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Chemical and Chemotaxonomical Studies of Filices. LXXVII.[1] Isolation and Structure of Novel Catechin and Proanthocyanidins from *Dennstaedtia distenta* MOORE[2]

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From the fronds of *Dennstaedtia distenta*, new flavan-3-ol (catechin) derivatives, distenin (**1**), its dimer (**2**) and its trimer (**3**), were isolated in addition to vitexin. On the basis of the spectroscopic and chemical evidence, their structures were established as (2*R*,3*R*)-flavan-3,5,7-triol (**1**), distenin-(4β→8)-distenin (**2**) and distenin-(4β→8)-distenin-(4β→8)-distenin (**3**), respectively.

Keywords——*Dennstaedtia distenta*; distenin; modified Horeau's method; proanthocyanidin; prodistenidin; flavan-3-ol; condensed tannin; thiolytic degradation

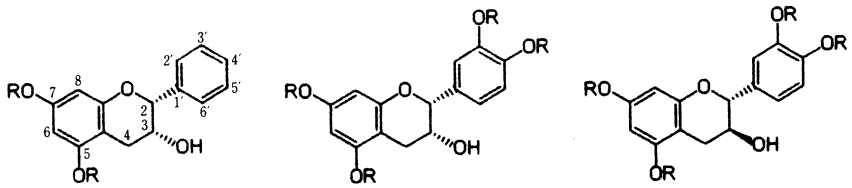
In the previous paper[2] we reported the presence of two monachosorin derivatives (pterosin-type dimeric dinorsesquiterpenoids) in the dried aerial fronds of *Dennstaedtia distenta* MOORE (Pteridaceae). Further, we have isolated a new flavan-3-ol compound (**1**) lacking the hydroxy groups on the B-ring, and its condensed dimeric (**2**) and trimeric (**3**) derivatives, together with vitexin from this fern. This paper deals with the isolation and the structural elucidation of these compounds.

Compound **1** was obtained as colorless needles, $C_{15}H_{14}O_4$, mp 202—203 °C, $[\alpha]_D^{24}$ −61.3° (EtOH). In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum (400 MHz, C_5D_5N) of **1**, besides two doublet signals at δ 6.70 and 6.71 (each 1H, J=3.2 Hz) which were assignable to *meta*-coupled aromatic protons, a triplet-triplet signal at δ 7.30 (1H, J=7.3, 1.8 Hz), a triplet signal at δ 7.39 (2H, J=7.3 Hz) and a doublet-doublet signal at δ 7.87 (2H, J=7.3, 1.8 Hz) due to a mono-substituted benzene ring, were seen.

Moreover, aliphatic proton signals were observed at δ 5.35 (1H), 4.68 (1H), 3.43 (1H) and 3.55 (1H) arising from the flavan C-ring. From these ^{1}H-NMR data, compound **1** was suggested to be a flavan-3,5,7-triol without any hydroxy groups on the B-ring. This suggestion was supported by the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (100 MHz, C_5D_5N) of **1** (Table I).

The coupling pattern of 2-H [δ 5.35 (br s, $W_{1/2}$=4.5 Hz)] in the ^{1}H-NMR and chemical shift of C-2 (δ 79.86) in the ^{13}C-NMR were close to those [2-H, δ 5.35 (br s, $W_{1/2}$=4.5 Hz): C-2, δ 79.85] of epicatechin (**5**)[3] (2,3-*cis*) rather than those [2-H, δ 5.21, (d, J=7.4 Hz): C-2, δ 82.94] of catechin (**6**)[4] (2,3-*trans*), so that **1** was indicated to possess a 2,3-*cis* stereochemistry.

In order to confirm the absolute configuration at C-3, the following experiment was done. The treatment of **1** with diazomethane (CH_2N_2) for protecting phenolic hydroxy groups gave the dimethylether (**4**). Compound **4** still showed infrared (IR) absorption due to a secondary hydroxy group at C-3. Authentic optically active **5** (3*R*)[3] and **6** (3*S*)[4] were



1 (distenin): R=H **5** ((−)-epicatechin): R=H **6** ((+)-catechin): R=H
4 : R=CH_3 **7** : R=CH_3 **8** : R=CH_3

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of **1**, **5** and **6** (C_5D_5N, δ)

Carbon atom	**1**[a]	**5**[b]	**6**[b]
2	79.86	79.85	82.94
3	66.54	66.80	68.10
4	29.58	29.41	29.36
5	158.45[c]	158.35[d]	158.02[e]
6	96.78	96.65	96.53
7	158.50[c]	158.46[d]	158.40[e]
8	95.70	95.73	95.40
9	157.10[c]	157.37[d]	157.00[e]
10	99.90	100.06	100.81
1′	140.67	131.91	131.91
2′	127.67	116.04	116.09[f]
3′	128.16	146.65[g]	146.81
4′	127.57	146.49[g]	146.81
5′	128.16	116.04	115.83[f]
6′	127.67	119.23	119.51

a) 100 MHz. *b*) 22.5 MHz. *c*—*g*) Assignments of chemical shifts may be reversed.

methylated with CH_2N_2 to give their tetramethylethers, **7** and **8**, respectively. Compounds **4**, **7** and **8** were treated by a modified Horeau's method.[5,6]

From these results (shown in Table II), the configuration at C-3 was concluded to be *R*. On the basis of the above spectral and chemical evidence, compound **1** was determined to be (2*R*,3*R*)-flavan-3,5,7-triol, and was named distenin.

Compound **2** was obtained as colorless needles, but it decomposed and became dark red immediately in air or on a dried silica gel plate. It gave an orange-red color with the anisaldehyde–sulfuric acid reagent. These findings suggested **2** to be a proanthocyanidin (condensed tannin). The mass spectrum (MS) of **2** did not show the molecular ion peak but gave a strong peak at *m/z* 258, which was the molecular ion of **1**. The field desorption mass spectrum (FD-MS) of **2** exhibited the M^+ ion peak at *m/z* 514, indicating a biflavanoid constitution for **2**.

In the ^{1}H-NMR spectrum of **2**, the coupling patterns of two flavan 2-H signals [δ 5.13, 4.94 (each s)] suggested that compound **2** consisted of flavan-3-ol units with 2,3-*cis* stereochemistry, and this was also supported by the ^{13}C-NMR chemical shifts [δ 77.03 (C-2), 79.26 (C-2′)] of the two flavan C-2 signals.[7] Acid catalyzed thiolytic degradation of **2** gave **1** (formed from the lower unit) and distenin-4-benzylthioether (**9**) (formed from the upper unit) which was identified by ^{1}H-NMR analysis and converted to **1** with Raney Ni, proving that compound **2** consisted only of **1**. The location of the interflavanoid linkage was confirmed to be $C_{(4)}$–$C_{(8)}$ on the basis of a comparison[8] of the chemical shift (δ 5.00) of the lower 2-H signal in the ^{1}H-NMR spectrum of **2**, with that (δ 4.96) of procyanidin B-2 (**11**, 4, 8 linkage) and that (δ 4.84) of procyanidin B-5 (**12**, 4, 6 linkage). Furthermore, the configuration of the inter-

TABLE II. Results Obtained by the Modified Horeau's Method

Compound	Configuration	Peak increment (*R*-acid, %)[a]
4	3*R*	−8.2
7	3*R*	−7.2
8	3*S*	+6.1

a) Obtained by subtraction of the corresponding value in the reaction with cyclohexanol.

TABLE III. ^{13}C-NMR Chemical Shifts of **2** and **3** (Acetone-d_6, δ)

Carbon atom	**2**[a]	**3**[b]
2	77.03	77.16
3	71.85	72.02[c]
4	37.10	37.71[d]
2′	79.26	77.59
3′	66.58	72.17[c]
4′	29.25	37.47[d]
2″		79.41
3″		66.74
4″		29.44

a) 22.5 MHz. *b*) 100 MHz. *c*, *d*) Assignments of chemical shifts may be reversed.

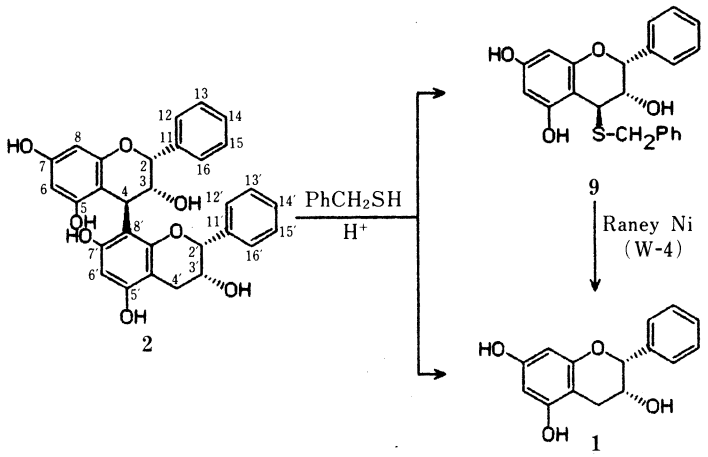


Chart 2

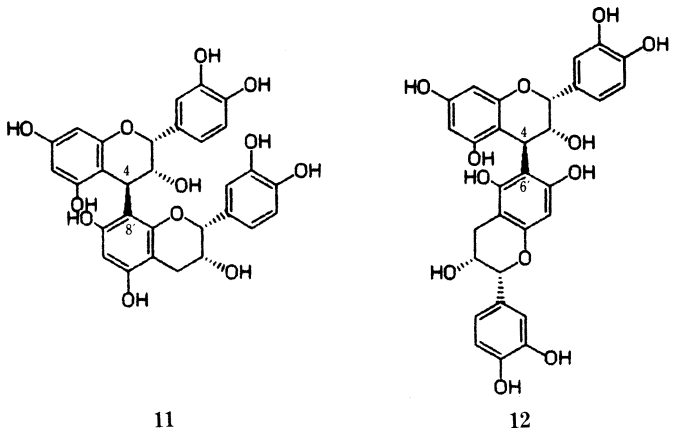


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Chart 3

flavanoid linkage was determined to be β from the coupling constant[8] ($J \fallingdotseq 0$ Hz) of the 4-H signal (upper unit, δ 4.71) in the ^{1}H-NMR and C-2 signal[7,8] (upper unit, δ 77.03) in the ^{13}C-NMR.

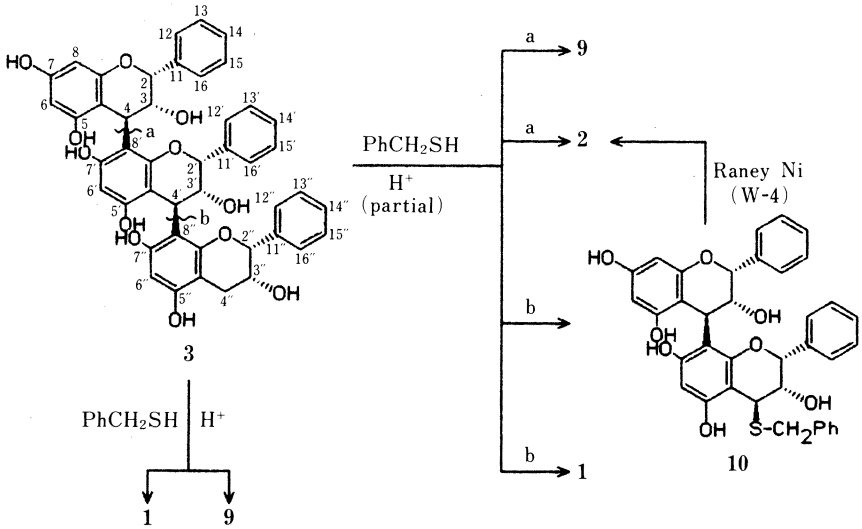

Chart 4

Based on these spectral and chemical results, **2** was characterized as distenin-(4β→8)-distenin, and named prodistenidin B-2.

Compound **3** was obtained as colorless needles, mp 228—231 °C, $[\alpha]_D^{24}$ +23.0° (EtOH), positive to the anisaldehyde–sulfuric acid (orange-red) reagent. The triflavanoid constitution of compound **3** was indicated by FD-MS analysis [M^+ *m/z* 770]. The occurrence of three distenin (**1**) units in the molecule was deduced from the ^{1}H-NMR and ^{13}C-NMR data (Table III). This was also supported by the thiolytic degradation of **3**, to give **1** and **9**.

The location and the configurations of the linkages between the component units were determined[8] as follows. Partial thiolytic degradation of **3** afforded, in addition to **1** and **9**, **2** and a thioether (**10**) which was characterized as the 4-benzylthioether of **2** by ^{1}H-NMR analysis and by its conversion to **2** with Raney Ni. These results indicated that all the component units were connected through 4β→8 linkages. Thus, compound **3** was concluded to be distenin-(4β→8)-distenin-(4β→8)-distenin, and was named prodistenidin C-1.

Experimental

The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus (values are uncorrected); optical rotations, JASCO DIP-360 automatic polarimeter; IR spectra, Hitachi 270-30 infrared spectrometer; ultraviolet (UV) spectra, Hitachi 150-20 spectrophotometer; FD-MS, Hitachi M-80 mass spectrometer at 20—25 mA; electron impact (EI)-MS and high-resolution MS, JEOL JMS new D-300 mass spectrometer with a direct inlet system at 70 eV; ^{1}H-NMR spectra, Hitachi R-600 FT-NMR spectrometer (60 MHz) and Bruker AM-400 FT-NMR spectrometer (400 MHz) using tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet); ^{13}C-NMR spectra, JEOL JMN FX-90Q FT-NMR spectrometer (22.5 MHz) and Bruker AM-400 FT-NMR spectrometer (100 MHz) using tetramethylsilane as an internal standard. Chromatography was carried out as follows: gas-liquid chromatography (GLC), with a Hitachi gas chromatograph model 163; thin layer chromatography (TLC), on pre-coated TLC plates (Merck, Kieselgel 60F-254), detection by spraying with anisaldehyde–sulfuric acid reagent followed by heating; column chromatography, activated charcoal (Wako Pure Chemical).

Extraction and Isolation——The air-dried *Dennstaedtia distenta* MOORE (560 g) collected in April 1986 and January 1987 in the greenhouse of Kobe Gakuin University, was extracted under reflux with MeOH (2.5 l) 5 times for 6 h. The combined extracts (12.5 l) were passed through an activated charcoal (60 g) packed in a column of 5.5 cm diameter and the column was further eluted with MeOH (25.4 l) and $CHCl_3$–MeOH (3 : 7, 18 l). The fraction eluted with $CHCl_3$–MeOH (3 : 7) was concentrated *in vacuo* to a syrup (32.2 g), which was partitioned into $CHCl_3$–MeOH–H_2O (4 : 4 : 3) mixture (550 ml). The bottom layer was concentrated *in vacuo* to a syrup (18.8 g), which was

chromatographed on Sephadex LH-20 (240 g) with $CHCl_3$–MeOH (1 : 2) twice to afford vitexin (55 mg) and a fraction containing **1**, **2** and **3**. The fraction was chromatographed over MCI gel CHP 20P with 10% aqueous MeOH, and further purified by chromatography on polyamide (Wako Pure Chemical C-100 and C-200 in half) with MeOH to give **1** (230 mg), **2** (105 mg) and **3** (110 mg).

Distenin (1)——Colorless needles from a mixture of *n*-hexane and ethylacetate, mp 202—203 °C, $[\alpha]_D^{24}$ −61.3° (c=2.0, EtOH). MS m/z (%): 258.0902 (27.6) (M^+, Calcd for $C_{15}H_{14}O_4$, 258.0892), 139 (100), 120 (19.8), 91 (20.3). UV λ_{max}^{MeOH} nm (log ε): 212 (4.70), 231 sh (4.13), 268 (3.22). IR ν_{max}^{KBr} cm^{-1}: 3600—3300 (br), 2916, 1626, 1512, 1498, 1472, 1452, 1276, 1108, 1054, 1040, 822, 730. ^{1}H-NMR (400 MHz, C_5D_5N) δ: 3.43 (1H, dd, J=16.5, 4.6 Hz, 4β-H), 3.55 (1H, dd, J=16.5, 3.1 Hz, 4α-H), 4.68 (1H, br s, $W_{1/2}$=8 Hz, 3-H), 5.35 (1H, br s, $W_{1/2}$=4.5 Hz, 2-H), 6.70, 6.71 (each 1H, d, J=3.2 Hz, 6-H, 8-H), 7.30 (1H, tt, J=7.3, 1.8 Hz, 4′-H), 7.39 (2H, t, J=7.3 Hz, 3′-H, 5′-H), 7.87 (2H, dd, J=7.3, 1.8 Hz, 2′-H, 6′-H). ^{13}C-NMR: Table I.

Distenin Dimethylether (4)——An excess of diazomethane in ether (15 ml) was added to a solution of **1** (15 mg) in MeOH (3 ml) and the solution was allowed to stand at 5 °C for 10 h. The reaction mixture was concentrated *in vacuo*, and the oily residue was chromatographed on Sephadex LH-20 ($CHCl_3$–MeOH, 1 : 2), to furnish **4** (13 mg) as a colorless syrup. $[\alpha]_D^{24}$: −38.9° (c=1.42, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 212 (4.88), 231 sh (4.22), 268 (3.28). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600—3400 (br), 2938, 1621, 1595, 1497, 1466, 1208, 1147, 1121, 1074. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.60—2.96 (2H, m, 4-H_2), 3.72 (6H, s, OCH_3×2), 4.20—4.50 (1H, m, 3-H), 5.01 (1H, br s, $W_{1/2}$=6.0 Hz, 2-H), 6.13, 6.18 (each 1H, d, J=3.8 Hz, 6-H, 8-H), 7.30—7.70 (5H, m, aromatic protons).

(2*R*,3*R*)-Epicatechin Tetramethylether (7) and (2*R*,3*S*)-Catechin Tetramethylether (8)——Methylation of **5** and **6** (each 20 mg) in the same way as for **1** followed by chromatography on Sephadex LH-20 ($CHCl_3$–MeOH, 1 : 2) gave **7** (15 mg) and **8** (16 mg), respectively. **7**: Colorless needles from EtOH, mp 138—140 °C, $[\alpha]_D^{24}$ −28.1° (c=2.5, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 216 (4.99), 229 sh (4.86), 277 (4.14), 284 sh (3.97). IR ν_{max}^{KBr} cm^{-1}: 3600—3350 (br), 3004, 2940, 1624, 1594, 1520, 1506, 1464, 1444, 1412, 1358, 1328, 1256, 1236, 1158, 1118, 1018, 822, 780. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.90—2.98 (2H, m, 4-H_2), 3.80, 3.90 (each 6H, s, OCH_3×2), 4.20—4.40 (1H, m, 3-H), 4.98 (1H, br s, $W_{1/2}$=4.5 Hz, 2-H), 6.15, 6.22 (each 1H, d, J=2.2 Hz, 6-H, 8-H), 6.95—7.20 (3H, m, 2′-H, 5′-H, 6′-H). **8**: Colorless needles from EtOH, mp 122—123 °C, $[\alpha]_D^{24}$ −9.8° (c=3.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 216 (4.98), 229 sh (4.90), 277 (4.18), 285 sh (4.02). IR ν_{max}^{KBr} cm^{-1}: 3650—3300 (br), 1624, 1598, 1522, 1498, 1450, 1420, 1338, 1312, 1266, 1214, 1144, 1124, 1056, 806. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.52 (1H, dd, J=16.2, 8.6 Hz, 4α-H), 3.10 (1H, dd, J=16.2, 5.0 Hz, 4β-H), 3.75, 3.80 (each 3H, s, OCH_3), 3.90 (6H, s, OCH_3×2), 3.85—4.20 (1H, m, 3-H), 4.70 (1H, d, J=7.0 Hz, 2-H), 6.13 (2H, br s, $W_{1/2}$=6.0 Hz, 6-H, 8-H), 6.98 (3H, br s, $W_{1/2}$=8.0 Hz, 2′-H, 5′-H, 6′-H).

Modified Horeau's Method for 4, 7 and 8——A solution of **4** (4.6 mg, 16 μmol) and *dl*-2-phenylbutyric acid anhydride (10 mg, 48 μmol) in dry pyridine (0.1 ml) was allowed to stand in a sealed microtube under N_2 gas at 45 °C for 3 h, then (+)-(*R*)-phenylethylamine (6 μl) was added to the reaction mixture. The whole mixture was allowed to stand at the same temperature for 15 min. The mixture was cooled to room temperature (23 °C), and diluted with EtOAc (2 ml). The solution was subjected to GLC analysis [FID detector; carrier gas, N_2 5.0 kg/cm^2; column, Chemical Bonded glass capillary column with silicone OV-1 25 m × 0.25 mm i.d. (Gasukuro Kougyou); column temperature, 180 °C; injection temperature, 200 °C]. The relative proportions of the amides of (−)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid were calculated from the areas of their respective peaks. Compounds **7**, **8** and cyclohexanol (each 16 μmol) were treated in the same manner as for **4**, and results of these experiment are reported in Table II.

Prodistenidin B-2 (2)——Colorless needles from a mixture of *n*-hexane and ethylacetate, mp 167—169 °C, $[\alpha]_D^{24}$ +27.1° (c=2.70, EtOH). FD-MS m/z (%): 514 (M^+, 12.4), 258 (100). EI-MS m/z (%): 258 (100), 139 (32.8), 120 (73.8), 91 (76.0). *Anal.* Calcd for $C_{30}H_{26}O_8$: C, 70.03; H, 5.09. Found: C, 69.72; H, 5.09. UV λ_{max}^{MeOH} nm (log ε): 209 (5.11), 238 sh (4.57). IR ν_{max}^{KBr} cm^{-1}: 3700—3000 (br), 1624, 1602, 1498, 1450, 1286, 1256, 1204, 1150, 1048, 900, 702. ^{1}H-NMR (400 MHz, acetone-d_6) δ: 2.69 (1H, dd, J=16.9, 3.0 Hz, 4′α-H), 2.88 (1H, dd, J=16.9, 4.6 Hz, 4′β-H), 4.14—4.19, 4.24—4.29 (each 1H, m, 3-H, 3′-H), 4.71 (1H, s, 4-H), 5.00 (1H, s, 2′-H), 5.13 (1H, s, 2-H), 6.11 (1H, s, 6′-H), 6.13 (2H, s, 6-H, 8-H), 7.25—7.52 (10H, m, aromatic protons). ^{13}C-NMR (22.5 MHz, acetone-d_6) δ: 29.25 (t, C-4′), 37.10 (d, C-4), 66.58 (d, C-3′), 71.89 (d, C-3), 77.03 (d, C-2), 79.26 (d, C-2′), 96.05 (d, C-8), 96.43 (d, C-6), 96.80 (d, C-6′), 99.80 (s, C-10), 100.43 (s, C-10′), 107.59 (s, C-8′), 127.47 (d×4, C-12,16,12′,16′), 128.01 (d×2, C-14,14′), 128.50 (d×4, C-13,15,13′,15′), 140.09, 140.20 (each s, C-11,11′), 154.77, 155.10, 155.75, 157.32, 158.62, 159.05 (each s, C-5,7,9,5′,7′,9′).

Thiolytic Degradation of 2——A solution of **2** (50 mg), benzylmercaptan (1.5 ml) and acetic acid (2 ml) in EtOH (10 ml) was stirred under reflux for 27 h. The reaction mixture was concentrated under reduced pressure, and the oily residue was chromatographed over Sephadex LH-20 (EtOH), to yield distenin (10 mg) and a thioether (**9**, 14 mg). **9**: An off-white amorphous powder, $[\alpha]_D^{24}$ −3.1° (c=1.22, EtOH). *Anal.* Calcd for $C_{22}H_{20}O_4S \cdot 1/2\,H_2O$: C, 67.84; H, 5.43. Found: C, 67.61; H, 5.35. UV λ_{max}^{EtOH} nm (log ε): 215 (4.51). IR ν_{max}^{KBr} cm^{-1}: 3600—3000 (br), 1628, 1602, 1496, 1468, 1452, 1366, 1310, 1072, 1058, 826, 732. ^{1}H-NMR (400 MHz, acetone-d_6) δ: 4.03 (1H, s, 3-H), 4.05 (2H, s, –S–CH_2–), 4.13 (1H, d, J=1.88 Hz, 4-H), 5.44 (1H, s, 2-H), 5.96 (1H, d, J=2.2 Hz, 6-H), 6.05 (1H, d, J=2.2 Hz, 8-H), 7.20—7.50 (10H, m, aromatic protons).

Desulfurization of 9——The thioether (**9**, 10 mg) in EtOH–acetic acid (9 : 1, 1 ml) was stirred with Raney Ni (W-4) for 15 min at 50 °C. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure.

The residue was purified on SEP PAK C_{18} (MeOH) to give **1** (4 mg).

Prodistenidin C-1 (3)——Colorless needles from MeOH, mp 228—231 °C, $[\alpha]_D^{24}$ +23.0° (c=2.7, EtOH). FD-MS m/z (%): 770 (M^+, 2.3), 514 (15.8), 258 (100). EI-MS m/z (%): 258 (27.3), 228 (6.0), 139 (100), 120 (20.2), 91 (21.4). *Anal.* Calcd for $C_{45}H_{38}O_{12}\cdot 2H_2O$: C, 66.99; H, 5.24. Found: C, 66.92; H, 5.18. UV λ_{max}^{MeOH} nm (log ε): 214 (5.31), 238 sh (4.75). IR ν_{max}^{KBr} cm^{-1}: 3600—3000 (br), 1622, 1600, 1498, 1446, 1354, 1312, 1258, 1204, 1176, 1148, 1052, 702. ^{1}H-NMR (400 MHz, acetone-d_6) δ: 2.74 (1H, dd, J=16.5, 2.9 Hz, 4″-α-H), 2.90 (1H, dd, J=16.5, 4.5 Hz, 4″-β-H), 4.08—4.21 (3H, m, 3-H, 3′-H, 3″-H), 4.58 (1H, s, 4′-H), 4.73 (1H, s, 4-H), 4.96 (1H, s, 2″-H), 4.98 (1H, s, 2′-H), 5.05 (1H, s, 2-H), 6.05 (1H, s, 6″-H), 6.07 (1H, d, J=2.2 Hz, 6-H), 6.17 (1H, d, J=2.2 Hz, 8-H), 6.31 (1H, s, 6′-H), 7.22—7.53 (15H, m, aromatic protons). ^{13}C-NMR (100 MHz, acetone-d_6) δ: 29.44 (t, C-4″), 37.47 (d, C-4′), 37.71 (d, C-4), 66.74 (d, C-3″), 72.02 (d, C-3), 72.17 (d, C-3′), 77.16 (d, C-2), 77.59 (d, C-2′), 79.41 (d, C-2″), 96.19 (d, C-8), 96.77 (d, C-6), 96.88 (d×2, C-6′,6″), 98.98 (s, C-10), 100.61 (s×2, C-10′,10″), 108.06 (s×2, C-8′,8″), 128.01, 128.09, 128.29 (each d, C-14,14′,14″), 127.56, 127.57, 128.08 (each d×2, C-12,16,12′,16′,12″,16″), 128.52, 128.60, 128.63 (each d×2, C-13,15,13′,15′,13″,15″), 140.16, 140.26, 140.36 (each s, C-11,11′,11″), 154.90, 155.34, 157.69, 159.03 (each s×2), 159.64 (s) (C-5,7,9,5′,7′,9′,5″,7″,9″).

Thiolytic Degradation of 3——A solution of **3** (15 mg), benzylmercaptan (0.8 ml) and acetic acid (1 ml) in EtOH (10 ml) was refluxed for 21 h. The reaction mixture was worked up in the same way as described for **2** to afford **1** (3 mg) and **9** (8 mg).

Partial Thiolytic Degradation of 3——A solution of **3** (70 mg), benzylmercaptan (0.5 ml) and acetic acid (1 ml) in EtOH (10 ml) was refluxed for 4 h. The reaction mixture was concentrated under reduced pressure. The residue was repeatedly chromatographed on Sephadex LH-20 (EtOH) and MCI gel CHP 20P (20% aqueous MeOH) to give **1** (4 mg), **9** (13 mg), **2** (7 mg) and a prodistenidin B-2-4′-benzylthioether (**10**, 11 mg). **10**: An off-white amorphous powder, $[\alpha]_D^{24}$ +63.8° (c=0.84, EtOH). *Anal.* Calcd for $C_{37}H_{32}O_8S\cdot H_2O$: C, 67.87; H, 5.23. Found: C, 67.87; H, 5.33. UV λ_{max}^{EtOH} nm (log ε): 211 (5.07). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (br), 1624, 1602, 1498, 1452, 1254, 1206, 1148, 1025, 702. ^{1}H-NMR (400 MHz, acetone-d_6) δ: 4.01 (1H, br s, $W_{1/2}$=5.0 Hz, H-3), 4.07 (2H, s, –S–C$\underline{H}_2$–), 4.08—4.16 (2H, m, 4′-H, 3′-H), 4.71 (1H, s, 4-H), 5.20 (1H, s, 2′-H), 5.38 (1H, s, 2-H), 6.09 (1H, s, 6-H), 6.13 (2H, s, 6′-H, 8′-H), 7.19—7.48 (15H, m, aromatic protons).

Desulfurization of 10——The thioether (**10**, 8 mg) in EtOH–acetic acid (9 : 1, 1 ml) was stirred with Raney Ni (W-4) at 50 °C for 10 min. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure. The residue was chromatographed on MCI gel CHP 20P (10% aqueous MeOH) to give **2** (4 mg).

Vitexin——Yellowish powder, mp 263—265 °C, $[\alpha]_D^{24}$ −12.6° (c=1.38, C_5H_5N). UV λ_{max}^{MeOH} nm (log ε): 270 (4.77), 328 (4.70), $\lambda_{max}^{MeOH+NaOMe}$ nm: 280, 328, 393. IR ν_{max}^{KBr} cm^{-1}: 3500—3000 (br), 1652, 1616, 1568, 1504, 1428, 1390, 1364, 1294, 1178, 1042, 1012, 834. ^{1}H-NMR (60 MHz, DMSO-d_6) δ: 3.1—4.0 (6H, m, sugar protons), 4.75 (1H, d, J= 10 Hz, anomeric proton), 6.27 (1H, s, 6-H), 6.76 (1H, s, 3-H), 6.89 (2H, d, J=8.2 Hz, 3′-H, 5′-H), 8.02 (2H, d, J= 8.2 Hz, 2′-H, 6′-H), 8.53 (1H, br s, O$\underline{H}$), 10.32 (1H, br s, O$\underline{H}$), 13.15 (1H, s, 5-O$\underline{H}$). It was identical with an authentic sample by direct comparison (IR and mixed fusion).

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References and Notes

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