione A (**2**) was isolated as an amorphous solid. Its molecular formula was determined to be $C_{18}H_{26}O_3$ from the $[M+H]^+$ peak at *m*/*z* 291.1942 (calcd for $C_{18}H_{27}O_3$ 291.1915) in the HRESIMS. The IR spectrum of **2** indicated the presence of hydroxyl (3410 $cm^{-1}$) and α,β-unsaturated ketone (1670 $cm^{-1}$) groups. The presence of an α,β-unsaturated ketone was also implied by the UV absorption maximum at 230 nm (log $\varepsilon$ 3.86). The $^1$H NMR spectrum showed characteristic signals of one tertiary methyl group ($\delta$ 1.07, s, 3H) and a pair of cis-coupled olefinic protons ($\delta$ 5.93 and 7.03, each d, *J*=10.0 Hz) (Table 1). The $^{13}$C

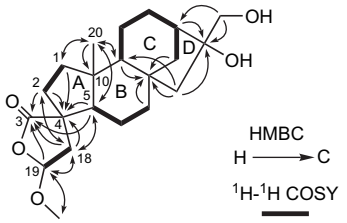



**Figure 2**. $^1$H–$^1$H COSY and selected HMBC correlations for **1**.

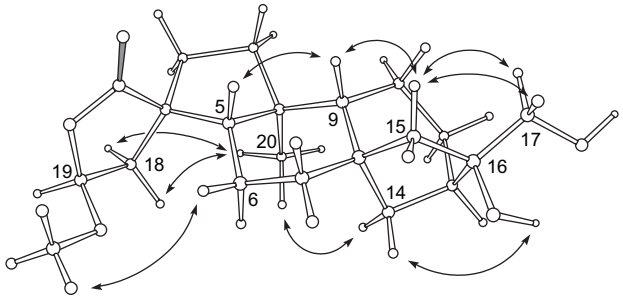


**Figure 3**. Selected NOE correlations for tricalysiolide H (**1**).

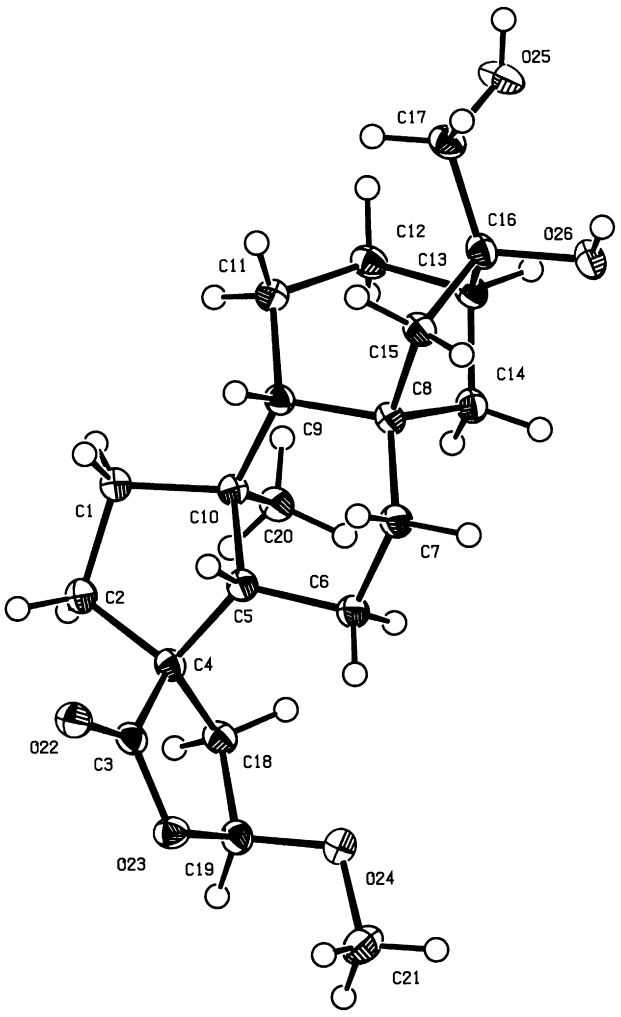



**Figure 4**. ORTEP representation of tricalysiolide H (**1**).

NMR spectrum gave signals of 18 carbons, comprising one methyl, eight methylenes, three methines, two olefinic methines, three quaternary carbons, and one ketone carbonyl carbon (Table 2). Analysis of the $^1$H–$^1$H COSY, HMQC, and HMBC spectra revealed that the B, C, and D ring structures of **2** were the same as those of cafestol (**4**) (Fig. 5). By the $^1$H–$^1$H COSY and HMQC spectra, C-4 ($\delta_C$ 41.3) of **2** was shown to be a methylene carbon. By the HMBC correlations

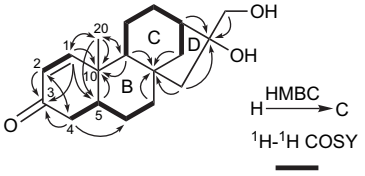



**Figure 5**. $^1$H–$^1$H COSY and selected HMBC correlations for **2**.

from an olefinic methine proton resonated at $\delta$ 7.03 to C-5 and C-10, one olefinic bond was placed at C-1/C-2, and by the correlations from H-1, H-2, and $H_2$-4 to the quaternary carbon at $\delta$ 199.3, a ketone was placed at C-3. By the NOE correlations between H-5/H-9, H-9/H-15b, and H-15b/$H_2$-17, H-5, H-9, H-15b, and the C-17 hydroxymethyl were shown to be β-oriented, whereas by the correlations between $H_3$-20/H-14b and H-14a/OH-16, the C-20 methyl, C-14 methylene, and the hydroxyl group at C-16 were shown to be α-oriented. Accordingly, tricalysione A (**2**) was determined to have a bisnor-kauranoid structure given in Figure 1.

Tricalysione B (**3**) was isolated as an amorphous solid. Its molecular formula was determined to be $C_{20}H_{26}O_5$ from the $[M+H]^+$ peak at *m*/*z* 347.1845 (calcd for $C_{20}H_{27}O_5$ 347.1858) in the HRESIMS. Its $^1$H NMR spectrum showed the presence of one allylic methyl group ($\delta$ 1.89, s, 3H) and three olefinic protons (Table 1). The $^{13}$C NMR spectrum of **3** displayed signals due to one methyl, seven methylenes, three methines, two quaternary carbons, three olefinic methines, three olefinic quaternary carbons, and a ketone carbonyl carbon (Table 2). By the analysis of the $^1$H–$^1$H COSY, HMQC, and HMBC spectra, **3** was shown to have the same C and D ring structures as cafestol (**4**) (Fig. 6) and an α,β-disubstituted furan ring ($\delta_H$ 7.06 and 7.69, each d, $J$=1.8 Hz; $\delta_C$ 111.2 d, 121.1 s, 142.2 d, 160.6 s). By the HMBC correlations from H-9 and $H_2$-11 to the olefinic quaternary carbon

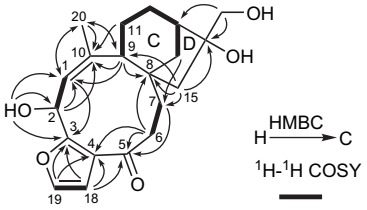
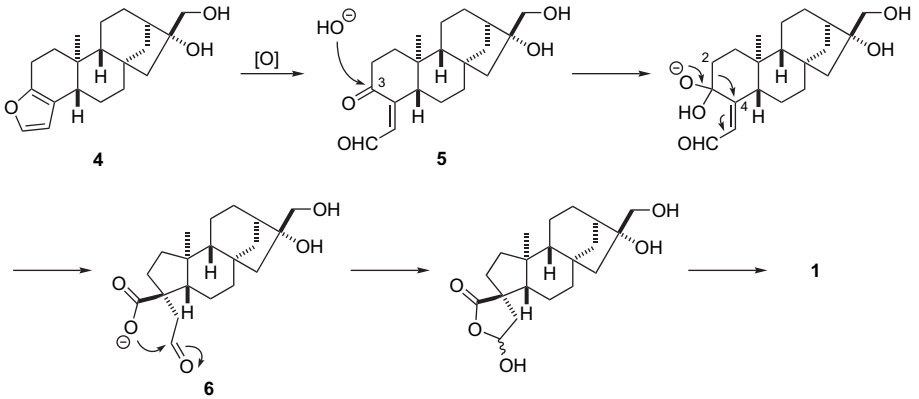



**Figure 6**. $^1$H–$^1$H COSY and selected HMBC correlations for **3**.




**Scheme 1**. A possible biosynthetic pathway from cafestol (**4**) to tricalysiolide H (**1**).

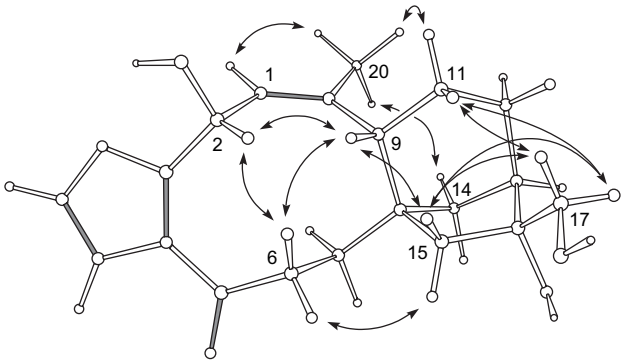



**Figure 7**. Selected NOE correlations for tricalysione B (**3**).

resonated at $\delta$ 140.3 and from H-9 to the olefinic methine carbon at $\delta$ 129.8, one olefinic bond was placed at C-1/C-10. By the correlation of H-2 with H-1 ($\delta$ 5.95) in the $^1$H–$^1$H COSY spectrum, the oxymethine proton at $\delta$ 6.61 was assigned to H-2. This oxymethine proton was also correlated with a hydroxyl proton at $\delta$ 7.66, thus indicating the presence of a hydroxyl group at C-2. The furan ring was considered to be fused at C-3/C-4, because one of the quaternary carbons of the furan ring at $\delta$ 160.6 was correlated with H-1 and OH-2, and the other quaternary carbon at $\delta$ 121.1 was correlated with H-6b in the HMBC spectrum. By the HMBC correlations from $H_2$-6, H-7a, and H-18 to the carbonyl carbon at $\delta$ 195.4, a ketone was placed at C-5 (Fig. 6). These observations and the fact that it had eight degrees of unsaturation as deduced from the molecular formula implied that compound **3** was a new diterpenoid having a 5,10-seco-kauranoid skeleton. The stereochemistry of **3** was determined on the basis of NOESY experiments in $CD_3OD$ (Fig. 7). By the NOE correlations between H-1 and $H_3$-20, the geometry of the C-1/C-10 double bond was assigned as *Z*. NOE correlations between H-2/H-6a, H-2/H-9, H-6a/H-9, H-9/H-15a, and H-15a/$H_2$-17 indicated that H-2, H-9, H-15a, and the C-17 hydroxymethyl were β-oriented; hence the hydroxyl group at C-2 was concluded to be α-oriented. Thus, the structure of tricalysione B (**3**) was determined to be as shown in Figure 1.

The three new diterpenoids, tricalysiolide H (**1**), and tricalysiones A (**2**) and B (**3**), isolated from the wood of *T. dubia* possess characteristic structural features. Tricalysiolide H (**1**) has a novel carbon framework, tricalysione A (**2**) is a 18,19-bisnor-kauranoid, not previously reported in natural products, and tricalysione B (**3**) contains a rare 5,10-seco-kauranoid skeleton.[5] The absolute configuration of compounds **1–3** remains to be established, but it may be reasonable to consider that these compounds are *ent*-derivatives, because the related kauranoids, tricalysiosides A–G,[1] tricalysiolides A–F,[3] and cafestol (**4**),[4,6] isolated from this plant, are all of *ent*-configuration.

## 3. Experimental

### 3.1. 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 on a Bruker DRX-500 spectrometer at 300 K. The $^1$H chemical shifts in $C_5D_5N$ and in $CD_3OD$ were referenced to the residual $C_5D_4HN$ resonance at 7.21 ppm and $CD_2HOD$ at 3.31 ppm, respectively, and the $^{13}$C chemical shifts in $C_5D_5N$ and in $CD_3OD$ were referenced to the solvent resonance at 135.5 ppm and 49.0 ppm, respectively. Mass spectra were obtained by using a Micromass LCT spectrometer. Preparative HPLC was carried out on a JASCO PU-986 pump unit equipped with a UV-970 UV detector ($\lambda$=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 ($\lambda$=0.71073 Å).

### 3.2. Plant material

Wood of *T. dubia* was collected in Iriomote Island, Okinawa, in March 2005, and the plant origin was identified by Dr. T. Kinoshita (Teikyo University, Japan). A voucher specimen has been deposited at the Herbarium, Medicinal Plant Garden, Teikyo University (Sagamiko-machi, Kanagawa).

### 3.3. 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) eluate (192 g) was subjected to silica gel (Merck Kieselgel 60, 230–400 mesh, 1.5 kg) column chromatography 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 $CHCl_3$ eluate (76.8 g) was subjected to aminopropyl-bonded silica gel (Chromatorex, 200–350 mesh, 700 g) column chromatography eluting sequentially with $CHCl_3$–MeOH (250:1), $CHCl_3$–MeOH (100:1), $CHCl_3$–MeOH (50:1), $CHCl_3$–MeOH (20:1), $CHCl_3$–MeOH (10:1), and MeOH (each 2 L) to give six fractions. The first fraction (4.2 g) was subjected to silica gel column chromatography eluting sequentially with toluene–MeOH (5:1) and MeOH to give eight fractions. The fifth fraction (1.6 g) of the toluene–MeOH (5:1) eluates was further separated by ODS HPLC using MeOH–$H_2O$ (45:55) to afford fractions A–E. Fraction D (395 mg) was separated by repeated ODS HPLC using MeCN–$H_2O$ (25:75) and then MeOH–$H_2O$ (40:60) to afford **2** (5.9 mg). Separation of fraction E (531 mg) by ODS HPLC using MeOH–$H_2O$ (50:50) and then MeCN–$H_2O$ (23:77) afforded **1** (4.2 mg).

The $CHCl_3$–MeOH (10:1) fraction (25.1 g) of the first silica gel column chromatography was subjected to aminopropyl-bonded silica gel column chromatography eluting sequentially with $CHCl_3$–MeOH (30:1), $CHCl_3$–MeOH (20:1), $CHCl_3$–MeOH (10:1), and MeOH to give seven fractions. The first fraction (994 mg) was subjected to silica gel column chromatography eluting sequentially with $CHCl_3$–MeOH (30:1), $CHCl_3$–MeOH (15:1), and MeOH to give nine fractions. The eighth fraction (294 mg), eluted with

$CHCl_3$–MeOH (15:1), was further purified by repeated ODS HPLC using MeOH–$H_2O$ (35:65) and then MeCN–$H_2O$ (19:81) to afford **3** (2.0 mg).

The residue of the MeOH eluate (209 g) of the HP-20 column chromatography was subjected to silica gel column chromatography eluting sequentially with EtOAc, toluene–MeOH (5:1), $CHCl_3$–MeOH (5:1), and MeOH (each 6 L). After evaporation, the EtOAc eluate (104 g) was subjected to aminopropyl-bonded silica gel column chromatography eluting with $CHCl_3$, $CHCl_3$–MeOH (100:1), and then MeOH to afford seven fractions. The third fraction (9.7 g), eluted with $CHCl_3$–MeOH (100:1), was subjected to silica gel column chromatography eluting with toluene–MeOH (12:1) and then MeOH to give eight fractions. The fourth fraction (2.4 g) was further separated by silica gel column chromatography using $CHCl_3$–MeOH (20:1), $CHCl_3$–MeOH (15:1), and then MeOH to give five fractions. The third fraction (1.0 g), eluted with $CHCl_3$–MeOH (20:1), was subjected to silica gel column chromatography using toluene–acetone–MeOH (15:1:1) to give five fractions. The second fraction (0.55 g) was separated by repeated ODS HPLC using MeOH–$H_2O$ (50:50) and then MeCN–$H_2O$ (35:65) to give **4** (157 mg).

### 3.4. Characteristics of each terpenoid

**3.4.1. Tricalysiolide H (1).** Colorless prisms (MeOH); mp 206–208 °C; $[\alpha]_D^{26}$ −14 (*c* 0.12, pyridine); IR (film) $\nu_{max}$ 3420, 2931, 2867, 1765, 1449, 1360, 1165, 1125, 1026 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, Tables 1 and 2; HRESIMS *m/z* 365.2286 $[M+H]^+$ (calcd for $C_{21}H_{33}O_5$ 365.2328).

**3.4.2. Tricalysione A (2).** Amorphous solid; $[\alpha]_D^{26}$ −75 (*c* 0.14, $CHCl_3$); IR (film) $\nu_{max}$ 3410, 2927, 2864, 1670, 1469, 1450, 1254, 1055, 1018 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$) 230 (3.86); $^1H$ and $^{13}C$ NMR data, Tables 1 and 2; HRESIMS *m/z* 291.1942 $[M+H]^+$ (calcd for $C_{18}H_{27}O_3$ 291.1915).

**3.4.3. Tricalysione B (3).** Amorphous solid; $[\alpha]_D^{25}$ −102 (*c* 0.10, pyridine); IR (film) $\nu_{max}$ 3377, 2925, 2855, 1651, 1566, 1463, 1408, 1260, 1030 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$) 206 (3.86), 267 (3.40); $^1H$ and $^{13}C$ NMR data in $C_5D_5N$, Tables 1 and 2; $^1H$ NMR (500 MHz, $CD_3OD$) $\delta$ 7.49 (1H, d, *J*=1.9 Hz, H-19), 6.70 (1H, d, *J*=1.9 Hz, H-18), 6.13 (1H, d, *J*=10.2 Hz, H-2), 5.53 (1H, d, *J*=10.2 Hz, H-1), 3.76 (1H, d, *J*=11.4 Hz, H-17a), 3.67 (1H, d, *J*=11.4 Hz, H-17b), 3.14 (1H, d, *J*=7.2 Hz, H-9), 3.08 (1H, t, *J*=12.4 Hz, H-6a), 2.43 (1H, ddd, *J*=12.2, 12.2, 9.0 Hz, H-6b), 2.10 (1H, s-like, H-13), 1.98 (3H, s, $H_3$-20), 1.93 (1H, m, H-11a), 1.81–1.70 (6H, m, H-7a, H-12a, H-12b, H-14a, H-14b, H-15a), 1.68 (1H, d, *J*=14.6 Hz, H-15b), 1.63 (1H, dd, *J*=14.8, 5.8 Hz, H-11b), 1.43 (1H, ddd, *J*=13.2, 12.7, 9.0 Hz, H-7b); $^{13}C$ NMR (125 MHz, $CD_3OD$) $\delta$ 198.1 (C, C-5), 160.8 (C, C-3), 143.2 (CH, C-19), 142.4 (C, C-10), 129.0 (CH, C-1), 121.6 (C, C-4), 111.3 (CH, C-18), 83.2 (C, C-16), 66.6 ($CH_2$, C-17), 64.9 (CH, C-2), 47.6 ($CH_2$, C-15), 46.3 (CH, C-13), 43.2 (CH, C-9), 40.5 ($CH_2$, C-14), 39.6 ($CH_2$, C-6), 36.5 ($CH_2$, C-7), 26.4 ($CH_2$, C-12), 24.8 ($CH_3$, C-20), 23.9 ($CH_2$, C-11); HRESIMS *m/z* 347.1845 $[M+H]^+$ (calcd for $C_{20}H_{27}O_5$ 347.1858).

### 3.5. Single-crystal X-ray crystallography of 1[7]

$C_{21}H_{32}O_5 \cdot CH_3OH$; *M*=396.51; 0.48×0.33×0.33 mm; monoclinic; space group $P2_1$; *a*=7.4610(5) Å; *b*=6.3650(2) Å; *c*=22.0660(14) Å; $\beta$=94.992(3)°; *V*=1044.03(10) $Å^3$; *Z*=2; $D_x$=1.261 Mg $m^{-3}$; $\mu$(Mo K$\alpha$)=0.090 $mm^{-1}$; 2442 measured reflections, 2442 unique reflections, 2199 observed reflections [$I>2\sigma(I)$], $R_1$=0.0332, $wR_2$=0.0856 (observed data), GOF=0.998; $R_1$=0.0363, $wR_2$=0.0864 (all data). The structure was solved by direct methods using the maXus crystallographic software package,[8] and refined by full-matrix least-squares on $F^2$ using the program SHELXL-97.[9] The absolute structure could not be determined crystallographically.

## Acknowledgements

Our thanks are due to Tropical Biosphere Research Center, Iriomote Station, University of the Ryukyus for providing us with the facilities during our collection of the plant material.

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7. Crystallographic data for tricalysiolide H (**1**) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, under the reference number CCDC-633038. 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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