Studies on the Constituents of Bark of *Parameria laevigata* MOLDENKE[1)]

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One new trimeric proanthocyanidin, epicatechin-(2β→*O*→7,4β→6)-epicatechin-(2β→*O*→7, 4β→8)-epicatechin (5) and two new tetrameric proanthocyanidins, epicatechin-(2β→*O*→7, 4β→8)-[epicatechin-(4β→6)]-epicatechin-(4β→8)-epicatechin, named as parameritannin A-1 (6), and epicatechin-(2β→*O*→5, 4β→6)-[epicatechin-(2β→*O*→7, 4β→8)]-epicatechin-(4β→8)-epicatechin, named as parameritannin A-2 (7), have been isolated from the bark of *Parameria laevigata* Moldenke (Apocynaceae) along with the two known dimers, proanthocyanidin A-2 (1) and proanthocyanidin A-6 (2), and two trimers, cinnamtannin B-1 (3) and aesculitannin B (4). These structures were elucidated by spectral and chemical evidence.

Key words *Parameria laevigata*; proanthocyanidin; parameritannin; doubly linked structure; thiolytic degradation; Apocynaceae

As a continuation of our studies on Jamu and the medicinal resources in Indonesia, we have investigated the chemical constituents of the bark of *Parameria laevigata* Moldenke. *P. laevigata* (Apocynaceae) is widely distributed from southern China to Malaysia and Indonesia. It is one of the drug materials of traditional folk-medicines, called "Jamu" in Indonesia; it is called "Kayu rapet" and has been traditionally used as an antiulcer and antidiarrhea medicine and to treat wounds. In the present paper, we describe the isolation and structural elucidation of seven proanthocyanidins including three new compounds from the bark of *P. laevigata.* The AcOEt and *n*-BuOH-soluble portion, obtained from the MeOH extract of the bark of *P. laevigata*, was subjected to repeated column chromatography on SiO_2, Sephadex LH-20, and Rp-18 to afford one new trimeric proanthocyanidin (**5**) and two new tetrameric proanthocyanidins (**6**, **7**), together with two known dimeric proanthocyanidins (**1**, **2**) and two known trimeric proanthocyanidins (**3**, **4**).

Compound **1** and **2** were obtained as pale yellow amorphous powder. The molecular formula of **1** and **2** were determined by the high-resolution (HR) negative-FAB mass spectrum to be $C_{30}H_{24}O_{12}$. The ^{1}H-NMR spectra of **1** and **2** closely resembled each other (Table1); the presence of the AB coupling systems due to the C-ring protons of A-type proanthocyanidins [at δ 4.06 and 4.41 (each d, J=3.4 Hz) in **1** and at δ 4.08 and 4.29 (each d, J=3.6 Hz) in **2**], *meta*-coupling doublets [at δ 6.01 and 6.07 (each d, J=2.4 Hz) in **1** and δ 6.09 and 6.04 (each d, J=2.2 Hz) in **2**] and the aromatic singlets [at δ 6.10 in **1** and δ 6.11 in **2**] were observed. Furthermore, two ABX coupling systems [(δ 6.81—7.15) in **1** and (δ 6.75—7.17) in **2**] of B- and E-ring were observed. These findings indicated that they were A-type proanthocyanidins composed of two 5,7,3′,4′-tetrahydroxy flavan-3-ol units. In the ^{13}C-NMR spectrum, the doubly linked structures of **1** and **2** were also evident from the characteristic ketal signals at δ 100.19 in **1** and δ 100.55 in **2**. From the above data, compound **1** and **2** were identified as proanthocyanidin A-2[2,3)] and proanthocyanidin A-6,[4)] respectively by direct comparison of their spectral data with literature.

Compound **3** showed a molecular formula of $C_{45}H_{36}O_{18}$ from the HR-negative-FAB mass spectrum, which was assumed to be a trimeric proanthocyanidin. The ^{1}H-NMR spectrum exhibited the presence of an A-type unit from the AB coupling system at δ 3.29 and 4.15 (each d, J=3.5 Hz). This doubly linked structure was also supported from the one ketal carbon signal at δ 99.95 in the ^{13}C-resonace. Then, treatment of **3** with benzylmercaptane/acetic acid in EtOH yielded proanthocyanidin A-2 4-benzylthioether (**3a**) and epicatechin (**3b**).[5)] The location of interflavonoid linkage was deduced to be Cβ-4/C-8 based on the comparison of the ^{1}H-resonances between **3** and degradation products (**3a**, **b**).[6)] This absolute configuration was also evident from the positive Cotton effect in the diagnostic wavelength region of the CD spectrum.[7)] Consequently, the structure of **3** was determined as epicatechin-(2β→*O*→7, 4β→8)-epicatechin-(4β→8)-epicatechin (cinnamtannin B-1), previously isolated from the bark of *Cinnamomum zeylanicum.*[8)]

Compound **4** gave the same molecular formula as that of **3**. Furthermore, the ^{1}H- and ^{13}C-NMR spectra were similar to those of **3**, suggesting it was also a trimeric proanthocyanidin possessing one doubly linked structure. However, the significant differences were observed in the chemical shifts of the heterocyclic protons and carbons (F-ring). In the ^{13}C-NMR spectrum, the presence of signals at δ 84.48 and 73.87 due to C-2 and C-3 of the F-ring, respectively, indicated a catechin middle unit. This was supported from the proton coupling constant ($J_{2,3}$=9.3 Hz). Besides, the 3,4 *trans*-relative configuration was also evident from the proton coupling constant ($J_{3,4}$=9.3 Hz). Upon consideration of the above results, **4** was identified as epicatechin-(2β→*O*→7, 4β→8)-*ent*-catechin-(4β→8)-epicatechin (aesculitannin B). This absolute structure was supported by the positive Cotton effect in the diagnostic wavelength region of the CD spectrum.[7)] This compound was first isolated from seed shells of *Aesculus hippocastanum* L.[4)]

Compound **5** showed a molecular formula of $C_{45}H_{34}O_{18}$ from the HR-negative-FAB-mass spectrum ($[M-H]^-$ ion at m/z 861.1649), which was two mass units lower than that of **4**. In the NMR spectra, two pairs of AB system [one at δ 4.68 and 3.99 (each d, J=3.1 Hz), and the other at δ 4.53 and 4.10 (each d, J=3.1 Hz) in ^{1}H resonance] and two ketal signals [at δ 99.99 and 100.72 in ^{13}C resonance] were observed. These findings suggested that **5** was a trimeric proanthocyanidin possessing two doubly linked structures. Further-

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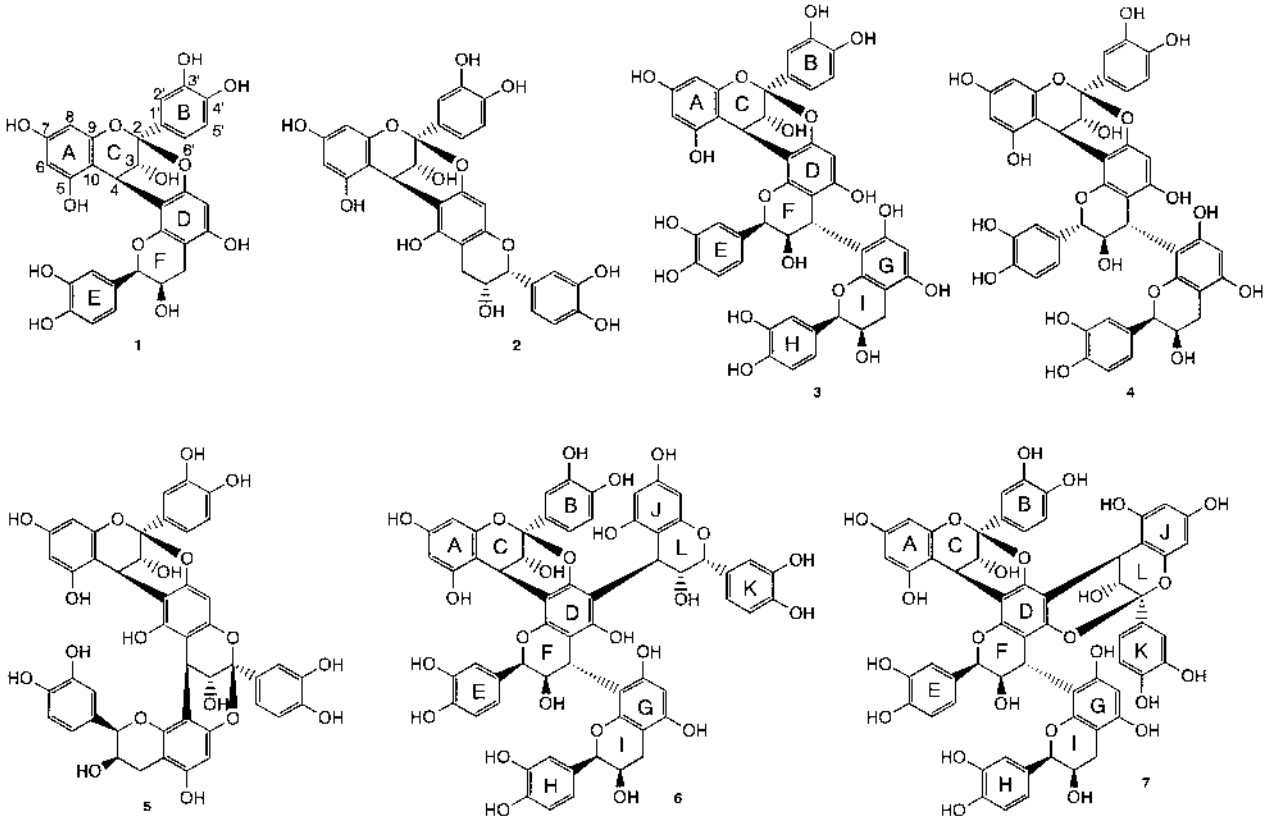

Fig. 1. Chemical Structures of Compounds **1**—**7**

more, the ^{1}H-NMR spectrum indicated the presence of three ABX coupling systems (δ 6.78—7.42), a pair of *meta*-coupled protons [δ 6.02 and 6.07 (each, d, J=2.4 Hz)], and two aromatic singlets (δ 6.06 and 6.16). By a combination of ^{1}H–^{1}H and ^{1}H–^{13}C shift correlation spectroscopy (COSY), and the hetero nuclear multiple-bond correlation spectroscopy (HMBC) (Fig. 2), a planar structure was established and the signals of proton and carbon can be definitively assigned (Table 2). In the ^{1}H- and ^{13}C-NMR spectral data, the signal patterns of the lower unit and the heterocyclic F-ring of **5** were quite close to those of the lower unit and the heterocyclic C-ring of **1**, suggesting that the middle and lower units were the same structurally as **1**. Moreover, the upper unit was determined to be epicatechin from the C-3 carbon signal at δ 68.66 (C-ring). The interflavonoid linkage between the upper and middle unit was confirmed to be C-4/C-6 on the basis of the carbon chemical shift for C-6 of the D-ring (δ 108.93). As to the C-6 and C-8 chemical shifts of the extending unit of A-type proanthocyanidin, the [2β→O→7, 4β→8]-interflavonoid linkage (*ca.* δ 107.0 for C-8) are distinguished from the [2β→O→7, 4β→6]-interflavonoid linkage (*ca.* δ 108.8 for C-6).[3] Moreover, the positive sign of the Cotton effect in the diagnostic wavelength region (220—240 nm) of the CD spectrum indicated β orientation of C-4 flavan-3-ol substituents.[7] Thus, the structure of **5** was designed as epicatechin-(2β→O→7, 4β→6)-epicatechin-(2β→O→7, 4β→8)-epicatechin. As for the trimeric proanthocyanidin possessing two doubly bonded interflavonoid linkages like compound **5**, aesculitannin C and D,[4] and pavetannin B7 and B8[9] have so far been found.

Compound **6** showed a molecular formula of $C_{60}H_{48}O_{24}$ from the HR-negative-FAB-mass spectrum ([M−H]$^{-}$ ion at *m/z* 1151.2450), which was consistent with tetrameric proanthocyanidin possessing one doubly linked interflavonoid linkage. The ^{13}C-NMR spectrum indicated the presence of four flavan-3-ol units from the signals at δ 66.84, 67.40, 71.41 and 72.44, attributable to C-3 of each flavan-3-ol unit, and one ketal carbon signal at δ 100.17 and three carbon signals at δ 76.61, 78.73 and 80.18 due to the C-2 of each flavan-3-ol unit. To determine the structure and the sequence of each flavan-3-ol unit, acid catalyzed thiolytic degradation was attempted.[5] Treatment of **6** with benzylmercaptane/acetic acid in EtOH yielded the trimeric proanthocyanidin (**6a**), epicatechin (**6c**) and two thioethers (**6b** and **6d**) (Chart 1). The trimeric proanthocyanidin (**6a**) was identified as **3**, and **6b** and **6c** were identified as **3a** and **3b**, which were degradation products of **3**, respectively, and further, **6d** was identified as epicatechin 4-benzylthioether from the spectroscopic data. This fact indicated that **6** was a coupling product of **3** and epicatechin. From these results, the two alternative structures were considered for **6**; for one structure it was considered that epicatechin was attached to C-6 (D-ring) of **3**, and for the other structure it was considered that epicatechin was attached to C-6 (G-ring) of **3**. To clarify the position of an interflavonoid linkage between **3** and epicatechin, the correlation spectroscopy *via* long-range coupling (COLOC) experiment of a nonadecaacetyl derivative (**6e**) derived from **6** was attempted and completely assigned all carbons and protons of **6e**. In the COLOC experiment (Fig. 3) of **6e**, the position of the interflavonoid linkage between **3** and epicatechin was confirmed as C-4/C-6 from the correlations between the proton signal at δ 4.64 (H-4, L-ring) of the remaining epicate-

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Dimeric proanthocyanidins **1** and **2** in CD_3OD

Ring	No.	**1**		**2**	
		^{13}C	^{1}H	^{13}C	^{1}H
Upper unit					
C	C-2	100.19		100.55	
	C-3	68.07	4.06 (d, 3.4)	67.61	4.08 (d, 3.6)
	C-4	29.26	4.41 (d, 3.4)	29.70	4.29 (d, 3.6)
A	C-5	157.00		155.32	
	C-6	98.33	6.01 (d, 2.4)	96.96	6.09 (d, 2.2)
	C-7	158.12		158.12	
	C-8	96.65	6.07 (d, 2.4)	96.62	6.04 (d, 2.2)
	C-9	154.24		154.41	
	C-10	104.28		104.25	
B	C-1′	132.46		132.22	
	C-2′	115.68	7.14 (d, 2.2)	115.80	7.17 (d, 2.1)
	C-3′	145.65		145.74	
	C-4′	146.76		146.79	
	C-5′	116.06	6.81 (d, 8.3)	115.67	6.83 (d, 8.3)
	C-6′	119.79	7.02 (dd, 8.3, 2.2)	119.96	7.05 (dd, 8.3, 2.1)
Lower unit					
F	C-2	81.77	4.92 (br s)	79.84	4.78 (br s)
	C-3	66.97	4.24 (m)	67.40	4.14 (m)
	C-4	29.89	2.95 (dd, 17.2, 4.9)	29.54	2.90 (dd, 17.0, 4.5)
			2.76 (dd, 17.2, 2.3)		2.79 (dd, 17.0, 3.0)
D	C-5	156.59		155.71	
	C-6	96.53	6.10 (s)	108.83	
	C-7	152.29		152.78	
	C-8	107.23		96.96	6.11 (s)
	C-9	152.12		151.73	
	C-10	102.45		103.07	
E	C-1′	131.19		132.07	
	C-2′	115.96	7.15 (d, 2.1)	115.25	6.95 (d, 1.7)
	C-3′	145.99		145.64	
	C-4′	146.30		145.89	
	C-5′	115.64	6.81 (d, 8.2)	115.91	6.75 (d, 7.8)
	C-6′	120.40	6.98 (d, 8.2, 2.1)	119.39	6.77 (dd, 7.8, 1.7)

All assignments are based on the HH-COSY, CH-COSY and COLOC spectral data. Coupling patterns and coupling constants (J) in Hz are given in parentheses.

chin and carbon signals at 148.95 (C-5) and 147.71 (C-7) in the D-ring. The CD spectrum of **6** revealed a high-amplitude positive Cotton effect in the diagnostic wavelength region (220—240 nm), reflecting β-orientation of 4-flavanyl substituents.[7] Accordingly, the structure of **6** was characterized as epicatechin-(2β→O→7, 4β→8)-[epicatechin-(4β→6)]-epicatechin-(4β→6)-epicatechin (named as parameritannin A-1).

Compound **7** showed a molecular formula of $C_{60}H_{46}O_{24}$ from the HR-negative-FAB mass spectrum ($[M-H]^-$ ion at m/z 1149.2290), which was 2 mass units lower than that of **6**. Furthermore, the presence of the ^{1}H resonances of two AB coupling systems [one at δ 3.45 and 4.25 (each, d, J= 3.5 Hz), and the other at δ 3.83 and 4.05 (each, d, J=3.6 Hz)] and ^{13}C resonances of two ketal carbons at δ 100.80 and 100.23 were indicated, suggesting it to be a tetrameric proanthocyanidin possessing two doubly linked structures. In the ^{13}C-NMR spectrum, the presence of four flavan-3-ol units were indicated by the signals at δ 66.43, 72.41, 67.57, and 67.96, each attributable to the C-3 in the heterocyclic ring (C-, F-, I-, and L-ring), respectively. By the combination of the HH- and CH-COSY and COLOC spectral analysis, all carbon and proton signals could be definitively assigned (Table 3). In the COLOC spectrum (Fig. 4), one AB doublet proton signal at δ 4.25 (C-4, C-ring) was correlated with quaternary oxygenated carbon signals at δ 145.54 and 150.27 (C-7 and C-9, D-ring), and the other at δ 4.01 (C-4, L-ring) was correlated with carbon signals δ 149.71 and 145.54 (C-5 and C-7, D-ring). These results indicated that two flavan-3-ol units possessing two doubly linked structures were linked to the D-ring. The remaining GHI unit was an epicatechin from the broad singlet proton signal at δ 4.40. Further correlations were observed by COLOC analysis between the methine proton signal at δ 4.37 (H-4, F-ring) and quaternary aromatic carbon signals at δ 155.85, 109.00 and 155.59 (C-7, 8 and 9, G-ring). Furthermore, in the ^{13}C-NMR spectrum, carbon signals of the upper and lower units and the heterocyclic F-ring of **7** were similar to those of **3**, suggesting that the sequence of the ABC–DEF–GHI unit of **7** was the same structure as that of **3**. Moreover, **7** showed a positive Cotton effect in the diagnostic wavelength region (220—240 nm), indicating β-orientation of each interflavonoid linkage.[7] This result was also supported from the presence of an upfield shifted H-6′ (K-ring) proton signal at δ 5.94 by the magnetic anisotropic effect of the aromatic G-ring.[6] Upon consideration of the above results, compound **7** was determined as epicatechin-(2β→O→5, 4β→6)-[epicatechin-(2β→O→7, 4β→8)]-epicatechin-(4β→8)-epicatechin (named as parameritannin A-2).

The proanthocyanidins possessing a branched chain like **6** and **7** were reported at first from natural sources.

Experimental

General Procedures and Plant Material Optical rotations were measured using a Jasco DIP-1000 digital polarimeter. CD spectra were recorded on a Jasco J-725 spectrometer. HR-FAB-MS were performed with a JEOL JMS-BU 20 spectrometer. IR and UV spectra were measured on a Shimadzu FT-IR 8300 infrared spectrometer and a Hitachi U-3000 spectrometer, respectively. The NMR spectra were recorded in CD_3OD or $CDCl_3$, on a Bruker DPX-400 instrument. TLC was performed on Merck precoated TLC plates (Kieselgel 60F_{254}, Rp-18F_{254}). Column chromatography was conducted with Kieselgel 60 (70—230 Mesh, Merck) and Sephadex LH-20 (Pharmacia). Medium pressure liquid chromatography (MPLC, micro pump KP-7, Kusano Scientific Co., Tokyo) was carried out on a CIG column [ODS (C-18)]. The bark of *P. laevigata* was purchased from JAMU factory in Jakarta and the plant was identified by Dr. Asmanizar, University of Indonesia. The herbarium specimen has been deposited at the Botanical Museum of Kobe Gakuin University.

Extraction and Isolation The dried bark of *P. laevigata* (4.7 kg) was extracted with hot MeOH (7h, 12 l×7). The solvent was evaporated off under reduced pressure to yield MeOH extract (522 g). The MeOH extract was suspended in a MeOH–H_2O (1 : 3 l) mixture and was extracted successively with $CHCl_3$, AcOEt, and *n*-BuOH (each 4 l×3 times). Each solvent was evaporated off under reduced pressure to yield $CHCl_3$ (89 g), AcOEt (65 g), *n*-BuOH (239 g), and H_2O (123 g) extract. The AcOEt extract was chromatographed on silica gel using a gradient of AcOEt, MeOH and H_2O system to give catechin-containing fractions. These fractions were subjected repeatedly to Sephadex LH-20 column chromatography using MeOH–H_2O (2 : 1) and to SiO_2 column chromatography using $CHCl_3$–MeOH (4 : 1) and finally were purified by CC on Rp-18 using MeOH–H_2O (1 : 3) to give compounds **1** (150 mg), **2** (35 mg), **3** (3000 mg), **4** (35 mg), and **5** (60 mg). The *n*-BuOH extract was chromatographed on silica gel using a gradient of AcOEt, MeOH and H_2O system to give catechin-containing fractions. These fractions were subjected repeatedly to Sephadex LH-20 column chromatography using MeOH–H_2O (2 : 1) and to SiO_2 column chromatography using $CHCl_3$–MeOH (4 : 1) and finally were purified by CC on Rp-18 using MeCN–H_2O (1 : 7) to give compounds **6** (800 mg) and **7** (30 mg).

Proanthocyanidin A-2 (1) Pale yellow amorphous powder; $[\alpha]_D^{21}$ +58.9° (MeOH, c=0.75); HR-negative-FAB-MS: m/z $[M-H]^-$ 575.1227 (Calcd for $C_{30}H_{23}O_{12}$: 575.1189); CD: $[\theta]_{241}$ +27300, $[\theta]_{222}$ +62600, $[\theta]_{206}$ −168400, $[\theta]_{196}$ −10500, $[\theta]_{188}$ −32400sh; IR ν_{max}^{KBr} cm^{-1}: 3219, 1616, 1522, 1448, 1286, 1069; UV λ_{max}^{MeOH} nm (log ε): 207 (4.90), 225sh (4.58),

237sh (4.32), 280 (4.09); ^{1}H- and ^{13}C-NMR (400 MHz, CD_3OD): see Table 1.

Proanthocyanidin A-6 (2) Pale yellow amorphous powder; $[\alpha]_D^{21}$ +17.3° (MeOH, c=1.68); HR-negative-FAB-MS: m/z [M−H]$^-$ 575.1189 (Calcd for $C_{30}H_{23}O_{12}$: 575.1189); CD: $[\theta]_{247}$ +49700, $[\theta]_{237}$ +27200sh, $[\theta]_{228}$ +5900, $[\theta]_{220}$ +27200, $[\theta]_{202}$ −81000, $[\theta]_{188}$ +19400; IR ν_{max}^{KBr} cm^{-1}: 3275, 1609, 1522, 1437, 1287, 1054; UV λ_{max}^{MeOH} nm (log ε): 223 (4.60), 280sh (4.05); ^{1}H- and ^{13}C-NMR (400 MHz, CD_3OD): see Table 1.

Cinnamtannin B-1 (3) Pale yellow amorphous powder; $[\alpha]_D^{21}$ +69.2° (MeOH, c=0.99); HR-negative-FAB-MS: m/z [M−H]$^-$ 863.1824 (Calcd for $C_{45}H_{35}O_{18}$: 863.1822); CD: $[\theta]_{242}$ +105600sh, $[\theta]_{230}$ +152800, $[\theta]_{221}$ +97000sh, $[\theta]_{208}$ −270000, $[\theta]_{199}$ +79800; IR ν_{max}^{KBr} cm^{-1}: 3275, 1609, 1522, 1437, 1287, 1054; UV λ_{max}^{MeOH} nm (log ε): 223 (4.60), 280sh (4.05); ^{1}H- and ^{13}C-NMR (400 MHz, CD_3OD): see Table 2.

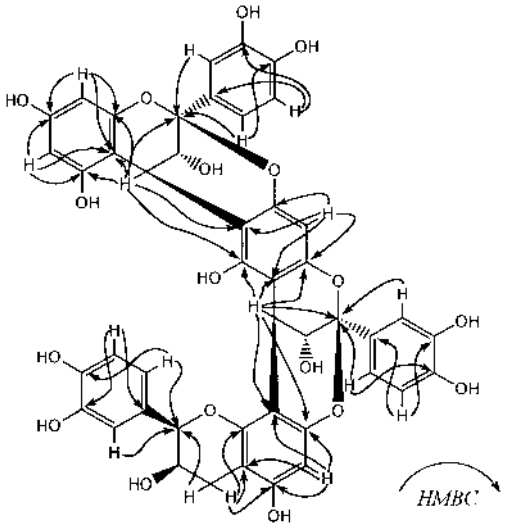

Fig. 2. HMBC Correlations of Compound **5**

Thiolytic Degradation of 3 Compound **3** (200 mg) was treated with benzylmercaptane (1.3 ml) and acetic acid (2 ml) in ethanol (8 ml) under reflux for 40 h. The reaction mixture was chromatographed on Sephadex LH-20 using MeOH to give proanthocyanidin A-2 4-benzylthioether (**3a**) (45 mg) and epicatechin (**3b**) (6 mg).

Proanthocyanidin A-2 4-benzylthioether (3a) Colorless amorphous powder; $[\alpha]_D^{21}$ +96.6° (MeOH, c=1.68); ^{1}H-NMR (400 MHz, CD_3OD) δ: 4.06 [1H, d, J=3.3 Hz, H-3 (C)], 4.38 [1H, d, J=3.3 Hz, H-4 (C)], 6.01 [1H, d, J=1.7 Hz, H-6 (A)], 6.08 [1H, d, J=1.7 Hz, H-8 (A)], 7.17 [1H, d, J=2.0 Hz, H-2′ (B)], 6.83 [1H, d, J=8.2 Hz, H-5′ (B)], 6.95 [1H, dd, J=8.2, 2.0 Hz, H-6′ (B)], 5.29 [1H, br s, H-2 (F)], 3.92 [1H, br d, J=2.0 Hz, H-3 (F)], 4.05 [1H, br d, J=2.0 Hz, H-4 (F)], 6.11 [1H, s, H-6 (D)], 7.13 [1H, d, J=2.1 Hz, H-2′ (E)], 6.81 [1H, d, J=8.3 Hz, H-5′ (E)], 7.02 [1H, dd, J=8.3, 2.1 Hz, H-6′ (E)], 3.97 [2H, s, S-CH_2], 7.40 [2H, br d, J=7.3 Hz, H-2″, 6″ (Benzyl SH)], 7.29 [2H, br t, J=7.3 Hz, H-3″, 5″ (Benzyl SH)], 7.20 [1H, br t, J=7.3 Hz, H-4″ (Benzyl SH)]; ^{13}C-NMR (400 MHz, CD_3OD) δ: 100.10 [C-2 (C)], 68.03 [C-3 (C)], 29.25 [C-4 (C)], 157.10 [C-5 (A)], 98.28 [C-6 (A)], 158.23 [C-7 (A)], 96.60 [C-8 (A)], 154.12 [C-9 (A)], 103.91 [C-10 (A)], 132.32 [C-1′ (B)], 116.14 [C-2′ (B)], 145.94 [C-3′ (B)], 146.73 [C-4′ (B)], 116.07 [C-5′ (B)], 120.52 [C-6′ (B)], 77.43 [C-2 (F)], 71.22 [C-3 (F)], 44.31 [C-4 (F)], 157.45 [C-5 (D)], 96.97 [C-6 (D)], 153.72 [C-7 (D)], 107.11 [C-8 (D)], 152.01 [C-9 (D)], 102.48 [C-10 (D)], 130.95 [C-1′ (E)], 115.55 [C-2′ (E)], 145.61 [C-3′ (E)], 146.35 [C-4′ (E)], 115.63 [C-5′ (E)], 119.74 [C-6′ (E)], 38.04 (CH_2S), [140.14, 130.03, 129.47, and 127.97 (C-1, C-2, 6, C-3, 5, and C-4 of benzyl SH ring)].

Epicatechin (3b) Pale yellow amorphous powder; $[\alpha]_D^{21}$ −53.8° (MeOH, c=1.03); ^{1}H-NMR (400 MHz, CD_3OD) δ: 4.82 (1H, br s, H-2), 4.19 (1H, m, H-3), 2.87 (1H, dd, J=16.8, 4.6 Hz, H-4), 2.73 (1H, dd, J=16.8, 2.9 Hz, H-4), 5.94 (1H, d, J=2.3 Hz, H-6), 5.97 (1H, d, J=2.3 Hz, H-8), 6.98 (1H, d, J=1.9 Hz, H-2′), 6.76 (1H, d, J=8.4 Hz, H-5′), 6.81 (1H, dd, J=8.4, 1.9 Hz, H-6′); ^{13}C-NMR (400 MHz, CD_3OD) δ: 79.88 (C-2), 67.49 (C-3), 29.26 (C-4), 158.00 (C-5), 96.38 (C-6), 157.67 (C-7), 95.88 (C-8), 157.37 (C-9), 100.06 (C-10), 132.28 (C-1′), 115.32 (C-2′), 145.78 (C-3′), 145.95 (C-4′), 115.88 (C-5′), 119.39 (C-6′).

Aesculitannin B (4) Pale yellow amorphous powder; $[\alpha]_D^{21}$ +125.3° (MeOH, c=1.92); HR-negative-FAB-MS: m/z [M−H]$^-$ 863.1800 (Calcd for

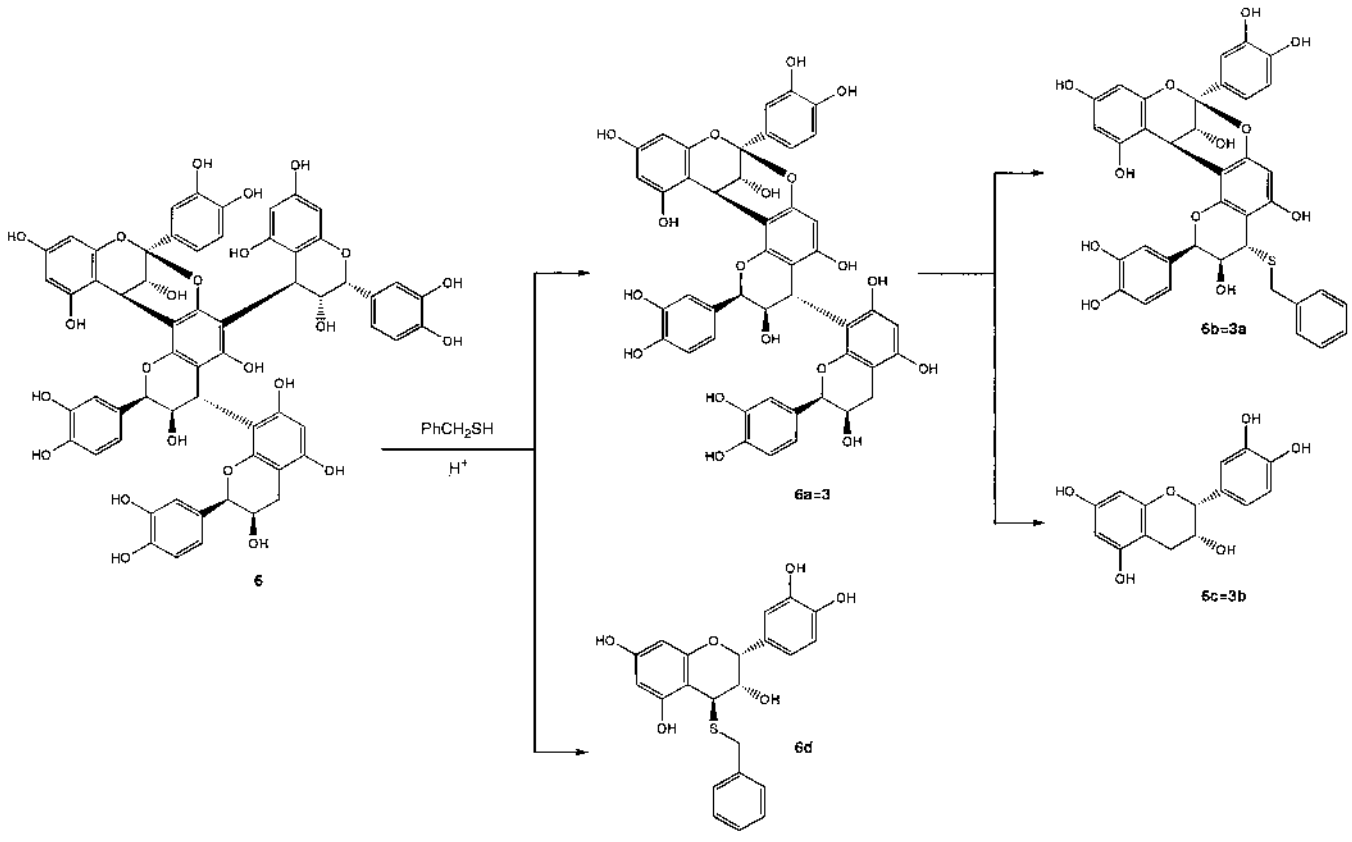

Chart 1. Thiolytic Degradation of Compound **6**

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data for Trimeric Proanthocyanidins **3**, **4** and **5** in CD_3OD

Ring	No.	**3**		**4**		**5**	
		^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
Upper unit							
C	C-2	99.95		100.11		99.99	
	C-3	67.17	3.29 (d, 3.5)	67.05	3.31 (d, 3.5)	68.66	3.99 (d, 3.1)
	C-4	28.86	4.15 (d, 3.5)	28.91	3.95 (d, 3.5)	29.78	4.68 (d, 3.1)
A	C-5	156.74		156.49		158.14	
	C-6	98.33	5.97 (d, 2.4)	97.86	5.85 (d, 2.3)	97.86	6.02 (d, 2.4)
	C-7	157.80		157.95		157.80	
	C-8	96.60	6.02 (d, 2.4)	96.48	6.00 (d, 2.3)	96.43	6.07 (d, 2.4)
	C-9	154.14		153.93		154.36	
	C-10	104.96		104.06		103.70	
B	C-1′	132.44		132.24		132.70	
	C-2′	115.75	7.03 (d, 2.0)	115.68	7.02 (d, 2.1)	115.42	7.09 (d, 2.2)
	C-3′	145.46		145.21		146.04	
	C-4′	146.59		145.50		146.72	
	C-5′	116.18	6.84 (d, 8.1)	115.98	6.83 (d, 8.3)	115.65	6.78 (d, 8.3)
	C-6′	119.90	6.86 (dd, 8.1, 2.0)	119.82	6.86 (dd, 8.3, 2.1)	119.63	6.98 (dd, 8.3, 2.2)
Middle unit							
F	C-2	78.86	5.70 (br s)	84.48	4.62 (d, 9.3)	100.72	
	C-3	72.55	4.13 (br d, 1.4)	73.87	4.57 (t, 9.3)	68.09	4.10 (d, 3.1)
	C-4	38.27	4.56 (br s)	39.04	4.51 (d, 9.3)	29.83	4.53 (d, 3.1)
D	C-5	155.76		155.37		149.07	
	C-6	96.09	5.80 (s)	97.21	5.80 (s)	108.93	
	C-7	151.08		151.20		155.98	
	C-8	106.42		106.86		98.47	6.16 (s)
	C-9	151.78		152.21		153.36	
	C-10	106.73		108.93		105.95	
E	C-1′	131.76		131.06		132.52	
	C-2′	116.72	7.32 (d, 2.0)	116.52	7.20 (d, 2.0)	115.52	7.42 (d, 2.1)
	C-3′	145.89		146.10		145.63	
	C-4′	146.27		146.68		146.89	
	C-5′	115.79	6.82 (d, 8.2)	116.34	6.90 (d, 8.2)	115.74	6.88 (d, 8.9)
	C-6′	121.36	7.19 (dd, 8.2, 2.0)	121.14	7.15 (dd, 8.2, 2.0)	120.24	7.41 (dd, 8.9, 2.1)
Lower unit							
I	C-2	80.27	4.38 (br s)	79.61	4.37 (br s)	81.40	4.93 (br s)
	C-3	65.71	3.85 (br t, 3.8)	67.61	4.07 (br d, 3.5)	67.10	4.26 (m)
	C-4	29.84	2.83 (m)	30.07	2.87 (dd, 17.0, 4.8)	30.01	2.96 (dd, 17.2, 4.8)
					2.77 (br d, 17.0)		2.81 (dd, 17.2, 1.8)
G	C-5	155.99		156.19		156.64	
	C-6	96.51	6.10 (s)	96.57	6.08 (s)	96.23	6.06 (s)
	C-7	155.53		156.16		152.68	
	C-8	108.85		108.55		107.75	
	C-9	155.78		155.26		151.76	
	C-10	100.08		100.97		102.46	
H	C-1′	133.17		132.92		131.52	
	C-2′	115.48	6.82 (d, 1.7)	115.27	6.98 (d, 1.8)	115.67	7.17 (d, 2.0)
	C-3′	145.31		145.87		145.65	
	C-4′	145.74		146.65		146.65	
	C-5′	116.03	6.76 (d, 8.2)	115.68	6.80 (d, 8.3)	116.07	6.82 (d, 8.2)
	C-6′	119.45	6.72 (dd, 8.2, 1.7)	119.26	6.88 (dd, 8.3, 1.8)	120.02	7.02 (dd, 8.2, 2.0)

All assignments are based on the HH-COSY, CH-COSY and COLOC spectral data. Coupling patterns and coupling constants (J) in Hz are given in parentheses.

$C_{45}H_{35}O_{18}$: 863.1822); CD: $[\theta]_{241}$ +151900sh, $[\theta]_{232}$ +203400, $[\theta]_{224}$ +149800sh, $[\theta]_{208}$ −193600, $[\theta]_{200}$ +51100, $[\theta]_{189}$ −79800; IR ν_{max}^{KBr} cm^{-1}: 3244, 1609, 1522, 1447, 1285, 1076; UV λ_{max}^{MeOH} nm (log ε): 208 (5.07), 234sh (4.67), 281 (4.09); ^{1}H- and ^{13}C-NMR (400 MHz, CD_3OD): see Table 2.

Epicatechin-(2β→*O*→7, 4β→6)-epicatechin-(2β→*O*→7, 4β→8)-epicatechin (5) Pale yellow amorphous powder; $[\alpha]_D^{21}$ +184.9° (MeOH, c=1.08); HR-negative-FAB-MS: m/z $[M-H]^-$ 861.1649 (Calcd for $C_{45}H_{33}O_{18}$: 863.1666); CD: $[\theta]_{227}$ +474200, $[\theta]_{208}$ −461400, $[\theta]_{196}$ −113700; IR ν_{max}^{KBr} cm^{-1}: 3291, 1622, 1522, 1437, 1286, 1069; UV λ_{max}^{MeOH} nm (log ε): 213 (4.88), 226sh (4.80), 280 (4.09); ^{1}H- and ^{13}C-NMR (400 MHz, CD_3OD): see Table 2.

Parameritannin A-1 (6) Pale yellow amorphous powder; $[\alpha]_D^{21}$ +50.1° (MeOH, c=1.33); HR-negative-FAB-MS: m/z $[M-H]^-$ 1151.2450 (Calcd for $C_{60}H_{47}O_{24}$: 1151.2456); CD: $[\theta]_{243}$ +135600, $[\theta]_{237}$ +152800, $[\theta]_{233}$ +146400, $[\theta]_{211}$ −218500, $[\theta]_{198}$ +114200; IR ν_{max}^{KBr} cm^{-1}: 3204, 1607, 1518, 1449, 1283, 1067; UV λ_{max}^{MeOH} nm (log ε): 218 (5.00), 281 (4.24); ^{1}H-NMR (400 MHz, CD_3OD): δ 4.0—5.7 (H-2 of both extending and terminating units), δ 3.3—4.1 (H-3 of both extending and terminating units), δ 2.7—2.9 (H-4 of terminating unit), δ 4.2—4.5 (H-4 of extending unit), δ 5.8—6.2 (H-6 and -8 of A, G and J-ring), δ 6.6—7.3 (H-2′, 5′ and 6′ of B, E, H and K-ring); ^{13}C-NMR (400 MHz, CD_3OD) δ: 100.17, 78.73, 80.18 and 76.61 (C-2 of C, F, I and L-ring, respectively), 66.84, 72.44, 67.40 and 71.41 (C-3 of C, F, I and L-ring, respectively), 28.90, 38.38, 29.72 and 37.64 (C-3 of C, F, I and L-ring, respectively), 98.34, 107.77, 96.68 and 96.84 (C-6 of A, D, G and J-ring), 96.55, 106.94, 108.84 and 96.17 (C-8 of A, D, G and J-ring), 104.92, 107.24, 100.01 and 99.41 (C-10 of A, D, G and J-ring), 115.47, 115.82, 115.93, 116.00, 116.15, 116.17, 116.24 and 116.76 (C-2′ and 5′ of B, E, H and K-ring), 119.28, 119.95, 120.69 and 121.43 (C-6′ of B, E, H and K-ring), 131.663, 131.75, 132.32 and 132.85 (C-1′ of B, E, H

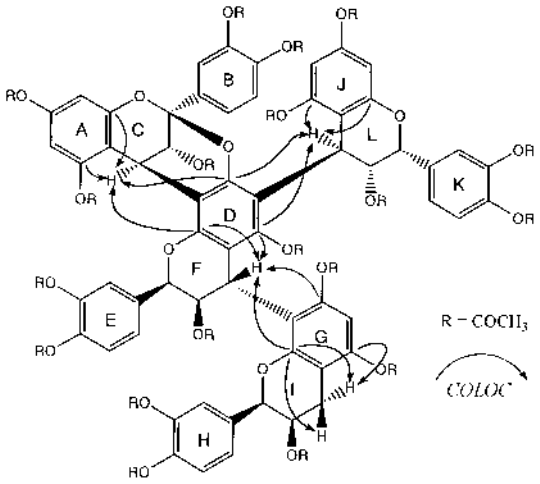

Fig. 3. COLOC Correlations of Compound **6e**

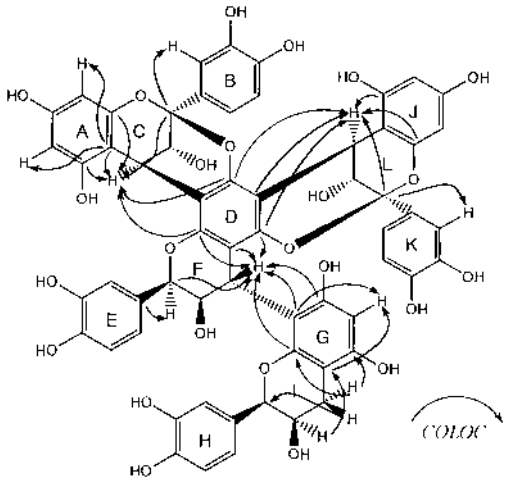

Fig. 4. COLOC Correlations of Compound **7**

Table 3. ^{1}H- and ^{13}C-NMR Spectral Data for Tetrameric Proanthocyanidins **6e** in $CDCl_3$ and **7** in CD_3OD

Ring	No.	**6e** ^{13}C	**6e** ^{1}H	**7** ^{13}C	**7** ^{1}H
Upper unit					
C	C-2	98.82		100.80	
	C-3	67.84	5.12 (d, 4.2)	66.43	3.45 (d, 3.5)
	C-4	27.61	4.74 (d, 4.2)	28.86	4.25 (d, 3.5)
A	C-5	148.87		157.76	
	C-6	110.2	6.58 (d, 2.2)	98.69	5.92 (d, 2.4)
	C-7	150.13		157.90[b]	
	C-8	106.59	6.80 (d, 2.2)	96.78	6.04 (d, 2.4)
	C-9	153.38		153.66	
	C-10	113.46		104.19	
B	C-1′	134.44		131.24	
	C-2′	122.57	7.43 (d, 2.0)	115.62	7.12 (d, 2.2)
	C-3′	141.35[a]		145.68	
	C-4′	141.63[a]		147.01	
	C-5′	125.05	7.14 (d, 8.5)	116.11	6.87 (d, 8.4)
	C-6′	125.29	7.50 (dd, 8.5, 2.0)	119.77	6.94 (dd, 8.4, 2.2)
Middle unit					
F	C-2	75.24	5.45 (br s)	78.78	5.59 (br s)
	C-3	69.66	5.14 (br t, 1.7)	72.14	4.09 (br d, 1.6)
	C-4	33.30	4.37 (br s)	38.81	4.50 (br t, 1.6)
D	C-5	148.95		149.71	
	C-6	114.02		107.04	
	C-7	147.71		145.54	
	C-8	108.73		108.08	
	C-9	151.48		150.27	
	C-10	109.01		107.64	
E	C-1′	134.56		131.52	
	C-2′	124.38	7.29 (d, 2.0)	116.66	7.29 (d, 2.0)
	C-3′	141.65[a]		145.81	
	C-4′	141.72[a]		146.26	
	C-5′	122.77	7.13 (d, 8.4)	116.11	6.80 (d, 8.3)
	C-6′	125.97	7.26 (dd, 8.4, 2.0)	121.29	7.17 (dd, 8.3, 2.0)
Lower unit					
I	C-2	76.79	5.10 (br s)	80.56	4.40 (br s)
	C-3	66.36	5.46 (m)	67.57	3.85 (m)
	C-4	26.26	2.98 (m)	29.97	2.94 (dd, 17.4, 4.5) 2.88 (dd, 17.2, 1.6)
G	C-5	148.63		156.32	
	C-6	110.98	6.51 (s)	97.41	6.09 (s)
	C-7	147.36		155.85	
	C-8	118.24		109.00	
	C-9	151.75		155.59	
	C-10	110.29		100.20[c]	
H	C-1′	135.12		132.89	
	C-2′	121.47	7.10 (d, 1.8)	115.29	6.81 (d, 1.9)
	C-3′	142.02[a]		145.74	
	C-4′	142.47[a]		145.45	
	C-5′	122.70	7.12 (d, 8.4)	116.02	6.78 (d, 8.2)
	C-6′	123.13	7.07 (dd, 8.4, 1.8)	119.10	6.67 (dd, 8.2, 1.9)
Branched unit					
L	C-2	74.09	5.67 (br s)	100.23[c]	
	C-3	69.80	5.54 (br t, 1.7)	67.96	3.80 (d, 3.6)
	C-4	32.61	4.64 (br s)	29.45	4.01 (d, 3.6)
J	C-5	150.18		156.20	
	C-6	107.65	6.72 (d, 2.2)	98.42	5.81 (d, 2.4)
	C-7	149.76		157.87[b]	
	C-8	107.15	6.62 (d, 2.2)	96.57	5.93 (d, 2.4)
	C-9	154.35		154.06	
	C-10	109.19		103.87	
K	C-1′	136.84		131.85	
	C-2′	121.95	7.43 (d, 2.0)	116.54	6.92 (d, 2.2)
	C-3′	142.50[a]		144.73	
	C-4′	143.09[a]		146.19	
	C-5′	123.20	7.19 (d, 8.6)	115.96	6.50 (d, 8.3)
	C-6′	125.21	7.56 (dd, 8.6, 2.0)	119.60	5.94 (dd, 8.3, 2.2)

All assignments are based on the HH-COSY, CH-COSY and COLOC spectral data. Coupling patterns and coupling constants (*J*) in Hz are given in parentheses. Symbol *a*—*c*) in each column may be interchanged.

and K-ring), 145.44, 145.56, 145.80, 145.90, 146.30, 146.32, 146.37 and 146.74 (C-3′ and 4′ of B, E, H and K-ring), 148.44, 150.30, 150.90, 154.24, 155.47, 155.58, 156.08, 156.73, 157.89, 157.93, 159.31 and 159.44 (C-5, 7 and 9 of A, D, G and J-ring).

Thiolytic degradation of 6 Compound **6** (100 mg) was treated with benzylmercaptane (1.3 ml) and acetic acid (2 ml) in ethanol (8 ml) under reflux for 45 h. The reaction mixture was chromatographed on Sephadex LH-20 using MeOH to give cinnamtannin B-1 (**6a**=**3**, 6.4 mg), epicatechin 4-benzylthioether (**6d**, 21.8 mg), proanthocyanidin A2-4-benzylthioether (**6b**=**3a**, 10.7 mg) and epicatechin (**6c**=**3b**, 7.4 mg).

Epicatechin 4-benzylthioether (6d) Colorless amorphous powder; $[\alpha]_D^{21}$ −22.7° (MeOH, *c*=1.09); ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.41 (2H, dd, *J*=7.4, 1.3 Hz, H-2″, 6″), 7.30 (2H, t, *J*=7.4 Hz, H-3″, 5″), 7.21 (1H, tt, *J*=7.4, 1.3 Hz, H-4″), 6.93 (1H, d, *J*=2.0 Hz, H-2′), 6.75 (1H, d, *J*=8.1 Hz,

H-5′), 6.68 (1H, dd, J=8.1, 2.0 Hz, H-6′), 5.96 (1H, d, J=2.3 Hz, H-6), 5.89 (1H, d, J=2.3 Hz, H-8), 5.22 (br s, H-2), 4.05 (1H, d, J=2.4 Hz, H-4), 3.96 (2H, s, S-CH_2), 3.85 (1H, br d, J=2.4 Hz, H-3); ^{13}C-NMR (400 MHz, CD_3OD) δ: 75.58 (C-2), 71.65 (C-3), 43.93 (C-4), 159.10 (C-5), 96.83 (C-6), 158.90 (C-7), 95.76 (C-8), 157.29 (C-9), 100.19 (C-10), 132.08 (C-1′), 115.27 (C-2′), 145.75 (C-3′), 145.97 (C-4′), 115.92 (C-5′), 119.20 (C-6′), 140.44 (C-1″), 130.03 (C-2″, 6″), 129.50 (C-3″, 5″), 127.92 (C-4″), 37.92 (S-CH_2).

Acetylation of 6 Compound **6** (200 mg) was acetylated with Ac_2O–pyridine and the product was purified by column chromatography on Sephadex LH-20 using $CHCl_3$–MeOH (2 : 1) to yield nonadecaacetate **6e** (200 mg). **6e**: Colorless amorphous powder; ^{1}H- and ^{13}C-NMR (400 MHz, $CDCl_3$): see Table 3, methyl protons of –$COCH_3$ δ: 2.30, 2.29, 2.28, 2.27 (×2), 2.26 (×2), 2.25, 2.23 (×2), 2.20, 2.04, 2.01, 1.80, 1.75, 1.74, 1.71, 1.55, 1.48 (each 3H, s), carbonyl carbons of –$COCH_3$ δ: 170.13, 170.03, 169.81, 169.27, 169.02, 168.49, 168.46, 168.34, 168.33, 168.16, 168.07, 168.05, 167.93, 167.89, 167.88, 167.85, 167.74, 167.49, 167.16, methyl carbons of –$COCH_3$ δ: 21.13, 21.04, 20.93, 20.75, 20.69, 20.60, 20.58, 20.56, 20.54 (×2), 20.52, 20.36, 20.32, 20.29, 20.25, 20.16, 20.01, 19.92, 19.40.

Parameritannin A-2 (7) Pale yellow amorphous powder; HR-negative-FAB-MS: m/z $[M-H]^-$ 1149.2290 (Calcd for $C_{60}H_{45}O_{24}$: 1149.2300); CD: $[\theta]_{255}$ −22700, $[\theta]_{241}$ +116700, $[\theta]_{230}$ +64000, $[\theta]_{220}$ +101300, $[\theta]_{207}$ −264900, $[\theta]_{197}$ +61800; IR ν_{max}^{KBr} cm^{-1}: 3369, 1614, 1522, 1447, 1286, 1065; UV λ_{max}^{MeOH} nm (log ε): 212 (4.97), 231sh (4.79), 280 (4.16); ^{1}H- and ^{13}C-NMR (400 MHz, CD_3OD): see Table 3.

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