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Phenolic constituents from *Grevillea robusta*

Amany S. Ahmed[a], Norio Nakamura[a], Meselhy R. Meselhy[a], Makhboul A. Makhboul[b], Nasr El-Emary[b], Masao Hattori[a,*]

[a]*Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt*

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Abstract

Seven phenolic compounds were isolated from a MeOH extract of the leaves of *Grevillea robusta*. Their structures were determined by various spectral methods including 2D NMR spectroscopy.

Keywords: *Grevillea robusta*; Proteaceae; 5-Alkylresorcinols; Macrocyclic phenols; Grevirobstols A–C; Robustasides A–D

1. Introduction

Silk oak (*Grevillea robusta* A. CUNN) is a tropical ornamental tree native to Australia and now spread to other countries (Hoffman, Hausen & Adams, 1985). Its timber is commercially valuable for making furniture (Ritchie, Taylor & Vautin, 1965). 5-Alkylresorcinols, macrocyclic phenols, and cinnamic acid derivatives have so far been isolated from *G. robusta* (Ritchie et al., 1965; Ridley, Ritchie & Taylor, 1968; Ridley, Ritchie & Taylor, 1970; Cannon, Chow, Fuller, Hamilton, Metcalf & Power, 1973; Varma, Manju & Parthasarathy, 1976). Resorcinols have been shown to inhibit a number of enzymes, including tryptophan peroxidase (Freiden, Westmark & Schor, 1961) and tyrosinase (Tsukamoto & Taniguchi, 1958), while topical application of these compounds has been reported to cause dermatitis (Hoffman et al., 1985; Ritchie et al., 1965). In our preliminary study, an extract of the leaves of *G. robusta* displayed appreciable inhibitory effects on HIV-1 protease, which prompted us to further investigate the constituents of the plant.

In the present paper, we report the isolation and characterization of 13 phenolic compounds. Structure elucidation of these compounds was achieved mainly by 2D NMR and mass spectroscopy.

2. Results and discussion

A MeOH extract of the leaves of *G. robusta* was suspended in H_2O and fractionated into hexane, $CHCl_3$, EtOAc and BuOH soluble fractions. Repeated column chromatography of the $CHCl_3$ soluble fraction afforded nine long-chain alkylphenols (**1**–**9**); the BuOH-soluble fraction yielded four new derivatives (**11**–**14**) of arbutin (**10**). The spectroscopic data of **4**–**9** were in excellent agreement with those reported for bis-norstriatol (**4**), robustol (**5**) and a related compound (**6**), dehydrorobustols A (**7**) and B (**8**) (Cannon et al., 1973; Varma et al., 1976), and 5-[14′-(3″,5″-dihydroxyphenyl)-*cis*-tetradec-6′-en-1-yl]benzene-1,3-diol (**9**) (Varma et al., 1976; Scannell, Barr, Murty, Reddy & Hecht, 1988; Morimoto, Kawamatsu & Sugihara, 1968; Lytollis et al., 1995) (Fig. 1). The structures of the new compounds were determined as follows.

Compound **1** was assigned the molecular formula $C_{28}H_{38}O_4$ on the basis of the FAB MS (positive ion mode) spectrum at m/z 399 $[M+H]^+$. The ^{1}H-NMR spectrum (see Section 3) analysed with the aid of ^{1}H^{1}H COSY showed signals for long-chain methylenes at δ

* Corresponding author. Fax: +81-764-34-5060.
E-mail address: saibo421@ms.toyama-mpu.ac.jp (M. Hattori).

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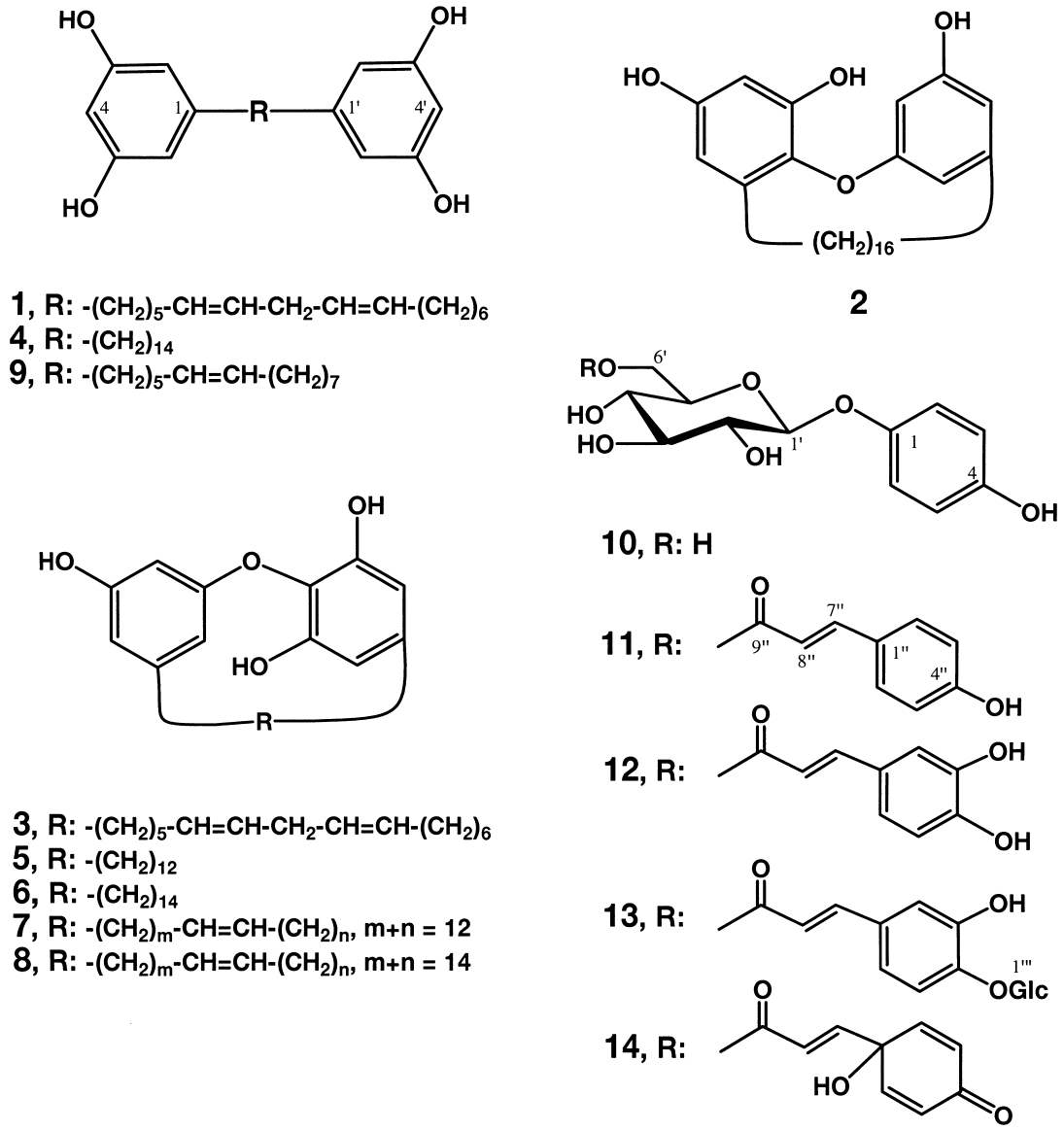

Fig. 1. Phenolic compounds isolated from *G. robusta*.

1.24–2.14 (9 × C$\underline{H}_2$) (a signal due to a terminal methyl group was not seen), two benzylic methylenes at δ 2.43 and 2.44 (each *t*, J = 8.1 Hz), allylic methylene at δ 2.78 (*t*, J = 6.3 Hz), four vinyl protons at δ 5.28–5.38 and two AB_2 aromatic protons at δ 6.08 and 6.12 (*d*, J = 2.4 Hz). The ^{13}C-NMR and the HMQC spectra of **1** (see Section 3) showed signals for 12 methylene carbons (including two benzylic methylenes (δ 36.8 and 37.0), six aromatic methines (δ 100.9–107.9), four vinyl methines (δ 129.0–131.0), and six aromatic carbons (δ 146.2 and 159.3). These data suggested an *n*-alkylresorcinol derivative related to bis-norstriatol (**4**) (Varma et al., 1976; Scannell et al., 1988; Morimoto et al., 1968; Lytollis et al., 1995), with an unsaturated polymethylene chain connecting two resorcinol residues.

The MS/MS spectrum of the parent ion peak (*m*/*z* 439) demonstrated a characteristic fragmentation pattern of an *n*-alkenylresorcinol (Occolowitz, 1964) with a fragment ion at *m*/*z* 124 (base peak). Fragments of *m*/*z* 301, 287 and 259 indicated stepwise loss of 14 amu from *m*/*z* 315. Intense fragment ions at *m*/*z* 233, 215 $[233\text{-}H_2O]^+$ and 205 placing one of the double bonds at C-6(7) and the other at C-9(10), and a –CH_2–CH=CH–CH_2–CH=CH–CH_2– moiety, could be assigned (Fig. 2). The fragment ion at *m*/*z* 246 might have originated from allylic cleavage of the two double bonds. Both double bonds were established to be in the *Z*-form by considering the chemical shifts of the allylic carbons (δ 28.2, C-5 and C-11) (Yasuda, Takeya & Itokawa, 1981; Tang, Feng & Huang, 1996; Sugimoto, Miyase, Kuroyanagi & Ueno, 1988; Coppola, 1985). In the light of this evidence, **1**

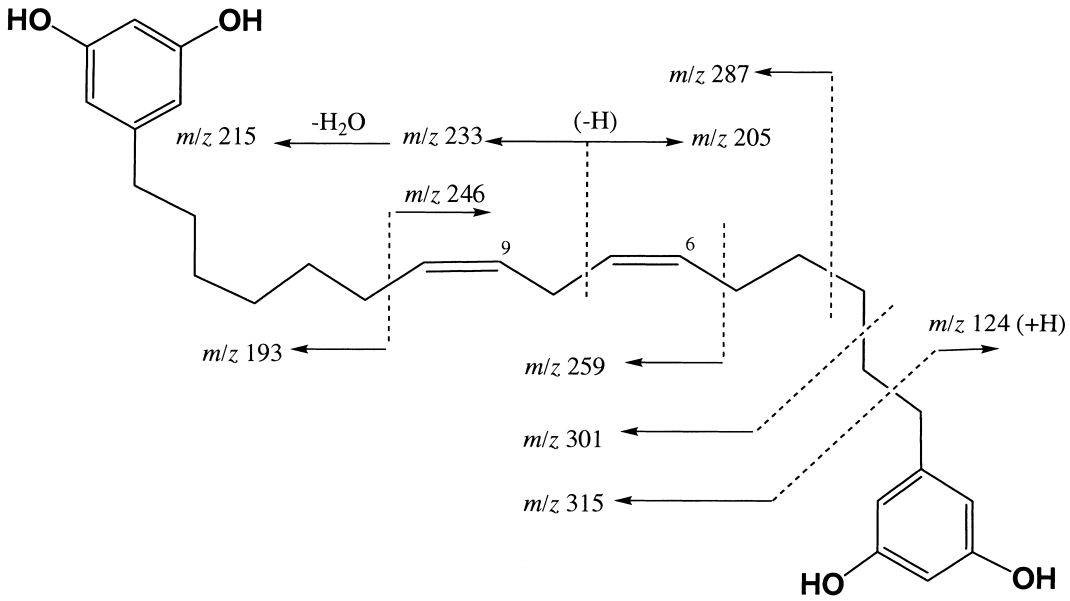

Fig. 2. Proposed fragmentation pattern of **1** as deduced from MS/MS experiment.

appeared to be a new alkenylresorcinol derivative subsequently named grevirobstol A.

Compound **2** (named grevirobstol B) was assigned the molecular formula $C_{28}H_{40}O_4$ by FAB MS (positive ion mode) at *m/z* 441 $[M+H]^+$ (base peak) (Ridley et al., 1970). The ^{1}H-NMR and ^{1}H^{1}H COSY spectra showed signals for long-chain methylenes at δ 1.2–1.6 (28 H) (the signal for a terminal methyl group was absent) and two benzylic methylenes at δ 2.35 and 2.51. In addition, two spin systems of AB- and ABC-aromatic protons at δ 6.28 and 6.39, and at δ 6.02, 6.33 and 6.46, respectively, were also observed. This was further confirmed by ^{13}C-NMR spectroscopic analysis which showed signals for 16 long-chain methylenes (including two benzylic methylenes at δ 26.6–35.9) and two (tri- and tetrasubstituted) benzene rings. On the basis of these observations, a macrocyclic phenol, similar to **6**, was suggested (Cannon et al., 1973) The coupling constant (J = 2.8 Hz) of the AB-aromatic protons established the tetrasubstitution pattern seen in **2** against that in **6** (two equivalent protons at δ 6.44) as further confirmed by the HMQC experiment. Accordingly, the structure of **2** was determined as shown in Fig. 1.

The structure of **3** (grevirobstol C) was based on comparison of its FAB MS and ^{1}H-NMR spectra with those of **1**, **5** and **6**. A molecular ion at *m/z* 437 $[M+H]^+$ denoted the base peak (Ridley et al., 1970), and fission of the polymethylene chain followed by allylic cleavage yielded a fragment ion at *m/z* 246 (Varma et al., 1976; Occolowitz, 1964; Rasmussen, Ridley, Ritchie & Taylor, 1968). The chemical shift of vinyl protons and allylic methylene protons matched those of **1**. Moreover, five aromatic protons at δ 5.98 (*br s*), 6.38 (*br s*), 6.43 (2H, *s*) and 6.60 (*d*, J = 2.4 Hz) suggested a substitution pattern similar to that of **5** and **6** (Varma et al., 1976). From these data and on biogenetic grounds (Varma et al., 1976), the geometry

Table 1
^{13}C-NMR spectral data (125 MHz, CD_3OD) for compounds **10**–**14**

C	**10**	**11**	**12**	**13**	**14**
Arbutin					
1	153.7 *s*	153.7 *s*	153.7 *s*	153.8 *s*	153.7 *s*
2, 6	119.3 *d*	119.4 *d*	119.5 *d*	119.6 *d*	119.4 *d*
3, 5	116.5 *d*	116.4 *d*	116.6 *d*	116.6 *d*	116.6 *d*
4	152.3 *s*	152.1 *s*	152.3 *s*	152.2 *s*	152.1 *s*
1′	103.5 *d*	103.5 *d*	103.7 *d*	103.7 *d*	103.0 *d*
2′	74.9 *d*	74.7 *d*	74.9 *d*	74.8 *d*	74.7 *d*
3′	77.9 *d*	77.7 *d*	77.8 *d*	78.1 *d*	77.7 *d*
4′	71.3 *d*	71.6 *d*	71.7 *d*	71.5 *d*	71.6 *d*
5′	77.9 *d*	75.3 *d*	75.4 *d*	75.3 *d*	75.1 *d*
6′	62.5 *t*	64.5 *t*	64.7 *t*	64.7 *t*	64.9 *t*
Phenyl propanoid					
1″		126.8 *s*	122.7 *s*	126.5 *s*	70.8 *s*
2″		131.1 *d*	114.7 *d*	113.5 *d*	128.6 *d*
3″		116.8 *d*	151.6 *s*	154.0 *s*	151.1 *s*
4″		161.4 *s*	151.2 *s*	151.1 *s*	186.0 *s*
5″		116.8 *d*	117.9 *d*	120.0 *d*	151.1 *s*
6″		131.1 *d*	120.3 *d*	119.9 *d*	128.6 *d*
7″		146.7 *d*	142.5 *d*	141.7 *d*	148.0 *d*
8″		114.6 *d*	117.7 *d*	118.4 *d*	122.7 *d*
9″		168.8 *s*	169.2 *s*	169.0 *s*	167.1 *s*
Glucose					
1‴				103.8 *d*	
2‴				74.8 *d*	
3‴				78.0 *d*	
4‴				71.2 *d*	
5‴				77.8 *d*	
6‴				62.5 *t*	

of both double bonds was proposed to be in the *Z*-form, and the structure of **3** was assigned as shown in Fig. 1.

The HR-FAB MS spectrum of **11** (*m/z* 419.1378 $[M+H]^+$) established the molecular formula $C_{21}H_{22}O_9$, and the UV spectrum (λ_{max} 297 and 312 nm) implied the presence of a conjugated chromophore. The ^{1}H-NMR spectrum of **11**, with the aid of ^{1}H^{1}H COSY, showed signals for two sets of AB-aromatic protons (δ 7.46 and 6.82, J = 8.5 Hz) and (δ 6.65 and 6.95, J = 9.0 Hz), *trans*-olefinic protons (J = 16.0 Hz) at δ 6.35 and 7.64, and sugar protons (anomeric proton at δ 4.78). The ^{13}C-NMR (Table 1) and HMQC spectra of **11** showed 17 signals assigned for 21 carbon atoms in the molecule, including signals for a sugar moiety, 12 aromatic and 2 olefinic carbons and an ester carbonyl. The sugar moiety was identified as glucose by comparing ^{13}C-NMR spectral data with those reported for methyl-*O*-glucosides and by considering the glycosidation effect (Agrawal, Jain, Gupta & Thakur, 1985; Agrawal & Bansal, 1989; Yoshimoto, Itatani & Tsuda, 1980). These spectral data (Table 1) suggested an arbutin residue (**10**) acylated at C-6′ of the sugar moiety (δ 4.34 and 4.53/δ_c 62.5) with a *p*-hydroxycinnamate (Occolowitz, 1964). Fragment ions at *m/z* 309 $[M-109]^+$ and 147 (cinnamoyl) supported the partial structure of **11**. The coupling constant of H-1′ (J = 7.5 Hz) indicated a β configuration of the sugar moiety. The structure of **11** (named robustaside A) was finally established by HMBC as 6′-(4″- hydroxycinnamoyl)arbutin.

Compound **12** (named robustaside B) had one more oxygen atom than **11** (positive ion FAB MS *m/z* 435 $[M+H]^+$). From the ^{1}H- and ^{13}C-NMR spectra of **12** (Table 1), it was obvious that its structure was very similar to **11**, except that an AB-spin system in **11** was replaced by an ABC-system [a broad singlet at δ 6.93 (δ_c 114.7, C-2″) and a multiplet at 6.72 (δ_c 117.9, 120.3, C-5″ and C-6″) in **12**], and the carbon signals at δ 116.8 (C-3″) and 161.4 (C-4″) in **11** were shifted to δ 151.6 and 151.2, respectively, in **12**. This finding was further confirmed by analysis of the HMQC spectrum. Similarly, fragment ions at *m/z* 325 and 163 supported the structure of **12** as shown in Fig. 1. Accordingly, the structure of **12** was established as 6′-(3″,4″-dihydroxycinnamoyl)arbutin.

The FAB MS spectrum (negative ion mode) of **13** (named robustaside C) showed a quasi-molecular ion peak at *m/z* 595 $[M-H]^-$, which was consistent with the molecular formula $C_{27}H_{32}O_{15}$. By comparing the ^{1}H- and ^{13}C-NMR spectra of **13** (Table 1) with those of **10** and **11**, an arbutin moiety, a phenylpropanoid moiety (with ABC-aromatic protons) and a hexose moiety were deduced. The sugar moiety was similarly identified as β-D-glucose (J = 7.7 Hz, H-1‴) (Agrawal et al., 1985; Agrawal & Bansal, 1989; Yoshimoto et al., 1980). HMBC correlations between C-4″ (δ 151.1) and proton signals at δ 4.78 (H-1‴), 7.04 (H-2″) and 6.83 (H-6″) suggested the presence of a glucose moiety at C-4″. The structure of **13** was finally determined as 6′-(4″-*O*-β-glucopyranosyl-3″-hydroxycinnamoyl)arbutin.

Compound **14** (named robustaside D) was assigned the molecular formula $C_{21}H_{22}O_{10}$ (positive ion FAB MS *m/z* 457 $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectra of **14** (Table 1) showed signals for an arbutin moiety acylated at C-6′ with a phenylpropanoid moiety. Since H-7″ and H-8″ were shifted upfield (relative to those in **11** and **12**), and characteristic signals for a conjugated ester (δ 167.1, C-9″), a conjugated ketone (δ 186.0, C-4″, IR ν_{max} 1668 cm^{-1}), and an oxygen-bearing quaternary carbon (δ 70.8, C-1″) were observed in the ^{13}C-NMR spectrum of **14**, a different acyl moiety was suggested. Significant correlations between C-1″ and H-2″, H-7″ and H-8″, and C-4″ and H-2″ and H-3″ in the HMBC spectrum of **14** supported the proposed acyl moiety. From the above mentioned data, the structure of **14** was established as illustrated in Fig. 1.

To the best of our knowledge compounds **1**–**3** and **11**–**14** are isolated here for the first time from nature.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO DIP-360 automatic polarimeter at 25°. UV spectra were taken on a Shimadzu UV-2200 UV-VIS spectrometer. IR spectra were measured on a JASCO FT/IR 230 spectrometer. NMR spectra (^{1}H: 500 and ^{13}C: 125 MHz) on a Varian Unity Plus 500 MHz spectrometer and δ values (in ppm) with TMS as int. standard. 2D NMR experiments were performed using standard Varian pulse sequences. FAB and HR-FAB MS spectra were taken on a JEOL-JMS700 spectrometer with Xe atoms of energy of 6–8 kV and glycerol/NBA as matrix. FAB MS/MS of **1** was taken on a JEOL-SX102A tandem-mass spectrometer with accelerated voltage of 10 kV and glycerol as matrix. Preparative HPLC was performed on YMC-Pack ODS-AP (10 × 250 mm, YMC, Japan). MPLC: LiChroprep RP-18 (Merck, Germany). CC: Kieselgel 60 (Merck), Cosmosil 140 C_{18}-OPN (Nacalai Tesque, Japan), and Sephadex LH-20 (Pharmacia, Sweden) were used, and TLC: Kieselgel 60 F_{254} and RP-18 F_{254} S (Merck) plates were used. Spots were observed under UV light and after spraying with anisaldehyde-H_2SO_4 reagent, followed by heating.

3.2. Plant material

The leaves of *Grevillea robusta* A Cunn. were collected in Assiut, Egypt in May 1994 and identified by Prof. Dr. Ibrahim Hassan, Department of Horticulture, Faculty of Agriculture, Assiut University, Assiut, Egypt. A voucher specimen is deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

3.3. Extraction and isolation of compounds

The air-dried leaves (2.75 kg) of *G. robusta* was extracted with boiling MeOH (12 l × 3) for 3 h. The combined MeOH extracts were evaporated in vacuo to give a residue (605 g). A part of the MeOH extract (388 g) was suspended in water (1.5 l) and extracted with hexane, $CHCl_3$, EtOAc and *n*-BuOH in this order, to give the respective fractions (14.6 g, 50.0 g, 50.4 g and 56.0 g) after evaporation. The $CHCl_3$ soluble fraction (40 g) was applied to a column of silica gel and elution was started with C_6H_6 containing increasing amounts of EtOAc. Fractions eluted with C_6H_6–EtOAc (17:3) were subjected to repeated column chromatography and preparative HPLC (80% CH_3CN and/or 50% CH_3CN) to yield compounds **2** (3 mg), **3** (4 mg), **5** (5 mg), **6** (3 mg), **7** (5 mg) and **8** (13 mg). Similarly, fractions eluted with C_6H_6–AcOEt (13:7) gave **1** (5 mg), **4** (10 mg) and **9** (4 mg).

Column chromatography of the *n*-BuOH soluble fraction (38 g) over Sephadex LH-20 (8 × 18.5 cm, H_2O–MeOH, 0–100%) gave four fractions. Column chromatography/RP-18 (20% aq. MeOH) of Fr. # 2 afforded robustaside D (**14**, 40 mg). Fr. # 3 gave rutin (20 mg) after concentration and myricetin 3-*O*-rutinoside (3 mg) (Ridley et al., 1970) after MPLC/RP-18 (40% aq. MeOH). Repeated column chromatography/silica gel of Fr. #4 (elution with C_6H_6–Me_2CO) and MPLC/RP-18 (40–50% aq. MeOH) gave robustasides A (**11**, 55 mg), B (**12**, 50 mg) and C (**13**, 10 mg).

3.4. Grevirobstol A (*1*)

Yellowish oil. ^{1}H-NMR spectral data (CD_3OD) δ: 1.24–1.42 (10H), 1.52–1.62 (4H), 2.02–2.14 (4H), 2.43 (2H, *t*, *J* = 8.1 Hz), 2.44 (2H, *t*, *J* = 8.1 Hz), 2.78 (2H, *t*, *J* = 6.3 Hz), 5.28–5.38 (4H), 6.08 (2H, *t*, *J* = 2.4 Hz), 6.12 (4H, *t*, *J* = 2.4 Hz). ^{13}C-NMR spectral data (CD_3OD) δ: 26.6 (=CH–$\underline{C}H_2$–CH=), 28.2 (=CH–$\underline{C}H_2$– × 2), 30.3 (–$\underline{C}H_2$– × 2), 30.5 (–$\underline{C}H_2$–), 30.7 (–$\underline{C}H_2$– × 2), 32.0 and 32.4 (Ar–CH_2–$\underline{C}H_2$–), 36.8 and 37.0 (Ar–$\underline{C}H_2$–), 100.9 and 101.0 (C-4, C-4′), 107.9 (C-2, C-6, C-2′, and C-6′), 129.0 and 129.2 (–$\underline{C}$=C–), 130.8 and 131.0 (–C=$\underline{C}$–), 146.2 (C-1, C-1′), 159.3 (C-3, C-5, C-3′, and C-5′). FAB MS (positive ion mode) *m*/*z*: 440 $[M+2H]^+$, 439 $[M+H]^+$, 177, 163, 149, 137, 124, 123; (negative ion mode) *m*/*z*: 438 $[M]^-$, 437$[M-H]^-$, 339, 325, 311, 135, 122. HR-FAB MS (positive ion mode) *m*/*z*: 440.2919, calculated for $C_{28}H_{40}O_4$: 440.2927.

3.5. Grevirobstol B (*2*)

Amorphous powder. UV (MeOH) λ_{max}: 279 nm. ^{1}H-NMR spectral data ($CDCl_3$) δ: 1.20–1.35 (24H), 1.42–1.48 (4H), 2.35 (2H, *t*, *J* = 8.0 Hz), 2.51 (2H, *t*, *J* = 7.7 Hz), 6.02 (1H, *t*, *J* = 2.2 Hz), 6.28 (1H, *d*, *J* = 2.8 Hz), 6.33 (1H, *br s*), 6.39 (1H, *d*, *J* = 2.8 Hz), 6.46 (1H, *br s*). ^{13}C-NMR spectral data ($CDCl_3$) δ: 26.6–35.9 ($\underline{C}H_2$ × 16), 98.9, 100.9, 108.1, 108.3, 109.5, 110.6, 125.5, 144.2, 146.4, 156.5, 159.0. FAB MS (positive ion mode) *m*/*z*: 441 $[M+H]^+$ (base peak). HR-FAB MS (positive ion mode) *m*/*z*: 441.2977, calculated for $C_{28}H_{41}O_4$: 441.3005.

3.6. Grevirobstol C (*3*)

Yellowish oil. ^{1}H-NMR spectral data ($CDCl_3$) δ: 1.16–1.36 (10H), 1.40–1.64 (4H), 1.97–2.05 (4H), 2.44 (2H, *t*, *J* = 8.1 Hz), 2.54 (2H, *t*, *J* = 6.7 Hz), 2.74 (2H, *t*, *J* = 6.0 Hz), 5.27–5.43 (4H), 5.98 (1H, *br s*), 6.38 (1H, *br s*), 6.43 (2H, *s*), 6.60 (1H, *t*, *J* = 2.4 Hz). FAB MS (positive ion mode) *m*/*z*: 437 $[M+H]^+$ (base peak), 246, 231, 163, 139, 123.

3.7. Robustaside A (*11*)

Amorphous powder. $[\alpha_D]$ −34.3° (MeOH, *c* 1.61). UV (MeOH) λ_{max}: 297, 312 nm. IR (film) ν_{max}: 1690 cm^{-1}. ^{1}H-NMR spectral data (CD_3OD) δ: 3.40 (1H, *t*, *J* = 7.5 Hz, H-4′), 3.44 (1H, *t*, *J* = 7.5 Hz, H-2′), 3.47 (1H, *t*, *J* = 7.5 Hz, H-3′), 3.65 (1H, *ddd*, *J* = 7.5, 6.8, 2.4 Hz, H-5′), 4.34 (1H, *dd*, *J* = 11.9, 6.8 Hz, H-6′), 4.53 (1H, *dd*, *J* = 11.9, 2.4 Hz, H-6′), 4.78 (1H, *d*, *J* = 7.5 Hz, H-1′), 6.35 (1H, *d*, *J* = 16.0 Hz, H-8″), 6.65 (2H, *d*, *J* = 9.0 Hz, H-3, H-5), 6.82 (2H, *d*, *J* = 8.5 Hz, H-3″, H-5″), 6.95 (2H, *d*, *J* = 9.0 Hz, H-2, H-6), 7.46 (2H, *d*, *J* = 8.5 Hz, H-2″, H-6″), 7.64 (1H, *d*, *J* = 16.0 Hz, H-7″). ^{13}C-NMR spectral data (CD_3OD) (see Table 1). FAB MS (positive ion mode) *m*/*z*: 419 $[M+H]^+$, 309, 147 (base peak). HR-FAB MS (positive ion mode) *m*/*z*: 419.1378, calculated for $C_{21}H_{23}O_9$: 419.1343.

3.8. Robustaside B (*12*)

Amorphous powder. $[\alpha_D]$ −49.9° (MeOH, *c* 6.95). UV (MeOH) λ_{max}: 280, 359 nm. IR (film) ν_{max}: 1695 cm^{-1}. ^{1}H-NMR spectral data (CD_3OD) δ: 3.42 (1H, *t*, *J* = 7.3 Hz, H-4′), 3.45 (1H, *t*, *J* = 7.3 Hz, H-2′), 3.48 (1H, *t*, *J* = 7.3 Hz, H-3′), 3.67 (1H, *ddd*, *J* =

7.3, 6.6, 2.2 Hz, H-5′), 4.36 (1H, *dd*, *J* = 12.0, 6.6 Hz, H-6′), 4.54 (1H, *dd*, *J* = 12.0, 2.2 Hz, H-6′), 4.73 (1H, *d*, *J* = 7.3 Hz, H-1′), 6.55 (1H, *d*, *J* = 16.0 Hz, H-8″), 6.68 (2H, *d*, *J* = 9.0 Hz, H-3, H-5), 6.72 (2H, *m*, H-5″, H-6″), 6.93 (1H, *br s*, H-2″), 6.96 (2H, *d*, *J* = 9.0 Hz, H-2, H-6), 7.98 (1H, *d*, *J* = 16.0 Hz, H-7′). ^{13}C-NMR spectral data (CD_3OD) (see Table 1). FAB MS (positive ion mode) *m*/*z*: 435 $[M+H]^+$, 325, 163. HR-FAB MS (positive ion mode) *m*/*z*: 435.1303, calculated for $C_{21}H_{23}O_{10}$: 435.1219.

3.9. Robustaside C (*13*)

Amorphous powder. $[\alpha_D]$ −57.2° (MeOH, *c* 0.58). UV (MeOH) λ_{max}: 279, 339 nm. IR (film) ν_{max}: 1695 cm^{-1}. ^{1}H-NMR spectral data (CD_3OD) *δ*: 3.34–3.53 (7H, H-2′, H-3′, H-4′, H-2‴, H-3‴, H-4‴, H-5‴), 3.66 (1H, *ddd*, *J* = 7.5, 6.0, 2.1 Hz, H-5′), 3.70 (1H, *dd*, *J* = 12.2, 5.2 Hz, H-6‴), 3.88 (1H, *dd*, *J* = 12.2, 2.2 Hz, H-6‴), 4.40 (1H, *dd*, *J* = 11.9, 6.0 Hz, H_b-6′), 4.53 (1H, *dd*, *J* = 11.9, 2.1 Hz, H_a-6′), 4.74 (1H, *d*, *J* = 7.7 Hz, H-1′), 4.78 (1H, *d*, *J* = 7.7 Hz, H-1‴), 6.48 (1H, *d*, *J* = 16.2 Hz, H-8″), 6.66 (2H, *d*, *J* = 9.2 Hz, H-3, H-5), 6.83 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-6″), 6.94 (2H, *d*, *J* = 9.2 Hz, H-2, H-6), 7.04 (1H, *d*, *J* = 3.0 Hz, H-2″), 7.15 (1H, *d*, *J* = 9.0 Hz, H-5″), 8.21 (1H, *d*, *J* = 16.2 Hz, H-7″). ^{13}*C*-NMR spectral data (CD_3OD) (see Table 1). FAB MS (positive ion mode) *m*/*z*: 619 $[M+Na]^+$, 597$[M+H]^+$; (negative ion mode) *m*/*z*: 595 $[M-H]^-$, 433. HR-FAB MS (negative ion mode) *m*/*z*: 595.1586, calculated for $C_{27}H_{31}O_{15}$: 595.1663.

3.10. Robustaside D (*14*)

Gum. $[\alpha_D]$ −46.1° (EtOH, *c* 1.84). UV (EtOH) λ_{max}: 284 nm. IR (film) ν_{max}: 3360 (OH), 1711 (ester C=O), 1668 (C=O) cm^{-1}. ^{1}H-NMR spectral data (CD_3OD) *δ*: 3.37 (1H, *t*, *J* = 7.3 Hz, H-4′), 3.43 (1H, *t*, *J* = 7.3 Hz, H-2′), 3.45 (1H, *t*, *J* = 7.3 Hz, H-3′), 3.61 (1H, *ddd*, *J* = 2.1, 6.8, 7.3 Hz, H-5′), 4.32 (1H, *dd*, *J* = 11.9, 6.8 Hz, 1H, H-6′), 4.47 (1H, *dd*, *J* = 11.9, 2.1 Hz, H-6′), 4.71 (1H, *d*, *J* = 7.3 Hz, H-1′), 6.25 (2H, *dd*, *J* = 9.6, 1.8 Hz, H-2″, H-6″), 6.27 (1H, *d*, *J* = 15.6 Hz, H-8″), 6.67 (1H, *d*, *J* = 15.6 Hz, H-7″), 6.68 (2H, *d*, *J* = 8.9 Hz, H-3, H-5), 6.84 (2H, *d*, *J* = 9.6 Hz, H-3″, H-5″), 6.91 (2H, *d*, *J* = 8.9 Hz, H-2, H- 6). ^{13}C-NMR spectral data (CD_3OD) (see Table 1). FAB MS (positive ion mode) *m*/*z*: 457 $[M+Na]^+$, 434 $[M]^+$. HR-FAB MS (positive ion mode) *m*/*z*: 434.1235, calculated for $C_{21}H_{22}O_{10}$: 434.1213.

References

Agrawal, P. K., & Bansal, M. C. (1989). In P. K. Agrawal, *Carbon-13 NMR of Flavonoids* (p. 283). Amsterdam: Elsevier.

Agrawal, P. K., Jain, D. C., Gupta, R. K., & Thakur, R. S. (1985). *Phytochemistry*, *24*, 2479.

Cannon, J. R., Chow, P. W., Fuller, M. W., Hamilton, B. H., Metcalf, B. W., & Power, A. J. (1973). *Aust. J. Chem*, *26*, 2257.

Coppola, G. M. (1985). *J. Heterocycl. Chem*, *22*, 491.

Freiden, E., Westmark, G. W., & Schor, J. M. (1961). *Arch. Biochem. Biophys*, *92*, 179.

Hoffman, T. E., Hausen, B. M., & Adams, R. M. (1985). *J. Am. Acad. Dermatology*, *13*(5 pt 1), 778.

Lytollis, W., Scannel, R. T., An, H., Murty, V. S., Reddy, S., Barr, J. R., & Hecht, S. M. (1995). *J. Am. Chem. Soc*, *117*, 12683.

Morimoto, H., Kawamatsu, Y., & Sugihara, H. (1968). *Chem. Pharm. Bull*, *16*, 2282.

Occolowitz, J. L. (1964). *Analyt. Chem*, *36*, 2177.

Rasmussen, M., Ridley, D. D., Ritchie, E., & Taylor, W. C. (1968). *Aust. J. Chem*, *21*, 2989.

Ridley, D. D., Ritchie, E., & Taylor, W. C. (1970). *Aust. J. Chem*, *23*, 147.

Ridley, D. D., Ritchie, E., & Taylor, W. C. (1968). *Aust. J. Chem*, *21*, 2979.

Ritchie, E., Taylor, W. C., & Vautin, T. K. (1965). *Aust. J. Chem*, *18*, 2015.

Scannell, R. T., Barr, J. R., Murty, V. S., Reddy, K. S., & Hachet, S. M. (1988). *J. Am. Chem. Soc*, *110*, 3650.

Sugimoto, T., Miyase, T., Kuroyanagi, M., & Ueno, A. (1988). *Chem. Pharm. Bull*, *36*, 4453.

Tang, Y.-Q., Feng, X.-Z., & Huang, L. (1996). *Phytochemistry*, *43*, 719.

Tsukamoto, C., & Taniguchi, T. (1958). *J. Invest. Dermatol*, *30*, 305.

Varma, R. S., Manju, M. S., & Parthasarathy, M. R. (1976). *Phytochemistry*, *15*, 1418.

Yasuda, I., Takeya, K., & Itokawa, H. (1981). *Chem. Pharm. Bull*, *29*, 564.

Yoshimoto, K., Itatani, Y., & Tsuda, Y. (1980). *Chem. Pharm. Bull*, *28*, 2065.