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Tannins and Related Compounds. LXXII.[1] Isolation and Characterization of Mongolicanin (Procyanidino-ellagitannin), Mongolinin A, Acutissimin C and Vescalagin Carboxylic Acid, Novel Tannins from *Quercus mongolica* var. *grosseserrata*

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A novel procyanidino-ellagitannin, mongolicanin (**1**), has been isolated, together with three new tannins, mongolinin A (**4**), acutissimin C (**7**) and vescalagin carboxilic acid (**8**), from the bark of *Quercus mongolica* var. *grosseserrata* (Fagaceae), and the structures of these compounds were established on the basis of physicochemical data.

Keywords——*Quercus mongolica* var. *grosseserrata*; Fagaceae; mongolicanin; procyanidino-ellagitannin; mongolinin A; acutissimin C; vescalagin carboxylic acid; flavano-ellagitannin; *C*-glycosylated ellagitannin; open-chain-form glucose

In the preceding paper, we described the isolation and structural elucidation of a novel class of flavono-ellagitannins, mongolicins A and B,[1] which possess a hydrolyzable tannin [vescalagin (**2**)] moiety and a flavonoid [(+)-taxifolin 3-*O*-β-D-glucopyranoside] moiety in each molecule, from the bark of *Quercus mongolica* var. *grosseserrata* (Japanese name: mizunara). Mizunara, one of the richest sources of tannins in Fagaceous plants, has also been found to produce a variety of flavano-ellagitannins such as stenophyllanins A and B,[2] acutissimins A and B,[3] and mongolicains A and B,[4] which consist of a flavan-3-ol (catechin) unit connected to a hydrolyzable tannin (vescalagin, stachyurin, *etc.*) moiety. In a continuing chemical examination of polyphenolic constituents in the bark and acorn of this plant, we have now isolated a novel tannin, mongolicanin (**1**), in which a condensed tannin [procyanidin B-3 (**3**)][5] moiety is connected to a hydrolyzable tannin [vescalagin (**2**)][6] moiety through a carbon–carbon linkage. In addition, we have isolated two novel flavano-ellagitannins, mongolinin A (**6**) and acutissimin C (**7**), and a new ellagitannin, vescalagin carboxylic acid (**8**), from this plant material. In this paper, we present details of the isolation and structure elucidation of these compounds (**1**, **6**, **7** and **8**), and propose the name of "procyanidino-ellagitannins" for the class of tannin such as mongolicanin (**1**).

The 80% aqueous acetone extract of the fresh bark was fractionated by chromatography over Sephadex LH-20 (H_2O–MeOH–acetone)[7] into five fractions.[1] Among these fractions, the first fraction was repeatedly chromatographed on various reverse-phase gels to afford compound **6**, while the second fraction yielded compounds **1** and **7**. Similarly, fractionation of the 80% aqueous acetone extract of the fresh acorn yielded five fractions as previously reported,[4] and subsequent purification of fraction III by Sephadex LH-20 and MCI-gel CHP-

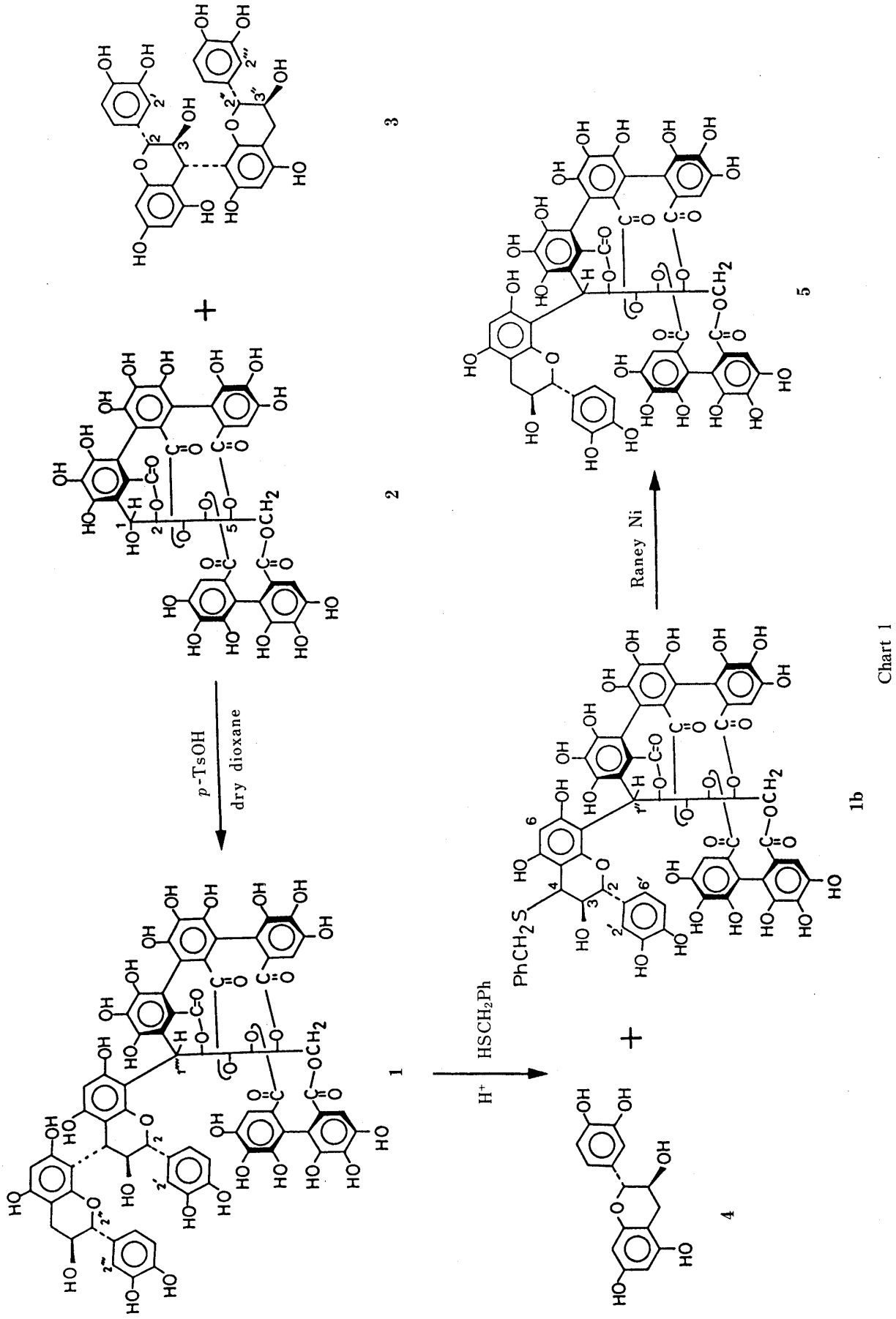


Chart 1

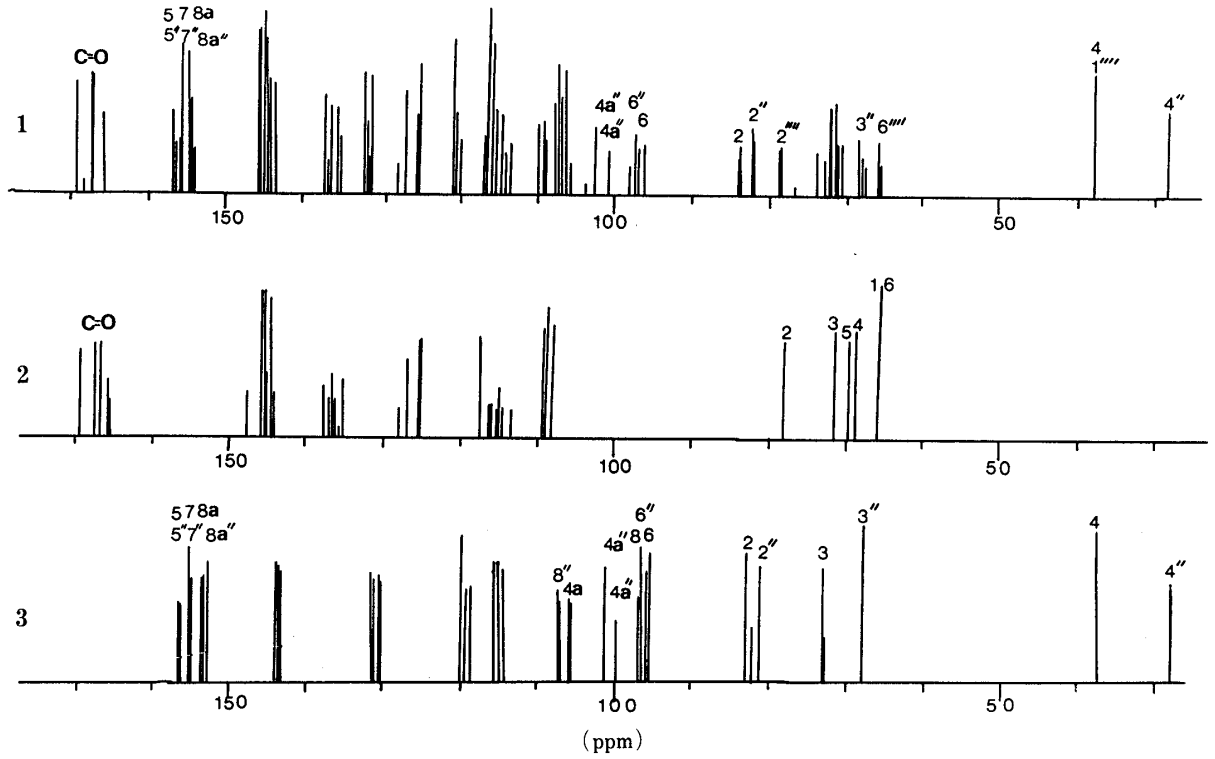


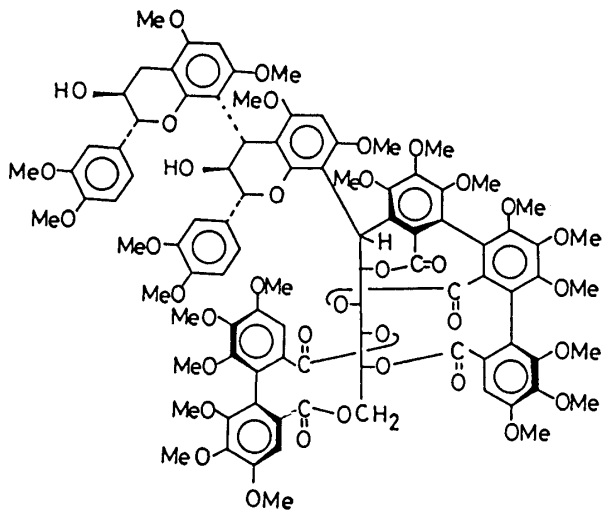
Fig. 1. ^{13}C-NMR Spectra of **1**, **2**, and **3** (25.05 MHz; in Acetone-d_6 + D_2O)

20P chromatographies gave compound **8**.

Compound **1** (mongolicanin) gave an orange color (characteristic of procyanidins) on treatment with the anisaldehyde–sulfuric acid reagent[7] and a brown coloration (characteristic of ellagitannins) in the sodium nitrite–acetic acid test.[8] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1**, measured at room temperature, showed a complicated signal pattern probably caused by conformational isomerism, the complication being commonly observed in 4,8-linked procyanidins with 4α-configuration such as procyanidins B-3 (**3**) and B-4.[5] The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, although similarly complicated, showed a signal pattern which is closely correlated with the combined signal patterns of **2** and **3** (Fig. 1). In particular, the signals in the aromatic region almost coincided with those of **2** plus **3**. Among the aliphatic signals, the chemical shifts (δ 81.9 and 83.3) of the signals, assignable to the flavan C-2, as well as the observation of one C-4 methine signal at δ 37.8, were diagnostic for the presence of two catechin units, that is, a procyanidin B-3 moiety, while the moderate upfield shift (δ 37.8) of the polyalcohol C-1 signal, as compared with that of **2**, indicated that the C-1 position is substituted through a carbon-to-carbon linkage. These ^{13}C-NMR observations suggested that **1** possesses a procyanidin B-3 (**3**) moiety to which a vescalagin (**2**) moiety is connected at the C-1 position. Furthermore, the negative fast atom bombardment mass spectrum (FAB-MS), exhibiting the $(M-H)^-$ peak at m/z 1493, was consistent with the expected structure.

On methylation with dimethyl sulfate and potassium carbonate in dry acetone, **1** afforded the tricosamethyl ether (**1a**) [field desorption mass spectrum (FD-MS) m/z: 1816 $(M)^+$ (base peak)]. In the ^{1}H-NMR (270 MHz) spectrum of **1a**, duplicated signals attributable to the polyalcohol C-1 proton appeared as broad singlets at δ 4.42—4.44 (1H in total), and the small coupling constant, similar to that (J = 2 Hz) of **2**, indicated the configuration of the C-1 atom to be the same as that of **2**.[6a]

On the other hand, thiolytic degradation of **1** with benzylmercaptan in the presence of



1a

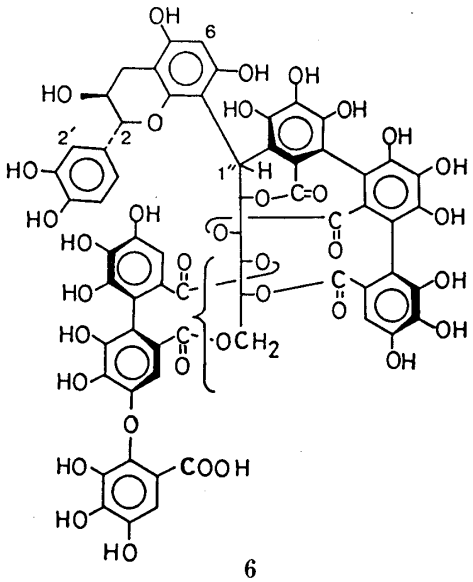


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TABLE I. ^{1}H-NMR Spectral Data for Compounds **2** and **5**—**8** (δ-Values)

	6[a]	**5**[a]	**7**[a]	**8**[a]	**2**[b]
Catechin					
H-2	5.13 (d, J=8)	5.48 (br s)	4.82 (d, J=8)		
H-3	3.96 (m)	4.56 (m)	—[c]		
H-4	2.55 (dd, J=16, 8)	2.40 (br d, J=16)	2.50—2.84 (m)		
	2.82 (dd, J=16, 5)	2.90 (br d, J=16)			
H-6	6.06 (s)	6.32 (s)	6.03 (s)		
H-2′	6.69 (br s)	6.90 (br s)	6.90 (br s)		
H-5′	6.72 (d, J=8)	6.76 (d, J=8)	6.86 (d, J=8)		
H-6′	6.60 (br d, J=8)	6.94 (br d, J=8)	6.78 (br d, J=8)		
Polyalcohol					
H-1	4.52 (br s)	4.84 (s)	4.66 (s)	3.78 (d, J=1)	4.92 (d, J=2)
H-2	5.12 (br s)	5.20 (s)	5.33 (s)	5.98 (br s)	5.30 (t, J=2)
H-3	4.76 (d, J=8)	4.76 (d, J=8)	4.64 (d, J=8)	4.93 (d, J=8)	4.59 (dd, J=8, 2)
H-4	5.13 (m)[d]	5.28 (t, J=8)	—[c]	5.19 (t, J=8)	5.23 (t, J=8)
H-5	5.56 (d, J=8)	5.60 (d, J=8)	5.16 (m)	5.72 (dd, J=3, 8)	5.66 (dd, J=3, 8)
H-6	4.10 (d, J=12)	4.12 (d, J=12)	—[c]	4.13 (d, J=13)	4.08 (d, J=13)
	4.65 (m)[d]	4.60 (d, J=12)		5.10 (dd, J=13, 3)	5.08 (dd, J=13, 3)
Arom.-H					
	6.63 (s)	6.56 (s)	6.80 (s)	6.69 (s)	6.67 (s)
	6.71 (s)	6.76 (s)		6.79 (s)	6.82 (2H, s)
	7.01 (s)	7.08 (s)		6.85 (s)	
	7.30 (br s)				

a) 100 MHz; in acetone-d_6 + D_2O. *b*) 400 MHz; in acetone-d_6. *c*) Overlapped with an HOD signal which lies in the range of δ 4.0—4.5. *d*) Due to the overlap of signals, the exact coupling constant could not be calculated.

acetic acid[5] yielded (+)-catechin (**4**) (formed from the lower unit of the procyanidin moiety) and the benzylthioether (**1b**) (negative FAB-MS m/z 1327 $(M-H)^-$). Subsequent desulfurization of **1b** with Raney nickel gave a product found to be identical with acutissimin A (**5**), indicating clearly that the vescalagin moiety is attached to the C-8 position of the upper unit of the procyanidin B-3 moiety.

Final structural confirmation was done by preparing **1** from **2** and **3**; stirring of a mixture of **2** and **3** in dry dioxane containing *p*-toluenesulfonic acid at 55 °C, followed by repeated

Sephadex LH-20 and MCI-gel CHP-20P chromatographies, afforded **1**, together with acutissimins A (**5**) and B (**9**) as the major products.[9]

From the above-mentioned spectroscopic and chemical evidence, the structure of mongolicanin was established unequivocally as **1**.

Compound **6** (mongolinin A) gave a dark blue coloration (characteristic of hydrolyzable tannins) with the ferric chloride reagent, and a reddish pink coloration with the anisaldehyde–sulfuric acid reagent, the latter suggesting the presence of a flavan skeleton. The ^{1}H-NMR spectrum (Table I) of **6** exhibited three aromatic ABX-like signals at δ 6.60 (br d, J=8 Hz), 6.69 (br s) and 6.72 (d, J=8 Hz), and a one-proton singlet signal at δ 6.06, which were assignable from their chemical shifts to the protons of catechol and phloroglucinol rings, respectively. In addition, the appearance of a doublet (δ 5.13, J=8 Hz), a multiplet (δ 3.96) and benzylic methylene signals (δ 2.55, dd, J=16, 8 Hz and δ 2.82, dd, J=16, 5 Hz) characteristic of the C-ring of 2,3-*trans*-flavan-3-ol, suggested the occurrence of a catechin moiety. In the aliphatic proton region, the chemical shifts and coupling patterns of the low-field signals arising from a polyalcohol moiety were analogous to those found in the flavano-ellagitannin, acutissimin A (**5**). However, the aromatic signals from the ellagitannin moiety appeared as four singlets at δ 6.63, 6.71, 7.01 and 7.30, three of which (δ 6.63, 7.01 and 7.30) were consistent with those of a valoneayl group.[10] On the other hand, the ^{13}C-NMR spectrum of **6** indicated more clearly the presence of a catechin moiety (δ 82.1: C-2; δ 68.3: C-3; δ 27.3: C-4; δ 96.6: C-6; δ 101.0: C-4a) and a polyalcohol moiety with a substitution system similar to that of **5**. Based on these spectral observations coupled with the fact that **6** exhibited a prominent $(M-H)^-$ peak at m/z 1373 in the negative FAB-MS, **6** was assumed to be structurally related to **5** except for having a valoneayl ester group in place of a hexahydroxydiphenoyl ester group.

On methylation with dimethyl sulfate and potassium carbonate in dry acetone, **6** afforded the docosamethyl ether (**6a**) [FD-MS m/z 1682 $(M)^+$ (base peak)], which, on subsequent alkaline methanolysis, gave trimethyl octamethylvaloneate (**6c**) and the methanolysate (**6b**). The structure of **6b** was confirmed by comparison of the physical and spectral data with those

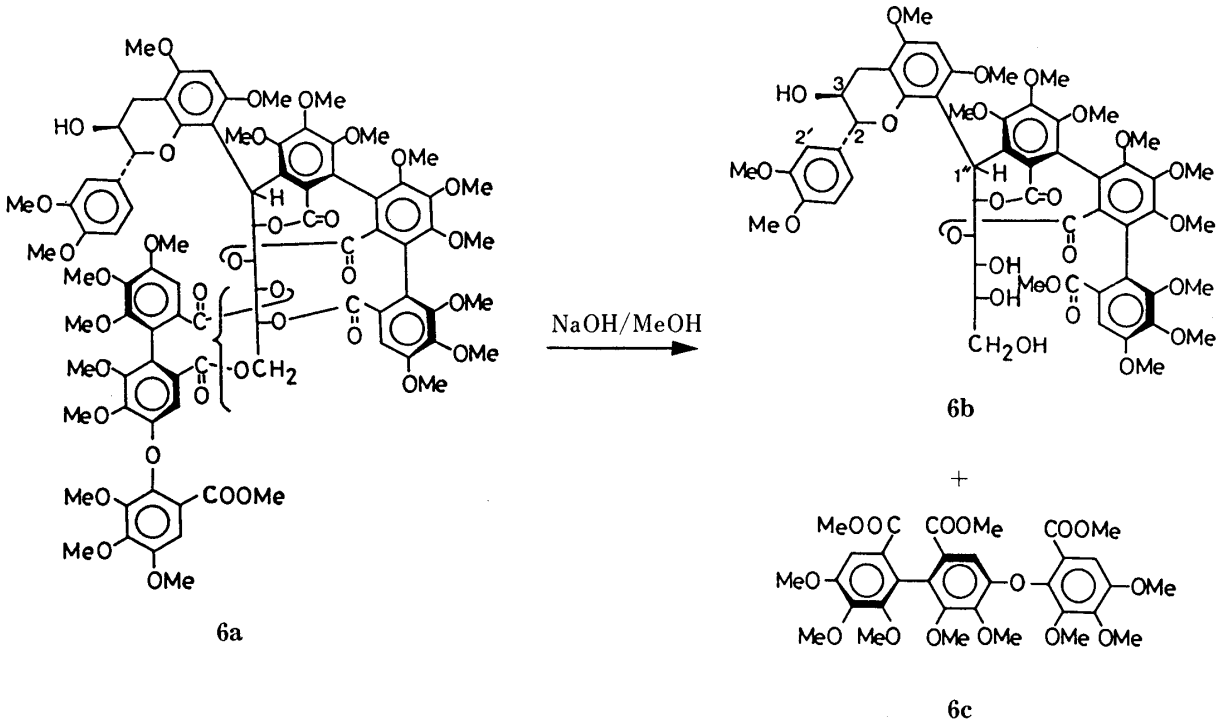
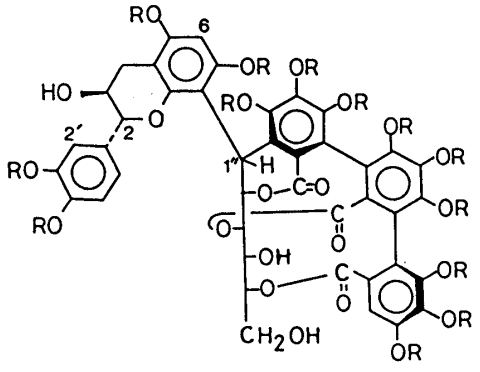


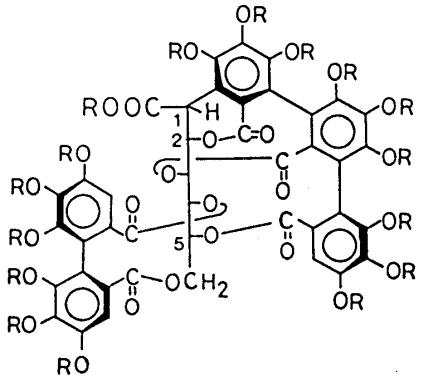
Chart 2

of a sample prepared in a similar way from **5**.[3] The negative sign of the specific optical rotation [−18.7° (acetone)] of **6c** confirmed the atropisomerism to be in the *S*-series.[11]

From this physicochemical evidence, mongolinin A was concluded to be represented by the formula **6**. The orientation of the valoneayl group remains unclarified.



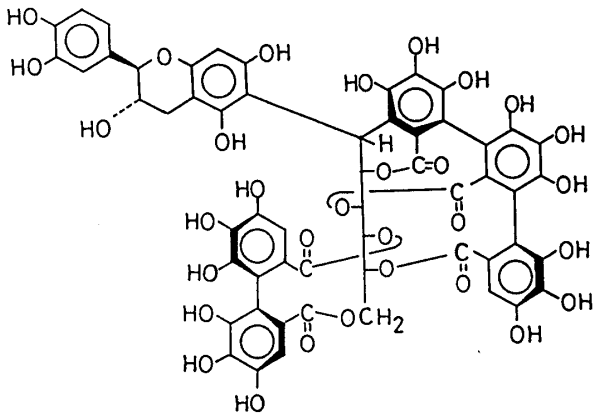
7 : R=H
7a : R=Me



8 : R=H
8a : R=Me

Compound **7** (acutissimin C) gave a coloration (reddish pink) similar to that of **6** with the anisaldehyde–sulfuric acid reagent. The ^{1}H-NMR spectrum (Table I) of **7** was analogous to that of **5** except for the upfield shifts of signals due to polyalcohol C-4 and C-6 protons and for the absence of two aromatic singlets.

The negative FAB-MS of **7** showed an intense $(M-H)^-$ ion peak at *m/z* 903 which corresponded to the loss of one hexahydroxydiphenoyl group from the molecule of **5**.



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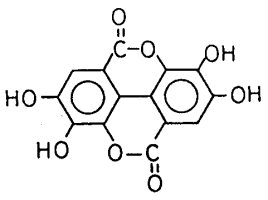

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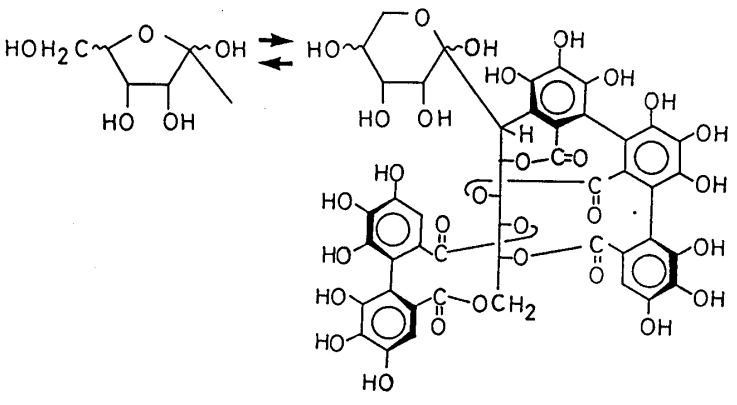

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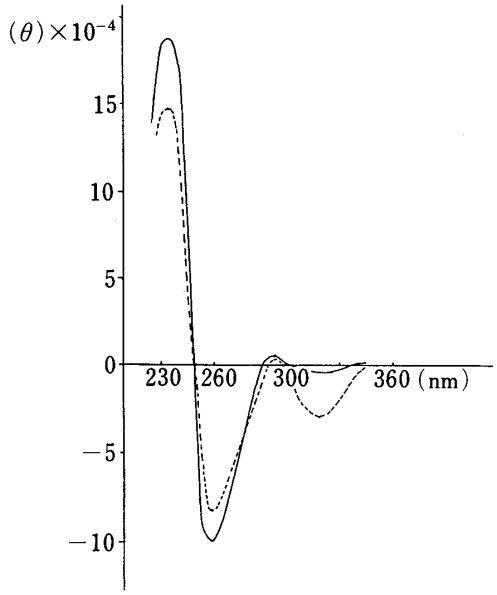

Fig. 2. CD Spectra of **2** and **8** (MeOH)
----, **2**; ——, **8**.

Furthermore, the observation of the prominent $(M)^+$ peak at *m/z* 1086 in the FD-MS of the methyl ether (**7a**) was consistent with the deshexahydroxydiphenoyl structure.

Final structural confirmation was obtained by partial hydrolysis of **5** with tannase, which afforded, together with ellagic acid (**10**), a hydrolysate shown to be identical with **7**.

The ^{1}H-NMR spectrum (Table I) of compound **8** was closely related to that of **2**, except for the upfield (δ 3.78) and lowfield (δ 5.98) shifts of the polyalcohol C-1 and C-2 proton signals, respectively.

The ^{13}C-NMR spectrum was also similar to that of **2**, but differed in the significant upfield shift (δ 50.2) of the C-1 atom and the appearance of a carboxylic acid signal at δ 176.4, suggesting that the C-1 position has an extra carbon atom of carboxylic acid.

The MS analyses were consistent with the expected structure **8**. Namely, the negative FAB-MS of **8** showed the $(M-H)^-$ peak at 961, in agreement with its molecular mass, while the observation of the $(M)^+$ peak at *m/z* 1186 in the FD-MS of the methyl ether (**8a**) was consistent with the hexadecamethyl structure.

The atropisomerisms of the hexahydroxydiphenoyl and triphenoyl ester groups in **8** were concluded to be in the *S*- and *S*,*S*-series,[6b] respectively, based on the observation [in the circular dichroism (CD) spectrum (Fig. 2)] of a positive Cotton effect at 235 nm and a negative one at 260 nm, similar to those found in the case of **2**.

From these results, compound **8** was concluded to be represented by the formula **8**, and was named vescalagin carboxylic acid. From the biosynthetic point of view, the location of the extra carbon atom (carboxylic acid) is rather unusual. However, taking into account the co-occurrence of grandinin (**11**)[12] in this plant material, compound **8** is presumed to be biosynthetically formed from **11** by degradation of the cyclic polyalcohol moiety.

Experimental

The instruments and chromatographic conditions used throughout this work were the same as described in the preceding paper[1] except in the following respects. High-performance liquid chromatography (HPLC) was carried out on a Toyo Soda apparatus equipped with an SP-8700 solvent delivery system, a UV-8 model II spectrometer and a Cosmosil 5C_{18}-P column (4.6 mm i.d. ×250 mm).

Isolation of 1, 6, 7 and 8——a) From the bark of *Quercus mongolica* var. *grosseserrata*: Details of the fractionation of the 80% aqueous acetone extract of the fresh bark (18 kg) were given in the preceding paper,[1] and the following fraction numbers correspond to those appearing in that paper. Fraction I-1 was chromatographed over

Fuji-gel ODS-G3 (H_2O–MeOH) and Sephadex LH-20 (EtOH) to afford **6** (2 g). Fraction II was applied to a Sephadex LH-20 column using EtOH with increasing amounts of H_2O–acetone (1 : 1) to give a further five fractions (frs. II-1—II-5). Fraction II-4 was purified on a column of Sephadex LH-20 (80% MeOH) to give **7** (250 mg). Repeated chromatography of fraction II-5 on MCI-gel CHP-20P (H_2O–MeOH) and Fuji-gel ODS-G3 (H_2O–MeOH) afforded **1** (230 mg). b) From the acorn of *Quercus mongolica* var. *grosseserrata*: The fractionation of the 80% aqueous acetone extract of the fresh acorn (12.3 kg) was described in the previous paper,[4] and the fraction described here corresponds to that of the previous work. Fraction III was subjected to column chromatography on Sephadex LH-20 (H_2O–MeOH) to afford further four fractions (frs. III-1—III-4). Fraction III-1 was rechromatographed over Sephadex LH-20 (60% MeOH) and MCI-gel CHP-20P (H_2O–MeOH) to yield **8** (185 mg).

Mongolicanin (1)——An off-white amorphous powder, $[\alpha]_D^{23}$ −50.9° (c=0.53, MeOH). *Anal.* Calcd for $C_{71}H_{50}O_{37}\cdot 9H_2O$: C, 51.45; H, 4.13. Found: C, 51.40; H, 3.97. Negative FAB-MS m/z: 1493 $(M-H)^-$. ^{1}H-NMR (100 MHz, acetone-d_6 + D_2O) ppm: 2.20—3.00 (1H, m, H-4″), 4.20—5.75 (polyalcohol-H), 5.83—6.36 (2H, m, arom.-H), 6.53—7.10 (9H, m, arom.-H). ^{13}C-NMR (25.05 MHz, acetone-d_6 + D_2O) ppm: 37.8 (C-1‴″, 4), 56.8 (C-6‴″), 68.1 (C-3″), 70.5, 71.3, 71.9, 78.5 (C-2‴″, 3‴″, 4‴″, 5‴″), 73.5 (C-3), 81.9 (C-2″), 83.3 (C-2), 96.0, 96.9 (C-6, 6″), 100.4, 102.1, 106.0 (C-4a, 4″a), 165.9, 167.3, 167.4, 168.0, 169.4 (C=O).

Methylation of 1——A mixture of **1** (30 mg), dimethyl sulfate (0.5 ml) and anhydrous potassium carbonate (700 mg) in dry acetone (10 ml) was heated under reflux for 2 h with stirring. After removal of the inorganic salts by filtration, the filtrate was concentrated under reduced pressure, and subjected to silica gel chromatography. Stepwise elution with benzene containing increasing proportions of acetone furnished the tricosamethyl ether (**1a**) (8 mg), colorless needles (MeOH), mp 234 °C, $[\alpha]_D^{23}$ −90.0° (c=0.20, acetone). *Anal.* Calcd for $C_{94}H_{96}O_{37}\cdot 2H_2O$: C, 60.90; H, 5.43. Found: C, 60.67; H, 5.36. FD-MS m/z: 1816 $(M)^+$. ^{1}H-NMR (270 MHz, $CDCl_3$) ppm: 2.49 (dd, J=16, 9 Hz, 4″-H), 2.62 (dd, J=16, 9 Hz, 4″-H), 2.93 (dd, J=5, 16 Hz, 4″-H), 3.03 (dd, J=5, 16 Hz, 4″-H), 3.34—4.25 (23 × OMe, 6‴″, 3-H), 4.30 (d, J=9 Hz, 4-H), 4.42, 4.44 (1H in total, each s, 1‴″-H), 4.50, 4.55 (1H in total, each d, J=13 Hz, 6‴″-H), 4.65, 4.71, 4.73 (each d, J=8 Hz, 3‴″, 3″, 2-H), 5.02, 5.17 (1H in total, each d, J=8 Hz, 2″-H), 5.13, 5.22 (1H in total, each s, 2‴″-H), 5.30, 5.34 (1H in total, each t, J=8 Hz, 4‴″-H), 5.71 (1H, br d, J=8 Hz, 5‴″-H), 5.88, 6.17, 6.24, 6.42 (1H in total, each s, arom.-H), 6.58—7.10 (10H, m, arom.-H).

Thiolytic Degradation of 1——A mixture of **1** (48 mg), benzylmercaptan (6 ml) and acetic acid (3 ml) in EtOH (15 ml) was refluxed for 36 h with stirring. The reaction mixture was concentrated under reduced pressure to give an oily residue, which was chromatographed over Sephadex LH-20 (EtOH), affording (+)-catechin (**4**) (5 mg), colorless needles, mp 144—145 °C, $[\alpha]_D^{23}$ +11.6° (c=0.5, acetone), and a thioether (**1b**) (16 mg), an off-white amorphous powder, $[\alpha]_D^{23}$ −53.4° (c=0.58, MeOH). *Anal.* Calcd for $C_{63}H_{44}O_{31}S\cdot 9H_2O$: C, 50.74; H, 4.19. Found: C, 50.33; H, 4.20. Negative FAB-MS m/z: 1327 $(M-H)^-$. ^{1}H-NMR (100 MHz, acetone-d_6 + D_2O) ppm: 4.00—4.20 (4H, m, 6″, 4-H, S–CH_2), 4.50 (1H, m, 3-H), 4.64 (1H, br s, 1″-H), 4.70—5.00 (3H, m, 3″, 6″, 2-H), 5.08 (1H, br s, 2″-H), 5.28 (1H, t, J=7 Hz, 4″-H), 5.64 (1H, br d, J=7 Hz, 5″-H), 6.02 (1H, s, 6-H), 6.67, 6.83 (each 1H, s, HHDP-H), 6.90—7.40 (9H, m, arom.-H). ^{13}C-NMR (25.05 MHz, acetone-d_6 + D_2O) ppm: 37.7 (S–CH_2), 38.9 (C-1″), 44.3 (C-4), 65.9 (C-6″), 69.7 (C-3), 71.2, 72.0, 78.4 (C-2″, 3″, 4″, 5″, 2), 96.6 (C-6), 101.2 (C-4a), 107.2, 109.3, 110.0 (arom.-C), 108.7 (C-8), 155.1, 158.7 (C-5, 7, 8a), 166.3, 167.2, 167.6, 169.6 (C=O).

Desulfurization of 1b——A solution of **1b** (8 mg) in acetic acid (0.1 ml) and EtOH (0.9 ml) was treated with Raney nickel (W-4) at room temperature for 20 min. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure, and the residue thus obtained was dissolved in MeOH. Analysis by HPLC (Cosmosil 5C_{18}-P; solvent, 20% aqueous acetonitrile; flow rate, 1 ml/min) showed a peak (t_R 4.3 min) corresponding to acutissimin A (**5**).

Preparation of 1, 5 and 9——A mixture of procyanidin B-3 (**3**) (500 mg) and vescalagin (**2**) (650 mg) in dry dioxane (20 ml) containing *p*-toluenesulfonic acid (20 mg) was heated at 55 °C for 15 min with stirring. The reaction mixture, after concentration (to *ca.* 5 ml) under reduced pressure, was applied to a Sephadex LH-20 column. Elution with EtOH containing increasing amounts of H_2O–acetone (1 : 1) gave condensation products, which were repeatedly chromatographed over MCI-gel CHP-20P (H_2O–60% MeOH) and Sephadex LH-20 (60% MeOH) to yield **1** (25 mg), **5** (130 mg) and **9** (130 mg).

Mongolinin A (6)——Colorless needles, mp 185 °C (dec.), $[\alpha]_D^{29}$ −68.6° (c=0.70, MeOH). *Anal.* Calcd for $C_{63}H_{42}O_{36}\cdot 10H_2O$: C, 48.65; H, 4.01. Found: C, 48.52; H, 3.68. Negative FAB-MS m/z: 1373 $(M-H)^-$. ^{13}C-NMR (25.05 MHz, acetone-d_6 + D_2O) ppm: 38.4 (C-1″), 66.2 (C-6″), 68.3 (C-3), 70.6, 71.0, 72.3, 77.6 (C-2″, 3″, 4″, 5″), 82.1 (C-2), 96.6 (C-6), 101.0 (C-4a), 105.5 (C-8), 107.2, 108.6, 109.3 (arom.-C), 155.3 (C-5, 7, 8a), 165.9, 166.8, 167.2, 169.3, 170.0 (C=O).

Methylation of 6——A mixture of **6** (100 mg), dimethyl sulfate (1.5 ml) and anhydrous potassium carbonate (1.5 g) in dry acetone (20 ml) was heated under reflux for 1.5 h with stirring. After removal of the inorganic salts by filtration, the filtrate was concentrated under reduced pressure to a syrup, which was chromatographed over silica gel (benzene–acetone, 8 : 1—3 : 1) to afford **6a** (80 mg) as an off-white amorphous powder, $[\alpha]_D^{29}$ −142.3° (c=1.21, acetone). *Anal.* Calcd for $C_{85}H_{86}O_{36}\cdot H_2O$: C, 59.99; H, 5.21. Found: C, 59.65; H, 5.18. FD-MS m/z: 1682 $(M)^+$. ^{1}H-NMR (100 MHz, $CDCl_3$) ppm: 4.62 (1H, br d, J=12 Hz, 6″-H), 4.70 (1H, s, 1″-H), 4.72 (1H, d J=7 Hz, 3″-H), 5.04 (1H, s, 2″-H), 5.16 (1H, d, J=7 Hz, 2-H), 5.20 (1H, t, J=7 Hz, 4″-H), 5.72 (1H, d, J=7 Hz, 5″-H), 6.02, 6.14 (1H in

total, each s, 6-H), 6.60—7.34 (7H, m, arom.-H). ^{13}C-NMR (25.05 MHz, $CDCl_3$) ppm: 27.4 (C-4), 36.4 (C-1″), 55.4, 55.8, 56.2, 56.6, 59.8, 60.3, 60.9, 61.0, 61.3, 61.5 (OMe), 65.4 (C-6″), 68.3, 70.0, 70.2, 71.3, 76.3 (C-2″, 3″, 4″, 5″, 3), 81.4 (C-2), 88.8 (C-6), 101.9 (C-4a), 104.8, 107.3, 108.1, 110.9, 111.4, 114.2, 118.2 (arom.-C), 157.6, 158.7 (C-5, 7, 8a), 163.8, 164.2, 164.9, 165.7, 167.6 (C=O).

Alkaline Methanolysis of 6a——A solution of **6a** (40 mg) in 2.5% sodium hydroxide/MeOH (2 ml) was left at room temperature for 12 h. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form) resins, and the solvent was evaporated off under reduced pressure. The residue was chromatographed on silica gel. Elution with benzene–acetone (8 : 1—1 : 1) yielded trimethyl (*S*)-octamethylvaloneate (**6c**) (14 mg) and the methanolysate (**6b**) (15 mg). **6c**: $[\alpha]_D^{29}$ −18.7° (*c*=0.31, acetone). ^{1}H-NMR (100 MHz, $CDCl_3$) ppm: 3.49, 3.58, 3.60, 3.68, 3.78(×2), 3.94(×3), 3.98, 4.08 (OMe×11), 6.93, 7.30, 7.35 (each 1H, arom.-H). **6b**: $[\alpha]_D^{29}$ −32.0° (*c*=0.50, $CHCl_3$), FD-MS *m/z*: 1118 $(M)^+$.

Acutissimin C (7)——An off-white amorphous powder, $[\alpha]_D^{23}$ −23.2° (*c*=0.76, MeOH). *Anal.* Calcd for $C_{42}H_{32}O_{23}\cdot 6H_2O$: C, 49.81; H, 4.37. Found: C, 49.81; H, 3.99. Negative FAB-MS *m/z*: 903 $(M-H)^-$. ^{13}C-NMR (25.05 MHz, acetone-d_6+D_2O) ppm: 27.1 (C-4), 38.1 (C-1″), 62.2 (C-6″), 67.6 (C-3), 70.0, 74.4, 74.7, 77.1 (C-2″, 3″, 4″, 5″), 81.6 (C-2), 96.5 (C-6), 100.0 (C-4a), 105.5 (C-8), 108.8 (unsubstituted arom.-C), 132.0 (C-1′), 153.9, 155.4, 156.3 (C-5, 7, 8a), 166.6, 168.4 (C=O).

Methylation of 7——A mixture of **7** (50 mg), dimethyl sulfate (0.7 ml) and anhydrous potassium carbonate (1 g) in dry acetone (10 ml) was refluxed for 1.5 h. The reaction mixture was worked up in the same way as described for **6** to give the tridecamethyl ether (**7a**) (16 mg) as an off-white amorphous powder, $[\alpha]_D^{23}$ −8.0° (*c*=0.40, acetone). *Anal.* Calcd for $C_{55}H_{58}O_{23}\cdot 5/2H_2O$: C, 58.35; H, 5.60. Found: C, 58.19; H, 5.50. FD-MS *m/z*: 1086 $(M)^+$. ^{1}H-NMR (270 MHz, $CDCl_3$) ppm: 2.70 (2H, br d, *J*=5 Hz, 4-H), 3.42, 3.46, 3.52, 3.63, 3.66, 3.72, 3.84, 3.86, 3.91, 3.98, 4.05, 4.15 (OMe), 4.60 (1H, br s, 1″-H), 4.63, 4.81 (1H in total, each d, *J*=7 Hz, 3″-H), 5.03 (1H, d, *J*=5 Hz, 2-H), 5.21 (1H, m, 5″-H), 5.25, 5.40 (1H in total, each br s, 2″-H), 6.01, 6.23 (1H in total, each s, 6-H), 6.83 (1H, d, *J*=8 Hz, 5′-H), 6.85 (1H, s, arom.-H), 6.88 (1H, d, *J*=2 Hz, 2′-H), 6.96 (1H, dd, *J*=8, 2 Hz, 6′-H).

Vescalagin Carboxylic Acid (8)——Colorless needles, mp 180 °C (dec.), $[\alpha]_D^{23}$ −59.3° (*c*=0.60, MeOH–H_2O, 3 : 7). *Anal.* Calcd for $C_{42}H_{26}O_{27}\cdot 13/2H_2O$: C, 46.71; H, 3.64. Found: C, 46.26; H, 3.65. Negative FAB-MS *m/z*: 961 $(M-H)^-$. ^{13}C-NMR (100 MHz, acetone-d_6) ppm: 50.2 (C-1), 66.1 (C-6), 69.9 (C-4), 71.7 (C-3), 71.8 (C-5), 73.7 (C-2), 107.8, 108.1, 109.1 (unsubstituted arom.-C), 166.3, 166.8, 167.2, 168.0, 169.9 (C=O), 176.4 (COOH). CD (*c*=0.69×10^{-5}, MeOH) $[\theta]^{23}$ (nm): −100000 (260), 0 (249), +186000 (235). UV λ_{max}^{MeOH} nm (log ε): 230 (4.60).

Methylation of 8——A mixture of **8** (55 mg), dimethyl sulfate (0.9 ml) and anhydrous potassium carbonate (1 g) in dry acetone (10 ml) was refluxed for 2 h. The reaction mixture was worked up as described above to give the hexadecamethyl ether (**8a**) (20 mg), colorless needles, mp 223—224 °C, $[\alpha]_D^{20}$ −89.7° (*c*=0.68, acetone). *Anal.* Calcd for $C_{58}H_{58}O_{27}$: C, 58.68; H, 4.92. Found: C, 58.18; H, 4.96. FD-MS *m/z*: 1186 $(M)^+$. ^{1}H-NMR (100 MHz, $CDCl_3$) ppm: 4.76 (1H, d, *J*=8 Hz, 3-H), 5.02 (1H, dd, *J*=12, 2 Hz, 6-H), 5.26 (1H, t, *J*=8 Hz, 4-H), 5.70 (2H, m, 2, 5-H), 6.76, 6.88, 6.92 (each 1H, s, arom.-H).

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