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The unusual canangafruticosides A–E: Five monoterpene glucosides, two monoterpenes and a monoterpene glucoside diester of the aryldihydronaphthalene lignan dicarboxylic acid from leaves of *Cananga odorata* var. *fruticosa*

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ABSTRACT

From the leaves of *Cananga odorata* var. *fruticosa*, five unusual monoterpene glucosides, named canangafruticosides A–E (**1**–**5**), along with two unusual non-glucosidic monoterpenes (**6**, **7**) were isolated. An aryldihydronaphthalene-type lignan dicarboxylate (**8**) was also isolated, with two moles of canangafruticoside A (**1**) on its ester moiety. This lignan also showed strong blue fluorescence emission under basic conditions. The structures of these compounds were elucidated by means of spectroscopic methods, with their absolute configurations determined by application of the modified Mosher's method to a compound chemically derived from canangafruticoside E.

1. Introduction

Cananga odorata (Lamarck) Hooker f. & Thomson var. *fruticosa* (Craib) Corner (Annonaceae) is known as ylang–ylang and grows in tropical Asia. Its leaves are long, smooth and glossy. Its flowers are greenish yellow and curly, each one resembling a starfish, and they yield a highly fragrant essential oil. The latter evokes a feeling of deep and languid calm that melts away anxiety, tension and stress, and thus is used in the field of aromatherapy. Thus, investigation of its essential oil has been reported (Katague and Kirch, 1963; Gaydou et al., 1986; Olivero et al., 1997). The isolation of some other components, such as a guaiapyridine sesquiterpene alkaloid and eudesmane sesquiterpenes from its fruit (Hsieh et al., 2001), and alkaloids from its stem bark, have also been reported (Leboeuf et al., 1975; Leboeuf and Cavé, 1976; Rao et al., 1986). This phytochemical investigation herein was thus intended to examine the non-volatile constituents from the plant part, which has not yet been used as a medicinal.

Although the International Plant Names Index (IPNI) lists only one species, *C. odorata*, there are obviously two different varieties of *C. odorata*. One is called shrubby cananga [*C. odorata* (Lamarck) Hooker f. & Thomson var. *fruticosa* (Craib) Corner] and the other one, fragrant cananga or wild cananga [*C. odorata* (Lamarck) Hooker f. & Thomson var. *odorata*] (Porcher, 2004). These two varieties are cultivated in the Botanical Gardens of the Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand. The former is a small shrubby tree of 5 m in height and the latter is a tall, longer, tree of about 30 m in height. This paper deals with investigation of constituents of the leaves of shrubby cananga.

2. Results and discussion

Air-dried leaves of *C. odorata* var. *fruticosa* were extracted with MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The **1**-BuOH-soluble fraction was separated by means of various chromatographic procedures including column chromatography (CC) on a highly-porous synthetic resin (Diaion HP-20), and then normal silica gel and reversed-phase octadecyl silica gel (ODS) CC, droplet counter-current chromatography (DCCC), and high-performance liquid chromatography (HPLC), respectively, to afford 10 compounds (**1**–**10**). The details and yields are given in Section 4. The structures of the new unusual monoterpene glucosides, named canangafruti-

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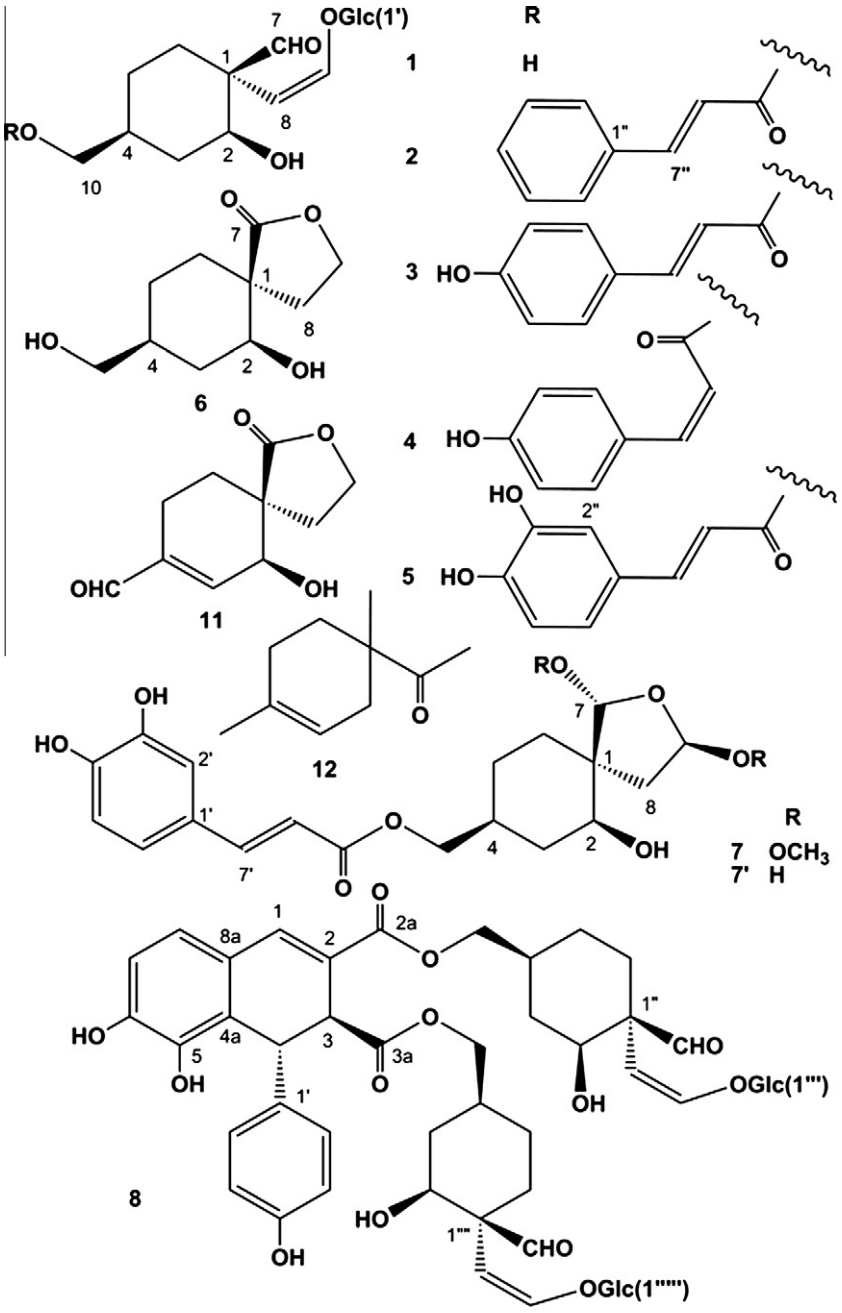


Fig. 1. Structures of interest.

cosides A–E (**1**–**5**), the unusual monoterpenes (**6**, **7**), and the unusual monoterpene diester of the lignan dicarboxylic acid (**8**) were elucidated on the basis of spectroscopic evidence (Fig. 1), and the known compounds were identified as corchoionoside C (**9**) (Yoshikawa et al., 1997) and (+)-syringaresinol 4-*O*-β-D-glucopyranoide (**10**) (Shahat et al., 2004) by comparison of spectroscopic data with those reported in the literature. From *C. odorata* f. *genuina*, an unusual monoterpene, called canangone (**11**) has been isolated (Caloprisco et al., 2002) and its absolute configuration was determined by synthesis of optically active (+)-canangone (Koripelly et al., 2007).

Canangafruticoside A (**1**), $[\alpha]_D^{25}$ +23.8, was isolated as an amorphous powder and its molecular formula was calculated to be $C_{16}H_{26}O_9$ from the results of high resolution (HR)-electrospray ionization (ESI) MS. The IR spectrum exhibited strong absorptions for hydroxyl groups (3363 and 1074 cm^{-1}) and a carbonyl functional group (1713 cm^{-1}). In the ^1H NMR spectrum, signals assignable to an anomeric proton (δ_H 4.47), olefinic protons on a *cis*-double bond [δ_H 6.44 (1H, *d*, *J* = 7 Hz, H-9) and 4.60 (1H, *d*, *J* = 7 Hz, H-8)] and an aldehyde (δ_H 9.81) were observed. The ^{13}C NMR spectrum with the DEPT experiments showed six signals assignable to β-glucopyranose and 10 resonances to three methylenes, one oxymethylene, one methine, one oxymethine, one disubstituted double bond, one carbonyl carbon (δ_C 207.1) with a hydrogen atom, and one quaternary carbon (Table 1). A highly deshielded olefinic carbon signal (δ_C 146.1) implied that the double bond was involved in an enol system. The absolute structure of glucose was determined to be of the D-series using a chiral detector. Analysis of the ^1H–^1H COSY and HSQC correlation spectra established proton sequences from the oxymethine proton (δ_H 3.80) on C-2 to methy-

Table 1
^{13}C NMR spectroscopic data for canangafruticosides A–E (**1**–**5**) and related compounds (**6** and **7**) (CD_3OD).

C	1[a]	2[b]	3[b]	4[b]	5[b]	6[a]	7[a]
1	56.6	56.3	56.3	56.3	56.3	49.6	51.2
2	76.2	75.7	75.8	75.7	75.8	76.2	76.2
3	36.1	35.9	35.9	35.9	35.9	34.7	35.6
4	40.5	37.3	37.4	37.3	37.4	40.4	37.0
5	26.3	26.1	26.2	26.2	26.1	25.2	27.4
6	31.6	31.1	31.1	31.1	31.1	34.5	32.1
7	207.1	206.8	206.9	206.8	206.8	181.3	107.1
8	110.4	110.0	110.1	110.1	110.0	36.5	45.5
9	146.1	146.2	146.2	146.2	146.2	67.2	105.2
10	68.0	69.7	69.5	69.4	69.3	68.1	69.6
1′	104.3	104.2	104.3	104.3	104.2		
2′	74.7	74.6	74.6	74.6	74.6		
3′	78.6	78.5	78.5	78.6	78.5		
4′	71.4	71.3	71.3	71.3	71.3		
5′	78.0	78.0	78.0	78.0	78.0		
6′	62.7	62.6	62.7	62.7	62.6		
1″ (1′)		135.7	127.2	127.8	127.8		(127.8)
2″ (2′)		129.2	131.2	133.4	115.2		(115.2)
3″ (3′)		130.0	116.9	115.9	146.9		(146.8)
4″ (4′)		131.5	161.3	160.0	149.6		(149.6)
5″ (5′)		130.0	116.9	115.9	116.6		(116.6)
6″ (6′)		129.2	129.2	133.4	123.0		(122.9)
7″ (7′)		146.3	146.6	146.2	147.0		(146.9)
8″ (8′)		118.9	115.9	115.2	115.2		(115.2)
9″ (9′)		168.5	169.3	168.4	169.3		(169.3)
$-OCH_3$							55.5
$-OCH_3$							56.1

[a] At 150 MHz.
[b] At 100 MHz.

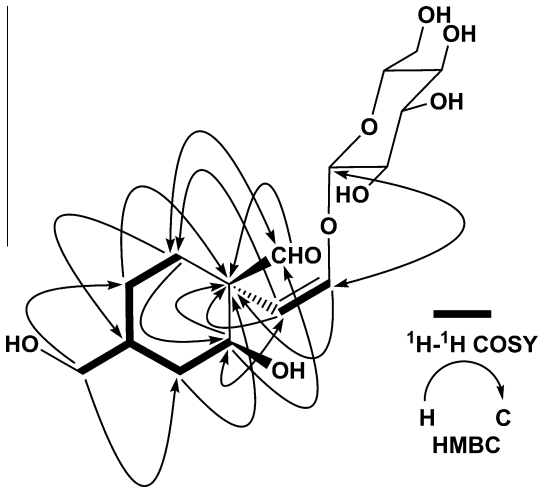


Fig. 2. ^{1}H–^{1}H COSY and diagnostic HMBC correlations for **1**. Dual arrowhead curves denote that HMBC correlations were observed both ways.

lene protons (δ_H 2.39 and 1.33) on C-6, and from the oxymethylene protons (δ_H 3.40 and 3.37) on C-10 to the methine proton (δ_H 1.60) on C-4 (Fig. 2). HMBC correlation cross-peaks between H-2 (δ_H 3.80) and C-1 (δ_C 56.6), and H-6a (δ_H 2.39) and C-2 (δ_C 76.2) established the formation of a six membered-ring through the quaternary carbon, to which, thus, the aldehyde and the double bond were attached. The cross-peaks between H-7 (δ_H 9.81) and C-1, and H-8 (δ_H 4.60) and C-1 confirmed the substituents on the quaternary carbon. The enol was stabilized by a sugar linkage, which was substantiated by cross-peaks between an anomeric proton (δ_H 4.47) and the enol carbon (δ_C 146.1, C-9), and H-9 (δ_H 6.44) and an anomeric carbon (δ_C 104.3) in the HMBC spectrum. A large coupling constant for H-2 and H-3 in the ^{1}H NMR spectrum enabled the location of the hydroxyl group in the equatorial position, and the cross-peak of H-2 with H-8 observed in the PS-NOESY experiment location of the double bond was also in the equatorial position. The C-10 primary alcohol was placed in the equatorial orientation based on the coupling constants of H-4 (J = 12 Hz) with adjacent axial protons. Therefore, the structure of canangafruticoside A (**1**) was elucidated to be (1*S**,2*S**,4*S**)-2-hydroxyl-4-(hydroxymethyl)-1-[(*Z*)-2-hydroxylvinyl]cyclohexanecarbaldehyde 9-*O*-β-D-glucopyranoside, as shown in Fig. 1. This *cis*-geometry of the C-8–C-9 double bond was finally confirmed by analysis of the PS-NOESY spectrum, in which H-8 and H-9 protons showed a significant correlation cross-peak. The absolute configuration of canangafruticoside A (**1**) will be discussed later.

Canangafruticoside B (**2**), $[\alpha]_D$ +15.0, was isolated as an amorphous powder, and its elemental composition was determined to be $C_{25}H_{32}O_{10}$ by HRESIMS. The IR spectrum indicated the presence of an aromatic ring (1606 and 1512 cm^{-1}), and the UV spectrum supported the above evidence. In the ^{1}H NMR spectrum, all the protons observed in that of canangafruticoside A (**1**), were similarly present, and further five finely coupled aromatic protons and two protons from a *trans* double bond [δ_H 7.68 (1H, *d*, J = 16 Hz) and 6.50 (1H, *d*, J = 16 Hz)] were represented by extra signals. The ^{13}C NMR spectrum was also similar to that of **1**, except for the presence of seven resonances, comprising five sp^2 signals with a hydrogen atom, two of which were of double strength, one without a hydrogen atom, and a carbonyl carbon. Thus, the structure of the extra moiety was expected to be *trans* cinnamic acid. The H_2-10 (δ_H 4.06) and C-10 (δ_C 69.7) signals were obviously shifted downfield, when compared with the corresponding resonances of **1**. Thus, the ester position of the acyl moiety was presumed to be on the hydroxyl group at C-10 and this was confirmed by the correlation peak for H_2-10 and the carbonyl carbon signal (δ_C 168.5) in the HMBC spectrum. Therefore, the structure of canangafruticoside B (**2**) was elucidated to be 10-*O*-*E*-cinnamoyl canangafruticoside A, as shown in Fig. 1.

Judging from the NMR spectroscopic data, canangafruticosides C (**3**), $[\alpha]_D$ +29.5, D (**4**), $[\alpha]_D$ +19.1, and E (**5**), $[\alpha]_D$ +17.6, were similar to compound **2** with the respective elemental compositions of $C_{25}H_{32}O_{11}$, $C_{25}H_{32}O_{11}$, and $C_{25}H_{32}O_{12}$. The structure of the acyl moiety of canangafruticoside C (**3**) was established to be *E*-*p*-coumaric acid from the spectroscopic evidence that one more oxygen atom existed in it than in **2**, and typical *para*-disubsituted patterns of protons [7.45 (2H, *d*, J = 9 Hz) and 6.81 (2H, *d*, J = 9 Hz)] on the aromatic ring were observed in the NMR spectra. The structure of canangafruticoside D (**4**) was assigned as *Z*-*p*-coumaric acid from the coupling constants of protons [6.87 (1H, *d*, J = 13 Hz) and 5.77 (1H, *d*, J = 13 Hz)] on the double bond. Canangafruticoside E (**5**) possessed one more oxygen atom than **3** and **4**, and three aromatic protons were coupled in an ABX system [7.05 (1H, *d*, J = 2 Hz), 6.95 (1H, *dd*, J = 8, 2 Hz) and 6.79 (1H, *d*, J = 8 Hz)]. The acyl moiety of **5** was determined to be *E*-caffeic acid. Therefore, the structures of canangafruticosides C (**3**), D (**4**), and E (**5**) were elucidated to be 10-*O*-*E*-*p*-coumaroyl, 10-*O*-*Z*-*p*-coumaroyl, and 10-*O*-*E*-caffeoyl canangafruticoside A, respectively, as shown in Fig. 1.

Compound **6**, $[\alpha]_D$ −10.3, was isolated as an amorphous powder and its elemental composition was determined to be $C_{10}H_{16}O_4$ by HRESIMS. The IR spectrum indicated the presence of an γ-lactone (1748 cm^{-1}). The ^{1}H–^{1}H COSY spectrum exhibited the same two proton sequences from H-2 to H_2-6 and from H_2-10 to H-4 as the same as in **1** (Fig. 3). The NMR spectroscopic data indicated that the C-7–C-8 double bond in **1** was reduced to a single bond and the aldehyde was oxidized to carboxylic acid. The HMBC correlation cross-peaks between H_2-9 (δ_H 4.32 and 4.25) and C-7 (δ_C 181.3) supported the formation of a spiro-lactone ring. Therefore, the structure of compound **6** was elucidated to be as shown in Fig. 1. Although the numbering for **6** is not in accord with the for-

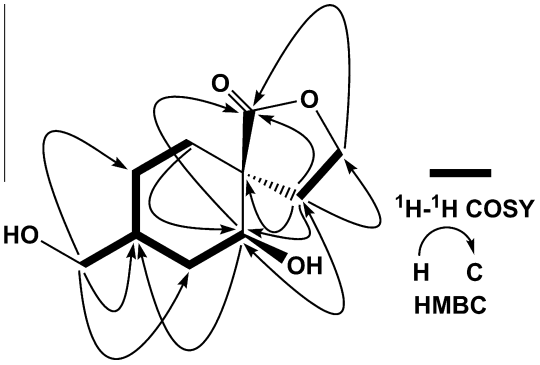


Fig. 3. ^{1}H–^{1}H COSY and diagnostic HMBC correlations for **6**. Dual arrowhead curve denotes HMBC correlations were observed both ways.

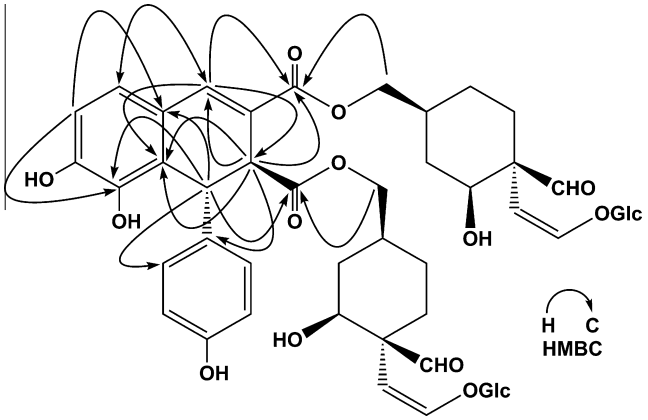


Fig. 4. Diagnostic HMBC correlations for **8**. Dual arrowhead curve denotes HMBC correlations were observed both ways.

mal one, for the readers' convenience, the same numbering as for the aforementioned compounds is used in Fig. 1 and Table 1.

Compound **7**, $[\alpha]_D$ −59.4, was isolated as an amorphous powder and its elemental composition was determined to be $C_{21}H_{28}O_8$ by HRESIMS. The NMR spectroscopic data indicated that the presence of a caffeoyl moiety, two hemiacetals (δ_C 107.1 with δ_H 4.98 and δ_C 105.2 with δ_H 5.09) and two methoxyl groups. Two methoxyl protons (δ_H 3.40 and 3.39) were correlated with two hemiacetalic carbons (δ_C 105.2 and 107.1, respectively) in the HMBC spectrum. The PS-NOESY correlation of the H-3 axial proton (δ_H 1.24) and H-7 (δ_H 4.98) placed the methoxyl group at the C-7 position in the α-orientation and the PS-NOESY correlation of H-8α (δ_H 2.10) and H-9 (δ_H 5.09) placed the methoxyl group at the C-9 position in the β-orientation. The formation mechanism for the two hemiacetals was assumed to comprise the hydrolysis of canangafruticoside E (**5**) in plants yielding a corresponding dialdehyde, the dialdehyde then being cyclized to give didemethyl compound **7′**. During extraction with MeOH, compound **7** might be formed from didemethyl compound **7′** as an artifact.

Compound **8**, $[\alpha]_D$ +78.3, was isolated as an amorphous powder and its fairly large molecular formula was calculated to be $C_{50}H_{62}O_{23}$ by HRESIMS. The IR spectrum exhibited two types of carbonyl functional groups (1710 and 1671 cm^{-1}), and the presence of aromatic ring(s) was indicated by the UV absorption at 330 nm. In the ^{13}C NMR spectrum, some signals appeared as pairs of two close resonances. These resonances showed good similarity to those of the monoterpene glucoside moiety of canangafruticoside B (**2**). The remaining 16 signals from 18 carbons comprised those of tetra- and *para*-substituted aromatic rings, one trisubstituted double bond, two methines and two carbonyl carbons. In the ^{1}H spectrum, two aromatic protons appeared as doublets with an *ortho* coupling constant and two pairs of two aromatic protons also as doublets with an *ortho* coupling constant. Two methine protons were coupled to each other with a small coupling constant and an olefinic proton appeared at δ_H 7.64 as a singlet. From the above evidence, 18 carbons were expected to form an aryldihydronaphthalene dicarboxylate skeleton. The diagnostic HMBC correlation cross peaks between H-8 (δ_H 6.86) and C-1 (δ_C 140.4), H-1 (δ_H 7.64) and C-2a (δ_C 168.7) and C-3 (δ_C 48.7), H-4 (δ_H 5.00) and C-2′ (δ_C 129.5), and H-4 and C-3a (δ_C 174.1) supported the structure (Fig. 4). Thus, the structure of **8** was established to be as shown in Fig. 1, and H_2-10″ (δ_H 4.00) and H_2-10‴ (δ_H 3.89) clearly showed HMBC cross-peaks with C-2a and C-3a, respectively. The small coupling constant of H_{3-4} indicated that H-3 and H-4 were in a *trans* relationship, and judging from the positive Cotton effect at 257 nm in the CD spectrum, the configurations at the 3- and 4-positions were assigned as *S* and *R*, respectively (Nishizawa et al., 1990).

A similar lignan, aryldihydronaphthalene dicarboxylic acid diester, to compound **8** was isolated from *Trigonotis peduncularis* and its fluorescent nature was examined (Otsuka et al., 2008). The compound isolated from *T. peduncularis* emitted yellow fluorescence at 526 nm on excitation with UV light at 435 nm, whereas compound **8** emitted blue fluorescence at 494 nm on excitation with UV light at 375 nm. The functional groups on the aromatic rings varied with the excitation and emission wavelengths. Similar to in the case of lignan from *T. peduncularis*, in neutral and acidic buffers, a limited fluorescence strength was observed, but under basic conditions, the fluorescence strength drastically increased (Fig. 5). The quantum yield (φ_x) of compound **8** in a carbonate buffer at pH 9 was 0.454. This value was comparable to that of well-known fluorescent reagent rhodamine B.

To determine the absolute configuration of the monoterpene moiety, compound **7** was expected to be the most plausible compound for the modified Mosher's method (Ohtani et al., 1991), although it was probably an artifact. Thus, compound **7** was first methylated with trimethylsilyldiazomethane, and then esterification was attempted using (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardodiimide hydrochloride (EDC) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP). However, no reaction occurred. A further attempt under different conditions using (*S*)- and (*R*)-MTPA chlorides/pyridine was also unsuccessful, probably due to steric hindrance of dimethoxyl dihemiacetalic spiro ring system. In the next attempt, the most abundant canangafruticoside E (**5**) was used as a candidate due to its quantity. On direct enzymatic

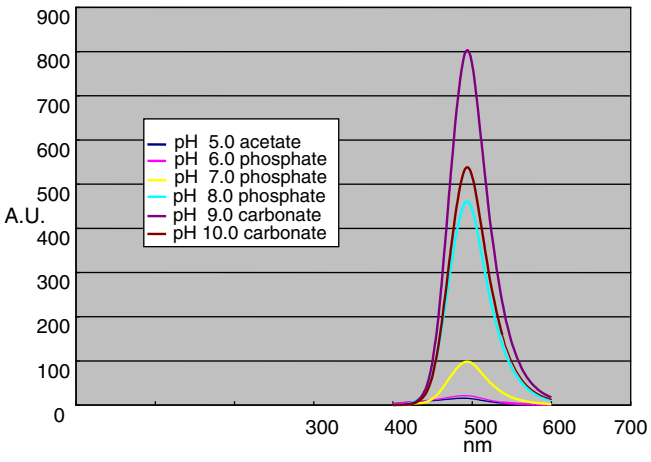
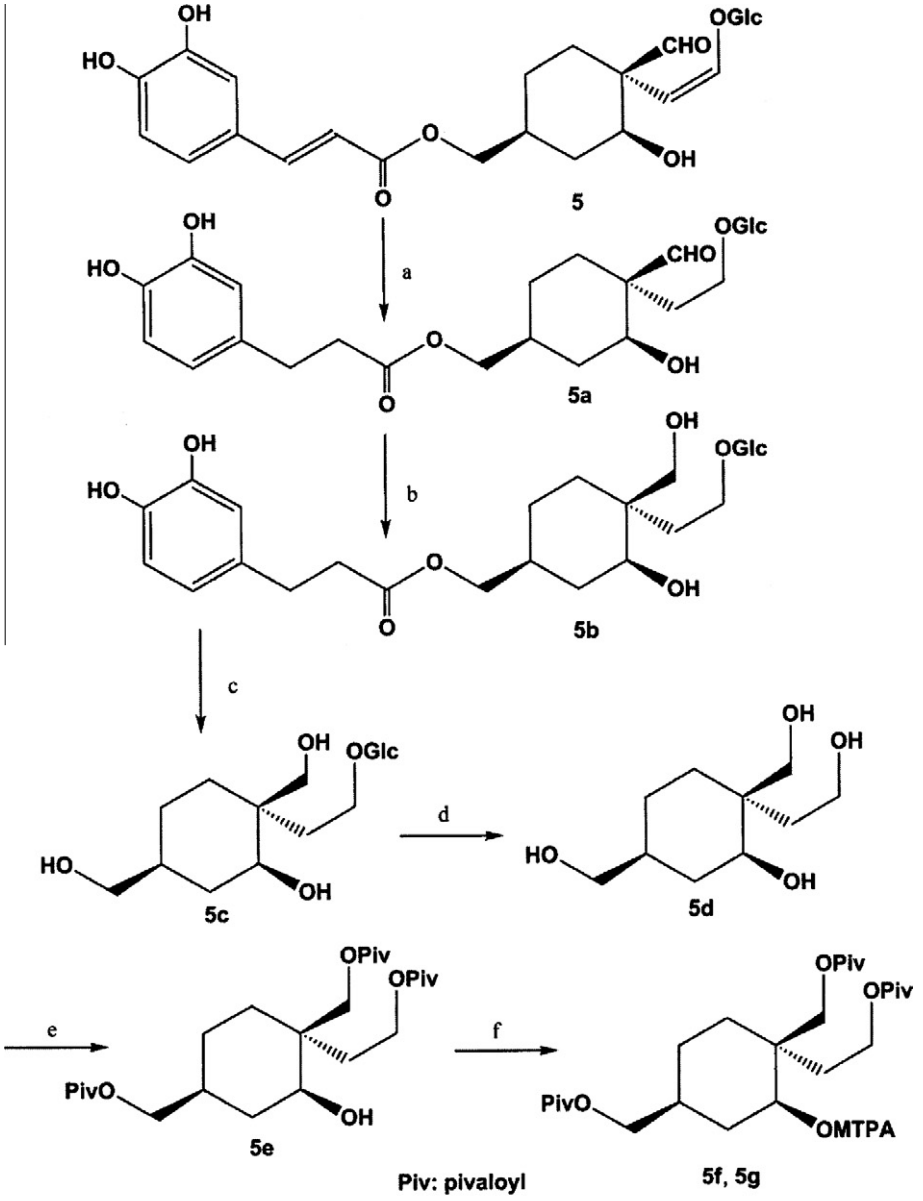


Fig. 5. Emission spectra for compound **5** at various pHs.



(a) PtO_2/H_2; (b) $NaBH_4$; (c) NaOMe: (d) β-glucosidase; (e) pivaloyl chloride, pyridine; (f) (*R*)- and (*S*)-MTPA, EDC, DMAP

Scheme 1. Conversion of canangafruticoside E (**5**) to its tripivaloyl MTPA derivatives (**5f**, **5g**).

hydrolysis of **5**, the free enol thus formed would be spontaneously converted to an aldehyde, and the resulting dialdehyde undergo cyclization to form a spiro ring system. To avoid spiro ring formation, a chain of reactions was initiated by catalytic reduction of the double bonds, and the a sequence of reactions being shown in Scheme 1. The final results with the modified Mosher's method in Fig. 6 showed that the unusual monoterpene moiety had the 1*S*,2*S*,4*S* configuration. Although the absolute stereochemistry of the remaining related compounds was not confirmed one by one, they were expected to have the same absolute stereochemistry as that of **5**.

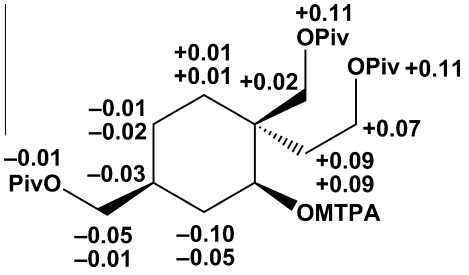


Fig. 6. Results of modified Mosher's method ($\Delta_{\delta S-\delta R}$).

3. Conclusions

It is noteworthy that the established monoterpene moiety does not observe the general isoprene rule. Before now, only two compounds with this carbon skeleton have been isolated, as previously stated, one from *C. odorata* f. *genuina* called canangone (**11**) (Caloprisco et al., 2002) and one from *Juniperus communis* called 1,4-dimethylcyclohex-3-enyl methyl ketone (**12**) (Thomas, 1973). Biosynthetic investigation of these unusual monoterpenes is a

subject of deep interest. A larger molecule, compound **8**, was established to be a derivative of an aryldihydronephthalene-type lignan with two carboxylic acid terminals, to which one molecule of canangafruticoside A (**1**), respectively, was attached through an ester bond. It is also interesting that under basic conditions, compound **8** emits strong blue fluorescence on irradiation with UV light at 375 nm.

4. Experimental

4.1. General experimental procedure

Optical rotations and CD spectra were measured on JASCO P-1030 and JASCO J-720 polarimeters, respectively, whereas IR and UV spectra were established using Horiba FT-710 and JASCO V-520 UV/vis spectrophotometers, respectively. Measurement of fluorescence spectra was performed on a Hitachi F-2500 fluorescence spectrophotometer. ^{1}H and ^{13}C NMR spectra were acquired on JEOL JNM α-400 and ECA-600 spectrometers at 400 and 600, and 100 and 150 MHz, respectively, with tetramethylsilane as an internal standard. Positive-ion HRESIMS was performed with an Applied Biosystem QSTAR XL system ESI (Nano Spray)-TOF-MS.

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) [Φ = 50 mm, L = 25 cm, linear gradient: MeOH–H_2O (1:9, 1.5 L) → (3:1, 1.5 L), fractions of 10 g being collected], respectively. Droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ = 2 mm, L = 40 cm), the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O-*n*-PrOH (9:12:8:2) being used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS-3 column (Inertsil; GL Science, Tokyo, Japan; Φ = 6 mm, L = 25 cm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor.

Hesperidinase was a gift from Tokyo Tanabe Pharmaceutical Co., Ltd. (Tokyo, Japan). (*R*)- and (*S*)-MTPAs were the products of Wako Pure Chemical Industry Co., Ltd. (Tokyo, Japan).

4.2. Plant material

Leaves of *C. odorata* var. *fruticosa* (Annonaceae) were collected in March, 2005, from cultivated species in the Botanical Garden, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand and a voucher specimen (05-COF-CMU-0308) was deposited in the Herbarium of Faculty of Pharmacy, Chiang Mai University.

4.3. Extraction and isolation

Dried leaves of *C. odorata* var. *fruticosa* (1.17 kg) were extracted with MeOH (4.5 × 3 L) at 25 °C for 1 week and then concentrated to 3 L *in vacuo*. The extract was washed with *n*-hexane (3 L, 36.3 g) and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in H_2O (3 L) and extracted with EtOAc (3 L) to give an EtOAc-soluble fraction (23.5 g). The aqueous layer was extracted with **1**-BuOH (3 L) to give a **1**-BuOH-soluble fraction (75.4 g), and the remaining aqueous-layer was concentrated to furnish a water-soluble fraction (173 g).

The **1**-BuOH-soluble fraction was subjected to a Diaion HP-20 column (Φ = 50 mm, L = 50 cm) using H_2O–MeOH [(4:1, 4 L), (2:3, 4 L), (3:2, 4 L), and (1:4, 4 L)], and MeOH (4 L), 1 L fractions being collected. The residue (6.36 g in fractions 6–8) of the 20–40% MeOH–H_2O eluent was subjected to silica gel (160 g) CC, with elution with $CHCl_3$ (1 L) and $CHCl_3$–MeOH [(99:1, 1 L), (97:3, 1 L), (19:1, 1 L), (37:3, 1 L), (9:1, 1 L), (7:1, 1 L), (17:3, 1 L), (33:7, 1 L), (4:1, 1 L) and (7:3, 1 L)], 200 mL fractions being collected. Combined fractions 22–25 (254 mg) were separated by DCCC and then the residue (141 mg) in fractions 1–72 was purified by HPLC (MeOH–H_2O, 1:9) to give **6** (3.8 mg) from the peak at 38 min.

The residue (19.9 g in fractions 9–12) obtained on Diaion HP-20 CC of the 40–60% MeOH–H_2O eluent was subjected to silica gel (500 g) CC, with elution with $CHCl_3$ (2 L) and $CHCl_3$–MeOH [(99:1, 3 L), (97:3, 3 L), (19:1, 3 L), (37:3, 3 L), (9:1, 3 L), (7:1, 3 L), (17:3, 3 L), (33:7, 3 L), (4:1, 3 L) and (7:3, 6 L)], 500 mL fractions being collected. Combined fractions 23–31 (606 mg) were separated by ODS open CC to give **10** (30.6 mg) in fractions 123–128. Combined fractions 32–39 (1.28 g) were separated by ODS open CC to give **9** (8.0 mg) in fractions 82–86 and a residue (126 mg) in fractions 164–170, which was then purified by DCCC to afford a residue (37.0 mg) in fractions 46–53. This was finally purified by HPLC (MeOH–H_2O, 23:27) to give **4** (7.1 mg) from the peak at 32 min. An aliquot of combined fractions 40–48 (2.49 g out of 2.71 g) was separated by ODS open CC to give a residue (224 mg) in fractions 247–255, which was then purified by DCCC to afford **3** (25.6 mg) in fractions 55–65. Combined fractions 49–54 (1.06 g) were separated by ODS open CC to give a residue (176 mg) in fractions 161–167, which was then purified by DCCC to afford **5** (93.0 mg) in fractions 31–39. An aliquot of combined fractions 55–68 (2.50 g out of 9.19 g) was separated by ODS open CC to give two residues in fractions 21–26 (25.7 mg) and 88–99 (688 mg). The former was purified by DCCC to give **1** (2.9 mg) in fractions 18–20. The latter was purified by DCCC to give a residue (121 mg) in fractions 26–31, which was then purified by HPLC (MeOH–H_2O, 1:1) to yield **8** (17.9 mg).

The residue (10.8 g in fractions 13–17) obtained on Diaion HP-20 CC of the 60–80% MeOH–H_2O eluent was subjected to silica gel (160 g) CC, with elution with $CHCl_3$ (1 L), $CHCl_3$–MeOH [(99:1, 1 L), (49:1, 1 L), (97:3, 1 L), (24:1, 1 L), (47:3 1 L), (19:1, 1 L), (23:2, 1 L), (17:3, 1 L) and (7:3, 1 L)] and $CHCl_3$–MeOH–H_2O (35:15:2, 1 L), 250 mL fractions being collected. Combined fractions 20–23 (300 mg) were separated by ODC open CC to give **7** (16.5 mg) in fractions 175–190. Combined fractions 37–41 (458 mg) were separated by ODS open CC to give **2** (29.6 mg) in fractions 217–223.

4.4. Canangafruticoside A (*1*)

Amorphous powder; $[\alpha]_D^{25}$ +23.8 (*c* 0.24, MeOH); IR (film) ν_{max} 3363, 2926, 1713, 1655, 1594, 1375, 1074 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ: 9.81 (1H, *s*, H-7), 6.44 (1H, *d*, *J* = 7 Hz, H-9), 4.60 (1H, *d*, *J* = 7 Hz, H-8), 4.47 (1H, *d*, *J* = 8 Hz, H-1′), 3.85 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.80 (1H, *dd*, *J* = 12, 4 Hz, H-2), 3.65 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.42–3.27 (3H, *m*, H-3′, 4′ and 5′), 3.40 (1H, *dd*, *J* = 11, 6 Hz, H-10a), 3.37 (1H, *dd*, *J* = 11, 6 Hz, H-10b), 3.23 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 2.39 (1H, *ddd*, *J* = 14, 4, 4 Hz, H-6a), 1.96 (1H, *ddd*, *J* = 13, 4, 4, 2 Hz, H-3a), 1.60 (1H, *dddddd*, *J* = 13, 12, 6, 6, 4, 4 Hz, H-4), 1.57 (1H, *ddddd*, *J* = 13, 4, 4, 4, 2 Hz, H-5a), 1.33 (1H, *ddd*, *J* = 14, 13, 4 Hz, H-6b), 1.22 (1H, *ddd*, *J* = 13, 12, 12 Hz, H-3b), 1.20 (1H, *dddd*, *J* = 13, 13, 12, 4 Hz, H-5b); for ^{13}C NMR (CD_3OD, 150 MHz) spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z*: 385.1461 [M+Na]$^+$ (Calcd. for $C_{16}H_{26}O_9Na$: 385.1469).

4.5. Canangafruticoside B (*2*)

Amorphous powder; $[\alpha]_D^{25}$ +15.0 (*c* 1.95, MeOH); IR (film) ν_{max} 3388, 2934, 1706, 1634, 1606, 1512, 1450, 1277, 1074 cm^{-1}; UV λ_{max} (MeOH): 322sh (3.75), 276 (4.06), 213 (4.17) nm (log ε); ^{1}H NMR (CD_3OD, 400 MHz) δ: 9.82 (1H, *s*, H-7), 7.68 (1H, *d*, *J* = 16 Hz, H-7″), 7.59 (2H, *m*, H-2″ and 6″), 7.39 (3H, *m*, H-3″, 4″

and 5″), 6.50 (1H, *d*, *J* = 16 Hz, H-8″), 4.38 (1H, *d*, *J* = 7 Hz, H-8), 6.45 (1H, *d*, *J* = 7 Hz, H-9), 4.48 (1H, *d*, *J* = 8 Hz, H-1′), 4.06 (2H, *s*, H_2-10), 3.84 (1H, *dd*, *J* = 11, 4 Hz, H-2), 3.84 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.66 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.41–3.31 (3H, *m*, H-3′, 4′ and 5′), 3.25 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 2.41 (1H, *dd*, *J* = 10, 3 Hz, H-6a), 1.98 (1H, *m*, H-3a), 1.88 (1H, *m*, H-4), 1.59 (1H, *m*, H-5a), 1.41–1.28 (3H, *m*, H-3b, 5b and 6b); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z*: 515.1879 $[M+Na]^+$ (Calcd. for $C_{25}H_{32}O_{10}Na$: 515.1887).

4.6. Canangafruticoside C (**3**)

Amorphous powder; $[\alpha]_D^{25}$ +29.5 (*c* 1.05, MeOH); IR (film) ν_{max} 3367, 2931, 1711, 1603, 1512, 1273, 1168, 1072 cm^{-1}; UV λ_{max} (MeOH): 310 (4.15), 211 (4.26) nm (log ε); ^{1}H NMR (CD_3OD, 400 MHz) δ: 9.82 (1H, *s*, H-7′), 7.61 (1H, *d*, *J* = 16 Hz, H-7), 7.45 (2H, *d*, *J* = 9 Hz, H-2′ and 6′), 6.81 (2H, *d*, *J* = 9 Hz, H-3′ and 5′), 6.45 (1H, *d*, *J* = 7 Hz, H-9), 6.31 (1H, *d*, *J* = 16 Hz, H-8′), 4.48 (1H, *d*, *J* = 8 Hz, H-1′), 4.38 (1H, *d*, *J* = 7 Hz, H-8), 4.06 (2H, *s*, H_2-10), 3.86 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.84 (1H, *dd*, *J* = 11, 4 Hz, H-2), 3.66 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.40–3.31 (3H, *m*, H-3′, 4′ and 5′), 3.24 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 1.98 (1H, *m*, H-3a), 1.88 (1H, *m*, H-4), 1.59 (1H, *m*, 5a), 2.41 (*dd*, *J* = 10, 3 Hz, H-6a), 1.41–1.28 (3H, *m*, H-3b, 5b and 6b); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z*: 531.1832 $[M+Na]^+$ (Calcd. for $C_{25}H_{32}O_{11}Na$: 531.1836).

4.7. Canangafruticoside D (**4**)

Amorphous powder; $[\alpha]_D^{25}$ +19.1 (*c* 0.46, MeOH); IR (film) ν_{max} 3367, 2931, 171, 1603, 1513, 1450, 1273, 1166, 1075 cm^{-1}; UV λ_{max} (MeOH): 310 (4.37), 227 (4.16) nm (log ε); ^{1}H NMR (CD_3OD, 400 MHz) δ: 9.82 (1H, *s*, H-7), 7.58 (2H, *d*, *J* = 9 Hz, H-2″ and 6″), 6.87 (1H, *d*, *J* = 13 Hz, H-7″), 6.75 (2H, *d*, *J* = 9 Hz, H-3″and 5″), 6.45 (1H, *d*, *J* = 7 Hz, H-9), 5.77 (1H, *d*, *J* = 13 Hz, H-8″), 4.60 (1H, *d*, *J* = 7 Hz, H-8), 4.47 (1H, *d*, *J* = 8 Hz, H-1′), 4.06 (2H, *s*, H_2-10), 3.86 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.81 (1H, *dd*, *J* = 11, 4 Hz, H-2), 3.66 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.38–3.28 (3H, *m*, H-3′, 4′ and 5′), 3.24 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 2.41 (1H, *dd*, *J* = 10, 3 Hz, H-6a), 1.98 (1H, *m*, H-3a), 1.88 (1H, *m*, H-4), 1.59 (1H, *m*, H-5a), 1.41–1.28 (3H, *m*, H-3b, 5b and 6b); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z*: 531.1832 $[M+Na]^+$ (Calcd. for $C_{25}H_{32}O_{11}Na$: 531.1836).

4.8. Canangafruticoside E (**5**)

Amorphous powder; $[\alpha]_D^{25}$ +17.6 (*c* 1.79, MeOH); IR (film) ν_{max} 3371, 2938, 1699, 1602, 1521, 1450, 1278, 1180, 1074 cm^{-1}; UV λ_{max} (MeOH): 324 (4.15), 300sh (4.07), 242sh (4.01), 215 (4.17) nm (log ε); ^{1}H NMR (CD_3OD, 400 MHz) δ: 9.82 (1H, *s*, H-7), 7.54 (1H, *d*, *J* = 16 Hz, H-7″), 7.05 (1H, *d*, *J* = 2 Hz, H-2″), 6.95 (1H, *dd*, *J* = 8, 2 Hz, H-6″), 6.79 (1H, *d*, *J* = 8 Hz, H-5″), 6.45 (1H, *d*, *J* = 7 Hz, H-9), 6.25 (1H, *d*, *J* = 16 Hz, H-8″), 4.61 (1H, *d*, *J* = 7 Hz, H-8), 4.48 (1H, *d*, *J* = 8 Hz, H-1′), 4.06 (1H, *dd*, *J* = 11, 6 Hz, H-10a), 4.02 (1H, *dd*, *J* = 11, 6 Hz, H-10b), 3.86 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.84 (1H, *dd*, *J* = 12, 4 Hz, H-2), 3.67 (1H, *dd*, *J* = 12, 5 Hz, H-6′b), 3.36–3.23 (3H, *m*, H-3′, 4′ and 5′), 3.25 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 2.41 (1H, *dd*, *J* = 10, 3 Hz, H-6a), 1.97 (1H, *ddd*, *J* = 12, 4, 4 Hz, H-3a), 1.87 (1H, *m*, H-4), 1.58 (1H, *m*, H-5a), 1.38 (1H, *m*, H-5b), 1.36 (1H, *d*, *J* = 10 Hz, H-6b), 1.36 (1H, *ddd*, *J* = 12, 12, 12 Hz, H-3b); for ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z*: 547.1793 $[M+Na]^+$ (Calcd. for $C_{25}H_{32}O_{12}Na$: 547.1785).

4.9. Compound **6**

Amorphous powder; $[\alpha]_D^{25}$ −10.3 (*c* 0.25, MeOH); IR (film) ν_{max} 3395, 2926, 1748, 1454, 1382, 1182, 1031 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ: 4.32 (1H, *ddd*, *J* = 12, 7, 6 Hz, H-9a), 4.25 (1H, *ddd*, *J* = 12, 7, 5 Hz, H-9b), 3.59 (1H, *dd*, *J* = 10, 4 Hz, H-2), 3.42 (2H, *m*, H_2-10), 2.55 (1H, *ddd*, *J* = 11, 7, 5 Hz, H-8a), 2.03 (1H, *ddd*, *J* = 11, 7, 6 Hz, H-8b), 1.98 (1H, *d*, *J* = 13 Hz, H-6a), 1.82–1.71 (2H, *m*, H_2-3), 1.63–1.53 (3H, *m*, H-4 and H_2-5), 1.52 (1H, *dd*, *J* = 13, 4 Hz, H-6b); for ^{13}C NMR (CD_3OD, 150 MHz) spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z*: 223.0931 $[M+Na]^+$ (Calcd. for $C_{10}H_{16}O_4Na$: 223.0940).

4.10. Compound **7**

Amorphous powder; $[\alpha]_D^{25}$ −59.4 (*c* 1.09, MeOH); IR (film) ν_{max} 3362, 2936, 1698, 1455, 1394, 1167, 1096, 1023 cm^{-1}; UV λ_{max} (MeOH): 325 (4.15), 297sh (4.09), 240sh (4.12), 217 (4.22) nm (log ε); ^{1}H NMR (CD_3OD, 600 MHz) δ: 7.04 (1H, *s*, H-2′), 6.94 (1H, *d*, *J* = 8 Hz, H-6′), 6.78 (1H, *d*, *J* = 8 Hz, H-5′), 7.54 (1H, *d*, *J* = 16 Hz, H-7′), 6.25 (1H, *d*, *J* = 16 Hz, H-8′), 5.09 (1H, *dd*, *J* = 6, 2 Hz, H-9), 4.98 (1H, *s*, H-7), 4.07 (1H, *dd*, *J* = 11, 7 Hz, H-10a), 4.03 (1H, *dd*, *J* = 11, 7 Hz, H-10b), 3.60 (1H, *dd*, *J* = 11, 4 Hz, H-2), 3.40 (3H, *s*, –OC*H*$_3$ on C-9), 3.39 (3H, *s*, –OC*H*$_3$ on C-7), 2.10 (1H, *dd*, *J* = 14, 6 Hz, H-8a), 2.00 (2H, *m*, H-3a and 6a), 1.90 (1H, *dd*, *J* = 14, 2 Hz, H-8b), 1.84 (1H, *m*, H-4), 1.54 (1H, *m*, H-5a), 1.30 (1H, *m*, H-6b), 1.24 (1H, *m*, H-3b), 1.15 (1H, *m*, H-5b); for ^{13}C NMR (CD_3OD, 150 MHz) spectroscopic data, see Table 1; HRESIMS (positive-ion mode) *m*/*z*: 431.1670 $[M+Na]^+$ (Calcd. for $C_{21}H_{28}O_8Na$: 431.1676).

4.11. Compound **8**

Amorphous powder; $[\alpha]_D^{25}$ +78.3 (*c* 1.13, MeOH); IR (film) ν_{max} 3373, 2931, 2871, 1710, 1671, 1615, 1509, 1452, 1281, 1205, 1073 cm^{-1}; UV λ_{max} (MeOH): 330 (4.20), 224 (4.40) nm (log ε); ^{1}H NMR (CD_3OD, 600 MHz) δ: 9.81 (1H, *s*, H-7‴), 9.79 (1H, *s*, H-7″), 7.64 (1H, *s*, H-1), 6.86 (1H, *d*, *J* = 8 Hz, H-8), 6.86 (2H, *d*, *J* = 9 Hz, H-2′ and 6′), 6.76 (1H, *d*, *J* = 8 Hz, H-7), 6.60 (2H, *d*, *J* = 9 Hz, H-3′ and 5′), 6.45 (1H, *d*, *J* = 7 Hz, H-9‴), 6.43 (1H, *d*, *J* = 7 Hz, H-9″), 5.00 (1H, *br d*, *J* = 2 Hz, H-4), 4.61 (1H, *d*, *J* = 7 Hz, H-8‴), 4.52 (1H, *d*, *J* = 7 Hz, H-8″), 4.48 (2H, *d*, *J* = 8 Hz, H-1‴ and 1⁗), 4.00 (2H, *m*, H_2-10″), 3.90 (1H, *d*, *J* = 2 Hz, H-3), 3.89 (2H, *m*, H_2-10‴), 3.86 (2H, *dd*, *J* = 12, 2 Hz, H-6‴a and 6⁗a), 3.81 (1H, *dd*, *J* = 11, 4 Hz, H-2⁗), 3.76 (1H, *dd*, *J* = 11, 4 Hz, H-2″), 3.67

Table 2
^{13}C NMR spectroscopic data for compound **8** (150 MHz, CD_3OD).

	8				
	C		C		C
1	140.4	1″	56.32	1⁗	56.32
2	122.2	2″	75.8	2⁗	75.5
2a	168.7	3″	35.7	3⁗	35.9
3	48.7	4″	37.35	4⁗	37.39
3a	174.1	5″	26.0	5⁗	26.1
4	40.4	6″	31.3	6⁗	31.4
4a	125.0	7″	206.89	7⁗	206.94
5	144.3	8″	110.17	8⁗	110.21
6	149.5	9″	146.1	9⁗	146.2
7	114.6	10″	69.8	10⁗	70.2
8	123.0				
8a	125.0				
1′	134.6	1‴	104.3	1⁗′	104.3
2′	129.5	2‴	74.6	2⁗′	74.6
3′	116.1	3‴	78.6	3⁗′	78.6
4′	157.0	4‴	71.3	4⁗′	71.3
5′	116.1	5‴	78.0	5⁗′	78.0
6′	129.5	6‴	62.7	6⁗′	62.7

(2H, *dd*, *J* = 12, 5 Hz, H-6‴b and 6⁗b), 3.38–3.25 (6H, *m*, H-3‴, 4‴, 5‴, 3⁗, 4⁗ and 5⁗), 3.26 (2H, *dd*, *J* = 9, 8 Hz, H-2‴and 2⁗), 2.35 (2H, *m*, H-6″a and 6‴a), 1.83 (1H, *m*, H-4″), 1.94–1.82 (2H, *m*, H-3″a and 3‴a), 1.67 (1H, *m*, H-4‴), 1.51 (1H, *m*, H-5‴b), 1.37–1.22 (6H, *m*, H-3″b, 5″a, 6″b, 3‴b, 5‴a and 6‴b), 1.10 (1H, *m*, H-5″b); for ^{13}C NMR (CD_3OD, 150 MHz) spectroscopic data, see Table 2; CD $\Delta\varepsilon$ (nm) −0.07 (361), +12.6 (309), +7.33 (257), −29.8 (229) (*c* 5.49 × 10^{-5} *m*, MeOH); HRESIMS (positive-ion mode) *m*/*z*: 1053.3579 $[M+Na]^+$ (Calcd. for $C_{50}H_{62}O_{23}Na$: 1053.3574).

4.12. Modified Mosher's method for canangafruticoside E (**5**)

4.12.1. Catalytic hydrogenation of canangafruticoside E (**5**)

Canangafruticoside E (**5**) (10.0 mg) was dissolved in 1 mL of MeOH and then reduced with PtO_2 (1 mg) under a H_2 atmosphere for 1 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness to give tetrahydrocanangafruticoside E (**5a)** (10.1 mg). Tetrahydrocanangafruticoside E (**5a**): amorphous powder; ^1H NMR (CD_3OD, 400 MHz) δ: 9.82 (1H, *s*, H-7), 6.66 (1H, *dd*, *J* = 8, 2 Hz, H-5″), 6.61 (1H, *d*, *J* = 2 Hz, H-2″), 6.50 (1H, *d*, *J* = 8 Hz, H-6″), 4.22 (1H, *d*, *J* = 8 Hz, H-1′), 4.02 (1H, *dd*, *J* = 10, 6 Hz, H-10a), 4.01 (1H, *dd*, *J* = 10, 6 Hz, H-10b), 4.00 (1H, *ddd*, *J* = 11, 6, 6 Hz, H-9a), 3.86 (1H, *m*, H-6′a), 3.68 (1H, *m*, H-2), 3.66 (1H, *m*, H-6′b), 3.60 (1H, *m*, H-9b), 3.33 (1H, *m*, H-3′), 3.33–3.25 (1H, *m*, H-4′), 3.25 (1H, *m*, H-5′), 3.13 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 2.76 (2H, *t*, *J* = 7 Hz, H_2-7″), 2.55 (2H, *t*, *J* = 7 Hz, H_2-8″), 2.26 (1H, *ddd*, *J* = 14, 7, 6 Hz, H-8a), 2.07 (1H, *ddd*, *J* = 14, 3, 3 Hz, H-6a), 1.86 (1H, *m*, H-3a), 1.81 (2H, *m*, H-4 and 8b), 1.54 (1H, *m*, H-5a), 1.22 (2H, *m*, H-5b and 6b), 1.16 (1H, *m*, H-3b); HRESIMS (positive-ion mode) *m*/*z*: 551.2091 $[M+Na]^+$ (Calcd. for $C_{25}H_{36}O_{12}Na$: 551.2098).

4.12.2. $NaBH_4$ reduction of tetrahydrocanangafruticoside E (**5a**)

Tetrahydrocanangafruticoside E (**5a**) (10.1 mg) was dissolved in EtOH (1 mL) and then cooled on an ice-bath. $NaBH_4$ (1 mg) in EtOH (500 μL) was added, followed by stirring for 30 min. Excess $NaBH_4$ was quenched by the addition of 500 μL of a 1% EtOH solution of acetic AcOH. The reduced product was purified by prep. TLC ($CHCl_3$–MeOH–H_2O, 15:6:1) to give tetrahydrocanangafruticoside E 7-ol (5.6 mg) (**5b**). Tetrahydrocanangafruticoside E 7-ol (**5b**): amorphous powder; ^1H NMR (CD_3OD, 400 MHz) δ: 6.66 (1H, *d*, *J* = 8 Hz, H-5″), 6.62 (1H, *d*, *J* = 2 Hz, H-2″), 6.51 (1H, *dd*, *J* = 8, 2 Hz, H-6″), 4.29 (1H, *d*, *J* = 8 Hz. H-1′), 4.10 (1H, *ddd*, *J* = 10, 7, 7, Hz, H-9a), 3.92 (2H, *d*, *J* = 6 Hz, H_2-10), 3.86 (1H, *dd*, *J* = 12, 1 Hz, H-6′a), 3.84 (1H, *d*, *J* = 11 Hz, H-7a), 3.76 (1H, *ddd*, *J* = 10, 7, 7 Hz, H-9b), 3.66 (1H, *m*, H-6′b), 3.60 (2H, *m*, H-2 and 7b), 3.35 (1H, *m*, H-3′), 3.33–3.25 (2H, *m*, H-4′ and 5′), 3.17 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 2.77 (2H, *t*, *J* = 7 Hz, H_2-7″), 2.56 (2H, *t*, *J* = 7 Hz, H_2-8″), 1.92 (1H, *m*, H-8a), 1.83 (1H, *ddd*, *J* = 15, 7, 7 Hz, H-8b), 1.72 (1H, *m*, H-6a), 1.71 (1H, *ddd*, *J* = 13, 3, 3 Hz, H-3a), 1.65 (1H, *m*, H-4), 1.37 (1H, *m*, H-5a), 1.31 (1H, *m*, H-6b), 1.16 (1H, *m*, H-3b), 1.11 (1H, *m*, 5b); HRESIMS (positive-ion mode) *m*/*z*: 553.2258 $[M+Na]^+$ (Calcd. for $C_{25}H_{38}O_{12}Na$: 551.2255).

4.12.3. Removal of acyl group from tetrahydrocanangafruticoside E 7-ol (**5b**)

Tetrahydrocanangafruticoside E 7-ol (**5b**) (5.6 mg) was dissolved in methanolic solution of 0.1 M $NaOCH_3$ (1 mL), followed by stirring for 30 min at 25 °C. The reaction solvent was neutralized with Amberlite IR-120B (H^+) and dihydrocanangafruticoside A 7-ol (**5c**) (2.2 mg) was purified by prep. TLC ($CHCl_3$–MeOH–H_2O, 15:6:1). Dihydrocanangafruticoside A 7-ol (**5c**): amorphous powder; ^1H NMR (CD_3OD, 400 MHz) δ: 4.29 (1H, *d*, *J* = 8 Hz, H-1′), 4.11 (1H, *ddd*, *J* = 10, 7, 7 Hz, H-9a), 3.87 (2H, *br d*, *J* = 12 Hz, H-7a and 6′a), 3.77 (1H, *ddd*, *J* = 10, 7, 7 Hz, H-9b), 3.66 (1H, *dd*, *J* = 12, 4 Hz, H-6′b), 3.63 (1H, *d*, *J* = 12 Hz, H-7b), 3.62 (1H, *m*, H-2), 3.41 (2H, *d*, *J* = 6 Hz, H_2-10), 3.34 (1H, *m*, H-3′), 3.32–3.25 (2H, *m*, H-4′ and 5′), 3.16 (1H, *dd*, *J* = 8, 8 Hz, H-2′), 1.93 (1H, *ddd*, *J* = 15, 7, 7 Hz, H-8a), 1.83 (1H, *ddd*, *J* = 15, 7, 7 Hz, H-8b), 1.82 (1H, *m*, H-3a), 1.72 (1H, *m*, H-6a), 1.52 (1H, *m*, H-4), 1.23–1.26 (1H, *m*, H-5a), 1.26 (1H, *m*, H-6b), 1.18 (1H, *m*, H-3b), 1.12 (1H, *dddd*, *J* = 13, 13, 13, 3 Hz, H-5b); HRESIMS (positive-ion mode) *m*/*z*: 389.1787 $[M+Na]^+$ (Calcd. for $C_{16}H_{30}O_9Na$: 389.1782).

4.12.4. Enzymatic hydrolysis of deacyldihydrocanangafruticoside A 7-ol (**5c**)

Dihydrocanangafruticoside A 7-ol (**5c**) (2.2 mg) was dissolved in 20 mM acetate buffer (1.2 mL) and then hesperidinase (1 mg) was added. The mixture was incubated for 18 h at 37 °C. The aglycone (**5d**) (0.9 mg) and glucose were purified by prep. TLC ($CHCl_3$–MeOH–H_2O, 15:6:1). Aglycone (**5d**): colorless oil; ^1H NMR (CD_3OD, 400 MHz) δ: 3.84 (1H, *d*, *J* = 11 Hz, H-7a), 3.76 (1H, *ddd*, *J* = 11, 8, 6 Hz, H-9a), 3.69 (1H, *ddd*, *J* = 11, 6, 5 Hz, H-9b), 3.64 (1H, *d*, *J* = 11 Hz, H-7b), 3.52 (1H, *dd*, *J* = 12, 5 Hz, H-2), 3.42 (1H, *dd*, *J* = 11, 6 Hz, H-10a), 3.41 (1H, *dd*, *J* = 11, 6 Hz, H-10b), 1.85–1.78 (3H, *m*, H-6a, 8a and 8b), 1.69 (1H, *ddd*, *J* = 15, 5, 5 Hz, H-3a), 1.53 (1H, *m*, H-4), 1.32–1.22 (2H, *m*, H-5a and 6b), 1.15–1.07 (2H, *m*, H-3b and 5b); HRESIMS (positive-ion mode) *m*/*z*: 227.1257 $[M+Na]^+$ (Calcd. for $C_{10}H_{20}O_4Na$: 227.1253). Glucose was analyzed with a chiral detector (JASCO OR-2090plus) on an amino column [Asahipak NH2P-50 4E, CH_3CN–H_2O (4:1), 1 mL/min], a peak at 14.5 min with a positive optical rotation sign being observed. Peak was identified by co-chromatography with authentic D-glucose.

4.12.5. Protection of the primary alcohols of aglycone (**5d**)

The aglycone (**5d**) (0.9 mg) was dissolved in pyridine (1 mL) and pivaloyl chloride (8 μL) was added. The reaction mixture was stirred for 3 h at 25 °C. To the reaction mixture, H_2O (1 mL) was added, followed by extracted with EtOAc (2 mL × 3). The residue from the dried (Na_2SO_4) organic layer was purified by prep. TLC [$CHCl_3$–$(CH_3)_2CO$, 20:1] to give tripivalate (1.2 mg) (**5e**). Tripivalate (**5e**): colorless oil; ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.28 (1H, *d*, *J* = 12 Hz, H-7a), 4.22 (2H, *t*, *J* = 7 Hz, H2–9), 4.18 (1H, *d*, *J* = 12 Hz, H-7b), 3.94 (1H, *dd*, *J* = 11, 6 Hz, H-10a), 3.91 (1H, *dd*, *J* = 11, 6 Hz, H-10b), 3.60 (1H, *dd*, *J* = 12, 5 Hz, H-2), 1.90 (1H, *ddd*, *J* = 14, 7, 7 Hz, H-8a), 1.85 (1H, *m*, H-3a), 1.84 (1H, *ddd*, *J* = 14, 7, 7 Hz, H-8b), 1.78 (1H, *m*, H-4), 1.76 (1H, *m*, H-6a), 1.35 (1H, *m*, H-3b), 1.35–1.10 (2H, *m*, H_2-5), 1.21 (1H, *m*, H-6b), 1.180, 1.184, 1.20 (each 9H, each *s*, CH_3 × 3); HRESIMS (positive-ion mode) *m*/*z*: 479.2984 $[M+Na]^+$ (Calcd. for $C_{25}H_{44}O_7Na$: 479.2979).

4.12.6. Preparation of (*R*)- and (*S*)-MTPA esters (**5f** and **5g**) of tripivalate (**5e**)

A solution of **5e** (0.6 mg) in dry CH_2Cl_2 (1 mL) was reacted with (*R*)-MTPA (50 mg) in the presence of EDC (40 mg) and 4-DMAP (25 mg), and then the mixture was occasionally stirred at 25 °C for 30 min and then at 40 °C for 5 min. After the addition of CH_2Cl_2 (1 mL), the solution was washed with H_2O (1 mL), 4 N HCl (1 mL), $NaHCO_3$-saturated H_2O, and then brine (1 mL), successively. The organic layer was dried (Na_2SO_4) and then evaporated under reduced pressure. The residue was purified by prep. TLC [silica gel (0.25 mm thickness), being applied for 18 cm, with development with $CHCl_3$–$(CH_3)_2CO$ (19:1) for 9 cm and then elution with $CHCl_3$–MeOH (9:1)] to furnish an MTPA ester, **5f** (0.5 mg). Through a similar procedure, the (*S*)-MTPA ester (**5g**) of tripivalate (0.6 mg) was prepared from **5e** (0.6 mg) using (*S*)-MTPA (44 mg), EDC (31 mg), and 4-DMAP (21 mg).

(*R*)-MTPA ester (**5f**): ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.43 (2H, *m*, aromatic protons), 7.33–7.31 (3H, *m*, aromatic protons), 4.98 (1H, *dd*, *J* = 11, 5 Hz, H-2), 4.43 (1H, *J* = 12 Hz, H-7a), 3.98 (2H, *m*, H_2-9), 3.87 (2H, *m*, H_2-10), 3.68 (1H, *d*, *J* = 12 Hz, H-7b), 3.47 (3H, *s*, –O*CH*$_3$), 2.00 (1H, *m*, H-3a), 1.89 (1H, m H-6a), 1.83 (1H, *m*, H-4),

1.66 (1H, *m*, H-8a), 1.51 (1H, *m*, H-5a), 1.46 (1H, *m*, H-8b), 1.40 (1H, *m*, H-3b), 1.24 (1H, *m*, H-6b), 1.10 (1H, *m*, H-5b), 1.13 (9H, *s*, $-CH_3 \times 3$), 1.01 (9H, *s*, $-CH_3 \times 3$), 1.00 (9H, *s*, $-CH_3 \times 3$); HRESIMS (positive-ion mode) *m*/*z* 695.3372 $[M+Na]^+$ (Calcd. for $C_{35}H_{51}O_9F_3Na$: 695.3377).

(*S*)-MTPA ester (**5g**): ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.42 (2H, *m*, aromatic protons), 7.34–7.32 (3H, *m*, aromatic protons), 4.96 (1H, *dd*, *J* = 11, 5 Hz, H-2), 4.39 (1H, *J* = 12 Hz, H-7a), 4.05 (2H, *m*, H_2-9), 3.86 (1H, *dd*, *J* = 11, 6 Hz, H-10a), 3.92 (1H, *dd*, *J* = 11, 6 Hz, H-10b)3.70 (1H, *d*, *J* = 12 Hz, H-7b), 3.41 (3H, *s*, $-OCH_3$), 1.95 (1H, *m*, H-3a), 1.90 (1H, m H-6a), 1.80 (1H, *m*, H-4), 1.75 (1H, *m*, H-8a), 1.50 (1H, *m*, H-5a), 1.55 (1H, *m*, H-8b), 1.30 (1H, *m*, H-3b), 1.25 (1H, *m*, H-6b), 1.09 (1H, *m*, H-5b), 1.122 (9H, *s*, $-CH_3 \times 3$), 1.119 (18H, *s*, $-CH_3 \times 6$); HRESIMS (positive-ion mode) *m*/*z* 696.3381 $[M+Na]^+$ (Calcd. for $C_{35}H_{51}O_9F_3Na$: 695.3377).

4.13. Fluorescence measurement of compound **9**

The buffer solutions, used, were as follows, 20 mM acetate buffer for pH 5.0, 20 mM phosphate buffer for pH 6.0, 7.0 and 8.0, 20 mM borate buffer for pH 8.0 and 9.0, and carbonate buffer for pH 9.0 and 10.0. The sample concentration was adjusted to 5.5 μM and measured at 25 °C. Maximum excitation wavelengths were determined by measurement of emission spectra.

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