Tannins and Related Compounds. CXIX.[1] Samarangenins A and B, Novel Proanthocyanidins with Doubly Bonded Structures, from *Syzygium samarangens* and *S. aqueum*

Gen-ichiro NONAKA,* Yukari AIKO, Kousuke ARITAKE, and Itsuo NISHIOKA

Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan. *Received April 16, 1992*

Two proanthocyanidins named samarangenins A (1) and B (2) have been isolated, together with a variety of flavan-3-ols, proanthocyanidins and hydrolyzable and complex tannins, from the leaves of *Syzygium samarangens* (BLUME) MERR. *et* PERRY and *S. aqueum* (BURM f.) ALSTON (=*Eugenia aquea* BURM.) (Myrtaceae). Compounds 1 and 2 were characterized on the basis of spectroscopic and chemical evidence as novel dimeric proanthocyanidins, in which two flavanoid units are doubly linked through ether and carbon–carbon bonds.

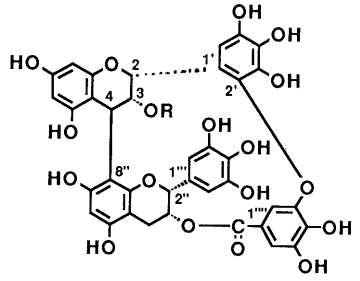
Keywords *Syzygium samarangens*; *Syzygium aqueum*; Myrtaceae; proanthocyanidin; prodelphinidin; samarangenin A; samarangenin B; (−)-epigallocatechin; (−)-epigallocatechin 3-*O*-gallate; tannin

As a part of our chemical examinations of tannins in Myrtaceous plants, we previously reported the isolation of ellagitannins and complex tannins from *Syzygium aromaticum* MERR. *et* PERRY,[2] *Eugenia grandis* WIGHT[3] and *Psidium guajava* L.[4] In a continuation of those studies, we have now examined *Syzygium samarangens* (BLUME) MERR. *et* PERRY and *S. aqueum* (BURM. f.) ALSTON (=*Eugenia aquea* BURM.), which are cultivated widely in Southeast Asia for their palatable fruits and medicinal uses. We have isolated two novel doubly linked proanthocyanidins named samarangenins A (**1**) and B (**2**), and we wish to describe herein the isolation and structure elucidation of these compounds.

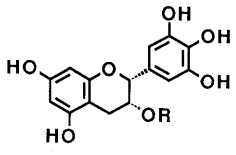
The air-dried leaves of *S. samarangens* and *S. aqueum*, collected in Taiwan and Indonesia, respectively, were extracted with 80% aqueous acetone. The extracts were repeatedly chromatographed on Sephadex LH-20, MCI-gel CHP 20P, Prep pak 500/C_{18} and Bondapak C_{18}/Porasil B to yield samarangenins A (**1**) and B (**2**) and structurally related compounds, (−)-epigallocatechin (**3**),[5] (−)-epigallocatechin 3-*O*-gallate (**4**)[5] and prodelphinidin B-2 3,3″-di-*O*-gallate (**5**),[5] together with a variety of hydrolyzable and complex tannins described in the experimental section.

Samarangenin A (**1**) gave, with the anisaldehyde–sulfuric acid reagent,[6] an orange coloration characteristic of a flavan skeleton. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** exhibited flavan H-2—H-4 signals at δ 4.52 (1H, s), 5.04 (1H, t-like, J=2 Hz), 3.10 (1H, br d, J=16 Hz) and 2.76 (1H, dd, J=2, 16 Hz), respectively, the coupling modes being similar to those of **4**. The lowfield shift of the H-3 signal indicated the presence of an acyl group at this position. Furthermore, the appearance of aliphatic signals at δ 6.22 (1H, s), 4.62 (1H, d-like, J=2 Hz) and 4.33 (1H, d, J=2 Hz), assignable to flavan H-2, H-4 and H-3, respectively, based on ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), indicated the presence of a 4-substituted flavan ring with 2,3-*cis* and 3,4-*trans* configurations.[7] These couplings were quite consistent with those of prodelphinidin B-2 3″-*O*-gallate (**6**).[5] In the aromatic field, the phloroglucinol A-ring signals appeared as a pair of *meta*-coupled doublets at δ 5.70 and 5.82 (each J=2 Hz) and a one-proton singlet at δ 6.08, suggesting that the two flavan units are linked at the C-4/C-8 or C-4/C-6 positions, analogous to those of common proanthocyanidins.[7] The flavan B-ring signals were observed as a two-proton singlet at δ 6.22 and a one-proton singlet at δ 6.84. These findings, coupled with the appearance of two mutually *meta*-coupled aromatic doublets at δ 7.04 and 7.58 (each J=2 Hz), suggested that one of the pyrogallol B-rings is connected through an ether linkage with the galloyl group located at the lower flavan C-3 position. This was supported by negative fast atom bombardment mass spectroscopy (FAB-MS) which showed the $(M-H)^-$ peak at m/z 759.

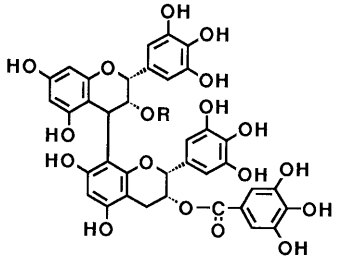
The ether linkage was determined to be located between the upper flavan C-2′ and the galloyl C-3 positions on the basis of the upfield shift of the C-1′ signal (δ 124.7) in the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum, and also by observation of a cross peak between the upper flavan H-2 signal (δ 6.22) and the aromatic singlet at δ 6.84 in the ^{1}H–^{1}H COSY spectrum. On the other hand, the position of the carbon–carbon bond between the two flavanoid units was confirmed by two-dimensional nuclear



1 : R=H
2 : R=galloyl



3 : R=H
4 : R=galloyl



5 : R=galloyl
6 : R=H

Chart 1

Overhauser effect (NOE) spectroscopy, which clearly showed a correlation between the flavan H-2 (δ 6.22) and the galloyl H-2 (δ 7.58) signals; examination of the Dreiding model showed that only in the case of the C-4/C-8 linkage is the approach of these protons possible. On the basis of these findings, the structure of samarangenin A was concluded to be as shown by the formula **1**. Evidence for the absolute configurations could not be obtained, but taking into account the negative sign of the specific optical rotation [$-218.4°$ (acetone)],[8] as well as the co-occurrence of compounds **3**, **4** and **5** in these plant materials, the formula **1** was considered to represent the absolute stereostructure.

The ^{1}H-NMR spectrum of samarangenin B (**2**) was closely related to that of **1**, but differed significantly in the fairly lowfield shift of the flavan H-3 signal (δ 5.56, br s), and also in the observation of a two-proton aromatic singlet at δ 7.00 attributable to a galloyl ester group. These findings clearly indicated that **2** possesses a galloyl group at the C-3 position in the upper flavan unit, and this was consistent with the negative FAB-MS, showing the $(M-H)^-$ peak at m/z 911.

Final structural confirmation was obtained by partial hydrolysis of **2** with tannase, which yielded **1** and gallic acid. The structure of samarangenin B was thus determined to be as represented by the formula **2**.

This is the first isolation of doubly bonded proanthocyanidins which possess an ether linkage formed by an oxidative pyrogallol–pyrogallol coupling. It should be noted that these compounds were not isolated from other Myrtaceous plants, *Syzygium cumini* and *Eugenia polyantha*.

Experimental

Melting points were determined on a micro-melting point apparatus (Yanagimoto) and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL FX-100 instrument, and ^{1}H–^{1}H COSY and NOESY spectra were measured with a JEOL GX-270 spectrometer. FAB-MS were recorded on a JEOL JMS DX-300 machine with glycerol as the matrix. Column chromatography was carried out with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP 20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.), Prep pak 500/C_{18} (37—75 μ, Waters Associates, Inc.) and Bondapak C_{18}/Porasil B (37—75 μ, Waters Associates, Inc.). Thin-layer chromatography was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck) with benzene–ethyl formate–formic acid (1 : 7 : 1), and spots were detected by the use of anisaldehyde–sulfuric acid and ferric chloride reagent sprays.

Isolation from *S. samarangens* The air-dried leaves (1.25 kg) of *S. samarangens*, collected in Ping-tung, Taiwan, were extracted five times with 80% aqueous acetone at room temperatue. After concentration of the extract, the resulting precipitates were removed by filtration, and the filtrate was subjected to Sephadex LH-20 chromatography. Elution first with water gave non-phenolic compounds, and stepwise gradient elution with water containing increasing amounts of methanol afforded three fractions consisting of tannins. The first fraction was rechromatographed over MCI-gel CHP 20P with 10% aqueous methanol to furnish grandinin (180 mg),[9] while the second fraction yielded castalagin[10] (210 mg) and vescalagin[10] (110 mg) on repeated chromatographies over Sephadex LH-20 (80% aqueous MeOH) and MCI-gel CHP 20P (20—30% aqueous methanol). Chromatography of the last fraction over Sephadex LH-20 with 80% aqueous methanol afforded samarangenin B (**2**) (60 mg) and a fraction containing a complex mixture of ellagitannins, flavan-3-ols and proanthocyanidins, which was repeatedly chromatographed over MCI-gel CHP 20P, Bondapak C_{18}/Porasil B and Prep pak 500/C_{18} with water containing an increasing proportion of methanol to give pedunculagin[11] (30 mg), (−)-epigallocatechin 3-*O*-gallate[5] (**4**) (31 mg), epicatechin 3-*O*-gallate[5] (13 mg), (−)-epigallocatechin[5] (**3**) (15 mg), prodelphinidin B-2 3,3″-di-*O*-gallate (**5**) (25 mg) and samarangenin A (**1**) (105 mg).

Isolation from *S. aqueum* The dried leaves (3.3 kg) of *S. aqueum*, collected at Desa Gekbrong, Cianjur, Indonesia, were extracted with 80% aqueous acetone at room temperature. The extract was concentrated *in vacuo*, and applied to a Sephadex LH-20 column. Elution in the same way as described above yielded three fractions containing tannins. The first fraction was sujected to chromatography over MCI-gel CHP 20P with aqueous methanol to give grandinin (418 mg). The second fraction was rechromatographed over MCI-gel CHP 20P (20% aqueous methanol) and then over Sephadex LH-20 with ethanol to furnish castalagin (3.6 g), vescalagin (460 mg), eugenigrandin A[4] (70 mg) and acutissimin A[12] (75 mg). Repeated chromatography of the third fraction over Bondapak C_{18}/Porasil B and Prep pak 500/C_{18} with water containing increasing proportions of methanol yielded pedunculagin (140 mg), eugeniin[2] (65 mg), 1-β-*O*-galloyl pedunculagin[13] (140 mg), samarangenins B (**2**) (122 mg) and A (**1**) (335 mg), prodelphinidin B-2 3,3″-di-*O*-gallate (**5**) (21 mg), (−)-epigallocatechin (**3**) (12 mg) and (−)-epigallocatechin 3-*O*-gallate (**4**) (17 mg).

Samarangenin A (1) A brown amorphous powder, $[\alpha]_D^{27}$ $-218.4°$ ($c=0.6$, acetone). *Anal.* Calcd for $C_{37}H_{28}O_{18}\cdot 2H_2O$: C, 55.78; H, 4.02. Found: C, 55.92; H, 4.05. Negative FAB-MS m/z: 759 $(M-H)^-$. ^{1}H-NMR (acetone-d_6+D_2O) δ: 2.76 (1H, dd, $J=2$, 16 Hz, H-4″), 3.10 (1H, br d, $J=16$ Hz, H-4″), 4.33 (1H, d, $J=2$ Hz, H-3), 4.52 (1H, s, H-2″), 4.62 (1H, d-like, $J=2$ Hz, H-4), 50.4 (1H, t-like, $J=2$ Hz, H-3″), 5.70 (1H, d, $J=2$ Hz, H-6), 5.82 (1H, d, $J=2$ Hz, H-8), 6.08 (1H, s, H-6″), 6.22 (2H, s, H-2‴, 6‴), 6.22 (1H, s, H-2), 6.84 (1H, s, H-6′), 7.04 (1H, d, $J=2$ Hz, H-6⁗), 7.58 (1H, d, $J=2$ Hz, H-2⁗). ^{13}C-NMR (acetone-d_6+D_2O) δ: 31.4 (C-4″), 36.4 (C-4), 70.4, 71.1 (C-3, 3″), 73.1 (C-2), 79.0 (C-2″), 95.7, 96.2 (C-6, 8, 6″), 98.8 (C-4a), 103.2 (C-4a″), 106.9 (C-2‴, 6‴), 107.7 (C-8″), 107.5, 111.9, 119.9 (C-6′, 2⁗, 6⁗), 121.3 (C-1⁗), 124.7 (C-1′), 130.2 (C-1‴), 132.7, 134.3, 138.5, 139.9, 142.5, 145.4 (×2), 147.3 (×2), 148.0 (C-2′, 3′, 4′, 5′, 3‴, 4‴, 5‴, 3⁗, 4⁗, 5⁗), 155.3, 155.5, 155.7, 155.9, 156.1, 157.1 (C-5, 7, 8a, 5″, 7″, 8a″), 166.0 (–COO–).

Samarangenin B (2) A brown amorphous powder, $[\alpha]_D^{27}$ $-246.9°$ ($c=0.7$, acetone). *Anal.* Calcd or $C_{44}H_{32}O_{22}\cdot 3H_2O$: C, 54.66; H, 3.93. Found: C, 54.48; H, 4.05. Negative FAB-MS m/z: 911 $(M-H)^-$. ^{1}H-NMR (acetone-d_6+D_2O) δ: 2.80 (1H, dd, $J=2$, 16 Hz, H-4″), 3.12 (1H, br d, $J=16$ Hz, H-4″), 4.58 (1H, s, H-2″), 4.64 (1H, br s, H-4), 5.08 (1H, t-like, $J=2$ Hz, H-3″), 5.56 (1H, br s, H-3), 5.74 (1H, d, $J=2$ Hz, H-6), 5.94 (1H, d, $J=2$ Hz, H-8), 6.09 (1H, s, H-6″), 6.24 (2H, s, H-2‴, 6‴), 6.44 (1H, s, H-2), 7.00 (2H, s, galloyl H), 7.04 (1H, d, $J=2$ Hz, -6⁗), 7.05 (1H, s, H-6′), 7.62 (1H, d, $J=2$ Hz, H-2⁗). ^{13}C-NMR (acetone-d_6+D_2O) δ: 34.5 (C-4), 70.6, 71.4 (C-3, 3″), 73.9 (C-2), 79.3 (C-2″), 95.7 (×2), 97.0 (C-6, 8, 6″), 99.3 (C-4a″), 101.6 (C-4a), 106.8 (C-8″), 107.1 (×2) (C-2‴, 6‴), 107.8, 112.4, 121.0 (C-6′, 2⁗, 6⁗), 110.2 (galloyl C-2, 6), 121.0 (C-1⁗, galloyl C-1), 123.5 (C-1′), 130.1 (C-1‴), 132.8, 134.4, 138.3, 139.4, 142.6, 145.6 (×3), 145.8, (×3), 147.1, 147.9 (C-2′, 3′, 4′, 5′, 3⁗, 4⁗, 5⁗, 3⁗⁗, 4⁗⁗, 5⁗⁗, galloyl C-3, 4, 5), 156.0, 156.5, 156.9 (each 2C, C-5, 7, 8a, 5″, 7″, 8a″), 165.7, 167.8 (–COO–). The C-4″ signal was overlapped with solvent signals.

Tannase Hydrolysis of 2 A solution of **2** (30 mg) in water (2 ml) was shaken with tannase at room temperature for 30 min. The mixture was concentrated to dryness, and the residue was treated with ethanol. The ethanol-soluble portion was subjected to Sephadex LH-20 chromatography with ethanol to give gallic acid (8 mg) and a hydrolysate (12 mg), which was found to be identical with samarangenin A (**1**) by physical and spectral comparisons.

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