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Three putrescine bisamides from the leaves of *Aglaia grandis*

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Abstract

Three putrescine (i.e. 1,4-butanediamine) bisamides were isolated from the leaves of *Aglaia grandis*. Their structures were elucidated by interpretation of spectral data.

Keywords: *Aglaia grandis*; Meliaceae; Leaf; Putrescine bisamide

1. Introduction

The genus *Aglaia* (Meliaceae) consists of about 130 species of dioecious trees and shrubs, mainly distributed in tropical and subtropical regions of the world. Several bisamides containing either putrescine (1,4-butanediamine) or the corresponding pyrrolidine ring as a diamine part have been characterized from these plants (Babidge, Massy-Westropp, Pyne, Shiengthong, Ungphakorn & Veerachat, 1980; Brader, Vajrodaya, Greger, Bacher, Kalchhauser & Hofer, 1998; Duh et al., 1993; Joshi, Chowdhury, Vishnoi, Shoeb & Kapil, 1987; Nugroho et al., 1999; Saifah, Puripattanavong, Likhitwitayawuid, Cordell, Chai & Pezzuto, 1993; Saifah & Suparakchinda, 1998), some of these compounds were shown to exhibit antiviral (Joshi et al., 1987) and cytotoxic (Saifah et al., 1993) activity. In our study of the same genus, we have previously reported the isolation of three pregnane-type steroids and two cycloartane-type triterpene hydroperoxides from the leaves of *A. grandis* Korth (Inada, Murata, Inatomi, Nakanishi & Darnaedi, 1997). Here we report three new putrescine bisamides (**1**–**3**) and a known aromadendrane-type sesquiterpene (**4**) (Beechan, Djerassi & Eggert, 1978; Bohlmann, Grenz, Jakupovic, King & Robinson, 1983) from the same source.

2. Results and discussion

After repeated column chromatography and HPLC separations of the EtOAc-soluble part of a MeOH extract of leaves of *A. grandis*, three putrescine bisamides, named grandiamides A (**1**), B (**2**), and C (**3**) were obtained, together with 4β,10α-dihydroxyaromadendrane (**4**). Identification of **4** was achieved by comparison with previously reported spectroscopic data (Beechan et al., 1978; Bohlmann et al., 1983). Grandiamide A (**1**), $[\alpha]_D$ − 108.8° ($CHCl_3$) exhibited a quasi-molecular ion peak $(M + H)^+$ of $C_{38}H_{45}N_2O_9$ by HR positive-FABMS and showed absorptions at 1750 (ester), 1665, 1620 (amide), and 1595 cm^{-1} (benzene ring) in the IR spectrum. The ^{1}H-NMR spectrum of **1** (Table 1) analyzed with the aid of 2D-NMR experiments (COSY, NOESY, and HETCOR) indicated the presence of three methoxy methyls (δ 3.67, 3.75, and 3.81), an acetoxyl methyl (δ 2.26), a tigloyl group [δ 1.75 (3H) 1.79 (3H), and 6.35 (1H)], four methylenes [(δ 1.35–1.5 (4H) and 3.0–3.2 (4H)], three methines (δ 3.69, 4.42, and 5.87), and aromatic protons. Beside these groups, the ^{13}C-NMR spectrum of **1** (Table 1)

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Table 1
^{1}H- and ^{13}C-NMR spectral data of grandiamide A (**1**) in CD_3OD[a]

Position	**1**		Position	**1**	
	δ_H	δ_C		δ_H	δ_C
la		154.7 *s*	19	1.75 *dq* (7.0,1.0)	13.9 *q*
2		89.3 *s*	20	1.79 *d* (1.0)	12.5 *q*
3	3.69 *d* (9.5)	57.3 *d*	1′		130.2 *s*
4	4.42 *d* (9.5)	66.7 *d*	2′	7.03 *d* (8.9)	130.8 *d*
5		81.3 *s*	3′	6.64 *d* (8.9)	113.9 *d*
5a		106.5 *s*	4′		160.5 *s*
6		160.0 *s*	5′	6.64 *d* (8.9)	113.9 *d*
7	6.17 *d* (2.2)	93.6 *d*	6′	7.03 *d* (8.9)	130.8 *d*
8		163.0 *s*	1″		142.5 *s*
9	6.10 *d* (2.2)	95.4 *d*	2″	7.05–7.14 *m*	130.9 *d*
10	5.87 *s*	81.4 *d*	3″	7.05–7.14 *m*	129.1 *d*
11		171.4 *s*	4″	7.05–7.14 *m*	127.5 *d*
12	3.0–3.2 *m*	40.3[b] *t*	5″	7.05–7.14 *m*	129.1 *d*
13	1.35–1.5 *m*	27.8[c] *t*	6″	7.05–7.14 *m*	130.9 *d*
14	1.35–1.5 *m*	27.9[c] *t*	4′–OMe	3.67 *s*	55.5 *q*
15	3.0–3.2 *m*	40.5[b] *t*	6–OMe	3.81 *s*	56.7 *q*
16		172.5 *s*	8–OMe	3.75 *s*	55.9 *q*
17		133.2 *s*	10–OAc	2.26 *s*	171.8 *s*
18	6.35 *qq* (7.0,1.0)	131.7 *d*			21.3 *q*

[a] Chemical shifts are expressed as δ-values; multiplicities and coupling constants (Hz) are given in parentheses.
[b,c] Assignments may be interchanged within each vertical column.

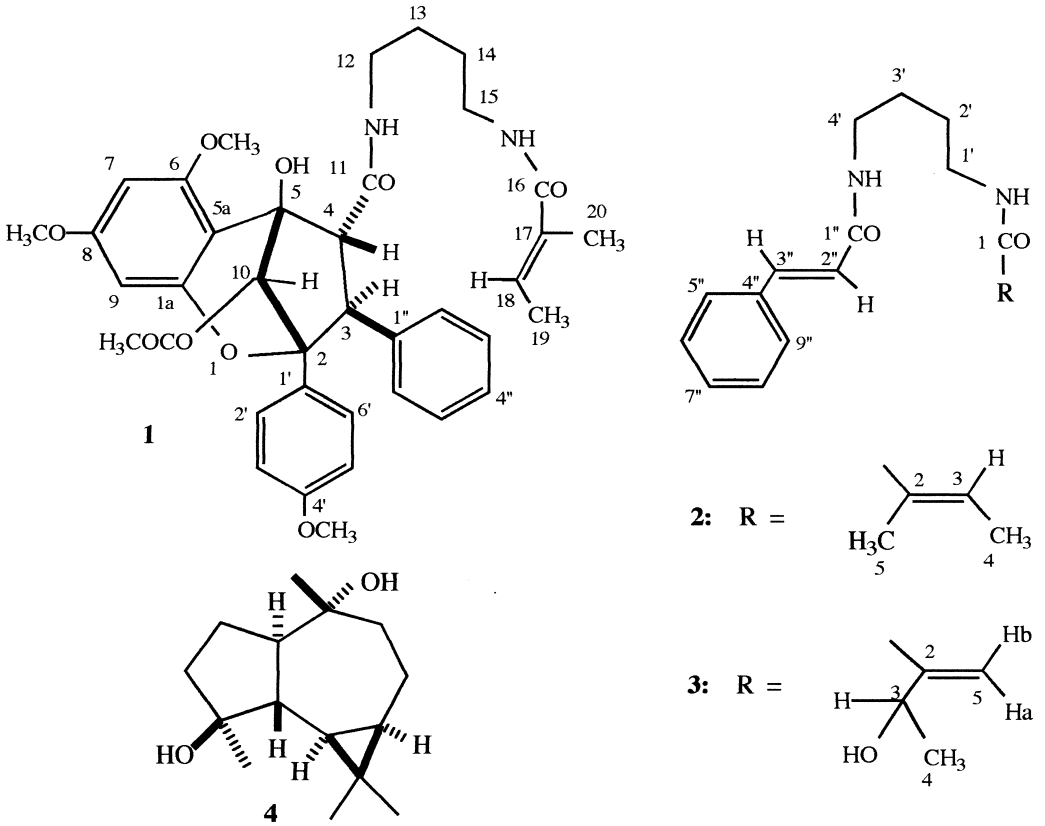

Fig. 1. Structures of grandiamides A, B, C (**1**–**3**) and 4β,10α-dihydroxyaromadendrane (**4**) isolated from the leaves of *A. grandis*.

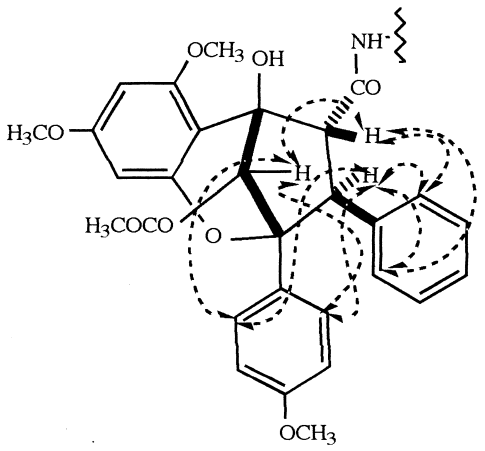

Fig. 2. Selected NOESY correlations of grandiamide A (**1**).

analyzed with the aid of HETCOR and HMBC experiments exhibited signals due to two amide carbonyl carbons (δ 171.4 and 172.5), an ester carbonyl carbon (δ 171.8), and two aliphatic quaternary carbons bearing an oxygen atom (δ 81.3 and 89.3). Recently, isolation of other bisamides, namely aglains A, B and C from *A. argentea* Bl., and aglaforbesins A and B from *A. forbesii* King., have been reported (Dumontet et al., 1996). These compounds have a characteristic bicyclic-structure with a cyclopentatetrahydrobenzopyran skeleton in the molecule (Fig. 1). The ^{1}H-NMR spectral data of **1** are similar to those of other bisamides, especially aglain C. However, signals due to a 2-methylbutyric acid moiety and a 2-aminopyrrolidine ring of aglain C were absent in **1**, being replaced by a tigloyl group [δ 1.75 (3H, *qd*, J = 7.0 and 1.0 Hz), 1.79 (3H, *d*, J = 1.0 Hz), and 6.35 (*qq*, J = 7.0 and 1.0 Hz)] and a putrescine group [(δ 1.35–1.50, 4H, *m*, –HN(CH_2)($\underline{CH_2}$)$_2$(CH_2)NH– and 3.0–3.2, 4H, *m*, –HN($\underline{CH_2}$)(CH_2)$_2$($\underline{CH_2}$)NH-], respectively. The ^{13}C-NMR spectroscopic results also confirmed the presence of these moieties. Hence, compound **1** is a putrescine bisamide having a bicyclic cyclopentatetrahydrobenzopyran moiety and a tigloyl moiety as acid portions. The relative stereochemistries of C-3 and C-4 in **1** were determined as follows. Among three methine protons in the ^{1}H-NMR spectrum, two methine protons (δ 3.69, *d* and 4.42, *d*) were assignable to H-3 and H-4 of the parent skeleton, and were coupled to each other with a large coupling constant (9.5 Hz), indicating a 3α-H/4β-H relative configuration in **1**. Selective NOESY correlations as shown in Fig. 2 confirmed this arrangement. The configuration of C-10 was determined from NOESY analysis, in which a methine proton at δ 5.87 (*s*), ascribable to 10-H, was adjacent to an acetoxyl group (δ 2.26), and significant correlations between 10-H/4β-H and between 10-H/2′, 6′-H_2 were observed. The stereochemistry of C-10 is shown in the figure. Finally, the unambiguous structure of **1** was established from HMBC experiments, which showed significant correlation peaks between H-10/C-3, C-4, and C-5a, H-4/C-2 and C-1″, and between H-3/C-5 and C-1″. Further correlations between H-3/C-11, H_3-

Table 2
^{1}H- and ^{13}C-NMR spectral data of grandiamides B (**2**) and C (**3**) in $CDCl_3$[a]

Position	**2**		**3**	
	δ_H	δ_C	δ_H	δ_C
1		169.8 *s*		168.2 *s*
2		131.8 *s*		146.4 *s*
3	6.44 *qq* (7.1, 1.1)	130.8 *d*	4.61 *q* (6.3)	68.8 *d*
4	1.74 *dq* (7.1, 1.1)	13.9 *q*	1.38 *d* (6.3)	22.0 *q*
5	1.84 *d* (1.1)	12.4 *q*	5.45 *s* (Ha), 5.83 *s* (Hb)	119.4 *t*
1′	3.34–3.43 *m*	39.3 [b] *t*	3.29–3.39 *m*	39.0 [b] *t*
2′	1.60–1.63 *m*	26.7 [c] *t*	1.50–1.60 *m*	26.87 [c] *t*
3′	1.60–1.63 *m*	27.3 [c] *t*	1.50–1.60 *m*	26.89 [c] *t*
4′	3.34–3.43 *m*	39.4 [b] *t*	3.29–3.39 *m*	39.4 [b] *t*
1″		166.2 *s*		166.5 *s*
2″	6.47 *d* (15.7)	121.1 *d*	6.48 *d* (15.6)	120.9 *d*
3″	7.61 *d* (15.7)	140.7 *d*	7.59 *d* (15.6.)	140.9 *d*
4″		135.0 *s*		134.9 *s*
5″, 9″	7.48–7.50 *m*	127.8 *d*	7.47–7.49 *m*	127.8 *d*
6″, 8″	7.32–7.36 *m*	128.8 *d*	7.33–7.36 *m*	128.8 *d*
7″	7.32–7.36 *m*	129.5 *d*	7.33–7.36 *m*	129.7 *d*
NH	6.02 *t*-like, 7.32–7.36 *m*		6.60 *t*-like, 7.29–7.32 *m*	
OH			4.09 *br. s*	

[a] Chemical shifts are expressed as δ-values; multiplicities and coupling constants (Hz) are given in parentheses.
[b,c] Assignments may be interchanged within vertical column.

20/C-16, and H-4/C-2″, 6″ indicated the connectivities of C-4 to an amide carbonyl carbon (C-11), C-17 to another amide carbonyl carbon (C-16), and C-3 to the non-substituted phenyl ring. Based on this evidence and detailed comparison of the spectral data of **1** with those of the aglains (Dumontet et al., 1996; Nugroho et al., 1999), the structure of grandiamide A was determined as **1**.

Grandiamide B (**2**) exhibited a molecular formula of $C_{18}H_{24}N_2O_2$ by high resolution EI MS, and showed absorptions due to an amide group and a benzene ring in the IR spectrum. The ^{1}H-NMR spectrum of **2** (Table 2) analyzed with the aid of 2D-NMR experiments showed the presence of a putrescine group [δ 1.60–1.63, 4H, *m*, –HN(CH_2)($\underline{CH_2}$)$_2$(CH_2)NH– and 3.34–3.43, 4H, *m*, –HN($\underline{CH_2}$)(CH_2)$_2$($\underline{CH_2}$)NH–]. The ^{13}C-NMR spectrum of **2** (Table 2) exhibited signals due to two amide carbonyl carbons (δ 166.2 and 169.8). Accordingly, **2** is a putrescine bisamide. From 2D-NMR spectroscopic analyses, two acid groups linked to putrescine through an amide bond were deduced to be cinnamic and tiglic acid, respectively. The stereochemistry of the double bond between C-2″ and C-3″ was deduced as *E* based on a large coupling constant (J = 15.7 Hz) and NOESY data. The presence of a cinnamoyl moiety in **2** was also suggested by the fragment ion at *m*/*z* 131 (base peak) in the EI MS (Senda et al., 1994). Based on this evidence and comparison to the spectral data of **2** and other analogous bisamides having cinnamic and tiglic acid moieties (Duh et al., 1993; Guggisberg & Hesse, 1983), the structure of grandiamide B was determined to be as shown by **2**.

Grandiamide C (**3**) exhibited a molecular formula of $C_{18}H_{24}N_2O_3$ by high resolution EI MS, and showed absorptions due to hydroxyl and amide groups, and a benzene ring in the IR spectrum. Compared with the ^{1}H-NMR spectrum (Table 2), grandiamides B (**2**), and C (**3**) showed similar chemical shifts and coupling constants relating to the cinnamoyl and putrescine moieties. Moreover, carbon chemical shifts due to amide carbonyl carbons in **3** (δ 166.5 and 168.2) were similar to those of **2** (Table 2). Hence, **3** is a putrescine bisamide having a cinnamoyl group in its structure. The presence of a cinnamoyl moiety in **3** was also suggested by the fragment ion at *m*/*z* 131 (base peak) in the EI MS (Senda et al., 1994); and the stereochemistry of a double bond between C-2″ and C-3″ was deduced as *E* based on a large coupling constant (J = 15.6 Hz) and NOESY data. The second acid moiety in **3** was determined as follows. In the ^{1}H-NMR spectrum, **3** showed signals due to a hydroxymethine proton (δ 4.61, *q*, J = 6.3 Hz), a secondary methyl (δ 1.38, *d*, J = 6.3 Hz), an exo methylene group (δ 5.45, *s* and 5.83, *s*), and a hydroxyl group (δ 4.09, *br. s*) as the second acid moiety. Connectivities of these groups were confirmed from the HMBC experiments, in which correlation peaks between H-3/C-1 and C-5, and between H_3-4/C-2 and C-5 suggested the presence of a 3-hydroxy-2-methylenebutyroyl group [–COC(=CH_2)-CH(OH)CH_3] (Wu et al., 1999) in **3**. By combination of the above evidence and ^{1}H- and ^{13}C-NMR spectroscopic analyses (Table 2), it was concluded that the structure of grandiamide C is **3**. Compound **3** did not show any optical rotation [α]$_D = \pm 0^\circ$ ($CHCl_3$)} and hence **3** seems to be a racemic mixture with respect to C-3.

3. Experimental

3.1. General

Mps are uncorr. ^{1}H-NMR: 600 MHz; ^{13}C-NMR: 150 MHz and TMS as int. standard. IR: in $CHCl_3$. HPLC: JAIODS-120T and JAIGEL-GS310 columns with a differential refractometer.

3.2. Plant materials

The leaves of *A. grandis* Korth. were harvested in 1993 at the Herbarium Bogoriense, Java, Indonesia and voucher specimens have been deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Setsunan University.

3.3. Extraction and isolation

The crushed leaves (460 g) were extracted with MeOH and the solvent was removed by evoporation in vacuo. The MeOH extract (68.0 g) was suspended with H_2O and the aqueous suspension was extracted with hexane and EtOAc, successively. The EtOAc extract (7.0 g) was chromatographed on silica gel with $CHCl_3$–MeOH containing an increasing MeOH concentration; a fraction containing **1** and **4** (0.3 g), and a fraction containing **2** and **3** (0.8 g) were separated in that order. Each fraction was further purfied with repeated HPLC to afford **1** (7.0 mg), **2** (12 mg), **3** (32 mg), and **4** (65 mg).

3.4. Grandiamide A (*1*)

Amorphous powder; $[\alpha]_D^{25} - 108.8^\circ$ ($CHCl_3$; *c* 0.25); IR ν_{max} cm^{-1}: 3450, 1750, 1665, 1620, 1595, 1520, and 1155 cm^{-1}; HR-FABMS (positive mode) *m*/*z* (%): 673.3129 [$(M + H)^+$, $C_{38}H_{45}N_2O_9$ requires 673.3125, 16]; EI MS *m*/*z* (%): 612 (M^+-AcOH, 4), 313 (48), 293 (38), 210 (33), 197 (12), 154 (29), 121 (90), 83 (100); ^{1}H- and ^{13}C-NMR spectral data (Table 1).

3.5. Grandiamide B (**2**)

Mp 99–102°C (Hexane–EtOH); IR ν_{max} cm^{-1}: 1660, 1620, 1560; EI and high resolution EI MS *m/z* (%): 300.1840 (M^+, $C_{18}H_{24}N_2O_2$ requires 300.1839, 9), 201 (48), 153 (33), 131 (100), 84 (85), 83 (60), 70 (68); ^{1}H- and ^{13}C-NMR spectral data (Table 2).

3.6. Grandiamide C (**3**)

Amorphous powder; $[\alpha]_D^{20} \pm 0°$ ($CHCl_3$; *c* 1.13); IR ν_{max} cm^{-1}: 3350, 1660, 1620, 1560; EI and high resolution EI MS *m/z* (%): 316.1791 (M^+, $C_{18}H_{24}N_2O_3$ requires 316.1787, 6), 301 (7), 298 [$(M-H_2O)^+$, 5], 201 (90), 151 (87), 131 (100), 70 (100); ^{1}H- and ^{13}C-NMR spectral data (Table 2).

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