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# AN HYDROLYSABLE TANNIN AND ACCOMPANYING POLYPHENOLS FROM *MELALEUCA LEUCADENDRON**

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**Key Word Index**—*Melaleuca leucadendron*; Myrtaceae; fruits; polyphenol; tannin; ellagitannin; 1,2-di-*O*-galloyl-3-*O*-digalloyl-4,6-*O*-hexahydroxydiphenoylglucose.

**Abstract**—A new hydrolysable tannin was isolated from dried fruits of *Melaleuca leucadendron* and characterized as 1,2-di-*O*-galloyl-3-*O*-digalloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucose, based on the chemical and spectral evidence. Nine known hydrolysable tannins, as well as known stilbene glycosides and triterpenes, were also isolated.

## INTRODUCTION

*Melaleuca leucadendron* L., whose leaves and fruits are known to be rich in essential oil (caju puti oil), has been used as a perfume and a popular remedy (Jamu medicine) for the treatment of colic, cholera, headaches, toothache and various skin diseases in Indonesia and Malaysia [2]. Besides volatile terpenoid constituents in the oil [3], the occurrence of sesqui- and triterpenoids, and several stilbene derivatives in the dried fruits has been reported [4]; their inhibitory effects on histamine release from mast cells has also been evaluated [4]. However, the tannin constituents of this species are partly documented. In our continuing study on tannins of myrtaceous plants, we have examined the tannins and related polyphenols of the fruits and succeeded in isolating a new hydrolysable tannin, together with 17 known compounds, including nine hydrolysable tannins.

## RESULTS AND DISCUSSION

Dried fruits were homogenized in aqueous acetone. Crystalline precipitates were obtained upon filtration and concentration of the homogenate. The concentrated filtrate was extracted with $Et_2O$, EtOAc and *n*-BuOH. Repeated column chromatography of the EtOAc extract over Toyopearl HW-40 or MCI-gel CHP-20P with aqueous MeOH, gave two stilbene glycosides and four hydrolysable tannins, which were identified as 1,2,3-tri-*O*-galloyl-β-D-glucose [5], 1,2,3,6-tetra-*O*-galloyl-β-D-glucose [5], tellimagrandin II (**1**) [6, 7] and rugosin D [8] by direct comparisons with authentic specimens. The stilbene glycosides were characterized as piceatannol-4′-*O*-β-D-glucopyranoside and its 6″-*O*-gallate, based on their physicochemical data [9]. The $Et_2O$ extract afforded piceatannol [9] and protocatechuic acid after column chromatography on MCI-gel CHP-20P. Oenothein B (**3**) [10], was isolated by column chromatography on Toyopearl HW-40 of the *n*-BuOH extract. Similar column chromatography of the water-soluble portion yielded brevifolincarboxylic acid and three *C*-glucosidic ellagitannins, castalagin [11], grandinin [12] and casuarinin [6, 13]. Column chromatography of the crystalline precipitates obtained from the 70% aqueous acetone homogenate on silica gel gave betulinic acid and its 3-*O*-caffeate (pyracrenic acid) [4, 14]. The other precipitates obtained upon concentration of the homogenate were subjected to a combination of column chromatography over MCI-gel CHP 20-P and prep. HPLC to afford ellagic acid, tellimagrandin II (**1**), 1,2,3,6-tetra- and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose [5] and the new compound (**2**).

It is noteworthy that oenothein B (**3**), which is a unique macro-cyclic dimer characteristic of oenotheraceous and lythraceous plants [15], was also found in a *Melaleuca* species, in addition to *Eucalyptus alba* of the Myrtaceae [1].

Compound **2** gave a peak at *m/z* 1113 due to $[M + Na]^+$ in the FAB-mass spectrum, which corresponded to the molecular formula $C_{48}H_{34}O_{30}$. The $^1H$ NMR spectrum exhibited three 2H-singlets and two 1H-singlets in the aromatic region, and sugar proton signals, most of which were duplicated in a ratio of *ca* 4:1. Besides these signals, two *meta*-coupled doublets ($J$ = 2 Hz) (each 4/5H) at δ 7.27 and 7.37, and a singlet (integrated to 2/5H) at δ 7.05 were also observed. These three aromatic signals are characteristic of *meta*-

*Part 2 in the series 'Tannins and Related Polyphenols from Myrtaceous Plants'. For Part 1, see ref. [1].

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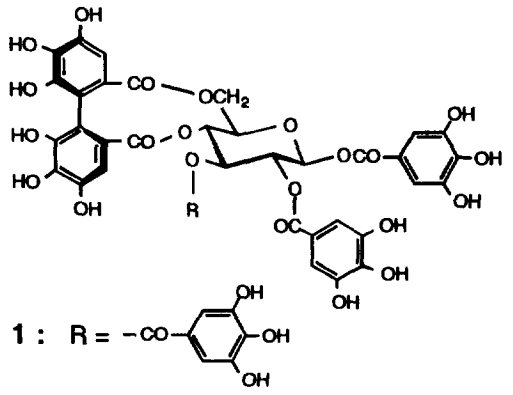
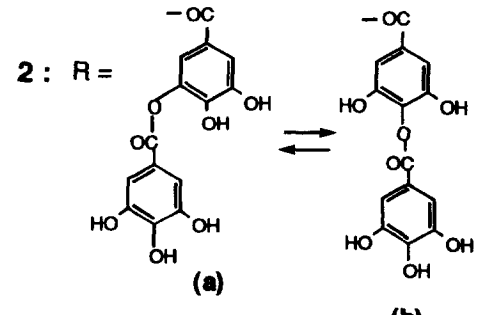
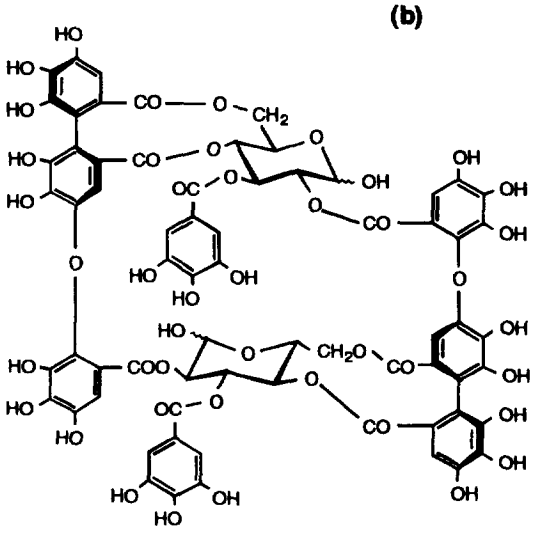


**1** : R = –CO– (digalloyl)



**2** : R = (a) ⇌ (b)



**3**

and *para*-digalloyl ester groups [5], indicating that the duplication of the signals is due to formation of an equilibrium mixture of *m*- and *p*-depside forms (a and b forms; 4:1) of the digalloyl group in the molecule. The chemical shifts and coupling patterns of the sugar proton signals of **2** were virtually identical to those of tellimagrandin II (**1**). Compound **2** was thus assumed to be a gallate of **1**, in which an additional galloyl group is linked depsidically to one of the galloyl groups at *O*-1, *O*-2 and *O*-3 of **1**. The presence of a depsidically-linked galloyl ester group in **2** was substantiated by mild methanolysis [5] in MeOH–acetate buffer (9:1) [5], which gave tellimagrandin II (**1**) and methyl gallate in an equivalent molar ratio as judged by HPLC. Upon comparison of the $^{13}C$ resonances of the sugar carbons of **2** with those of **1**, only the C-3 signal showed a split signal (doublet) which had a separation of 0.05 ppm. One half of the doublet was shifted to lower field by 0.6 ppm compared with that of **1**, while the remainder of the doublet due to C-3 and the other carbon signals were almost superimposable on each other (see Experimental), indicating that the galloyl group having the depside linkage in **2** is at *O*-3 of glucose core, as shown in the formula **2** [5]. Consequently, the tannin **2** was identified as 1,2-di-*O*-galloyl-3-*O*-digalloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucose.

Although the depsidically-linked galloyl group is a common unit of gallotannins [5], it is rarely found in ellagitannins. To the best of our knowledge, compound **2** is the third example of an ellagitannin possessing a depsidically-linked galloyl group, in addition to galloylgeraniin [16] and loropetalin C [17].

## EXPERIMENTAL

$^1H$ and $^{13}C$-NMR spectra were measured in $(CD_3)_2CO$–$D_2O$, and chemical shifts are given in $\delta$ values (ppm) relative to that of solvent [$(CD_3)_2CO$ ($\delta_H$ 2.04; $\delta_C$ 29.8)] on a TMS scale. FAB-MS were recorded using 3-nitrobenzylalcohol as matrix reagent. Normal-phase HPLC was conducted on a Superspher SI60 (Merck) column (4 × 125 mm) developed with *n*-hexane–MeOH–THF–$HCO_2H$ (55:33:11:1) containing oxalic acid (450 mg $l^{-1}$) (flow rate, 1.5 ml $min^{-1}$; detection 280 nm) at room temp. Prep. HPLC was performed using an ODS column (6 × 150 mm) developed with 10 mM $H_3PO_4$–10 mM $KH_2PO_4$–MeCN (9:9:2) (flow rate, 1.3 ml $min^{-1}$; detection 280 nm) at 40°. CC was carried out on Toyopearl HW-40 (Tosoh) and Dia-ion HP-20 and MCI-gel CHP-20P (Mitsubishi).

*Plant material.* Dried fruits were purchased from a market at Sukabumi, Cap Lonceng, Indonesia, and identified by comparison with the dried authentic plant specimens at Herbarium Bogoriense. A voucher specimen (AN-SKJ 288) is deposited at the Faculty of Pharmaceutical Sciences, Kyoto University.

*Extraction and isolation.* Dried fruits (2.7 kg) were homogenized in $Me_2CO$–$H_2O$ (7:3) × 3 (9 l × 3). The filtrate was kept standing overnight to give crystalline (6.9 g), a part (0.5 g) of which afforded betulinic acid (23 mg) and 3-*O*-caffeoylbetulinic acid (14 mg) after CC on silica gel with $CHCl_3$–MeOH. Concn of the filtrate to 2 l afforded further ppts (13.5 g). The filtrate was successively extracted with $Et_2O$, EtOAc and *n*-BuOH. CC of the $Et_2O$ extract (400 mg) on MCI-gel CHP-20P (1.1 cm i.d. × 39 cm) with aq. MeOH gave protocatechuic acid (4.3 mg) and piceatannol (45 mg). The EtOAc extract was submitted to CC over Toyopearl HW-40 (coarse grade) (2.2 cm i.d. × 39 cm) with MeOH–$H_2O$ (3:2 → 7:3) → MeOH – $Me_2CO$ – $H_2O$ (7:1:2 → 3:1:1) → $Me_2CO$–$H_2O$ (7:3) to give piceatannol-4′-*O*-β-D-glucopyranoside (136 mg), 1,2,

3-tri-*O*-galloyl-*β*-D-glucose (15 mg), piceatannol-4′-*O*-(6″-*O*-galloyl)-*β*-D-glucopyranoside (152 mg), 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose (192 mg), tellimagrandin II (**1**) (506 mg) and rugosin D (8.5 mg). The *n*-BuOH extract (10 g) was purified by a combination of CC over Toyopearl HW-40 (2.2 cm i.d. × 72 cm) and MCI-gel CHP-20P (1.1 cm i.d. × 37 cm) with aq. MeOH to yield oenothein B (**3**) (3 mg). The $H_2O$-sol. portion (75 g) was fractionated by CC over Dia-ion HP-20 with $H_2O$ including MeOH (20% → 30% → 40% → 60%). The 20% MeOH eluate was rechromatographed on Toyopearl HW-40 (2.2 cm i.d. × 44 m) with MeOH–$H_2O$ (3:2 → 7:3) and MeOH–$Me_2CO$–$H_2O$ (7:1:2) → 3:1:1) to give brevifolincarboxylic acid (66 mg), grandinin (61 mg) and castalagin (1.4g). The 40% MeOH eluate was similarly purified by CC to yield casuarinin (12 mg). The ppt obtained upon concn of the homogenate was treated with 70% MeOH to deposit ellagic acid (3.8 g). The mother liquor was directly subjected to CC over Toyopearl HW-40 (2.2 cm i.d. × 70 cm) and the eluate (118 mg) with MeOH–$Me_2CO$–$H_2O$ (7:1:2) was finally purified by prep. HPLC to give tellimagrandin II (**1**) (5.1 mg), 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose (3 mg), penta-*O*-galloyl-*β*-D-glucose (6.4 mg) and 1,2-di-*O*-galloyl-3-*O*-digalloyl-4,6-*O*-hexahydroxydiphenoyl-*β*-D-glucose (**2**) (8.3 mg).

1,2-*di*-O-*galloyl*-3-O-*digalloyl*-4,6-O-(S)-*hexahydroxydiphenoyl*-*β*-D-glucose (**2**). Light brown amorphous powder. $[\alpha]_D$ +50° (MeOH, *c* 1.0). $C_{48}H_{34}O_{30}.xH_2O$, FABMS: *m/z* 1113 $(M + Na)^+$. UV (MeOH) nm (log *ε*): 218 (5.01), 278 (4.62). $^1H$ NMR *δ*: (major *m*-depside form) 6.97, 7.12, 7.21 (each *s*, galloyl), 7.27, 7.37 (*d*, *J* = 2 Hz, *m*-depside galloyl), 6.65, 6.45 (each *s*, hexahydroxydiphenoyl), 6.22 [*d*, *J* = 8 Hz, glucose (Glc) H-1], 5.60 (*dd*, *J* = 8, 10 Hz, Glc H-2), 5.86 (*t*, *J* = 10 Hz, Glc H-3), 5.21 (*t*, *J* = 10 Hz, Glc H-4), 4.53 (*dd*, *J* = 6, 10 Hz, Glc H-5), 5.35 (*dd*, *J* = 6, 13 Hz, Glc H-6), 3.87 (*d*, *J* = 13 Hz, Glc H-6); 7.05 (*s*, *p*-depside galloyl). $^{13}C$ NMR *δ*: 93.6 (C-1), 71.7 (C-2), 73.2, 73.7 (C-3), 70.6 (C-4), 72.9 (C-5), 63.0 (C-6). Glucose signals of **1**: 93.6 (C-1), 71.7 (C-2), 73.1 (C-3), 70.6 (C-4), 72.9 (C-5), 63.0 (C-6).

*Methanolysis of* **2**. A soln of **2** (1 mg) in 0.2 M acetate buffer–MeOH (1:9) (1 ml) was left standing at 37° for 4 hr. After neutralization with HOAc followed by evapn of MeOH, the conc soln was passed through Bond-Elut C18 and washed with $H_2O$. The 20% and 30% MeOH eluates gave Me gallate and tellimagrandin II (**1**), respectively, which were identified by $^1H$ NMR spectral comparison and co-chromatography with authentic samples.

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