Tannins and Related Compounds. CXIX. 1) Samarangenins A and B, Novel Proanthocyanidins with Doubly Bonded Structures, from Syzygium samarangensis and S. aquum

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Two proanthocyanidins named samarangenins A (1) and B (2) have been isolated, together with a variety of flavan-3-ols, proanthocyanidins and hydroxylated and complex tannins, from the leaves of Syzygium samarangensis (BLUME) MERR. et PERRY and S. aquum (Burm. f.) ALSTON (= Eugenia aqua BURM.). 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 samarangensis; Syzygium aquum; Myrtaceae; proanthocyanidin; prodelphinidin; samarangenin A; samarangenin B; (−)-epigallocatechin; (−)-epigallocatechin 3-O-gallate; tannin

As a part of our chemical examinations of tannins in Myrtaceaeous plants, we previously reported the isolation of ellagittannins and complex tannins from Syzygium aromatcum MERR. et PERRY, 3) Eugenia grandis WIGHT and Psidium guajava L. 4) In a continuation of those studies, we have now examined Syzygium samarangensis (BLUME) MERR. et PERRY and S. aquum (Burm. f.) ALSTON (= Eugenia aqua 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 structural elucidation of these compounds.

The air-dried leaves of S. samarangensis and S. aquum, 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 C 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 hydroxylizable 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 flavon skeleton. The 1H-nuclear magnetic resonance (1H-NMR) spectrum of 1 exhibited flavan H-2–H-4 signals at δ 4.52 (1H, s), 5.04 (1H, t, 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 alphabetic signals at δ 6.22 (1H, s), 4.62 (1H, d, J = 2 Hz) and 4.33 (1H, d, J = 2 Hz), assignable to flavan H-2, H-4 and H-3, respectively, based on 1H–1H shift correlation spectroscopy (1H–1H 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). 3) 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 13C-nuclear magnetic resonance (13C-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 1H–1H COSY spectrum. On the other hand, the position of the carbon–carbon bond between the two flavanoid units was confirmed by two-dimensional nuclear
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 samaragenin 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 1H-NMR spectrum of samaragenin 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, brs), 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 samaragenin 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 Myrtaceae 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. 1H- and 13C-NMR spectra were obtained with a JEOL FX-100 instrument, and 1H-13C 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 (15−150 μ, Mitsubishi Chemical Industries, Ltd.), Prep pak 500 μ C18 (37−75 μ, Waters Associates, Inc.) and Bondapak C18 (37−75 μ, Waters Associates, Inc.). Thin-layer chromatography was performed on precoated Kieselgel 60F254 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 temperature. 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 11) (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 samaragenin 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 C18, Porasil B and Prep pak 500C18 with water containing an increasing proportion of methanol to give pedunculagin 11) (30 mg), (+)-epigallocatechin 3-O-gallate 5) (41 mg), epicatechin 3-O-gallate 5) (13 mg), (+)-epigallocatechin 5) (15 mg), prodelphinidin B-2 3,3'-di-O-gallate 5) (25 mg) and samaragenin A (1) (105 mg).

References


