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SYZYGININS A AND B, TWO ELLAGITANNINS FROM *SYZYGIUM AROMATICUM*

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Key Word Index—*Syzygium aromaticum*; Myrtaceae; leaves; tannins; tergallic acid.

Abstract—Two new ellagitannins, named syzyginins A and B, were isolated from the leaves of clove (*Syzygium aromaticum*.). Syzyginin A was characterized as 1,2,3-tri-*O*-galloyl-4,6-(*S*)-tergalloyl-β-D-glucopyranose. Syzyginin B was a novel hydrolysable tannin possessing a new aromatic acyl group, syzygyl, which has a dibenzo-1,4-dioxin structure probably derived from a tergalloyl group. Structures were established on the basis of chemical and spectroscopic evidence. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Previously, we reported the isolation and structural elucidation of a eugenol glucoside gallate, a chromone-*C*-glycoside, and galloyl and hexahydroxydiphenoyl (HHDP) esters of 2,4,6-trihydroxyacetophenone 3-*C*-β-D-glucopyranoside, from the leaves of clove (*Syzygium aromaticum*) [1]. The major phenolic constituent of this species is eugeniin (**3**) [2], which was accompanied by many structurally and biosynthetically related ellagitannins. In a continuing chemical examination of the tannins from clove, we have isolated two new ellagitannins, syzyginins A (**1**) and B (**2**), as minor constituents which have tergalloyl and a novel group, syzygyl, as component acyl groups. The present paper deals with the isolation and characterization of these tannins.

RESULTS AND DISCUSSION

Air-dried leaves collected in Indonesia were extracted with aqueous acetone. The extract was first fractionated by Sephadex LH-20 chromatography with water containing increasing proportions of methanol, to yield six fractions. The last fraction was further subjected to a combination of chromatography over MCI-gel CHP 20P, Avicel cellulose, Sephadex LH-20 and various ODS columns to yield syzyginin A, while similar chromatography of the third fraction provided syzyginin B.

Syziginin A (**1**) was isolated as a white amorphous powder and gave a dark blue coloration with ethanolic $FeCl_3$ reagent. The ^1H NMR spectrum was closely related to that of eugeniin (**3**), exhibiting two-proton aromatic singlets (δ 7.01, 7.02 and 7.13) due to three galloyl groups and aliphatic proton signals arising from a fully acylated glucopyranose moiety. However, three one-proton aromatic singlets were observed in contrast to the two singlets due to the HHDP group in the spectrum of compound **3**. In addition, the ^{13}C NMR spectrum of **1** exhibited signals arising from six aromatic rings accompanied by six carboxyl carbon signals. Furthermore, the negative FAB-mass spectrum showed a $[M-H]^-$ peak at *m/z* 1105 which is 168 mu larger than that of compound **3**. These observations suggested that compound **1** possessed an additional gallic acid moiety compared with compound **3**.

Methylation of **1** with Me_2SO_4–K_2CO_3 yielded methylate **1a** (*m/z* 1358, $[M]^+$), which was subsequently methanolysed to give compound **1b** together with methyl trimethoxybenzoate. Product **1b** was identified as trimethyl octamethyltergallate [3] by ^1H NMR comparison; its negative $[\alpha]_D$ value (−20°) indicated that the atropisomerism of the biphenyl bond is *S*. The location of the ester groups in compound **1** was determined on the basis of following spectroscopic evidence. In the C–H long-range COSY spectrum (5 Hz), the three two-proton singlets due to galloyl groups were correlated with the ester carbon signals at δ 164.9, 165.6 and 166.3, which was also coupled with glucose H-1 (δ 6.23), H-2 (δ 5.62) and H-3 (δ 5.89) signals, respectively, confirming the location of the galloyl groups. The carbon signal at δ 131.9 attributable to the C-2′ of tergalloyl B ring [3] was correlated with the aromatic proton signal at δ 6.64 (H-3′). This signal is correlated with the ester carbon signal at δ 167.8, which was coupled with glucose H-6 (δ 5.37). The remaining glucose H-4 signal (δ 5.28) showed a correlation peak between the ester carbon signal at δ 167.7, which was coupled with H-3 (δ 6.54) of the

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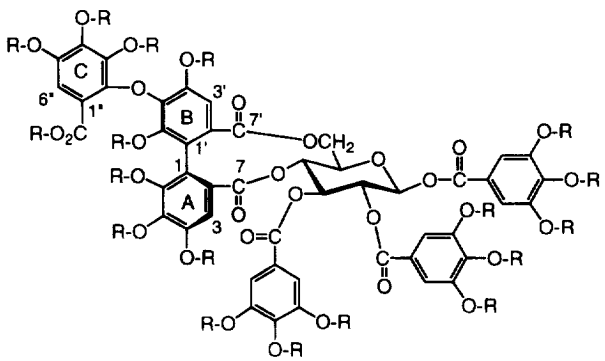
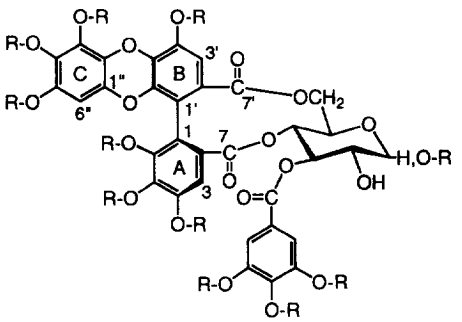
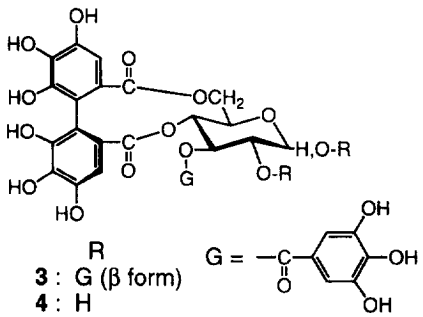


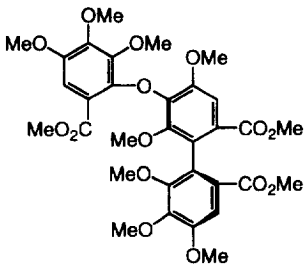
1 : R = H
1a : R = Me



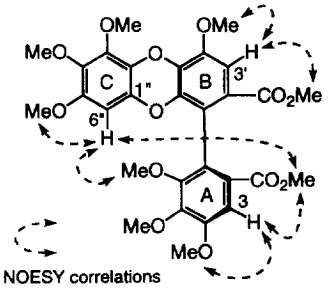
2 : R = H
2a : R = Me (α form)



R
3 : G (β form)
4 : H



1b



NOESY correlations

2b

tergalloyl A ring. The carbonyl carbon signal at δ 170.6 did not correlate with any protons, except for H-3″ of tergalloyl C ring. Thus, syzyginin A was determined to be 1,2,3-tri-*O*-galloyl-4,6-(*S*)-tergalloyl-β-D-glucopyranose, as shown in structure **1**.

Syzyginin B (**2**) was isolated as a white powder, and gave a reddish orange coloration with anisaldehyde–sulphuric acid and a dark blue coloration with $FeCl_3$ reagents. The ^1H NMR spectrum was closely related to that of 3-*O*-galloyl-4,6-(*S*)-HHDP-D-glucopyranose (gemin D, **4**) [4]; in particular, the signals arising from the sugar moiety were almost superimposable. The signals at δ 7.025 and δ 7.033 suggested the presence of a galloyl group and those at δ 6.45, 6.46, 6.69 and 6.70, resembled those due to the HHDP group of compound **4**. The most significant difference between the spectra of compounds **2** and **4** was the appearance of aromatic singlets at δ 5.836 and 5.842 in **2**. This proton signal showed ^2J, ^3J and ^4J correlation peaks with five aromatic carbons in the HMBC spectrum [5] (Table 1). The remaining aromatic protons were correlated with ester carboxyl and aromatic carbons (Table 1) indicating the partial structure related to the HHDP group. In addition, the M_r of **2**, which was revealed by the $[M-H]^-$ peak at m/z 755 in the negative FAB mass spectrum, was 122 mu larger than that of compound **5**. These observations suggested that compound **3** has an additional pentasubstituted aromatic ring, compared with the molecule of compound **4**.

Methylation of **2** with Me_2SO_4 and K_2CO_3 in acetone yielded an undecamethyl ether (**2a**), which was subsequently methanolysed with sodium methoxide in methanol to yield a methanolysate **2b**, along with methyl trimethoxybenzoate. The ^1H NMR spectrum of compound **2b** showed three aromatic singlets (δ 7.36, 7.31 and 5.94) and aliphatic singlets due to nine methoxyl groups (δ 3.62, 3.63(×2), 3.70, 3.80, 3.96(×2), 3.98 and 4.02). Taking into account the $[M]^+$ peak at m/z 586 in the EI-mass spectrum and the above-mentioned HMBC correlations in compound **2**,

Table 1. Long-range ^1H–^{13}C correlations (HMBC, $J = 8$ Hz) observed for the aromatic protons of compound **2** (in Me_2CO-d_6)

Proton signals (δ)	Correlated ^{13}C signals (ppm)
δ 5.836 and 5.842 (C-ring H-6″)	142.1(C-1″), 135.4(C-5″), 135.1(C-3″) 130.3(C-2″), 124.5(C-4″)
δ 6.45 and 6.46 (A-ring H-3)	167.8(C-7), 145.4(C-4), 136.0(C-5), 113.7(C-1)
δ 6.69 and 6.70 (B-ring H-3′)	167.4(C-7′), 145.2(C-4′), 132.8(C-5′), 115.7(C-1′)
δ 7.025 and 7.033 (galloyl-H)	166.8 and 166.5, 145.7, 138.6, 121.5

these observations indicated the presence of a dibenzo-1,4-dioxin partial structure in compound **2b**. There are four possible structures for compound **2b**. In the NOESY spectrum of compound **2b**, the aromatic signals at δ 7.31 and δ 7.36 (H-3 and 3′) showed strong NOE correlations with neighbouring methoxyl groups (δ 3.96 and δ 3.98, respectively) and weak ones with carbomethoxyl groups (δ 3.63 and δ 4.02, respectively), indicating that the positon of dioxane ring is C-5′,6′. Conversely, H-6″ (δ 5.94) showed a strong NOE correlation with the methoxyl signal at δ 3.70, due to the neighbouring methoxyl group, and two weak correlations with signals at δ 3.80 and δ 3.63. Although the two methyl signals overlapped at δ 3.63, the chemical shift coincided with that of the carbomethoxyl group attached to the A-ring, which correlated with H-3. These observations strongly suggested that H-6″ correlated with the carbomethoxyl and methoxyl groups attached to C-2 and C-6 of the A-ring in compound **2b**. Thus, the plane structure of this methanolysate was concluded to be that shown in compound **2b**. The atropisomerism of the biphenyl bond was deduced to be *S* on the basis of the negative $[\alpha]_D$ value (−17°), which is similar to that of **1b** and dimethyl hexamethoxydiphenate [6]. This free carboxylic acid is new and hence named syzygic acid. In the molecule of compound **2**, the location of the syzygyl esters was determined as shown in formula **2**, because the HMBC spectra of compound **2** showed long-range coupling between glucose H-4 [4.98 (α-glc), 5.01 (β-glc)] and syzygyl C-7 (δ 167.8), and between glucose H-6 [5.19 (α-glc), 5.21 (β-glc)] and syzygyl C-7′ (δ 167.4). From this spectroscopic and chemical evidence, the structure of syzyginin B was deduced to be that shown in formula **2**.

The syzygyl group is considered to be biosynthesized from the tergalloyl moiety; the occurrence of decarboxylation at the C ring of a tergalloyl group was previously observed [7] in weakly alkaline conditions. This group was, therefore, considered to be formed by subsequent intramolecular oxidative coupling between the C-6′ hydroxyl group and C-1″.

EXPERIMENTAL

General. ^{1}H and ^{13}C MMR spectra were recorded at 100, 270 and 500 MHz, and 25 and 125 MHz, respectively. Chemical shifts are given in δ (ppm) with TMS as int. standard. Negative FAB MS was measured at 1.5 kV (accelerating voltage) with MeOH–glycerol as matrix, EI MS as 30 eV (ionizing voltage). CC was carried out on Sephadex LH-20 (25–100 μm, Pharmacia). MCI-gel CHP20P (Mitsubishi Chemical Co.), TSK-gel Toyopearl HW40F(TOSOH), Avicel cellulose (Funakoshi), Bondapak C_{18}/Porasil B (Waters), Kiesel gel 60 (Merck), Cosmosil 75C_{18}-OPN (Nacalai tesque) and Chromatorex ODS (Fuji Silysia). TLC was conducted on precoated silica gel 60 F_{254} plates and precoated cellulose F_{254} plates; spots were detected under UV and by spraying with ethanolic $FeCl_3$ reagent (for phenolics) and dil. H_2SO_4, followed by heating (for Me ethers). Leaves of *S. aromaticum* (Merril et Perry) were collected in Cianjur, Indonesia.

Extraction and isolation. Dried and crushed leaves (4 kg) were extracted with Me_2CO–H_2O (7:3). After evapn of Me_2CO, the resulting ppt was removed by filtration. The filtrate was further concd and applied to a Sephadex LH-20 column with H_2O. Elution with H_2O containing increasing amounts of MeOH yielded six frs [1]. The third fr. was repeatedly chromatographed over MCI-gel CHP20P (H_2O–MeOH), Avicel cellulose (2% HOAc), Cosmosil 75C_{18}-OPN(H_2O–MeOH) and TSK gel Toyopearl HW40F (H_2O-MeOH) to yield compounds **2** (523 mg) and **4** (4.5 g). Similar chromatographic procedures on the last fr. gave compound **1** (390 mg).

Syzyginin A. Amorphous powder. $[\alpha]_D$ +21° (MeOH *c* 1.0). Negative FAB-MS *m/z*: 1105 $[M-H]^-$. ^{1}H NMR ($Me_2CO-d_6 + D_2O$): δ 3.93 (1H, *d*, *J* = 13 Hz, glc-H-6), 4.59 (1H, *dd*, *J* = 6, 10 Hz, glc-H-5), 5.28 (1H, *t*, *J* = 10 Hz, glc-H-4), 5.37 (1H, *dd*, *J* = 6, 13 Hz, glc-H-6), 5.62 (1H, *dd*, *J* = 8, 10 Hz, glc-H-2), 5.89 (1H, *t*, *J* = 10 Hz, glc-H-3), 6.23 (1H, *d*, *J* = 8 Hz, glc-H-1), 6.54 (1H, *s*, A-ring H-3), 6.64 (1H, *s*, B-ring H-3′), 6.98 (1H, *s*, C-ring H-6″), 7.01 (2H, *s*, 3-*O*-galloyl-H), 7.02 (2H, *s*, 2-*O*-galloyl-H), 7.13 (2H, *s*, 1-*O*-galloyl-H). ^{13}C NMR (Me_2CO-d_6): δ 63.4 (glc-C-6), 70.7 (glc-C-4), 71.8 (glc-C-2), 72.9 (glc-C-5), 73.2 (glc-C-3), 93.7 (glc-C-1), 108.0 (A-ring C-3), 108.7 (2C)(B-ring C-3′, C-ring C-6″), 110.2(2C), 110.4(4C)(galloyl C-2, 6), 114.3 (C-ring C-1″), 115.5 (B-ring C-1′), 116.0 (A-ring C-1), 119.8 (1-*O*-galloyl C-1), 120.4 (2-*O*-galloyl C-1), 120.5 (3-*O*-galloyl C-1), 125.2 (A-ring C-2), 131.9 (B-ring C-2′), 136.6 (B-ring C-5′), 137.4 (A-ring C-5), 139.2, 139.4(2C), 139.8, 140.0, 140.5, 142.3 (galloyl C-4, C-ring C-2″, 3″, 4″, 5″), 144.4 (A-ring C-6), 145.4 (A-ring C-4), 145.8(2C) (2-*O*-galloyl C-3, 5), 145.9(2C) (3-*O*-galloyl C-3, 5), 146.1(2C) (1-*O*-galloyl C-3, 5), 149.2 (B-ring C-4′), 149.6 (B-ring C-6′), 164.9 (1-*O*-galloyl C-7), 165.6 (2-*O*-galloyl C-7), 166.3 (3-*O*-galloyl C-7), 167.7 (A-ring C-7), 167.8 (B-ring C-7′), 170.6 (C-ring C-7″). (Found: C, 49.52; H, 3.37 $C_{48}H_{34}O_{31} \cdot 3H_2O$ requires: C, 49.67; H, 3.47%.)

Methylation of **1**. A mixt. of compound **1** (150 mg), Me_2SO_4 (2 ml), K_2CO_3 (2 g) in dry Me_2CO (20 ml) was heated under reflux for 1 hr. After removal of inorganic ppt, the soln was concd to a syrup which was applied to silica gel CC. Elution with benzene–Me_2CO (9:1) yielded the methylate **1a** (140 mg) as an amorphous powder. $[\alpha]_D$ +12° ($CHCl_3$; *c* 0.8). FAB-MS *m/z*: 1358 $[M]^+$. ^{1}H NMR($CDCl_3$): δ 3.47–3.90 (OMe), 4.07 (1H, *d*, *J* = 13 Hz, glc-H-6), 4.33 (1H, *dd*, *J* = 6, 10 Hz, glc-H-5), 5.43 (2H, *m*, glc-H-4, 6), 5.78 (2H, *m*, glc-H-2, 3), 6.12 (1H, *d*, *J* = 8 Hz, glc-H-1), 6.66, 6.80, 7.19 (each 1H, *s*), 7.17 (4H, *s*), 7.32 (2H, *s*).

Methanolysis of **1a**. Compound **1a** (30 mg) was treated with 2% NaOMe in MeOH (5 ml) at room temp. for 12 hr. The mixt. was neutralized with Amberlite IR 120B (H^+ form) and dried *in vacuo*. The residue

was separated by silica gel CC with benzene–Me_2CO (19:1–9:1) to yield Me trimethoxybenzoate (13 mg) and trimethyl octamethyltergallate (**1b**, 4.8 mg). Syrup. $[\alpha]_D$ −20° ($CHCl_3$; *c* 0.5). ^1H NMR ($CDCl_3$): δ 3.38, 3.61, 3.62, 3.63 (×2), 3.76, 3.81, 3.88 (×2), 3.91, 3.93 (each 3H, *s*, OMe), 7.18, 7.34, 7.39 (each 1H, *s*).

Syzyginin B (**2**). Powder (H_2O), mp 220° (decomp.). $[\alpha]_D$ +43° (MeOH; *c* 0.8). Negative FAB-MS *m/z*: 755 $[M-H]^-$. ^1H NMR (Me_2CO-d_6): δ 3.64 (2/5H, *dd*, *J* = 8, 9 Hz, β-glc-H-2), 3.78 (3/5H, *d*, *J* = 13 Hz, α-glc-H-6), 3.84 (3/5H, *dd*, *J* = 4, 10 Hz, α-glc-H-2), 3.85 (2/5H, *d*, *J* = 13 Hz, β-glc-H-6), 4.13 (2/5H, *dd*, *J* = 6, 10 Hz, β-glc-H-5), 4.58 (3/5H, *dd*, *J* = 6, 10 Hz, α-glc-H-5), 4.77 (2/5H, *d*, *J* = 8 Hz, β-glc-H-1), 4.98 (3/5H, *t*, *J* = 10 Hz, α-glc-H-4), 5.01 (2/5H, *t*, *J* = 10 Hz, β-glc-H-4), 5.19 (3/5H, *dd*, *J* = 6, 13 Hz, α-glc-H-6), 5.21 (2/5H, *dd*, *J* = 6, 13 Hz, β-glc-H-6), 5.29 (3/5H, *d*, *J* = 4 Hz, α-glc-H-1), 5.35 (2/5H, *dd*, *J* = 9, 10 Hz,m β-glc-H-3), 5.53 (3/2H, *t*, *J* = 10 Hz, α-glc-H-3), 5.836, 5.842 (1H in total, each *s*, C-ring H-6″), 6.45, 6.46 (1H in total, each *s*, A-ring H-3), 6.69, 6.70 (1H in total, each *s*, B-ring H-3′), 7.025, 7.033 (2H in total, each *s*, galloyl-H). ^{13}C NMR (Me_2CO-d_6): 64.2 (α,β-glc-C-6), 67.3 (α-glc-C-5), 71.3 (α,β-glc-C-4), 71.8 (β-glc-C-5), 72.1 (α-glc-C-2), 74.2 (α-glc-3), 74.8 (β-glc-2), 75.6 (β-glc-3), 94.0 (α-glc-1), 95.3 (C-ring C-6″), 99.0 (β-glc-C-1), 108.1 (A-ring C-3), 110.1 (2C) (galloyl C-2, 6), 110.3 (B-ring C-3′), 113.7 (A-ring C-1), 115.7 (B-ring C-1′), 121.5 (galloyl C-1), 124.5 (C-ring C-4″), 125.6 (A-ring C-2), 130.3 (C-ring C-2″), 130.7 (B-ring C-2′), 132.8 (B-ring C-5′), 135.1 (C-ring C-3″), 135.4 (C-ring C-5″), 136.0 (A-ring C-5), 138.7 (galloyl C-4), 142.0 (B-ring C-6′), 142.2 (C-ring C-1″), 145.2, 145.3 (B-ring C-4′, C-ring C-6), 145.4 (A-ring C-4), 145.7(2C) (galloyl C-3, 5), 166.5 (β-galloyl C-7), 166.8 (α-galloyl C-7), 167.4 (B-ring C-7′), 167.8 (A-ring C-7). (Found: C, 47.07; H, 3.66. $C_{33}H_{24}O_{21}\cdot 9/2H_2O$ requires: C, 47.32; H, 3.97%.)

Methylation of **2**. Compound **2** (45 mg) was methylated with Me_2SO_4 (1.5 ml), K_2CO_3 (1.5 g) in dry Me_2CO (20 ml) under reflux for 1.5 hr. The reaction mixt. was worked-up in the same way described for compound **1** to yield compound **2a** (α-methyl glucoside, 16 mg) and a mixture of α- and β-Me glucosides (30 mg). Compound **2a**. Amorphous powder. ^1H NMR($CDCl_3$): δ 3.51, 3.68, 3.73, 3.79(×2), 3.81, 3.84, 3.86, 3.93, 3.94, 4.02 (OMe), 4.35 (1H, *dd*, *J* = 6, 10 Hz, glc-H-5), 4.88 (1H, *d*, *J* = 4 Hz, glc-H-1), 5.19 (1H, *t*, *J* = 10 Hz, glc-H-4), 5.27 (1H, *dd*, *J* = 6, 12 Hz, glc-H-6), 5.45 (1H, *t*, *J* = 10 Hz, glc-H-3), 6.01, 6.64, 6.71 (each 1H, *s*), 7.27 (2H, *s*).

Methanolysis of **2a**. A mixt. of α- and β-forms of the methylate of compound **2** (30 mg) was treated with 2% NaOMe in MeOH (5 ml) at room temp. for 5 hr. The reaction mixt. was worked-up in the same way as described for compound **1a** to yield Me trimethoxybenzoate (7 mg) and compound **2b** (9 mg). Syrup. $[\alpha]_D$ −17° (Me_2CO; *c* 0.2). EI-MS *m/z* (rel. int.): 586 (100) $[M]^+$, 571(18), 450(44), 239(13). ^1H NMR($CDCl_3$): δ 3.62, 3.63(×2), 3.70, 3.80, 3.96(×2), 3.98, 4.02 (each *s*, OMe), 5.94, 7.31, 7.42 (each 1H, *s*).

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