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Acalyphidins M_1, M_2 and D_1, ellagitannins from *Acalypha hispida*[1]

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Abstract

Two new monomeric hydrolysable tannins, acalyphidins M_1 and M_2, together with fourteen known polyphenols were isolated from the leaves of *Acalypha hispida*, and characterized as 1-*O*-galloyl-3,6-(R)-hexahydroxydiphenoyl-4-*O*-brevifolincarboxyl-β-D-glucose, and an oxidative metabolite of geraniin, respectively, by spectroscopic and chemical methods. A new hydrolysable tannin dimer, acalyphidin D_1, which is composed of two moles of geraniin, was also isolated and characterized as an acetonyl derivative.

Keywords: *Acalypha hispida*; Euphorbiaceae; Tannins; Ellagitannins; Acalyphidin M_1; Acalyphidin M_2; Acalyphidin D_1

1. Introduction

Some *Acalypha* species of Euphorbiaceae have been used as folk medicines for treatment of diarrhea and skin complaints in Southeast Asia. *Acalypha hispida* Burm. f. widely distributed in Asia is one of them, and its leaves have been used as a remedy for thrush and boils in China and Indonesia (Perry, 1980). Although the medicinal value of these plants is thought to be a consequence of their tannin content, the polyphenol constituents in the plants have been little investigated. In our continuing studies on tannins of the euphorbiaceous plants, we have isolated sixteen polyphenols (see Figs. 1–3 for representative structures) including new ellagitannin monomers, named acalyphidins M_1 (**7**) and M_2 (**10**), from the leaf extract of this plant. A geraniin dimer, designated as acalyphidin D_1 (**12**), was also isolated as an acetonyl derivative (**12a**). This paper deals with the structural elucidation of these new tannins.

2. Results and discussion

After filtration of the precipitate deposited upon concentration of aqueous acetone homogenate of the dried leaves of *A. hispida*, the concentrated solution was extracted with Et_2O, EtOAc and *n*-BuOH, successively. The precipitate was chromatographed repeatedly over Diaion HP-20, Toyopearl HW-40 and/or MCI-gel CHP-20P to yield new tannin monomers, acalyphidins M_1 (**7**) and M_2 (**10**), together with five known compounds, phyllanthusiin C (Yoshida, Itoh, Matsunaga, Tanaka & Okuda, 1992), geraniin (**1**) (Okuda, Yoshida & Hatano, 1992), mallotusinin (Saijo, Nonaka & Nishioka, 1989), euphorbins A (4) and B (5) (Yoshida, Chen, Shingu & Okuda, 1988; Yoshida, Yokoyama, Namba & Okuda, 1991; Amakura & Yoshida, 1996). Similar chromatographic separation of the *n*-BuOH extract afforded acalyphidin M_2 (**10**) and a crude acalyphidin D_1 (**8**), along with

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[1] Part 16 in the series of Tannins and Related Polyphenols of Euphorbiaceous Plants. For Part 15, see Amakura, Kawada, Hatano, Agata, Sugaya, Nishibe, Okuda and Yoshida (1997).

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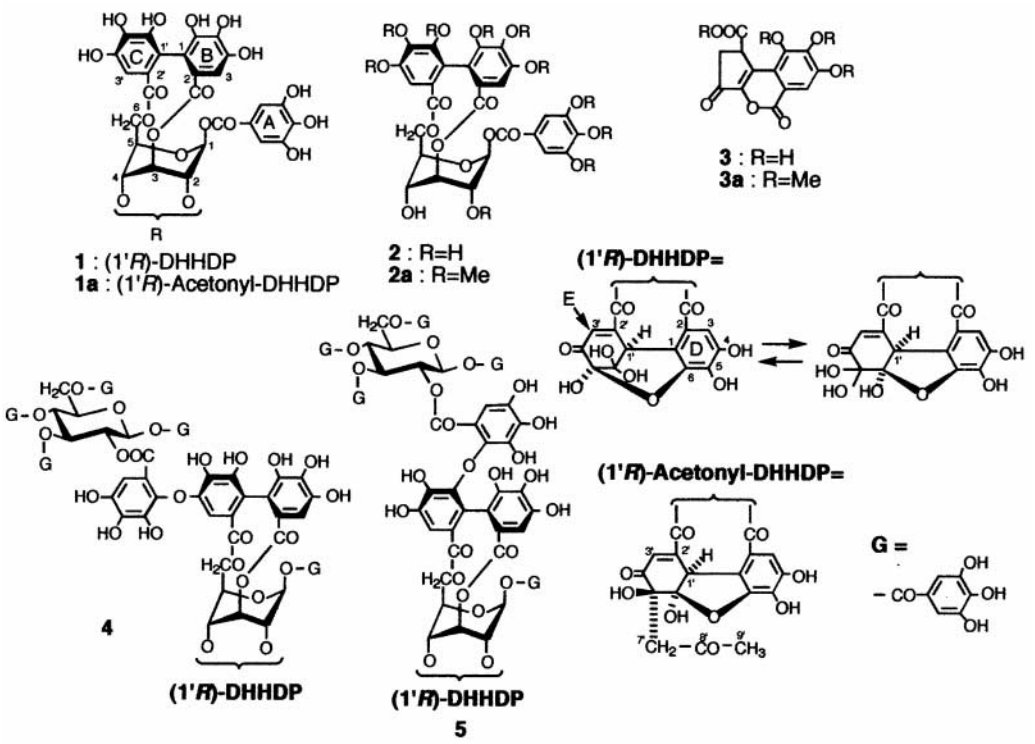

Fig. 1.

two flavonoids, rutin and kaempferol 3-rutinoside (Slimestad, Andersen, Francis, Marston & Hostettmann, 1995), and four tannins, corilagin (**2**) (Okuda et al., 1992), brevifolincarboxylic acid, (**3**) (Yoshida et al., 1992), excoecarianin (Lin, Tanaka, Nonaka, Nishioka & Chen, 1990) and euphorbin D (**12**) (Yoshida, Namba, Yokoyama & Okuda, 1989), which were identified by direct comparison with authentic samples. Furosin (Okuda, Hatano & Yazaki, 1982), 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose (Haddock, Gupta, Al-Shafi, Haslam & Magnolato, 1982) and repandinin A (**6**) (Saijo, Nonaka & Nishioka, 1987) were isolated by repeated column chromatography of the AcOEt extract.

Repandinin A was first isolated from *Macaranga repandus*, and its structure was proposed as **6** without assignment of orientation of *O*-3/*O*-6 acyl group (Saijo et al., 1987). The full characterization and NMR assignment of **6** have now been achieved. The ^{1}H NMR spectrum of **6** was very similar to that of geraniin (**1**) including duplication of signals arising from an equilibration between five and six-membered hemiacetal forms at the dehydrohexahydroxydiphenoyl (DHHDP) group. A remarkable difference of **6** from **1** was the presence of extra signals ascribable to an isolated methylene group in the former. Treatment of **6** with acetone in the presence of ammonium formate afforded an acetone adduct (**6a**) [ESI-MS *m*/*z* 1148

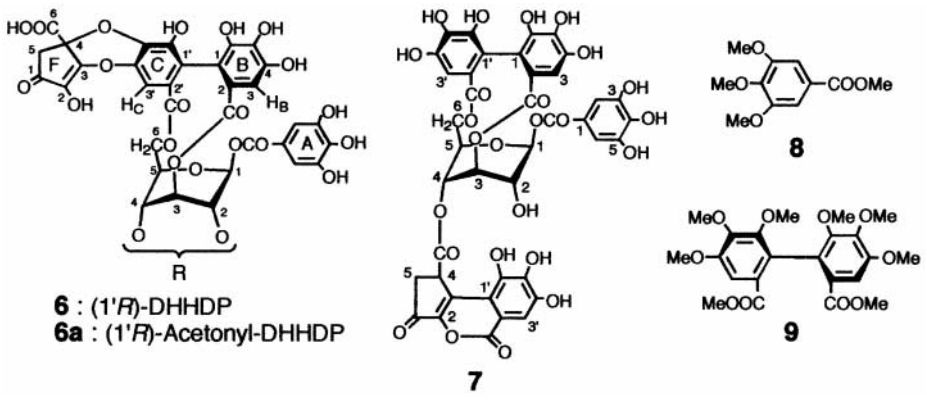

Fig. 2.

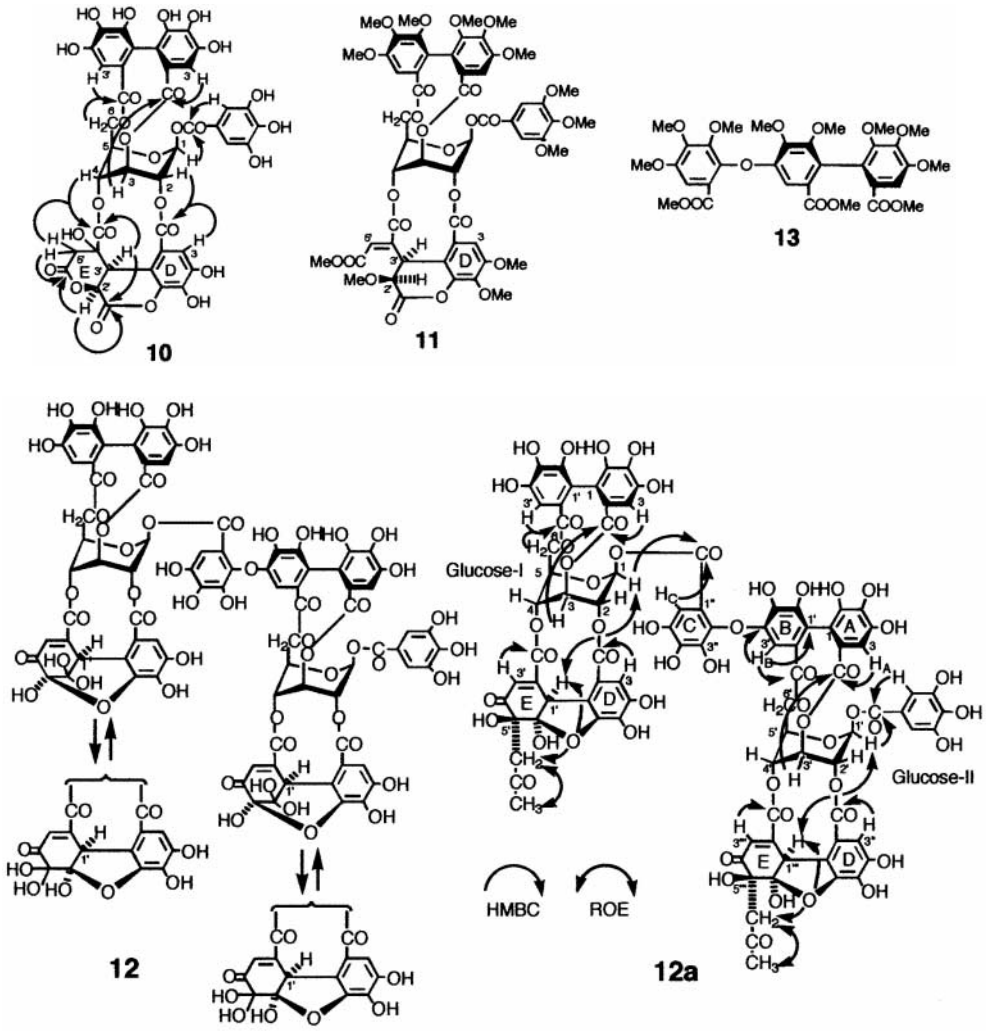

Fig. 3.

$(M + NH_4)^+$] at a DHHDP group in the molecule (Yoshida et al., 1992; Tanaka, Fujisaki, Nonaka & Nishioka, 1992). The presence of an oxygenated brevifolincarboxyl group in **6** was indicated by the ^{13}C NMR spectrum of **6a** which showed signals due to an α,β-unsaturated ketonic function (δ 195.0, 135.4 and 150.1), carboxyl carbon (δ 173.8), methylene carbon and an oxygen-bearing quarternary carbon (δ 76.9). In the HMBC spectrum of **6a**, the aromatic proton (H_B) signal at δ 7.15 was correlated with an ester carbonyl carbon resonance at δ 165.9 which in turn showed a three-bond correlation with the glucose H-3 (δ 5.46) moiety. The aromatic carbon resonances correlated through two- and three-bond couplings with the H_C signal were assignable to those of ring C bearing two ether linkages because their chemical shifts were significantly distinguished from those of the hexahydroxydiphenoyl (HHDP)-rings in the acetonyl derivative (**1a**) of geraniin (**1**). The gross structure of repandinin A was thus represented by **6**.

Acalyphidin M_1 (**7**) was obtained as a light brown amorphous powder, and showed a $[M + Na]^+$ ion peak at m/z 931 in the FABMS. The ^1H NMR spectrum of **7** showed signals attributable to a galloyl group [δ 7.16 (2H, s)] and an HHDP group [δ 6.84, 6.62 (each 1H, *s*)] in the aromatic region. The coupling patterns of the sugar proton signals, which were assigned on the basis of the ^1H-^1H COSY spectrum, were characteristic of glucopyranose in a skew boat conformation. Besides these signals, an aromatic singlet at δ 7.43 and aliphatic ABX-type signals at δ 2.69 (1H, *dd*, J = 2, 19 Hz), 3.00 (1H, *dd*, J = 8, 19 Hz) and 4.69 (1H, *dd*, J = 2, 8 Hz) attributable to a brevifolincarboxylic acid moiety, were observed. The presence of the brevifolincarboxyl group in **7** was also supported by the ^{13}C NMR spectra (δ 193.6, 161.1, 41.6, 37.9, 147.8, 150.1) (Yoshida et al., 1992). The polyphenolic acyl components of **7** assumed from the spectrum were confirmed by methylation of **7** followed by methanolysis yielding methyl tri-*O*-methylgallate (**8**), dimethyl hexamethoxydiphenate (**9**) and methyl tri-*O*-methylbre-

vifolincarboxylate (**3a**) (Yoshida et al., 1992). Partial hydrolysis of **7** with hot water yielded corilagin (**2**) and brevifolincarboxylic acid (**3**), thus establishing the locations of the galloyl and HHDP groups at *O*-1 and *O*-3/*O*-6, respectively, on the glucose core. The position of the brevifolincarboxyl group at *O*-4 was determined on the basis of a remarkable downfield shift of the H-4 signal (δ 5.60), compared with the corresponding signal of corilagin (**2**) (δ 4.45). The (*R*)-configuration at the chiral HHDP group was evidenced by the negative Cotton effect at 240 nm in the CD spectrum of **7** (Okuda, Yoshida, Hatano, Koga, Toh & Kuriyama, 1982). Acalyphidin M_1 was thus characterized as 1-*O*-galloyl-3,6-(*R*)-HHDP-4-*O*-brevifolincarboxyl-β-D-glucose (**7**), in which the configuration of methine carbon in the brevifolincarboxyl group remains to be determined. Although compound **7** has been reported to be obtainable upon treatment of geraniin (**1**) with triethylamine in acetonitrile (Tanaka, Nonaka & Nishioka, 1990), this is the first isolation from a natural source.

Acalyphidin M_2 (**10**) was obtained as a light brown amorphous powder, and gave a $[M + Na]^+$ ion peak at *m*/*z* 975 in FABMS which corresponds to the molecular formula $C_{41}H_{28}O_{27}$. Methylation of **10** and subsequent methanolysis yielded **8** and **9**. The ^{1}H NMR spectrum of **10** showed a 2H-singlet (δ 7.14) and two 1H-singlets (δ 7.05 and 6.60) due to a galloyl and an HHDP group. The chemical shifts and coupling patterns of sugar proton signals were very similar to those of geraniin (**1**), indicating that the glucose hydroxyl groups with $^1C_4$ conformation are fully acylated. These spectral features suggested that **10** has a corilagin unit as a partial structure. This assumption was substantiated by partial hydrolysis of **10** in hot water giving corilagin (**2**). Besides the proton signals due to the corilagin moiety in the ^{1}H NMR spectrum of **10**, a 1H-singlet at δ 7.46, methylene protons [δ 3.50 (*d*, *J* = 18 Hz), 2.87 (*d*, *J* = 18 Hz)], and two methine protons coupled to each other [δ 5.63 (*d*, *J* = 6 Hz), 5.16 (*d*, *J* = 6 Hz)] were also observed. These proton signals are thus ascribed to those of an acyl group at *O*-2/*O*-4. The carbon framework of this 2,4-acyl group was shown to involve four aliphatic, six aromatic and four carboxyl carbons (δ 40.9, 43.1, 72.9, 74.3, 114.2, 116.6, 119.2, 139.7, 142.1, 146.9, 164.9, 165.8, 167.1, 171.5) by subtracting the signals of the corilagin moiety from those in the ^{13}C NMR spectrum of **10**. The HMBC spectrum showed a three-bond long-range correlation between glucose H-2 (δ 5.56) and the ester carbonyl carbon (δ 164.9), the latter of which also correlated with D-ring H-3 (δ 7.46). The glucose H-4 (δ 5.32) showed a similar correlation with the ester carbonyl carbon signal at δ 171.5, which was coupled with methylene H-5′ (δ 3.50, 2.87) and methine H-3′ (δ 5.16) protons. The methylene H-5′ also exhibited correlation peaks with a lactone carbonyl carbon at δ 167.1, which in turn showed a cross peak with methine H-2′ (δ 5.63). The H-3′ showed a correlation through three-bond coupling with another lactone carbonyl carbon at δ 165.8, which coupled with the methine H-2′ signal. These results, taking the FABMS data into consideration, were consistent with a dilactone structure of the acyl unit attached to the glucose *O*-2/*O*-4 as shown in formula **10**. The other correlations in the HMBC are shown by arrows in (see structure **10**). The (*R*)-configuration at C-3′ in the *O*-2/*O*-4 acyl group was established by ROESY spectrum showing a definite NOE between H-3′ and the anomeric proton signal of the glucose core. The configuration at C-2′ was thus determined as illustrated in the formula on the basis of *cis*-arrangement between H-2′ and H-3′ as evidenced from their coupling constant (*J* = 6 Hz) (Yoshida, Okuda, Koga & Toh, 1982).

Methylation of **10** with Me_2SO_4 and K_2CO_3 in dry Me_2CO gave deca-*O*-methylcorilagin (**2a**) (Yoshida & Okuda, 1980) and a tridecamethyl derivative (**11**). The structure of **11** was deduced based on its FABMS (*m*/*z* 1135 $[M + H]^+$) and ^{1}H NMR spectra. Its formation is reasonably explainable by cleavage of one of the lactone rings accompanying β-elimination of a water molecule in the acyl group at *O*-2/*O*-4 during the methylation. The structure of acalyphidin M_2 was thus represented by **10**, although the stereochemistry at C-4′ remains undetermined.

The dimeric nature of acalyphidin D_1 (**12**) was shown by a large retention volume similar to that of euphorbin A (**4**) and other dimers on normal-phase HPLC. (Okuda, Yoshida & Hatano, 1996) However, extreme difficulty in the purification of **12** was encountered. Even though it gave a single peak in both normal- and reversed-phase HPLC, it was inevitably shown to be contaminated with a small amount of other dimers by the ^{1}H NMR spectrum. The ^{1}H NMR spectrum of the crude acalyphidin D_1, in spite of its complexity, suggested the presence of two DHHDP groups forming an equilibrium mixture of five- and six-membered hemiacetal structures as revealed by two set of methine and vinyl proton signals (δ 6.31, 6.21, 6.21, 6.20, 4.91, 4.91, 4.90, 4.87, each doublet of *J* = 2 Hz), characteristic of H-1′ and H-3′ of the DHHDP group. Then, derivatization at the DHHDP moiety was examined to facilitate its structure determination as well as purification. Acalyphidin D_1 (**12**) was obtained in a pure state as its acetonyl derivative (**12a**), ESIMS *m*/*z* 2000 $[M + NH_4]^+$, which was prepared by treatment with acetone in the presence of ammonium formate (Yoshida et al., 1992; Tanaka et al., 1992). The ^{1}H NMR spectrum of **12a** displayed a 2H-singlet (δ 7.12) and five ^{1}H-singlets (δ 7.13, 7.04, 7.02, 6.62, 6.25) ascribable to the galloyl, HHDP and valoneoyl groups. The assignment of these acyl groups was

Table 1
^{1}H NMR spectral data of **12a** and **1a** [500 MHz, Me_2CO-d_6 + D_2O (*J* in Hz)]

		12a		**1a**
		Glucose-I	Glucose-II	
Glucose				
	H-1	6.35 (*br s*)	6.52 (*br s*)	6.55 (*br s*)
	H-2	5.40 (*br s*)	5.54 (*br s*)	5.54 (*br s*)
	H-3	5.46 (*br s*)	5.46 (*br s*)	5.49 (*br s*)
	H-4	5.37 (*br s*)	5.37 (*br s*)	5.41 (*br s*)
	H-5	4.73 (*br t*, *J* = 8)	4.76 (*br t*, *J* = 8)	4.82 (*t*, *J* = 7.5)
	H-6	4.66 (*t*, *J* = 10.5)	4.66 (*t*, *J* = 10.5)	4.75 (*t*, *J* = 7.5)
		4.30 (*dd*, *J* = 8, 10.5)	4.36 (*dd*, *J* = 8, 10.5)	4.38 (*dd*, *J* = 7.5, 10.5)
Galloyl	H-2, 6	7.12 (2H, *s*)		7.15 (2H, *s*)
HHDP	H-3	7.04 (each 1H, *s*)		7.05 (each 1H, *s*)
	H-3′	6.62 (each 1H, *s*)		6.63 (each 1H, *s*)
Valoneoyl	H-A	7.02 (each 1H, *s*)		
	H-B	6.25 (each 1H, *s*)		
	H-C	7.13 (each 1H, *s*)		
Acetonyl-DHHDP				
Ring-D	H-3, 3″	7.22, 7.16 (each 1H, *s*)		7.20 (1H, *s*)
Ring-E	H-1′	4.93 (1H, *d*, *J* = 1)		4.89 (1H, *d*, *J* = 1.5)
	H-1‴	4.90 (1H, *d*, *J* = 1)		
	H-3′, 3‴	6.33, 6.23 (each 1H, *d*, *J* = 1)		6.28 (1H, *d*, *J* = 1.5)
	H-7′, 7‴	3.45, 3.22 (each 1H, *d*, *J* = 16		3.46
		2.99, 2.92 (each 1H, *d*, *J* = 16)		2.95 (each 1H, *d*, *J* = 15.5)
	H-9′, 9‴	2.14, 2.06 (each 3H, *s*)		2.17 (3H, *s*)

substantiated by production of **8**, **9** and trimethyl octa-*O*-methylvaloneate (**13**) upon methanolysis of a methylated derivative **12a**. Sugar proton signals showed close similarity to those of acetonyl derivative (phyllanthusiin D, **1a**) of geraniin (**1**) (Yoshida et al., 1992) in both the chemical shifts and coupling patterns. The presence of two acetonyl-DHHDP groups in **12a** was also indicated by comparison of the ring-D and E signals with those of **1a** as shown in Table 1. Based on these data, **12a** was regarded as a dimer of phyllanthusiin D (**1a**) (Yoshida et al., 1992) in which each monomer is linked through formation of the valoneoyl group. The ^{13}C NMR spectrum of **12a** was consistent with this assumption (Table 2). The location of each acyl group including the orientation of the valoneoyl group was established as follows. The HMBC spectrum of **12a** showed a correlation through a three-bond coupling between glucose H-6′ (δ 4.66) and ester carbonyl carbon (δ 168.3) which was also correlated with the aromatic proton signal at δ 6.25. This aromatic signal was assigned to H_B of the valoneoyl group based on its two-bond coupling with the signal attributed to C-4′ (δ 146.9) of the valoneoyl B-ring, and also by three bond coupling with C-1′ (δ 117.1). Connectivity between the valoneoyl B-ring and H-6′ of glucose core-II was thus established. The aromatic proton signal at δ 7.13 was correlated with the ester carbonyl carbon at δ 163.1, which showed a three-bond long-range coupling with the H-1 (δ 6.35) of glucose-I. Similarly, connectivities of galloyl and HHDP protons with H-1′ and H-6/H-3, respectively, were established as shown by arrows in the formula **12a**. In addition, the mode of linkage of the acetonyl-DHHDP unit to each glucose core was indicated to be the same as that of **1a**. The absolute configurations at C-1′ and C-5′ of each acetonyl group were also determined as illustrated in **12a** by ROE correlations of the H-1′ (H-1‴) of the ring-E with the acetonyl methylene and anomeric proton signals.

Based on these findings, the structure of the acetonyl derivative of acalyphidin D_1 was represented by the formula **12a**. Upon derivatization of a DHHDP group in a tannin into its acetone-adduct, the remainder of the molecule is proved to be unaffected as exemplified by conversion of **1** into **1a** (Yoshida et al., 1992). Therefore, the structure of acalyphydin D_1 was shown as formula **12**, which exists in an equilibrium mixture of four tautomers at two DHHDP moieties.

Although many hydrolysable tannin dimers including the geraniin part as a constituent monomer unit as represented by euphorbins have been hitherto isolated from various euphorbiaceous plants (Okuda et al., 1996), acalyphidin D_1 (**12**) is the first example of a dimer composed of two moles of geraniin.

Table 2
^{13}C NMR spectral data of **12a** and **1a** [126 MHz, Me_2CO-d_6 + D_2O]

	12a	1a		12a	1a
Glucose			HHDP		
C-1 (1′)	91.8, 92.0	91.9	C-1	117.4	117.3
C-2	70.1, 70.4	70.4	C-2	124.3*	124.3†
C-3	62.3, 62.5	62.4	C-3	110.0	110.3
C-4	66.4, 66.7	66.7	C-4	144.8§	144.7
C-5	72.8, 73.3	73.1	C-5	137.4	137.8
C-6	93.8, 63.9	62.9	C-6	144.9§	145.0‡
Galloyl			C-7	166.1	166.4
C-1	119.9	120.1	C-1′	115.4	115.3
C-2, 6	110.5 (2C)	110.6 (2C)	C-2′	125.1*	125.4†
C-3, 5	145.9 (2C)	146.1(2C)	C-3′	107.8	107.9
C-4	139.8	140.0	C-4′	145.6	145.5
C-7	165.1	165.3	C-5′	136.4	136.5
Valoneoyl			C-6′	145.3§	145.3‡
C-1	116.7		C-7′	168.7	168.7
C-2	123.7*		Acetonyl-DHHDP		
C-3	109.9		C-1, 1″	119.9 (2C)	120.1
C-4	144.6		C-2, 2″	116.7, 116.8	116.8
C-5	137.3		C-3, 3″	113.2, 113.4	113.4
C-6	145.0§		C-4, 4″	147.6, 147.7	147.7
C-7	166.3		C-5, 5″	137.7, 137.3	137.4
C-1′	117.1		C-6, 6″	146.7, 147.0	147.0
C-2′	125.1*		C-7, 7″	164.7, 164.8	164.8
C-3′	104.8		C-1′, 1‴	51.5, 51.8	52.0
C-4′	146.9		C-2′, 2‴	145.4 (2C)§	145.5
C-5′	137.0		C-3′, 3‴	126.5, 127.0	126.9
C-6′	145.1§		C-4′, 4‴	197.5, 187.8	197.8
C-7′	168.3		C-5′, 5‴	80.6, 80.8	80.9
C-1″	113.8		C-6′, 6‴	109.6, 109.8	109.8
C-2″	143.0		C-7′, 7‴	51.5, 51.8	50.0
C-3″	136.9		C-8′, 8‴	206.3, 206.4	206.4
C-4″	140.2		C-9′, 9‴	31.7, 31.8	32.0
C-5″	140.9		C-10, 10‴	165.4, 165.5	165.5
C-6″	110.2				
C-7″	163.1				

† , ‡, *, § Values are interchangeable.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded at ambient temperature (*ca.* 21°C) on a Varian VXR-500 instrument (500 MHz for ^1H and 125.7 MHz for ^{13}C), and chemical shifts are given in δ-values from tetramethylsilane. A sample [5–10 mg for ^1H (1D and 2D) and $20 \sim 30$ mg for ^{13}C (1D and 2D)] was dissolved in Me_2CO-d_6 or Me_2CO-d_6 + D_2O (0.6 ml). The standard pulse sequences programmed for the instrument VXR-500 were used for each 2D measurement. Average *J*-values for one-bond coupling and two- or three-bond coupling in HMQC and HMBC were set at 140 Hz and 6 or 8 Hz. Normal phase HPLC was conducted on a YMC-Pack SIL A-003 (YMC Co., Ltd.), column (4.6 × 250 mm) developed with *n*-hexane–MeOH–THF–HCO_2H (60:45:15:1) containing oxalic acid (500 mg l^{-1}) (flow rate, 1.5 ml min^{-1}; detection 280 nm) at room temp. Reversed-phase HPLC was performed on a YMC-Pack A312 (ODS) (YMC Co., Ltd.) column (4.6×150 mm) developed with 10 mM H_3PO_4 − 10 mM KH_2PO_4 − MeCN (41:41:18) (flow rate, 1.0 ml min^{-1}; detection 280 nm) at 40°. Other chromatographic methods and instruments employed in this work were the same as those described in the preceding paper (Amakura, Kawada, Hatano, Agata, Sugaya et al., 1997).

3.2. Plant material

The leaves of *Acalypha hispida* Burm. f. were collected in Taiwan. A voucher specimen was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Okayama University.

3.3. Extraction and isolation

The dried leaves (950 g) were homogenized (×3) in Me_2CO–H_2O (7:3) (12 l×3) and the homogenate was filtered. The filtrate was concd and extracted with Et_2O (1 l×3), EtOAc (1 l×5) and *n*-BuOH satd with H_2O (1 l×6). A part (8.5 g) of the precipitate (119 g) was chromatographed over Toyopearl HW-40 (fine) (2.2 cm i.d.×58 cm) with MeOH–H_2O (5:5 → 6:4 → 7:3) → MeOH–H_2O–Me_2CO (7:2:1 → 6:2:2) → Me_2CO–H_2O (7:3) in a stepwise gradient mode. The fractions showing similar HPLC patterns were combined and further purified by CC over Sephadex LH-20 with EtOH and/or MCI-gel CHP-20 P with aq. MeOH to afford geraniin (**1**) (180 mg), mallotusinin (4 mg), phyllanthusiin C (20 mg), euphorbin A (**4**) (12 mg), euphorbin B (**5**) (5 mg), and acalyphidin M_1 (**7**) (11 mg). In order to obtain additional crops of the new tannins, the rest (32 g) of the precipitate was chromatographed over Diaion HP-20 (5.5 cm i.d. × 45 cm) with H_2O → MeOH–H_2O (2:8 → 4:6 → 6:4) → MeOH in a stepwise gradient mode. The eluate with MeOH–H_2O (6:4) was similarly fractionated by repeated CC over Toyopearl HW-40 (fine), Sephadex LH-20 and MCI-gel CHP-20P to give fraction A [including acalyphidin D_1 (**12**)] (321 mg), acalyphidin M_1 (**7**) (20 mg) and acalyphidin M_2 (**10**) (16 mg).

A part (29 g) of the *n*-BuOH extract (35 g) was subjected to column chromatography over Diaion HP-20 (5.5 cm i.d. × 28 cm) and developed with H_2O → MeOH–H_2O (2:8 → 4:6 → 6:4) → MeOH in stepwise gradient mode. The eluate with MeOH–H_2O (4:6) was further chromatographed over Toyopearl HW-40 (fine) and MCI-gel CHP-20P to give fraction B [including acalyphidin D_1 (**12**)] (89 mg), rutin (34 mg), brevifolincarboxylic acid (**3**) (34 mg), excoecarianin (17 mg) and acalyphidin M_2 (**10**) (8.7 mg). The eluate with MeOH–H_2O (6:4) was similarly subjected to repeated column chromatographies to give kaempferol 3-rutinoside (120 mg), corilagin (**2**) (19 mg) and euphorbin D (52 mg).

A part (6.0 g) of the AcOEt extract (20 g) was chromatographed over Toyopearl HW-40 (coarse) (2.2 cm i.d.×50 cm) with aq. MeOH (60% → 70%) → MeOH–H_2O–Me2CO (7:2:1). The 60% MeOH eluate was rechromatographed over MCI-gel CHP-20P with aq. MeOH to give furosin(2.4 mg). The eluate of MeOH–H_2O–Me_2CO (7:2:1) was also rechromatographed over MCI-gel CHP-20P with aq. MeOH to give 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (12 mg) and repandinin A (**6**) (54 mg).

3.3.1. Repandinin A (*6*)

A brown amorphous powder, $[\alpha]_D$ −94° (*c* = 0.1, MeOH). UV λ_{max}(MeOH) nm (log ε): 221 (4.88), 284 (4.58). CD (MeOH) $[\theta]_{226}$ −8700, $[\theta]_{239}$ +26000, $[\theta]_{307}$ −73000. ESIMS *m/z*: 1108 $(M + NH_4)^+$. ^{1}H NMR (500 MHz, Me_2CO-d_6 + D_2O): δ 7.15, 7.16 (2H in total, *s*, ring A-H), 7.14, 7.12 (1H in total, *s*, ring B-H), 6.77, 6.75 (1H in total, *s*, ring C-H), 7.18, 7.20 (1H in total, *s*, ring D-H), 6.48 (*s*), 6.21 (*d*, *J* = 1 Hz) (1H in total, ring E H-3), 5.12 (*s*), 4.90 (*d*, *J* = 1 Hz) (1H in total, ring E H-1), 6.56, 6.54 (1H in total, *brs*, glucose (Glc) H-1), 5.58, 5.56, 5.53, 5.48, 5.43, 5.39 (3H in total, *m*, Glc H-2, 3, 4), 4.81 (*m*), 4.70 (*t*, *J* = 10 Hz) (1H in total, Glc H-5), 4.81 (1H, *m*, Glc H-6), 4.29 (*m*), 4.36 (*dd*, *J* = 8, 10 Hz) (1H in total, Glc H-6′), 2.93 (1H, *d*, *J* = 16.5 Hz, ring F-H), 2.54 (1H, *d*, *J* = 16.5 Hz, ring F-H). ^{13}C NMR (126 MHz, Me_2CO-d_6 + D_2O): δ 119.8 (C-1), 110.5, 110.4 (C-2), 146.93, 145.90 (C-3), 140.0, 139.9 (C-4), 146.93, 145.90 (C-5), 110.5, 110.4 (C-6) 165.2, 164.2 (C-7) [ring A], 116.47, 116.54 (C-1), 123.3, 123.0 (C-2), 111.7, 111.5 (C-3), 144.6, 144.5 (C-4), 139.43, 139.40 (C-5), 144.9 (C-6), 165.9 (C-7) [ring B], 115.8, 115.7 (C-1), 130.23, 130.20 (C-2), 109.1, 108.8 (C-3), 146.4, 146.3 (C-4), 132.89, 132.86 (C-5), 143.6 (C-6), 167.43, 167.41 (C-7) [ring C], 195.2 (C-1), 135.3 (C-2), 148.9 (C-3), 77.2 (C-4), 44.7 (C-5), 173.5 (C-6) [ring F], 115.6, 119.7 (C-1), 118.9, 116.7 (C-2), 113.35, 113.41 (C-3), 145.7, 147.6 (C-4), 139.1, 137.4 (C-5), 143.2, 146.9 (C-6), 165.4, 165.3 (C-7) [ring D], 45.9, 51.8 (C-1), 154.1, 150.3 (C-2), 128.7, 125.1 (C-3), 191.9, 194.7 (C-4), 96.1, 92.4 (C-5), 92.1, 109.1 (C-6), 165.6, 165.7 (C-7) [ring E], 91.0, 91.8 (C-1), 69.9, 70.4 (C-2), 62.3, 63.3 (C-3), 66.6, 65.7 (C-4), 72.2, 72.8 (C-5), 64.1, 64.4 (C-6) [Glc].

3.3.2. Acetonyl derivative (*6a*) of repandinin A (*6*)

A light brown amorphous powder, $[\alpha]_D$ −78° (*c* = 0.1, MeOH). UV λ_{max}(MeOH) nm (log ε): 221 (4.91), 284 (4.60). CD (MeOH) $[\theta]_{229}$ −7300, $[\theta]_{239}$ +26000, $[\theta]_{307}$ −82000. ESIMS *m/z*: 1148 $(M + NH_4)^+$. ^{1}H NMR (500 MHz, Me_2CO-d_6 + D_2O): δ 7.16 (2H, *s*, ring A-H), 7.15 (1H, *s*, ring B-H), 6.73 (1H, *s*, ring C-H), 7.18 (1H, *s*, ring D-H), 6.27 (1H, *d*, *J* = 1 Hz, ring E H-3), 4.87 (1H, *d*, *J* = 1 Hz, ring E H-1), 6.55 (1H, *brs*, Glc H-1), 5.57, 5.46, 5.45 (3H, *m*, Glc H-2, 3, 4), 4.81 (1H, *brdd*, *J* = 8, 11 Hz, Glc H-5), 4.73 (1H, *t*, *J* = 11 Hz, Glc H-6), 4.33 (1H, *dd*, *J* = 8, 11 Hz, Glc H-6), 2.92 (1H, *d*, *J* = 16.5 Hz, ring F-H), 2.54 (1H, *d*, *J* = 16.5 Hz, ring F-H), 3.45 (1H, *d*, *J* = 15.5 Hz, ring E H-7), 2.95 (1H, *d*, *J* = 15.5 Hz, ring E H-7′), 2.17 (3H, *s*, ring E H-9). ^{13}C NMR (126 MHz, Me_2CO-d_6 + D_2O): δ 119.8 (C-1), 110.4 (C-2), 145.9 (C-3), 140.0 (C-4), 145.9 (C-5), 110.4 (C-6) 165.3 (C-7) [ring A], 116.5 (C-1), 123.0 (C-2), 111.5 (C-3), 144.6 (C-4), 139.5 (C-5), 144.9 (C-6), 165.9 (C-7) [ring B], 115.8 (C-1), 130.2 (C-2), 109.1 (C-3), 146.4 (C-4), 132.9 (C-5), 143.5 (C-6), 167.4 (C-7) [ring C], 195.0 (C-1), 135.4 (C-2), 150.1 (C-3), 76.9 (C-4), 44.6 (C-5), 173.8 (C-6) [ring F], 119.8 (C-1), 116.6

(C-2), 113.3 (C-3), 147.6 (C-4), 137.3 (C-5), 146.9 (C-6), 164.6 (C-7) [ring D], 51.8 (C-1), 145.3 (C-2), 126.8 (C-3), 197.7 (C-4), 80.8 (C-5), 109.5 (C-6), 49.9 (C-7), 206.6 (C-8), 31.8 (C-9), 165.4 (C-10) [ring E], 91.7 (C-1), 70.2 (C-2), 62.2 (C-3), 66.4 (C-4), 72.7 (C-5), 64.3 (C-6) [Glc].

3.3.3. Acalyphidin M_1 (7)

A light brown amorphous powder. $[\alpha]_D$ −43° (MeOH; *c* 0.5). (Found: C, 48.04; H, 4.02. $C_{40}H_{28}O_{25}\cdot 5H_2O$ requires: C, 48.11, H, 3.50%). FABMS *m/z*: 931 $[M + Na]^+$. UV λ_{max}^{MeOH} nm (log ϵ): 220 (4.68), 280 (4.46), 294 (4.38), 360 (3.80). CD (MeOH): $[\theta]_{240}$ −94000, $[\theta]_{260}$ +45000, $[\theta]_{287}$ −88000, $[\theta]_{323}$ +1700, $[\theta]_{348}$ −5100. ^{1}H NMR (500 MHz, Me_2CO-d_6 + D_2O): δ 7.16 (2H, *s*, galloyl-H), 6.84, 6.62 (each 1H, *s*, HHDP-H), 7.43 [1H, *s*, brevifolincarboxyl (Brev.) H-3′], 6.22 (1H, *d*, *J* = 5, Glc H-1), 5.60 (1H, *br d*, *J* = 4, Glc H-4), 4.83 (1H, *br d*, *J* = 4, Glc H-3), 4.69 (1H, *t*, *J* = 8, Glc H-5), 4.69 (1H, *dd*, *J* = 2, 8 Hz, Brev. H-4), 4.45 (1H, *dd*, *J* = 8, 11, Glc H-6), 4.27 (1H, *dd*, *J* = 8, 11, Glc H-6), 4.18 (1H, *br d*, *J* = 5, Glc H-2), 3.00 (1H, *dd*, *J* = 8, 19 Hz, Brev. H-5), 2.69 (1H, *dd*, *J* = 2, 19 Hz, Brev. H-5), ^{13}C NMR (126 MHz, Me_2CO-d_6 + D_2O): δ 120.4 (C-1), 110.6 (C-2), 146.0 (C-3), 138.2 (C-4), 146.0 (C-5), 110.6 (C-6) 166.0 (C-7) [galloyl], 116.4, 115.9 (C-1, 1′), 125.2, 125.4 (C-2, 2′), 109.5, 109.7 (C-3, 3′), 145.2 (2C, C-4, 4′), 136.4, 137.0 (C-5, 5′), 144.8 (2C, C-6, 6′), 166.9, 168.4 (C-7, 7′) [HHDP], 193.6 (C-1), 147.8 (C-2), 150.1 (C-3), 41.6 (C-4), 37.9 (C-5), 115.0 (C-1′), 115.4 (C-2′), 108.2 (C-3′), 139.6 (C-4′), 140.5 (C-5′), 143.6 (C-6′), 161.1, 172.0 (C-6, 7′) [Brev], 94.4 (C-1), 70.7 (C-2), 72.9 (C-3), 65.9 (C-4), 74.3 (C-5), 64.6 (C-6) [Glc].

3.3.4. Partial hydrolysis of 7

A soln of **7** (0.5 mg) in H_2O (0.5 ml) was heated at 80° for 30 min. The reaction mixture showed peaks identical with those of corilagin (**2**) and brevifolin carboxylic acid (**3**) on reversed-phase HPLC.

3.3.5. Methylation of 7 followed by methanolysis

A mixture of **7** (2.5 mg), K_2CO_3 (100 mg) and Me_2SO_4 (0.01 ml) in Me_2CO (2 ml) was stirred overnight at room temp, and then refluxed for 2 hr. After removal of the inorganic material by centrifugation, the supernatant was evaporated to dryness. The reaction mixture was directly methanolyzed with 1% NaOMe (0.1 ml) in MeOH (1 ml) at room temp for 6 hr. After acidification with a few drops of HOAc, the solvent was removed in *vacuo*. The residue was subjected to prep. TLC to give methyl tri-*O*-methylgallate (**8**) (0.2 mg), dimethyl hexamethoxydiphenate (**9**) (0.1 mg) and methyl tri-*O*-methylbrevifolincarboxylate (**3a**) (0.2 mg), which were identified by direct comparison with authentic samples (TLC, HPLC and MS).

*3.3.6. Acalyphidin M_2 (**10**)*

A light brown amorphous powder. $[\alpha]_D$ −59° (MeOH; *c* 0.5). (Found: C, 45.98; H, 3.91. $C_{41}H_{28}O_{27}\cdot 7H_2O$ requires: C, 45.65, H, 3.92%). FABMS *m/z*: 975 $[M + Na]^+$. UV λ_{max}^{MeOH} nm (log ϵ): 224 (4.75), 280 (4.46). CD (MeOH): $[\theta]_{208}$ +32000, $[\theta]_{236}$ −59000, $[\theta]_{263}$ +23000, $[\theta]_{288}$ −42000, $[\theta]_{321}$ +27000. ^{1}H NMR (500 MHz, Me_2CO-d_6 + D_2O): δ 7.14 (2H, *s*, galloyl-H), 7.05, 6.60 (each 1H, *s*, HHDP-H), 7.46 (1H, *s*, ring-D H-3), 6.47 (1H, *br s*, Glc H-1), 5.70 (1H, *br s*, Glc H-3), 5.63 (1H, *d*, *J* = 6 Hz, H-2′), 5.56 (1H, *br s*, Glc H-2), 5.32 (1H, *br d*, *J* = 3, Glc H-4), 5.16 (1H, *d*, *J* = 6 Hz, H-3′), 4.74 (1H, *m*, glu. H-5), 4.74 (1H, *m*, Glc H-6), 4.36 (1H, *m*, Glc H-6), 3.50 (1H, *d*, *J* = 18 Hz, H-5′), 2.87 (1H, *d*, *J* = 18 Hz, H-5′). ^{13}C NMR (126 MHz, Me_2CO-d_6 + D_2O): δ 120.4 (C-1), 110.6 (C-2), 146.0 (C-3), 138.2 (C-4), 146.0 (C-5), 110.6 (C-6), 166.0 (C-7) [galloyl], 116.4, 115.9 (C-1, 1′), 125.2, 125.4 (C-2, 2′), 109.5, 109.7 (C-3, 3′), 145.2 (2C, C-4, 4′), 136.4, 137.0 (C-5, 5′), 144.8 (2C, C-6, 6′), 166.9, 168.4 (C-7, 7′) [HHDP-C], 114.2 (C-1), 119.2 (C-2), 116.6 (C-3), 146.9 (C-4), 139.7 (C-5), 142.1 (C-6), 164.9 (C-7) [ring-D], 165.8 (C-1′), 72.9 (C-2′), 43.1 (C-3′), 74.3 (C-4′), 40.9 (C-5′), 167.1 (C-6′), 171.5 (C-7′) [ring-E], 91.3 (C-1), 70.4 (C-2), 62.4 (C-3), 67.3 (C-4), 73.4 (C-5), 63.9 (C-6) [Glc].

*3.3.7. Partial hydrolysis of **10***

A soln of **10** (0.2 mg) in H_2O (1.0 ml) was heated at 80° for 10 hr. Reversed-phase HPLC of the reaction mixture showed peaks identical with that of corilagin (**2**).

*3.3.8. Methylation of **10***

A mixture of **10** (10 mg), Me_2SO_4 (0.02 ml) and K_2CO_3 (200 mg) in dry Me_2CO (3 ml) was stirred overnight at room temp and then refluxed for 2 hr. After removal of the inorganic material by centrifugation, the reaction mixture was subjected to prep. TLC (toluene–Me_2CO, 2:1), which gave deca-*O*-methylcorilagin (**2a**) (1.3 mg) and tridecamethylate (**11**) (1.8 mg). **2a**: A white amorphous powder, ^{1}H NMR (500 MHz, Me_2CO-d_6): δ 7.23 (2H, *s*, galloyl-H), 6.92, 6.81 (each 1H, *s*, HHDP-H), 3.94 (1H, *d*, *J* = 10 Hz, 4-OH), 3.90, 3.89, 3.88, 3.83, 3.68, 3.67 (6H), 3.66, 3.64, 3.16 (each 3H, *s*, OMe), glucose protons see Table 1. **11**: A white amorphous powder, $[\alpha]_D$ −21° (MeOH; *c* 0.5). FABMS *m/z* 1135 $[M + H]^+$, 1157 $[M + Na]^+$. ^{1}H NMR (500 MHz, Me_2CO-d_6): δ 7.25 (2H, *s*, galloyl-H), 7.10, 6.93 (each 1H, *s*, HHDP-H), 7.23 (1H, *s*, ring-D H-3), 5.74 (1H, *d*, *J* = 8 Hz, H-2′), 5.55 (1H, *d*, *J* = 8 Hz, H-3′), 6.39 (1H, *s*, H-5′), 4.01, 3.92, 3.90, 3.89, 3.87, 3.84, 3.79, 3.68, 3.66 (9H), 3.60, 3.25 (each 3H, *s*, OMe), glucose protons see Table 1.

*3.3.9. Methylation of **10** followed by methanolysis*

A mixture of **10** (0.5 mg), K_2CO_3 (50 mg) and Me_2SO_4 (0.01 ml) in dry Me_2CO (2.0 ml) was stirred overnight at room temp, and then refluxed for 2 hr. After removal of the inorganic material by centrifugation, the supernatant was evaporated to dryness. A soln of the mixture in MeOH (1 ml) and 1% NaOMe (0.1 ml) was kept standing at room temp for 6 hr. After acidification with HOAc, the solvent was removed *in vacuo*. The residue was re-dissolved in MeOH and analysed by normal-phase HPLC which showed the production of methyl tri-*O*-methylgallate (**8**) and dimethyl hexamethoxydiphenate (**9**).

*3.3.10. Preparation of the Me_2CO adduct of **12***

Crude acalyphidin D_1 (fraction A (321 mg) [and B (89 mg)] described in the isolation procedure of tannins) was treated with ammonium formate (150 mg) [45 mg in B] in Me_2CO (10 ml) [5 ml in B], and heated at 50° for 2 hr. The residue after removal of the solvent was subjected to column chromatography over MCI-gel CHP-20P with H_2O containing increasing amounts of MeOH. The 40% MeOH eluate gave an acetenyl derivative (**12a**) of acalyphidin D_1 (23 mg) [10 mg from B], as a light brown amorphous powder, $[\alpha]_D$ −43° (MeOH; *c* 0.42). ESIMS *m/z*: 2000 $[M + NH_4]^+$. UV λ_{max}^{MeOH} nm (log ϵ): 224 (4.75), 280 (4.46). CD (MeOH): $[\theta]_{208}$ +32000, $[\theta]_{236}$ −59000, $[\theta]_{263}$ +23000, $[\theta]_{288}$ −42000, $[\theta]_{321}$ +27000. ^{1}H NMR, see Table 1. ^{13}C NMR, see Table 2.

*3.3.11. Methylation of **12a** followed by methanolysis*

A mixture of **12a** (2 mg), K_2CO_3 (50 mg) and Me_2SO_4 (0.01 ml) in Me_2CO (2.5 ml) was stirred overnight at room temp, and then refluxed for 2 hr. A syrupy residue after removal of the inorganic material and solvent, was directly methanolyzed with 1% NaOMe (0.1 ml) in MeOH (1 ml), and subjected to prep. TLC (Kieselgel PF_{254}, *n*-hexane–$CHCl_3$–Me_2CO, 4:6:1) to give **8**, **9** and trimethyl octa-*O*-methylvalonate (**13**), which were identified by comparison authentic samples (TLC and HPLC).

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