Isolation and Structures of Procyanidins (Condensed Tannins) from *Rhaphiolepis umbellata* †

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Two new procyanidin trimers (1, 3) and one tetramer (2) were isolated in their free phenolic forms from the bark of *Rhaphiolepis umbellata*, which is used as the dyeing agent for Oshima Tsumugi. On the basis of 1H- and 13C-NMR spectral examinations and the results of partially acid-catalyzed degradation, 1, 2 and 3 were characterized as epicatechin-(4â†’8)-epicatechin-(4â†’6)-epicatechin, epicatechin-(4â†’8)-epicatechin-(4â†’8)-epicatechin-(4â†’6)-epicatechin and catechin-(4â†’6)-epicatechin-(4â†’8)-epicatechin, respectively. In addition, the occurrence of the known compounds, i.e., (−)-epicatechin (4), procyanidins B-1 (9), B-2 (5), B-5 (6) and C-1 (7), proanthocyanidin A-2 (10), cinnamtannins A₁ (8) and A₂ (14), cinchonains Ia (11) and IIb (12), and (−)-catechin 7-O-β-D-glucopyranoside (13) in the bark of *Rhaphiolepis umbellata* was confirmed.

*Rhaphiolepis umbellata* (Japanese name: Sharinbai) is an evergreen shrub native to the southern area of Japan, and is now widely cultivated throughout the country. The bark is known to contain polyphenolic compounds, so-called “tannins,” and has been used as the dyeing agent for Oshima Tsumugi, a well-known traditional textile in Japan. The tannins are considered to play an important role in this dyeing process, but no paper has been reported on the nature of the tannins in *R. umbellata*, except for those by Nishida† who demonstrated the presence of catechin. In an attempt to elucidate the mechanism of the dyeing system for Oshima Tsumugi, we first intended to clarify the chemical composition of tannins in *R. umbellata*, and identified two new procyanidin trimers (1 and 3) and one tetramer (2), together with a series of known dimeric, trimeric, tetrameric and pentameric procyanidins. This paper deals with the isolation and structural elucidation of these compounds.

The fresh bark of *Rhaphiolepis umbellata* was extracted with 80% aqueous acetone, and the extract was treated as shown in Chart 1 to yield an ethyl acetate-soluble portion which contained non-polar compounds (such as fatty acids, lipids, etc.) and a complicated mixture of lower-molecular-weight phenolics. After removing the lipids by extraction with benzene, the ethyl acetate extract was subjected to Sephadex LH-20 chromatography. Elution with ethanol and then with a mixture (9:1) of ethanold and water yielded fractions 1~3. Compounds 1 and 2 were isolated from fraction 3 by a combination of Sephadex LH-20 and high-porosity polystyrene gel (Diaion HP-20) chromatography with various solvent systems.² From fractions 2 and 3, (−)-epicatechin (4)³ and a series of procyanidin dimers [B-2 (5) and B-5 (6)],⁴ a trimer [C-1 (7)]⁴,⁵ and a tetramer [cinnamtannin A₁ (8)]⁶ were also

isolated, together with small quantities of procyanidin B-1 (9), proanthocyanidin A-2 (10), and cinchonains Ia (11) and IIb (12). These compounds were identified by comparing their spectral data with those of authentic samples. The aqueous layer, after removing the ethyl acetate-portion, was subjected to chromatography on Sephadex LH-20 and then on Diaion HP-20 to yield compound 3, (-)-catechin 7-O-β-D-glucopyranoside (13) and cinnamtannin A2 (14).

Compound 1, a pale-brown amorphous powder, [α]D +126.8° (acetone), C45H38O18·H2O, was positive to the ferric chloride and anisaldehyde-sulfuric acid reagents (dark green and orange colorations, respectively, typical of proanthocyanidins). The 1H-NMR spectrum of 1 showed three broad singlet signals at δ 4.84 (1H) and 5.10 (2H), which are ascribable to C2-H of flavan-3-ol frameworks. Moreover, these coupling constants suggest the presence of three epicatechin [C2, C3: cis] units. These findings are supported by the 13C-NMR examination which showed a pair of three sp3 signals due to C2 and C3 of the epicatechin moieties (Table I). Although the 1H-NMR signal pattern of 1 was closely correlated with that of procyanidin C-1 (7) con-
consisting exclusively of a chain of 4,8-linked epicatechin units, one of the C₂-H signals in 1 appeared at higher field (δ 4.84, Fig. 2), the chemical shift being consistent with those of (−)-epicatechin (4) and a 4,6-linked dimer, procyanidin B-5 (6). This upfield signal is assignable to C₂-H of the lower terminal unit, and indicates that the heterocyclic ring (C-ring) of the lower terminal unit is less affected magnetically by the aromatic rings in the upper units. Thus, 1 is considered to consist of three epicatechin moieties having 4,8- and 4,6-linkages, the latter existing in the lower two units. Further support for the structure was
Table I. $^{13}$C-NMR Spectral Data for Compounds 1 and 2a

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>$c_2$</td>
<td>79.1</td>
<td>79.0</td>
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<tr>
<td>$c_3$</td>
<td>66.7</td>
<td>66.7</td>
</tr>
<tr>
<td>$c_4$</td>
<td>___b</td>
<td>___b</td>
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<tr>
<td>$c_{4a}$</td>
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<td>100.4</td>
</tr>
<tr>
<td>$c_5$</td>
<td>76.6</td>
<td>76.5</td>
</tr>
<tr>
<td>$c_6$</td>
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<td>$c_8$</td>
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<td>$c_9$</td>
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<td>37.1</td>
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<tr>
<td>$c_{4a''''}$</td>
<td>100.7</td>
<td></td>
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</tbody>
</table>

a Spectra were run in acetone-$d_6$ + D$_2$O at 25.05 MHz.

obtained by the $^{13}$C-NMR analysis, showing a signal due to $c_{4a}$ of the terminal unit at a somewhat higher field ($\delta$ 100.0) than those of the upper units ($\delta$ 100.6, Table I).2)

On the basis of this evidence, compound 1 was concluded to be epicatechin-(4$\beta$-8)-epicatechin-(4$\beta$-6)-epicatechin.

Compound 2, a pale-brown amorphous powder, $[\alpha]_D + 100.7^\circ$ (acetone), C$_{60}$H$_{50}$O$_{24}$·3/2H$_2$O, showed colorations similar to those of compound 1 with the ferric chloride and anisaldehyde-sulfuric acid reagents. The $^1$H- and $^{13}$C-NMR spectra (see Fig. 2 and Table I) of 2 are closely related to those of compound 1, except for additional signals arising from one epicatechin unit, and suggest that 2 is a tetrameric procyanidin consisting entirely of epicatechin units. The chemical shift ($\delta$ 4.84) of $c_2$-H in the lower terminal unit was identical with that observed in 1, while those ($\delta$ 5.04 ~ 5.36) for three upper $c_2$-H signals were consistent with those ($\delta$ 5.00 ~ 5.25) in procyanidin C-1 (7). Thus, the structure of compound 2 is considered to be a tetrameric procyanidin in which the upper three units are bonded through 4,8-linkages, and the lower two through a 4,6-linkage. The points of the interflavanoid linkages were also shown by the $c_{4a}$ resonances which appeared at $\delta$ 100.7 (3C) and 100.4 (1C), the chemical shifts of the former being consistent with those of 4,8-linked procyanidins, and of the latter with that of a 4,6-linkage.2)

From these spectroscopic observations, compound 2 was characterized as epicatechin-(4$\beta$-8)-epicatechin-(4$\beta$-8)-epicatechin-(4$\beta$-6)-epicatechin.

Compound 3, a pale-brown amorphous powder, $[\alpha]_D - 38.0^\circ$ (acetone), C$_{45}$H$_{38}$O$_{18}$·3/2H$_2$O, showed almost the same Rf value on TLC as that of compound 1. The $^1$H-NMR spectrum was somewhat complicated due to the rotational isomerism caused by steric interaction between the interflavanoid linkages. However, owing to the existence of unequal proportions of conformers, signals arising from the major conformer were assignable. The appearance of two broad singlets at $\delta$ 5.05 and 5.27, and a doublet at $\delta$ 4.81 ($J = 10$ Hz), suggests that compound 3 is a procyanidin trimer having two epicatechin and one cate-
Procyanidins from *Rhaphiolepis umbellata*

![Diagram of compounds](image)

Fig. 3. Partial Thiolysis of Compound 3.

While procyanidins linked to [C2, C3: trans] moiety, these observations are also consistent with the 13C-NMR data which clearly showed the presence of two epicatechin C2-atoms (δ 78.9 and 76.8) and one catechin C2-atom (δ 82.9). In order to clarify the points of the interflavanoid linkages, acid-catalyzed partial degradation was attempted. Treatment of 3 with phenylmethanethiol in ethanolic acetic acid furnished two partial degradation products (3a and 3b, Fig. 3), together with 4-benzylthioethers of (-)-epicatechin and (+)-catechin. The products (3a and 3b) were found to be identical with procyanidin B-8 4-benzylthioether and procyanidin B-2, respectively, by comparing their physical and spectral data with those of authentic samples.

Thus, compound 3 was determined unequivocally to be catechin-(4α→6)-epicatechin-(4β→8)-epicatechin.

In conclusion, our chemical examination has shown that the bark of *R. umbellata* contains procyanidins (condensed tannins) consisting of (-)-epicatechin units, except for compound 3, procyanidin B-1 (9) and (-)-catechin 7-O-β-D-glucopyranoside (13). Taking the yield of each procyanidin into account, the tannins in *R. umbellata* seem to be composed of two major series of chains of linearly linked procyanidins in which the points of the interflavanoid linkages in the lower terminal units differ.

**EXPERIMENTAL**

Melting points determined on a Yanagimoto micro-melting point apparatus were uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter (cell length: 0.5 dm). 1H- and 13C-NMR spectra were recorded on JEOL PS-100 and JEOL FX-100 spectrometers, respectively, with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Abbreviations used: s, singlet; d, doublet; m, multiplet; br., broad. Column chromatography was carried out with Sephadex LH-20 (25 ~ 100 μ, Pharmacia Fine Chemicals Co., Ltd.), Diaion HP-20 (75 ~ 150 μ, Mitsubishi Chemical Industries Ltd.) or Kieselgel 60 (70 ~ 230 mesh, Merck). Thin-layer chromatography was conducted on precoated Kieselgel 60 F254 plates (0.20 mm, Merck) using (A) benzene-ethyl formate-formic acid (2:7:1), (B) benzene-ethyl formate-formic acid (1:7:1), (C) chloroform-ethyl acetate-formic acid (1:7:1), (D) chloroform-ethyl acetate-formic acid-isopropanol (1:7:1:1) or (E) benzene-acetone (4:1) as the solvent system, and spots on the plates were detected by a spray of FeCl3 reagent, p-anisaldehyde-H2SO4 reagent or 10% H2SO4.

**Isolation of proanthocyanidins.** Fresh bark of *Rhaphiolepis umbellata* (5.0 kg), collected in July 1981 from the campus of Kyushu University, was extracted at room temperature with 80% aqueous acetone. The acetone was removed by evaporation under reduced pressure, and the
resulting aqueous solution afforded precipitates, which were removed by filtration. The filtrate was successively extracted eleven times with equal volumes of ethyl acetate. The ethyl acetate layer was concentrated to dryness to yield a brown solid (154 g). This solid, after extraction with benzene, was applied to a column of Sephadex LH-20. Elution with EtOH and then with EtOH-H2O (9:1) yielded fractions 1 (20 g), 2 (85 g) and 3 (15 g).

Fraction 1 was negative to the FeCl3 reagent. Fraction 2 was recrystallized from THF-H2O. Elution with EtOH and then with EtOH-H2O (9:1) to give three further fractions: Fr. 2-1 (20.5 g), Fr. 2-2 (39.8 g) and Fr. 2-3 (23.5 g). Fraction 2-1 was negative to the FeCl3 reagent. Fraction 2-2 was applied to a column of Diaion HP-20, and elution with increasing amounts of MeOH in H2O (1:0~1:9) yielded ( )-epicatechin (4, 3.2 g) and cinchonoin A1 (11, 5.0 mg).

Fractions 2-3 and 3 were similarly chromatographed over Sephadex LH-20 with EtOH and EtOH-H2O (9:1) to give four further fractions: Fr. 3-1 was negative to the FeCl3 reagent, Fr. 3-2 (19.9 g), Fr. 3-3 (14.2 g) and Fr. 3-4 (5.8 g). Fraction 3-2 was chromatographed over Diaion HP-20, and followed by repeated Sephadex LH-20 chromatography using a variety of solvent systems (acetone, 80% MeOH and 60% MeOH) to give procyanidin B-1 (9, 135 mg), procyanidin B-2 (5, 5.05 mg), procyanidin B-5 (6, 344 mg) and proanthocyanidin A-2 (10, 119 mg). Fraction 3-3 was similarly chromatographed over Diaion HP-20 (H2O-MeOH) and Sephadex LH-20 (60% MeOH and EtOH) to yield procyanidin C-1 (7, 1.07 g), compound 1 (153 mg) and cinchonoin A1b (12, 43 mg). Cinnamminolin A1 (8, 56 mg) and compound 2 (58 mg) were obtained from Fr. 3-4 by Diaion HP-20 (H2O-MeOH) and Sephadex LH-20 (60% MeOH and EtOH) chromatography.

The aqueous layer (113 g), after concentration, was subjected to column chromatography on Sephadex LH-20. Elution first with H2O and then with H2O containing an increasing amount of MeOH yielded Frs. 1~9. Repeated chromatography of these fractions afforded compound 3 (73 mg), ( )-catechin 7-O-b-D-glucopyranoside (13, 78 mg) and cinnamminolin A2 (14, 11 mg) from Fr. 7, Fr. 2 and Fr. 8, respectively.

**Compound 1.** A pale-brown amorphous powder, [α]D+1

$\Delta \alpha = 26.8^\circ$ (c=1.15, acetone). Anal. Calcd. for C45H38O18-3/2H2O: C, 61.09; H, 4.33. Found: C, 61.24; H, 4.90. 1H-NMR (acetone-d6): 2.60~3.10 (2H, m, C2,-H), 4.00~4.30 (3H, m, C3,,-3_H), 4.70~4.80 (2H, m, C4,-H), 4.84 (1H, brs, C2,-H), 5.10 (2H, brs, C2,-H), 6.02~6.18 (4H in total, m, C6,6,,6,8,,-H), 6.64~7.18 (9H in total, m, aromatic H of B,B',B"-ring). 13C-NMR: Table I.

**Compound 2.** A pale-brown amorphous powder, [α]D+1

$\Delta \alpha = 100.7^\circ$ (c=1.13, acetone). Anal. Calcd. for C60H50O24-3/2H2O: C, 60.97; H, 4.26. Found: C, 61.29; H, 4.89. 1H-NMR (acetone-d6): 2.60~3.10 (2H, m, C2,-H), 4.00~4.16 (4H, m, C3,3,3,-3_H), 4.72~5.00 (3H, m, C4,4,4,-H), 4.84 (1H, brs, C2,-H), 5.04~5.36 (3H, m, C2,2,-H), 5.96~6.16 (5H in total, m, C6,6,,6,6,,-H), 6.60~7.24 (12H in total, m, aromatic H of B,B',B"-ring). 13C-NMR: Table I.

**Compound 3.** A pale-brown amorphous powder, [α]D+1

$\Delta \alpha = 38.0^\circ$ (c=0.76, acetone). Anal. Calcd. for C60H50O24-3/2H2O: C, 60.47; H, 4.62. Found: C, 60.61; H, 5.06. 1H-NMR (acetone-d6): 2.60~3.04 (2H, m, C2,-H), 3.98 (1H, brs, C2,-H), 4.39 (1H, m, C3,-H), 4.54 (1H, brs, C2,-H), 4.66 (1H, d, J=8 Hz, C2,-H), 4.78 (1H, brs, C2,-H), 4.81 (1H, d, J=10 Hz, C2,-H), 5.05 (1H, brs, C2,-H), 5.27 (1H, brs, C2,-H), 5.88~6.20 (4H in total, m, C6,6,,6,,-H), 6.60~7.20 (9H in total, m, aromatic H of B, B', B"-ring). 13C-NMR (acetone-d6 + D2O): 36.3 (C2), 38.1 (C2), 66.0 (C3), 72.4 (C3,3,3,3,3,7), 76.8 (C2,2,2,2), 78.9 (C2,2,2), 82.9 (C2,2,2), 96.0, 97.3 (C6,6,8,8,8-). 100.2 (C4a,4a,4a), 106.3, 106.9 (C6,6,8,8,8-).

Partial degradation of compound 3. Compound 3 (73 mg) was treated with phenylmethanethiol (1 ml) and acetic acid (1 ml) in EtOH (8 ml) under reflux for 3.5 hr. Removal of the solvent under reduced pressure left an oily residue, which was chromatographed on a column of Sephadex LH-20. Elution with chloroform-MeOH (3:1) and then with chloroform–EtOH (3:1) furnished compounds 3a (0.15 mg) and 3b (0.9 mg) as a pale-brown amorphous powder. Compound 3b was characterized as procyanidin B-2 by 1H-NMR comparison. 3a was found to be identical with procyanidin B-8 4-benzylthioether by comparison of the 1H-NMR spectrum. 1H-NMR (acetone-d6 + D2O): 3.60 (1H, m, C3,-H), 4.02 (1H, m, C3,-H), 4.09 (1H, m, C3,-H), 4.14 (2H, s, -CH2S-), 4.52 (1H, d, J=10Hz, C2,-H), 4.66 (1H, d, J=8 Hz, C2,-H), 4.78 (1H, brs, C2,-H), 4.81 (1H, d, J=10 Hz, C2,-H), 5.05 (1H, brs, C2,-H), 5.27 (1H, brs, C2,-H), 5.88~6.20 (4H in total, m, C6,6,8,8,8-). 13C-NMR: (acetone-d6 + D2O): 36.3 (C2), 38.1 (C2), 66.0 (C3), 72.4 (C3,3,3,3,3,7), 76.8 (C2,2,2,2), 78.9 (C2,2,2,2), 82.9 (C2,2,2,2), 96.0, 97.3 (C6,6,8,8,8-). 100.2 (C4a,4a,4a), 106.3, 106.9 (C6,6,8,8,8-).

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