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Tricalysiosides H–O: *Ent*-kaurane glucosides from the leaves of *Tricalysia dubia*

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Abstract

Eight *ent*-kaurane glucosides, named tricalysiosides H–O (**1**–**8**), were isolated from *Tricalysia dubia*. Tricalysioside H (**1**) possessed a hydroxyl group at the 1-position, to which the glucose moiety was attached. The structure was first elucidated by means of spectroscopic data analysis and finally confirmed by X-ray crystallography. Since acid hydrolysis of **1** gave D-glucose, the aglycone was proved to have an *enantio*-kaurane type skeleton. The structures of tricalysiosides I–O (**2**–**8**) were mainly elucidated from analysis of spectroscopic evidence.

Keywords: *Tricalysia dubia*; Rubiaceae; *Ent*-kaurane glucoside; Tricalysiosides H–O; X-ray analysis

1. Introduction

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 (Xiao and Lu, 1987).

Tricalysia dubia (Lindl.) Ohwi (Rubiaceae) is an evergreen shrub or tree that grows to a height of about 2–4 m. It is distributed in the south of China, Taiwan and the southern part of Japan (Hatusima, 1975). In the course of our study of Okinawa's promising resource plants, constituents of the title plant, *T. dubia*, were investigated.

In a previous paper (He et al., 2002), the structural elucidation of seven rearranged *ent*-kaurane glucosides, tricalysiosides A–G, from *T. dubia* were reported. Investigation of the same plant afforded a further eight *ent*-kaurane glucosides, named tricalysiosides H–O (**1**–**8**). This paper deals with their structural elucidation.

2. Results and discussion

Air-dried leaves of *T. dubia* were extracted with MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The *n*-BuOH-soluble fraction was separated by various chromatographic procedures including column chromatography (CC) on a highly porous synthetic resin (Diaion HP-20), then normal silica gel and reversed-phase octadecyl silica gel (ODS) CC, and droplet counter-current chromatography (DCCC) to afford eight diterpenoid glucosides, named tricalysiosides

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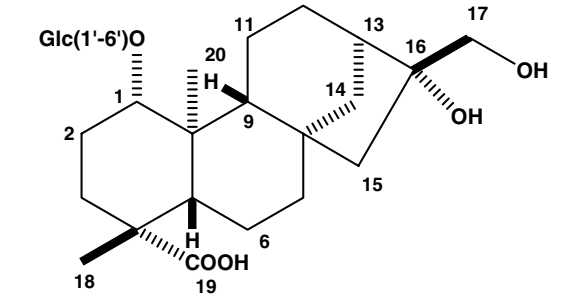

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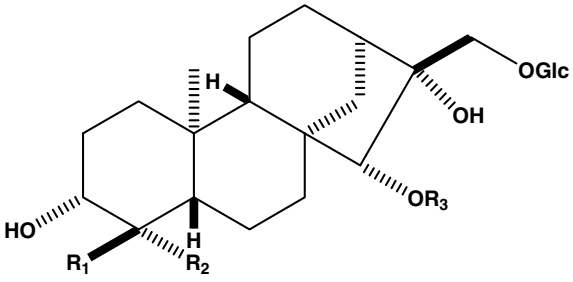
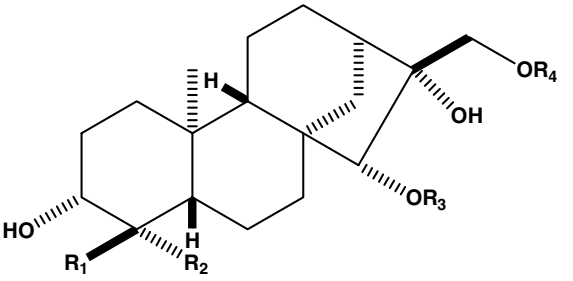


	R_1	R_2	R_3
2	CH_3	CHO	H
3	CH_3	CHO	Ac
4	CHO	H	Ac



	R_1	R_2	R_3	R_4
5	CH_3	CH_2OH	H	Glc
6	CH_3	CH_2OGlc	H	H
7	CH_3	CH_2OH	Ac	Glc
8	CH_2OH	CH_2OH	Ac	Glc

Fig. 1. Structures.

H–O (**1**–**8**) (Fig. 1). The details and yields are given in Section 4. The structure of tricalysioside H (**1**) was determined by spectroscopic methods and finally confirmed by X-ray crystallographic analysis, whereas the structures of tricalysiosides I–O (**2**–**8**) were elucidated from spectroscopic evidence.

Tricalysioside H (**1**), $[\alpha]_D$ −22.8°, was isolated as colorless rods and its elemental composition determined to be $C_{26}H_{42}O_{10}$ by negative-ion high-resolution (HR)-FAB-MS. The IR spectrum showed that compound **1** had a glycosidic feature (3407 and 1076 cm^{-1}), with an absorption band for a carboxyl group (1701 cm^{-1}). The ^{13}C NMR spectrum (Table 1) together with ^1H NMR, DEPT and two-dimensional spectra showed the presence of six signals assignable to β-glucopyranose, with the remaining 20 signals comprised those of one carboxyl carbon (δ_C 179.9), two singlet methyls, nine methylenes, one of which was assigned as a primary carbinol from its chemical shifts (δ_H 3.99 and 4.10 on δ_C 66.4), four methines, one of which was with an oxygen atom (δ_C 92.6), and four quartenary carbons, one of which bore an oxygen functional group (δ_C 82.1). From these functionalities together with co-occurrence of tricalysiosides A–G, compound **1** was presumed to be a diterpenoid glucoside with a kaurane skeleton. In the HMBC spectrum, since one (δ_H 1.31) of the methyl proton signals correlated with the carboxyl carbon signal, the carboxyl functional group must be at a geminal position as to the methyl group. One (δ_H 3.99, H-17a) of the methylene protons on the primary alcohol correlated with δ_C 82.1 (s), suggesting that the other methyl signal was located on C-10 as H_3-20 (δ_H 1.54), and then it correlated with the methine signal at δ_C 92.6, and the proton signal on it also correlated with the anomeric carbon signal. These results indicated that there was a hydroxyl group at C-1, to which the glucose moiety was attached. To confirm this assumption, X-ray crystal analysis was attempted using a suitable crystal. Fig. 2 shows a perspective drawing of tricalysioside H (**1**). As expected, functional groups were only located on rings A and D. On hydrolysis, **1** gave D-glucose. Thus, the aglycone was found to be in an *enantio*-kaurane form, as shown in Fig. 2. Therefore, the structure of

Table 1
^{13}C NMR spectroscopic data for tricalysiodies H–O (**1**–**8**) (100 MHz, pyridine-d_5)

C	1	2	3	4	5	6	7	8
1	92.6	36.0	35.9	35.3	36.0	36.0	36.4	36.3
2	28.3	28.6	28.6	27.2	28.5	28.6	28.4	28.2
3	36.7	76.4	76.1	71.6	80.3	79.4	80.0	74.3
4	43.6	53.4	53.3	56.3	43.3	43.3	43.3	47.1
5	56.0	56.8	56.4	47.2	56.1	56.4	55.6	48.3
6	22.8	20.4	20.2	22.0	20.2	21.2	20.1	20.3
7	43.7	38.0	38.2	38.5	38.9	39.5	38.7	38.8
8	46.0	47.4	47.3	47.6	47.6	48.0	47.5	47.5
9	55.7	54.6	54.1	55.2	56.0	56.3	55.6	55.8
10	45.8	39.3	39.3	38.3	39.3	39.6	39.3	39.2
11	21.2	19.0	19.0	18.8	18.9	19.1	18.9	19.0
12	27.4	26.0	25.7	25.8	26.1	26.3	25.8	25.9
13	45.7	43.7	44.7	44.7	43.8	43.6	44.8	44.9
14	39.0	36.5	36.6	36.6	37.0	37.5	36.5	36.5
15	53.7	82.6	83.8	83.7	82.7	82.7	83.7	83.9
16	82.1	80.6	81.1	81.1	80.6	81.3	81.2	81.2
17	66.4	74.8	75.3	75.2	74.8	66.3	75.5	75.4
18	29.3	21.5	21.5	207.1	23.8	24.5	23.7	63.8
19	179.9	207.5	207.5	9.4	64.4	72.6	64.4	63.1
20	13.0	18.4	18.2	18.2	18.5	17.9	18.4	18.3
1′	104.6	106.2	106.8	105.9	106.2	105.5	105.9	106.6
2′	75.8	75.4	75.5	75.4	75.4	74.9	75.6	75.5
3′	79.1	78.6	78.6	78.5	78.6	78.9	78.6	78.6
4′	71.8	71.7	71.7	71.7	71.7	71.8	71.7	71.7
5′	78.5	78.6	78.3	78.3	78.6	78.6	78.3	78.3
6′	62.9	32.8	62.7	62.7	62.8	62.8	62.7	62.7
CH_3 CO			21.1	21.1			21.1	21.1
CH_3 CO			171.2	171.2			171.2	171.1

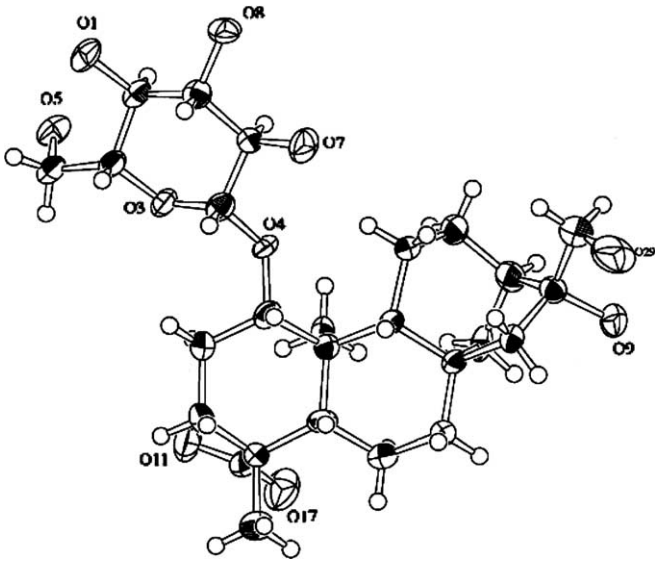


Fig. 2. Perspective ORTEP drawing of tricalysioside H (**1**). Oxygens have crystallographic numbering.

tricalysioside H (**1**) was elucidated to be *ent*-kaurane-1β, 16β, 17-triol-19-oic acid 1-*O*-β-D-glucopyranoside.

Tricalysioside I (**2**), $[\alpha]_D$ −28.8°, was isolated as colorless needles and its elemental composition was determined to be $C_{26}H_{42}O_{10}$ by negative-ion HR-FAB-MS. The IR spectrum showed that compound **2** had a glycosidic feature (3458–3429 and 1078 cm^{-1}), and an absorption band for a carbonyl group (1712 cm^{-1}) was observed. The NMR spectra showed the presence of an aldehyde functional group (δ_H 10.42 and δ_C 207.5), instead of the carboxyl group in the case of **1**. Another 19 carbon signals from the aglycone moiety and six carbon signals for the glucose moiety were also observed. In the HMBC spectrum (Fig. 3), the aldehyde proton correlated with the one of the methyl (δ_C 21.5) and methine C-5 (δ_C 56.8) carbons, and the protons (δ_H 1.43) on it also correlated with the aldehyde carbon. The methyl protons also correlated with the secondary alcohol methine carbon (δ_C 76.4 with δ_H 3.58). Similar to for tricalysioside H, one (δ_H 4.05, H-17a) of the methylene protons on the primary alcohol correlated with the quarternary carbon at δ_C 80.6 (s). Thus, the other methyl signal was placed on C-10 as H_3-20 (δ_H 0.90). Since both the protons on the primary alcohol correlated with an anomeric carbon at δ_C 106.2 and the anomeric proton (δ_H 5.02, *d*, $J = 8$ Hz) with the carbon signal (δ_C 74.8) of the primary alcohol, the glucose moiety was concluded to be linked to the primary alcohol in the β-mode. The remaining secondary alcohol was placed on C-15 from the fact that the proton (δ_H 3.76) on C-15 appeared as a singlet and was correlated to δ_C 74.8 (C-17) in the HMBC spectrum. The correlation observed between H-17b (δ_H 4.48) and C-15 also supported that the location of the hydroxyl group was C-15. Since the proton on C-3 appeared as *dd* with coupling constants of 13 and 5 Hz, it must be in an axial orientation. In the phase-sensitive (PH)-NOESY spectrum (Fig. 3), H-15 (δ_H 3.76) showed cross-peaks with H-9 (δ_H 1.14) and H-17b (δ_H 4.48), and thus these three protons were expected to be in the same face as the β-orientation. The correlation peak between the aldehyde proton and methyl protons at δ_H 0.90 enabled us to deduce that the methyl group at the C-4 position was in an equatorial β-orientation and the aldehyde group in an axial α-orientation. Therefore, the structure of tricalysioside I (**2**) was elucidated to be as shown in Fig. 1. Although the absolute configurations of the aglycone and glucose have not been determined yet, from the co-occurrence of tricalysioside H, they must be in an *enatio*-kaurane form and in D-series, respectively.

Tricalysioside J (**3**), $[\alpha]_D$ −20.8°, was isolated as colorless needles and its elemental composition was determined to be $C_{28}H_{44}O_{11}$ by negative-ion HR-FAB-MS. The NMR spectra indicated that tricalysioside J was the same compound as tricalysiodise I, except for the presence of an acetyl group (δ_H 2.17, and δ_C 21.1 and 171.2). Due to the formation of an ester linkage, a significant downfield shift, observed for the H-15 proton from δ_H 3.76 in **2** to 5.14 in **3**, and a downfield shift, also in C-15 from δ_C 82.6 to 83.8, the acetyl moiety must be on the secondary hydroxyl group at C-15. Therefore, the structure of tricalysioside J (**3**) was concluded to be the 15-*O*-acetate of tricalysioside I, as shown in Fig. 1.

Tricalysioside K (**4**), $[\alpha]_D$ −30.8°, was isolated as an amorphous powder and its elemental composition determined by

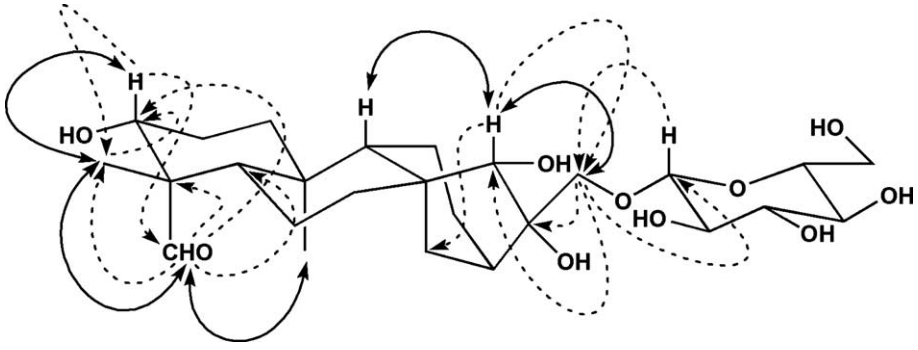


Fig. 3. Diagnostic HMBC and PH-NOEY correlations for tricalysioside I (**2**). Solid lines show phase-sensitive NOE correlations. Dotted lines show HMBC correlations. Arrowheads denote carbon atoms and arrowtails proton(s).

negative-ion HR-FAB-MS was the same as that of tricalysioside J. The NMR spectra also showed the same functionality as that of tricalysioside J. Since in the PH-NOESY spectrum, the aldehyde proton (δ_H 9.54) had a cross-peak with the H-3 proton (δ_H 4.06), and H_3-19 methyl protons (δ_H 1.33) correlated with H_3-20 protons (δ_H 0.98), the structure of tricalysioside K was concluded to be an epimeric compound of tricalysioside J as to the C-4 position.

Tricalysioside L (**5**), $[\alpha]_D$ −23.7°, was isolated as colorless needles and by negative-ion HR-FAB-MS, its elemental composition was determined to be $C_{26}H_{44}O_{10}$, which has one less degree of unsaturation than that of tricalysioside I (**2**). In the IR spectrum, no absorption band, attributable to a ketonic functional group was observed. The NMR spectroscopic data for the C and D rings were essentially the same as those of **2**. Instead of the signal assignable for an aldehyde group, which was seen in the NMR spectra of **2**, ^1H and ^{13}C NMR signals for one more primary alcohol were observed at δ_H 3.64 and 4.47, and at δ_C 64.4, respectively. These data suggested that **5** was an alcoholic derivative, e.g., the aldehyde in **2** could have been reduced to a primary alcohol. In the PH-NOESY spectrum, the cross-peak observed between H_2-19 (δ_H 3.64 and 4.47) and H_3-20 (δ_H 0.97) confirmed that the newly formed primary alcohol was in the axial α-orientation. Therefore, the structure of **5** was elucidated to be as shown in Fig. 1.

Tricalysioside M (**6**), $[\alpha]_D$ −39.2°, was isolated as colorless needles and by negative-ion HR-FAB-MS, its elemental composition was determined to be $C_{26}H_{44}O_{10}$, which was the same as that of **5**, and NMR spectroscopic analysis indicated that **6** was a similar compound to **5**. In the HMBC spectrum, cross-peaks were observed between the anomeric proton (δ_H 4.85) and the primary alcohol carbon (δ_C 72.6), whose protons (δ_H 3.91 and 4.68) then correlated with C-3 (δ_C 79.4), C-4 (δ_C 43.3), and C-18 (δ_C 24.5). PH-NOESY correlation was also observed between H_2-19 and H_3-20 (δ_H 1.14). This evidence led to the conclusion that **6** was the positional isomer of **5** as to the glucose moiety, and then its structure was elucidated to be as shown in Fig. 1.

Tricalysioside N (**7**), $[\alpha]_D$ −23.4°, was isolated as colorless needles and by negative-ion HR-FAB-MS, its elemental composition was determined to be $C_{28}H_{46}O_{11}$. The NMR spectroscopic signals for the A and B rings were essentially the same as those of tricalysioside L (**5**), and signals (δ_H 2.29 and, δ_C 21.1 and 171.2) assignable to an acetyl group were observed. A significant downfield shift ($\Delta\delta + 1.31$ ppm) observed for the H-15 (δ_H 5.07) signal, when compared with that of **5** (δ_H 3.76), and the HMBC correlation between H-15 and the acetyl carboxyl carbon supported the location of the acetyl moiety at the hydroxyl of C-15. Therefore, the structure of tricalysioside N (7) was elucidated to be 15-*O*-acetyl tricalysioside L, as shown in Fig. 1.

Tricalysioside O (**8**), $[\alpha]_D$ −23.0°, was isolated as colorless needles and by negative-ion HR-FAB-MS, its elemental composition was determined to be $C_{28}H_{46}O_{12}$, which was one more oxygen atom than that of **7**. The NMR spectroscopic signals of the B, C and D rings were essentially the same as those of **7**, and instead of only one methyl signal (δ_H 1.05 and δ_C 18.3), one more primary alcohol functional group was observed. From the chemical shifts, the methyl group at the C-10 position was expected to remain intact and thus both of the methyl groups at the C-4 position were presumed to be oxidized to primary alcohols. From the PH-NOESY correlations between δ_H 3.93 (*d*, $J = 11$ Hz, H-19a) and 4.56 (*d*, $J = 11$ Hz, H-19b), and δ_H 1.05 (H_3-20), these geminal protons were concluded to be on the axial carbinol carbon atom and then the carbon signal at δ_C 63.1 was judged to be in the axial position, such as C-19, in the HSQC experiment. Finally, observed cross-peaks between the protons on C-19 and C-18 (δ_C 63.8), and the protons on C-18 (δ_H 4.17 and 4.75) and 19 and C-3 (δ_C 74.3) in the HMBC spectrum supported the assumption. Therefore, the structure of tricalysioside O (**8**) was elucidated to be as shown in Fig. 1.

3. Conclusion

From Rubiaceous plant, *T. dubia*, eight new *ent*-kaurane glucosides, named tricalysiosides H–O (**1**–**8**), were isolated. *Ent*-kauranes and *ent*-kaurenes have relatively wide distribution in monocotyledons and dicotyledons, including Taxodiaceae (Vernin et al., 1990), Cupressaceae (Lin et al., 1999), Graminae (Kono et al., 1991), Liliaceae (Ruan et al., 2002), Araliaceae (Harinantenaina et al., 2002), Euphorbiaceae (Phan et al., 2005), Labiatae (Takeda and Otsuka, 1995; Takeda et al., 1997), Leguminosae (Murakami et al., 2000) and Compositae (Kohda et al., 1976), and a liverwort, Hepaticae (Nagashima et al., 2002). Almost all the plants, which belong to Rubiaceae family, are known to contain various types of iridoid glycosides (Inouye et al., 1988). It is interesting that only two genera of Rubiaceous plants, *Caffea* and *Tricalysia*, contain structurally rare rearranged *ent*-kauranes, cafestol (Wettstein et al., 1943) and furokaurane (Kaufmann and Sen Gupta, 1963), and their glycosides (He et al., 2002; Richter and Spiteller, 1979).

4. Experimental

4.1. General

Melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Union Giken PM-101 digital polarimeter. IR spectra were measured on a Horiba FT-710 spectrophotometer. ^1H and ^{13}C NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. Negative-ion HR-FAB-MS were taken on a JEOL JMS SX-102 spectrometer and PEG-400 was used as a calibration matrix.

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

4.2. 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 Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (90-TD-Okinawa-0822).

4.3. Extraction and fractionation

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

The *n*-BuOH-soluble fraction was separated first by CC on Diaion HP-20 ($\Phi = 5.0$ cm, $L = 60$ cm), with MeOH–H_2O [20% (8 L), 40% (8 L), 60% (8 L), and 80% (8 L) in water, successively], 500 mL factions being collected. The fraction eluted with 20%–40% MeOH (55.9 g in fractions 13–18) was subjected to a column of silica gel (1.0 kg) using $CHCl_3$ (3 L) and $CHCl_3$–MeOH [99:1 (3 L), 97:3 (3 L), 19:1 (3 L), 37:3 (3 L), 9:1 (6 L), 7:1 (6 L), 17:3 (6 L), 33:7 (6 L), 4:1 (6 L), 3:1 (6 L), and 7:3 (6 L); fractions of 500 mL being collected] as a solvent system. The residue of fractions 24–26 (1.64 g, 10% MeOH eluate) was subjected to ODS CC, fractions of 10 g being collected, and the residue (528 mg) of fractions 138–163 was purified by DCCC to afford 213 mg of crystalline compound **3** in fractions 138–165. A portion (1.94 g) of the residue (7.16 g in fractions 27–34, 10–12.5% MeOH eluate) was similarly subjected to ODS CC. The residues of fractions 133–147 (438 mg) and 148–153 (93.1 mg) were purified by DCCC to give 13.5 mg of **2** in fractions 85–92 in a crystalline state and 27.1 mg of **4** in fractions 104–125, respectively. A portion (1.79 g) of the residue (5.16 g in fractions 35–39, 15% MeOH eluate) was similarly subjected to ODS CC. The residue of fractions 116–124 (196 mg) was purified by DCCC to give 129 mg of **8** in fractions 39–49. Recrystallization of the residue (97.7 mg) of fractions in 135–141 gave 23.4 mg of compound **5**. A portion (1.91 g) of the residue (7.20 in fractions 46–53, 20–30% MeOH eluate) was subjected to ODS (110 mg in fractions 112–120) and DCCC to afford 40.8 mg of crystalline compound **6** in fractions 46–61.

The 40% eluate (26.6 g in fractions 13–18) on Diaion HP-20 CC was subjected to a column of silica gel (500 g) using $CHCl_3$ (3 L) and $CHCl_3$–MeOH [99:1 (3 L), 49:1 (3 L), 97:3 (3 L), 24:1 (3 L), 19:1 (2 L), 47:3 (3 L), 23:2 (3 L), 9:1 (4.5 L), 7:3 (4.5 L), 17:3 (4.5 L), 33:7 (4.5 L), 4:1 (3 L), 3:1 (3 L), and 7:3 (3 L); fractions of 500 mL being collected] as a solvent system. A portion (1.97 g) of the residue (6.00 g in fractions 42–49, 8–10% MeOH eluate) was similarly subjected to ODS CC. The residue of fractions 115–126 (436 mg) was purified by DCCC to give 294 mg of crystalline compound **7** in fractions 85–103. A portion (1.97 g) of the residue (2.75 g in fractions 64–72, 12.5–15% MeOH eluate) was similarly subjected to ODS CC. The residue of fractions 127–137 (439 mg) was purified by DCCC to give 274 mg of residue in fractions 31–48, which was then crystallized from MeOH to yield 190 mg of **1**.

4.4. Characterization data

4.4.1. Tricalysioside H (*1*)

Colorless rods, mp. 204–208 °C; $[\alpha]_D^{21}$ −22.8° (*c* 1.36, pyridine); IR ν_{max} (KBr) cm^{-1}: 3407, 2971, 2930, 2882, 1701, 1413, 1372, 1317, 1163, 1076, 1042; ^1H NMR (pyridine-d_5) δ: 1.10 (1H, *dd*, $J = 14$, 2 Hz, H-5), 1.21 (1H, *ddd*, $J = 14$, 14, 4 Hz, H-3a), 1.31 (3H, *s*, H_3-18), 1.51 (1H, *m*, H-7a), 1.54 (3H, *s*, H_3-20), 1.62 (1H, *m*, H-12a), 1.65 (1H, *br d*, $J = 8$ Hz, H-9), 1.81 (1H, *br d*, $J = 14$ Hz, H-7b), 1.87 (2H, *s*, H_2-15), 1.91 (1H, *m*, H-12b), 2.04 (1H, *dd*, $J = 14$, 2 Hz, H-6a), 2.10 (2H, *br s*, H_2-14), 2.10 (1H, *m*, H-11a), 2.33 (1H, *br ddd*, $J = 14$, 14, 14 Hz, H-6b), 2.40 (1H, *br s*, H-13), 2.47 (2H, *m*, H-2a and 3b), 2.64 (1H, *m*, H-2b), 3.50 (1H, *dd*, $J = 16$, 8 Hz, H-11b), 3.76 (1H, *dd*, $J = 12$, 4 Hz, H-1), 3.99 (1H, *d*, $J = 11$ Hz, H-17a), 4.00 (1H, *dd*, $J = 8$, 8 Hz, H-2′), 4.00 (1H, *m*, H-5′), 4.10 (1H, *d*, $J = 11$ Hz, H-17b), 4.21 (1H, *dd*, $J = 8$, 8 Hz, H-4′), 4.23 (1H, *dd*, $J = 8$, 8 Hz, H-3′), 4.38 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 4.53 (1H, *dd*, $J = 12$, 3 Hz, H-6′b), 4.98 (1H, *d*, $J = 8$ Hz, H-1′); For ^{13}C NMR (pyridine-d_5) spectrum, see Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 513.2685 $[M - H]^-$(calcd for $C_{26}H_{41}O_{10}$: 513.2700).

4.4.2. Tricalysioside I (*2*)

Colorless needles, mp. 243–247 °C; $[\alpha]_D^{21}$ −28.8° (*c* 0.87, pyridine); IR ν_{max} (KBr) cm^{-1}: 3458–3429, 3371, 3348, 2937, 2887, 1712, 1496, 1417, 1367, 1279, 1167, 1119, 1078, 1022; ^1H NMR (pyridine-d_5) δ: 0.90 (3H, *s*, H_3-20),

0.94 (1H, *dd*, $J = 14$, 4 Hz, H-7a), 1.05 (1H, *br d*, $J = 12$ Hz, H-5), 1.14 (1H, *br s*, H-9), 1.43 (3H, *s*, H_3-18), 1.46 (4H, *m*, H-6a, 11a, 11b and 12a), 1.73 (2H, *m*, H-1a and 14a), 1.77 (1H, *m*, H-12b), 1.81 (1H, *br d*, $J = 14$ Hz, H-7b), 1.90 (3H, *m*, H-1b, 6a and 14b), 1.96 (1H, *m*, H-6b), 2.07 (1H, *m*, H-2a), 2.26 (1H, *dddd*, $J = 13$, 13, 13, 4 Hz, H-2b), 2.44 (1H, *d-like*, $J = 3$ Hz, H-13), 3.58 (1H, *dd*, $J = 13$, 5 Hz, H-3), 3.76 (1H, *s*, H-15), 3.91 (1H, *m*, H-5′), 4.05 (1H, *d*, $J = 11$ Hz, H-17a), 4.07 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 4.20 (1H, *dd*, $J = 9$, 9 Hz, H-4′), 4.23 (1H, *dd*, $J = 9$, 9 Hz, H-3′), 4.31 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 4.48 (1H, *d*, $J = 11$ Hz, H-17b), 4.50 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 5.02 (1H, $J = 8$ Hz, H-1′), 10.42 (1H, *s*, H-19); For ^{13}C NMR (pyridine-d_5) spectrum, see: Table 1; HR-FAB-MS *m/z*: 513.2706 $[M - H]^-$ (calcd for $C_{26}H_{41}O_{10}$: 513.2700).

4.4.3. Tricalysioside J (**3**)

Colorless needles, mp. 189–190 °C; $[\alpha]_D^{23}$ −20.8° (*c* 1.59, pyridine); IR ν_{max} (KBr) cm^{-1}: 3433, 2937, 2871, 1721, 1649, 1448, 1375, 1252, 1164, 1078, 1041; ^{1}H NMR (pyridine-d_5) δ: 0.89 (3H, *s*, H_3-20), 0.94 (1H, *dd*, $J = 13$, 4 Hz, H-7a), 1.03 (1H, *br d*, $J = 11$ Hz, H-5), 1.28 (1H, *br s*, H-9), 1.33 (1H, *ddd*, $J = 13$, 13, 3 Hz, H-1a), 1.43 (1H, *m*, H-6a), 1.44 (3H, *s*, H_3-18), 1.48 (3H, *m*, H-11a, 11b and 12a), 1.51 (2H, *m*, H_2-2), 1.78 (4H, *m*, H-1b, 7b, 12b and 14a), 1.90 (1H, *v br d*, $J = 13$ Hz, H-6b), 2.07 (1H, *m*, H-14b), 2.17 (3H, *s*, –COCH_3), 2.42 (1H, *d-like*, $J = 3$ Hz, H-13), 3.59 (1H, *dd*, $J = 12$, 5 Hz, H-3), 3.92 (1H, *ddd*, $J = 9$, 5, 2 Hz, H-5′), 3.98 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 4.02 (1H, *d*, $J = 10$ Hz, H-17a), 4.15 (1H, *dd*, $J = 9$, 9 Hz, H-4′), 4.19 (1H, *dd*, $J = 9$, 9 Hz, H-3′), 4.37 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 4.40 (1H, *d*, $J = 11$ Hz, H-17b), 4.54 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.92 (1H, *d*, $J = 8$ Hz, H-1′), 5.14 (1H, *s*, H-15), 10.43 (1H, *s*, H-19); For ^{13}C NMR (pyridine-d_5) spectrum, see: Table 1, HR-FAB-MS *m/z*: 555.2786 $[M - H]^-$ (calcd for $C_{28}H_{43}O_{11}$: 555.2805).

4.4.4. Tricalysioside K (**4**)

Colorless amorphous powder; $[\alpha]_D^{24}$ −30.8° (*c* 1.59, pyridine); IR ν_{max} (KBr) cm^{-1}: 3431–3373, 2893, 2935, 2871, 1726, 1450, 1373, 1302, 1250, 1076, 1043; ^{1}H NMR (pyridine-d_5) δ: 0.92 (1H, *dd*, $J = 13$, 4 Hz, H-7a), 0.98 (3H, *s*, H_3-20), 1.04 (1H, *m*, H-6a), 1.30 (1H, *br s*, H-9), 1.33 (3H, *s*, H_3-19), 1.36 (1H, *br d*, $J = 13$ Hz, H-5), 1.39 (1H, *m*, H-1a), 1.40 (1H, *m*, H-6b), 1.50 (3H, *m*, H-11a, 11b and 12a), 1.68 (2H, *m*, H-1b and 7b), 1.80 (1H, *m*, H-12b), 1.85 (1H, *d*, $J = 12$ Hz, H-14a), 1.92 (2H, *m*, H_2-2), 2.10 (1H, *dd*, $J = 12$, 4 Hz, H-14b), 2.17 (3H, *s*, –COCH_3), 2.47 (1H, *d-like*, $J = 2$ Hz, H-13), 3.91 (1H, *ddd*, $J = 9$, 6, 3 Hz, H-5′), 3.98 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 4.06 (1H, *dd*, $J = 10$, 6 Hz, H-3), 4.17 (1H, *dd*, $J = 9$, 9 Hz, H-4′), 4.19 (1H, *dd*, $J = 9$, 9 Hz, H-3′), 4.24 (1H, *d*, $J = 11$ Hz, H-17a), 4.35 (1H, *dd*, $J = 12$, 6 Hz, H-6′a), 4.41 (1H, *d*, $J = 11$ Hz, H-17b), 4.52 (1H, *dd*, $J = 12$, 3 Hz, H-6′b), 4.91 (1H, *d*, $J = 8$ Hz, H-1′), 5.13 (1H, *s*, H-15), 9.54 (1H, *s*, H-18); For ^{13}C NMR (pyridine-d_5) spectrum, see Table 1; HR-FAB-MS *m/z*: 555.2793 $[M - H]^-$ (calcd for $C_{28}H_{43}O_{11}$: 555.2805).

4.4.5. Tricalysioside L (**5**)

Colorless needles, mp. 277–281 °C; $[\alpha]_D^{21}$ −23.7° (*c* 1.14, pyridine); IR ν_{max} (KBr) cm^{-1}: 3473–3344, 2935, 2870, 1408, 1363, 1280, 1254, 1200, 1165, 1119, 1078; ^{1}H NMR (pyridine-d_5) δ: 0.83 (1H, *ddd*, $J = 14$, 14, 4 Hz, H-7a), 0.91 (1H, *br d*, $J = 11$ Hz, H-5), 0.97 (3H, *s*, H_3-20), 1.03 (1H, *br s*, H-9), 1.30 (2H, *m*, H-1a and 6a), 1.47 (2H, *m*, H_2-11), 1.50 (3H, *s*, H_3-18), 1.75 (5H, *m*, H-1b, 6a, 7b, 12a and 12b), 1.88 (1H, *d*, $J = 12$ Hz, H-14a), 1.94 (2H, *m*, H_2-2), 2.04 (1H, *m*, H-14b), 2.46 (1H, *br s*, H-13), 3.61 (1H, *dd*, $J = 12$, 4 Hz, H-3), 3.64 (1H, *d*, $J = 11$ Hz, H-19a), 3.76 (1H, *s*, H-15), 3.97 (1H, *ddd*, $J = 9$, 5, 2 Hz, H-5′), 4.05 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 4.20 (1H, *dd*, $J = 9$, 9 Hz, H-4′), 4.22 (1H, *d*, $J = 11$ Hz, H-17a), 4.24 (1H, *dd*, $J = 9$, 9 Hz, H-3′), 4.37 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 4.47 (1H, *d*, J=11 Hz, H-19b), 4.49 (1H, *d*, J=11 Hz, H-17b), 4.54 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 5.02 (1H, *d*, $J = 8$ Hz, H-1′); For ^{13}C NMR (pyridine-d_5) spectrum, see Table 1; HR-FAB-MS *m/z*: 515.2821 $[M - H]^-$ (calcd for $C_{26}H_{43}O_{10}$: 515.2856).

4.4.6. Tricalysioside M (**6**)

Colorless needles, mp. (1) 248.5–249.0 °C, mp. (2) >300 °C; $[\alpha]_D^{21}$ −39.2° (*c* 1.20, pyridine); IR ν_{max} (KBr) cm^1: 3421, 3377, 3317, 2933, 2873, 1161, 1076, 1041; ^{1}H NMR (pyridine-d_5) δ: 0.86 (1H, *ddd*, $J = 11$, 11, 4 Hz, H-7a), 0.91 (1H, *br d*, $J = 11$ Hz, H-5), 1.09 (1H, *br s*, H-9), 1.14 (1H, *s*, H_3-20), 1.48 (3H, *s*, H_3-18), 1.52 (1H, *m*, H-12a), 1.52 (2H, *m*, H_2-11), 1.70 (1H, *m*, H-14a), 1.74 (1H, *m*, H-6a), 1.78 (2H, *m*, H-7b and 12b), 1.85 (1H, *br d*, $J = 11$ Hz, H-6b), 1.91 (1H, *m*, H-2a), 1.93 (3H, *m*, H-1a, 1b and 14b), 2.09 (1H, *dddd*, $J = 13$, 13, 13, 3 Hz, H-2b), 2.49 (1H, *s*, H-13), 3.47 (1H, *dd*, $J = 13$, 5 Hz, H-3), 3.79 (1H, *s*, H-15), 3.88 (1H, *ddd*, $J = 9$, 5, 2 Hz, H-5′), 3.91 (1H, *d*, $J = 10$ Hz, H-19a), 4.01 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 4.11 (2H, *s*, H_2-17), 4.15 (1H, *dd*, $J = 9$, 9 Hz, H-4′), 4.22 (1H, *dd*, $J = 9$, 9 Hz, H-3′), 4.31 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 4.50 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.68 (1H, *d*, $J = 10$ Hz, H-19b), 4.85 (1H, *d*, $J = 8$ Hz, H-1′); For ^{13}C NMR (pyridine-d_5) spectrum, see Table 1; HR-FAB-MS *m/z*: 515.2874 $[M - H]^-$ (calcd for $C_{26}H_{43}O_{10}$: 515.2856).

4.4.7. Tricalysioside N (**7**)

Colorless needles, mp. 198–202 °C; $[\alpha]_D^{21}$ −23.4° (*c* 0.81, pyridine); IR ν_{max} (KBr) cm^{-1}: 3429, 3411, 3348, 2935, 2871, 1718, 1375, 1254, 1165, 1078, 1041; ^{1}H NMR (pyridine-d_5) δ: 0.80 (1H, *ddd*, $J = 13$, 13, 4 Hz, H-7a), 0.88 (1H, *br d*, $J = 10$ Hz, H-5), 0.90 (1H, *d*, $J = 6$ Hz, H-9a), 0.92 (3H, *s*, H_3-20), 1.23 (1H, *br s*, H-9b), 1.30 (2H, *m*, H-1a and 6a), 1.47 (2H, *m*, H_2-11), 1.47 (3H, *s*, H_3-18), 1.69 (2H, *m*, H-6b and 7b), 1.80 (3H, *m*, H-1b, 12a and 12b), 1.83 (1H, *d*, $J = 12$ Hz, H-14a), 1.90 (2H, *m*, H_2-2), 2.09 (1H, *dd*, $J = 12$, 4 Hz, H-14b), 2.29 (3H, *s*, –COCH_3),

2.52 (1H, *d*, $J = 4$ Hz, H-13), 3.59 (1H, *dd*, $J = 11$, 4 Hz, H-3), 3.62 (1H, *d*, $J = 10$ Hz, H-19a), 3.85 (1H, *ddd*, $J = 9$, 5, 2 Hz, H-5′), 4.00 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 4.15 (1H, *dd*, $J = 9$, 9 Hz, H-4′), 4.20 (1H, *dd*, $J = 9$, 9 Hz, H-3′), 4.25 (1H, *d*, $J = 10$ Hz, H-17a), 4.31 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 4.42 (1H, *d*, $J = 10$ Hz, H-19b), 4.44 (1H, *d*, $J = 10$ Hz, H-17b), 4.51 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.89 (1H, *d*, $J = 8$ Hz, H-1′), 5.07 (1H, *s*, H-15); For ^{13}C NMR (pyridine-d_5) spectrum, see Table 1; HR-FAB-MS *m*/*z*: 557.2948 $[M - H]^-$ (calcd for $C_{28}H_{45}O_{11}$: 557.2962).

4.4.8. Tricalysioside O (**8**)

White amorphous powder; $[\alpha]_D^{21}$ −23.0° (*c* 1.13, pyridine); IR ν_{max} (KBr) cm^{-1}: 3782, 3433, 3402, 3373, 2933, 2875, 1720, 1373, 1255, 1164, 1076, 1039; ^{1}H NMR (pyridine-d_5) δ: 0.85 (1H, *m*, H-7a), 1.05 (3H, *s*, H_3-20), 1.27 (1H, *d*, $J = 7$ Hz, H-9), 1.48 (4H, *m*, H_2-11, 12a and 14a), 1.52 (1H, *m*, H-1a), 1.64 *d* (1H, $J = 10$ Hz, H-5), 1.69 (1H, *br d*, $J = 13$ Hz, H-7b), 1.81 (2H, *m*, H-1b and 12b), 1.86 (1H, *d*, $J = 11$ Hz, H-14b), 2.01 (1H, *m*, H-2a), 2.02 (2H, *s*, H_2-6), 2.10 (1H, *m*, H-2b), 2.11 (3H, *s*, –COCH_3), 2.52 (1H, *s*, H-13), 3.90 (1H, *m*, H-5′), 3.93 (1H, *d*, $J = 11$ Hz, H-19a), 3.99 (1H, *dd*, $J = 9$, 8 Hz, H-2′), 4.12 (1H, *dd*, $J = 9$, 9 Hz, H-4′), 4.17 (1H, *d*, $J = 11$ Hz, H-18a), 4.18 (1H, *dd*, $J = 9$, 9 Hz, H-3′), 4.25 (1H, *d*, $J = 11$ Hz, H-17a), 4.30 (1H, *m*, H-3), 4.32 (1H, *dd*, $J = 12$, 5 Hz, H-6′a), 4.42 (1H, *d*, $J = 11$ Hz, H-17b), 4.50 (1H, *dd*, $J = 12$, 2 Hz, H-6′b), 4.56 (1H, *d*, $J = 11$ Hz, H-19b), 4.75 (1H, *d*, $J = 11$ Hz, H-18b), 4.88 (1H, *d*, $J = 8$ Hz, H-1′), 5.04 (1H, *s*, H-15); For ^{13}C NMR (pyridine-d_5) spectrum, see Table 1; HR-FAB-MS *m*/*z*: 573.2881 $[M - H]^-$ (calcd for $C_{28}H_{45}O_{12}$: 573.2911).

4.4.9. Acidic hydrolysis of **1**

Tricalysioside H (**1**) (55 mg) was hydrolyzed with 20 mL of 2N H_2SO_4 under reflux for 2 h. The turbid hydrolysate was neutralized with $Ba(OH)_2$ and then the precipitate was removed by filtration. The filtrate was concentrated and subjected to silica gel (20 g) column chromatography [$CHCl_3$ (50 mL), $CHCl_3$–MeOH (9:1 50 mL), (4:1 100 mL), and (7:3 200 mL), and $CHCl_3$–MeOH–H_2O (7:3:0.5 200 mL); fractions of 15 mL being collected] to give 12.6 mg of D-glucose in fractions 22–30. D-glucose, $[\alpha]_D^{28}$ +50.0° (*c* 0.84, H_2O, 24 h after dissolving in H_2O).

4.4.10. X-ray structure determination of **1**

A suitable crystal (0.40 × 0.40 × 0.20 mm) was used for analysis. All data were obtained with a Rigaku AFC-5S automated four circle diffractometer with graphite-monochromated Mo Kα radiation. Crystal data: $C_{26}H_{42}O_{10}$, $M_r = 514.61$, orthorhombic, space group $P2_12_12_1$, $a = 19.768$ (6) Å, $b = 20.396$ (9) Å, $c = 13.586$ (6) Å, $V = 5478$ (3) Å^3, $Z = 4$, $D_x = 1.316$ Mg m^{-3}, $F(000) = 2336$, and μ(Mo Kα) = 1.012 cm^{-1}. Unit cell parameters were determined by least-square refinement of optimized setting angles of 25 reflections in the range of $10.0° < \theta < 12.5°$. The intensities were measured in the ω-scan mode, $2\theta < 55°$. Three standard reflections were monitored every 150 measurements. The data were corrected for Lorentz and polarization factors. Correction for secondary extinction was applied (coefficient = 0.9435×10^{-7}). Absorption correction was applied (Ψ-scan, transmission factor = 0.974 − 0.996) (North et al., 1968). Out of the 6922 independent reflections that were collected, 6890 with $I > 2.00\sigma(I)$ were used for the structural determination and refinement. The structure was solved by the direct method using the teXsan crystallographic software package (teXsan, 2000). All non-hydrogen atoms were found in the Fourier map. The refinement of atomic parameters was carried out by full matrix least-squares using anisotropic temperature factors for all non-H atoms. All H atoms, but not O atoms, were located geometrically and not refined. The final refinement converged with $R_1 = 0.056$ and $R_w = 0.169$ for 695 parameters. Atomic scattering factors were taken from international tables for X-ray crystallography (International Tables for X-ray Crystallography, 1992).

The final atom coordinates, and a list of the temperature factors and final structure factors have been deposited at the Cambridge Crystallographic Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB12 1EW, UK.

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References

Harinantenaina, L., Kasai, R., Yamasaki, K., 2002. *ent*-Kaurane diterpenoid glycosides from a Malagasy endemic plant, *Cussonia vantsilana*. Phytochemistry 61, 367–372.

Hatusima, S., 1975. Flora of Ryukyus, Added and Corrected. The Biological Society of Okinawa, Japan, p. 577.

He, D.-H., Otsuka, H., Hirata, E., Shinzato, T., Bando, M., Takeda, Y., 2002. Tricalysiosides A–G: rearranged *ent*-kaurane glycosides from the leaves of *Tricalysia dubia*. J. Nat. Prod. 65, 685–688.

Inouye, H., Takeda, Y., Nishimura, H., Kanomi, A., Okuda, T., Puff, C., 1988. Chemotaxonomic studies of rubiaceae plants containing iridoid glycosides. Phytochemistry 27, 2591–2598.

International Tables for X-ray Crystallography, 1992. vol. C, Kynoch Press, Birmingham, England.

Kaufmann, H.P., Sen Gupta, A.K., 1963. Terpenes as constituents of fats. II. Constitution of Kahweol.. Chem. Ber. 96, 2489–2498.

Kohda, H., Kasai, R., Yamasaki, K., Murakami, K., Tanaka, O., 1976. New sweet diterpene glucosides from *Stevia rebaudiana*. Phytochemistry 15, 981–983.

Kono, Y., Uzawa, J., Kobayashi, K., Suzuki, Y., Uramoto, M., Sakurai, A., Watanabe, M., Teraoka, T., Hosokawa, D., 1991. Structures of oryzalides A and B, and oryzalic acid A, a group of novel

antimicrobial diterpenes, isolated from healthy leaves of a bacterial leaf blight-resistant cultivar of rice plant. Agri. Biol. Chem. 55, 803–811.

Lin, T.-C., Fang, J.-M., Cheng, Y.-S., 1999. Terpenes and lignans from leaves of *Chamaecyparis formosensis*. Phytochemistry 51, 793–801.

Murakami, T., Kohno, K., Kishi, A., Matsuda, H., Yoshikawa, M., 2000. Medicinal foodstuffs. XIX. Absolute stereostructures of canavalioside, a new *ent*-kaurane-type diterpene glycoside, and gladiatosides A1, A2, A3, B1, B2, B3, C1, and C2, new acylated flavonol glycosides, from sword bean, the seeds of *Canavalia gladiata*. Chem. Pharm. Bull. 48, 1673–1680.

Nagashima, F., Kondoh, M., Uematsu, T., Nishiyama, A., Saito, S., Sato, M., Asakawa, Y., 2002. Cytotoxic and apoptosis-inducing *ent*-kaurane-type diterpenoids from the Japanese liverwort *Jungermannia truncata* Nees. Chem. Pharm. Bull. 50, 808–813.

North, A.C., Phillips, D.C., Mathews, F.S., 1968. A semi-empirical method of absorption correction. Acta Cryst. A24, 351–359.

Phan, M.G., Phan, S.T., Hamada, Y., Otsuka, H., 2005. Cytotoxic diterpenoids from Vietnamese medicinal plant *Croton tonkinensis* GAGNEP. Chem. Pharm. Bull. 53, 296–300.

Richter, H., Spiteller, G., 1979. A new furokaurane glycosides from green coffee-beans. Chem. Ber. 112, 1088–1092.

Ruan, H.-L., Zhang, Y.-H., Wu, J.-Z., S, H.-D., Fujita, T., 2002. Two new diterpenoid dimers, fritillebinide D and E, from bulbs of *Fritillaria ebeiensis*. J. Asian Nat. Prod. Res. 4, 309–314.

Takeda, Y., Otsuka, H., 1995. Recent advances in the chemistry of diterpenoids from *Rabdosia* species. In: Studies in Natural Product Chemistry. In: Atta-ur-Rahman (Ed.), Structure and Chemistry (Part C). Elsevier, Amsterdam, pp. 111–185.

Takeda, Y., Futatsuishi, Y., Matsumoto, T., Masuda, T., Terada, H., Otsuka, H., 1997. Longirabdolides E, F and G, 6,7-*seco*-*ent*-kauranoids from *Rabdosia longituba*. Phtytochemistry, 785–789.

teXsan, 2000. Molecular Structure Corporation and Rigaku Corporation. Single Crystal Structure Analysis Software. Version 1.11. 32000 Research Forest Drive, The Woodlands, TX 77381, USA., and 3-9-12 Matsubara-cho, Akishima-shi 196-8666, Tokyo, Japan.

Vernin, G., Faure, R., Pieribattesti, J.C., 1990. Two ent-kauranoid diterpenes constituents of *Cryptomeria japonica* D. Don: α-kaurene and 16-hydroxy-α-kaurane. J. Essent. Oil Res 2, 211–214.

Wettstein, A., Hunziker, F., Miescher, K., 1943. Cafestol V. Helv. Chim. Acta 26, 1197–1218.

Xiao, C.-H., Lu, Y.-R. (Eds.), 1987. Chinese Medicinal Chemistry. Shanghai Science Technology Press, Shanghai, China, pp. 1–7.