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Immunosuppressive constituents from *Saussurea medusa*

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Abstract

The methanol extract of *Saussurea medusa* Maxim afforded two lignans: 2α-guaicyl-4-oxo-6α-catechyl-3,7-dioxabicyclo [3.3.0]octane and 1α-hydroxy-2α,4α-guaicyl-3,7-dioxabicyclo[3.3.0]octane; two chlorophyll derivatives: 13-*epi*-phaeophorbide-a and 13-*epi*-phaeophorbide-a methyl ester; one megastigmane derivative: 3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one, along with 19 known compounds. Their structures were established on the basis of spectroscopic studies.

Keywords: *Saussurea medusa*; Compositae; Lignan; Phaeophorbide; Immunosuppressive activity

1. Introduction

Saussurea medusa Maxim (Compositae), a rare Chinese medicinal herb grown in Tibet province of China, has been used for the treatment of rheumatic arthritis and gynopathy (Yang et al., 1997). In our search for pharmacologically active compounds from crude drugs of plant origin, we found that a methanol extract of *S. medusa* exhibited inhibitory effects on cytokine production. This paper deals with the isolation and structure elucidation of five new and 19 known compounds, as well as their immunosuppressive activities.

2. Results and discussion

Repeated column chromatography of the ethyl acetate soluble fraction from the methanol extract of *S. medusa* Maxim yielded two new lignans (**1** and **2**), two new chlorophyll derivatives (**3** and **4**), one megastigmane derivative (**5**), along with 19 known compounds (**5–24**).

Compound **1** had a molecular formula $C_{20}H_{20}O_8$ from analysis of its HR EIMS. Its IR spectrum showed hydroxyl and ester carbonyl bond (3470 and 1757 cm^{-1}) absorbances and the UV spectrum revealed the presence of an aromatic ring (280 and 232 nm). The ^1H NMR spectral data of **1** showed two oxygenated methine [δ_H 5.37 (1H, *d*, $J=3.9$ Hz), 5.18 (1H, *d*, $J=3.6$ Hz)], an oxygenated methylene [δ_H 4.26 (1H, *dd*, $J=9.4$, 6.9 Hz), 4.03 (1H, *dd*, $J=9.4$, 4.1 Hz)], a 1,3,4,5-tetrasubstituted benzene ring [δ_H 6.65 (2H, *s*)], and a 1,3,4-trisubstituted benzene ring [δ_H 6.83 (1H, *d*, $J=1.8$ Hz), 6.72 (1H, *dd*, $J=8.1$, 1.8 Hz), 6.76 (1H, *d*, $J=8.1$ Hz)]. Its ^{13}C NMR spectral data revealed a carboxyl carbon (δ_C 182.4), an oxygenated methylene (δ_C 76.4), two oxygenated methine (δ_C 89.9 and 87.8), and the carbon signals among the downfield region indicated the presence of two benzene groups. From the above observations, compound **1** was assumed to be a lignan of the 3,7-dioxabicyclo[3.3.0]octane type. From the ^1H–^1H COSY and HMBC spectra, two partial structures [-O-CHCHCOO– and –CH_2CHCH–O–] were obtained, and could be assigned at positions C-4, 5, 6 and C-1, 2, 8 of the 3,7-dioxabicyclo[3.3.0]octane framework. The two-aryl groups were concluded to be guaiacyl (4-hydroxy-3-methoxyphenyl) and catechol (3,4-dihydroxyphenyl) moieties, respectively, from analysis of the coupling pattern and the NOESY spectrum. In the HMBC spectrum of **1**, the proton signal at δ_H 5.37 (H-2) correlated with the carbon signals at δ_C 134.4 (C-1′), 107.0 (C-2′ and 6′), 182.4 (C-4) and 76.4 (C-8), and the signal at δ_H

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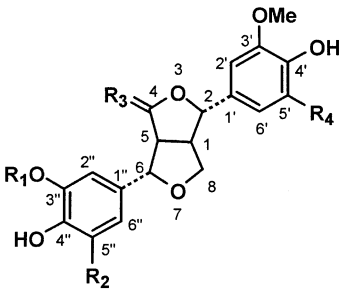
5.18 (H-6) correlated with the signals at δ_C 136.0 (C-1″), 121.0 (C-6″), 76.4 (C-8) and 182.4 (C-4). Thus, the guaiacyl and catechol groups were assigned at positions C-2 and C-6, respectively. The coupling constants of H-2 ($J=3.9$ Hz) and H-6 ($J=3.6$ Hz) indicated that both were axial protons. In the NOESY spectrum, the proton signal at δ_H 4.03 (H-8β) correlated with the signals at δ_H 5.37 (H-2) and 5.18 (H-6), while the proton signal at δ_H 3.32 (H-1) correlated with the signals at δ_H 4.26 (H-8α) and 3.62 (H-5). Therefore, compound **1** was 2α-guaicyl-4-oxo-6α-catechyl-3,7-dioxabicyclo[3.3.0]octane (Fig. 1).

Compound **2**, $C_{20}H_{22}O_7$, had two 1,3,4-trisubstituted benzene rings [δ_H 7.05 (2H, *br s*), 6.87 (1H, *dd*, $J=7.8$, 1.5 Hz), 6.85 (1H, *dd*, $J=7.8$, 1.5 Hz), 6.79 (1H, *d*, $J=7.8$ Hz), 6.78 (1H, *d*, $J=7.8$ Hz)], two oxygenated methines [δ_H 4.84 (1H, *d*, $J=5.2$ Hz), 4.67 (1H, *s*)], and two oxygenated methylenes [δ_H 4.46 (1H, *dd*, $J=9.1$, 8.6 Hz), 3.76 (1H, *dd*, $J=9.1$, 6.3 Hz); 4.03, 3.85 (each 1H, *d*, $J=9.3$ Hz)]. It was also a lignan of the 3,7-dioxabicyclo[3.3.0]octane type, and the position C-1 would be substituted by a hydroxy group due to a quaternary carbon (δ_C 95.5, C-1) and the coupling pattern of H-2 became a singlet. In the HMBC spectrum of **2**, the proton signal at δ_H 4.84 (H-4) correlated with the carbon signals at δ_C 136.3 (C-1″), 123.2 (C-6″), 65.1 (C-5), 95.5 (C-1) and 92.0 (C-2), while the proton signal at δ_H 4.67 (H-2) correlated with the carbon signals at δ_C 131.8 (C-1′), 124.3 (C-6′), 95.5 (C-1) and 78.8 (C-8). Furthermore, the proton signal at δ_H 4.84 (H-4) showed a NOESY correlation with the signal at δ_H 4.67 (H-2). So the two-aryl groups were assigned to positions C-2 and C-4. In the NOESY spectrum, the proton signal at δ_H 4.84 (H-4) correlated with the signals at δ_H 3.76 (H-6β) and 4.67 (H-2), the signal at δ_H 4.46 (H-6α) with the signal at δ_H 3.05 (H-5), while the proton signal at δ_H 4.03 (H-8β) correlated with the signals at δ_H 4.67 (H-2) and 3.76 (H-6β). Thus, the configurations of two aryl groups were determined as 2α and 4α. The other proton and carbon assignments were determined by 2D NMR spectra including NOESY. Therefore, compound **2** was elucidated as 1α-hydroxy-2α,4α-guaicyl-3,7- dioxabicyclo[3.3.0]octane.

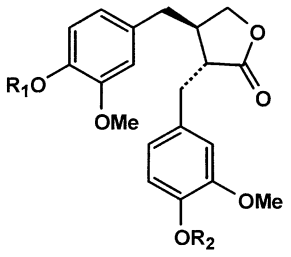
Compound **3** was obtained as a black-green solid and had a molecular formula $C_{35}H_{36}O_5N_4$ from HR FABMS. The UV spectrum showed absorptions of a chlorophyll derivative at 318, 421, 533, 566, 605 and 657 nm (Chan et al., 1999). Its ^{1}H NMR spectrum revealed the presence of three olefinic methyl groups [δ_H 3.12, 3.34 and 3.63 (each 3H, *s*)], one vinyl group [δ_H 7.88 (1H, *dd*, $J=17.8$, 11.5 Hz), 6.22 (1H, *d*, $J=17.8$ Hz), 6.12 (1H, *d*, $J=11.5$ Hz)], three olefinic protons [δ_H 9.40, 9.23 and 8.53 (each 1H, *s*)], and one ethyl group [δ_H 3.57 (2H, *q*, $J=7.2$ Hz), 1.64 (3H, *t*, $J=7.2$ Hz)]. In additional, three aliphatic protons and one secondary methyl group were observed. Compound **3** was a chlorophyll derivative, its ^{13}C NMR spectral data were very similar to those of phaeophorbide-a methyl ester (**17**) (Wray et al., 1979), and deduced to be a 13^2 or 17^2-oic acid of phaeophorbide-a (Fig. 1). In the HMBC spectrum of **3**, the proton signals at δ_H 6.12 (Ha-3^2) and 3.34 (H_3-2^1) correlated with the carbon signal at δ_C 136.1 (C-3), the proton signal at δ_H 2.62 (H-17^{1a}) with the signal at δ_C 176.1 (C-17^3), while the signals at δ_H 6.27 (H-13^2) and 3.87 (-OMe) correlated with the signal at δ_C 169.8 (C-13^3). Thus, the vinyl group and carboxylic acid were assigned at positions C-3 and C-17^2, respectively. In the NOESY spectrum, the proton signal at δ_H 4.45 (H-18) correlated with the signal at δ_H 2.26 (H-17^{1b}), while the proton signal at δ_H 4.19 (H-17) correlated with the signal at δ_H 1.81 (H_3-18^1) and 6.27 (H-13^2). Therefore, the structure of **3** was elucidated as 13^2-*epi*-phaeophorbide-a (Fig. 1).

Compound **4** had a molecular formula $C_{36}H_{38}O_5N_4$ from HR FABMS. The ^{1}H NMR spectrum of **4** showed three olefinic methyl groups [δ_H 3.68, 3.38 and 3.14 (each 3H, *s*)], one vinyl group [δ_H 7.91 (1H, *dd*, $J=17.1$, 11.7 Hz), 6.25 (1H, *d*, $J=17.1$ Hz) and 6.14 (1H, *d*, $J=11.7$ Hz)], three olefinic protons [δ_H 9.43, 9.26 and 8.56 (each 1H, *s*)], and one methoxy group [δ_H 3.60 (3H, *s*)]. It was also a chlorophyll derivative, its ^{1}H and ^{13}C NMR spectral data closely matched that of phaeophorbide-a methyl ester (**17**) (Wray et al., 1979; Nakatani et al., 1981). The difference of the ^{1}H NMR spectra between **4** and **17** was that the proton signal of H-13^2 revealed downfield signal (δ_H 6.28, in **4**) than that of **17** (δ_H 6.25, in **17**). So, compound **4** was deduced to be 13^2-epimer of **17**. In the NOESY spectrum of **4**, the proton signal at δ_H 4.23 (H-17) correlated with the signal at δ_H 6.28 (H-13^2) and 1.86 (H_3-18^1). Therefore, the structure of **4** was determined as shown (Fig. 1).

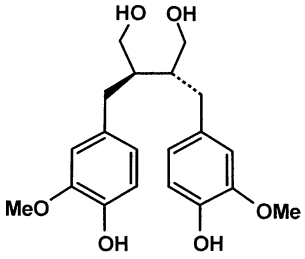
Compound **5** showed a $[M+Na]^+$ ion peak at m/z 247 in the positive FAB mass spectrum and had a molecular formula $C_{13}H_{20}O_3$ as deduced from analysis of its HR FABMS spectrum. The ^{1}H NMR spectrum revealed two coupled olefinic protons [δ_H 7.03 and 6.29 (each 1H, *d*, $J=15.6$ Hz), an oxygenated methine proton [δ_H 3.91 (1H, *m*)], and four methyl groups [δ_H 2.28, 0.98 (each 3H, *s*) and 1.20 (6H, *s*)]. Its ^{13}C NMR spectral data showed 13 carbon signals: a conjugate ketone (δ_C 197.5), one double bond [δ_C 142.4 (*d*) and 132.7 (*d*)] and an oxygenated methine (δ_C 64.1). An additional four methyl groups, two methylences and three quaternary carbons were also observed. Except for one double bond, a conjugate ketone and four methyl groups, compound **5** has a six membered ring and was assumed to be a megastigmane derivative (Takeda et al., 1997). In the HMBC spectrum, the proton signal at δ_H 6.29 (H-8) correlated with the carbon signals at δ_C 69.6 (C-6) and 197.5 (C-9), while the methyl proton signals at δ_H 0.98 (H_3-11) and 1.20 (H_3-13) could be correlated with the carbon signal at δ_C 69.6 (C-6). Thus, the 3-oxobutenyl group was assigned at position C-6. In the same



	R_1	R_2	R_3	R_4
1	H	H	O	OMe
6	Me	OMe	2H	OMe
7	Me	H	2H	H
8	Me	H	2H	OMe



	R_1	R_2
9	H	H
10	Me	H
11	Me	Glu



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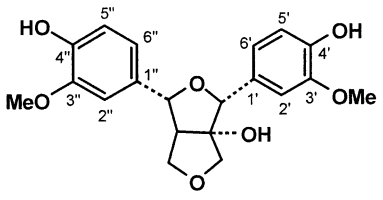

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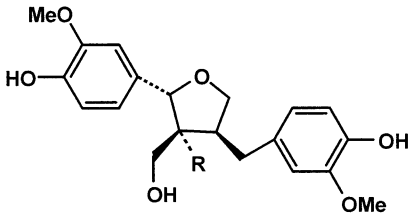
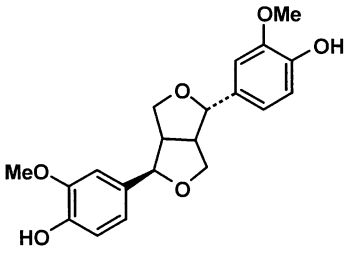


	R
12	OH
13	H



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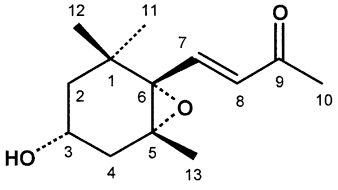

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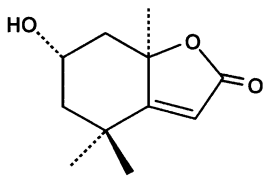

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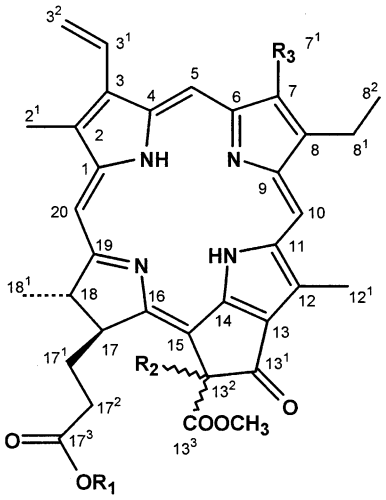

	R_1	R_2	R_3
3	H	α-H	Me
4	Me	α-H	Me
16	H	β-H	Me
17	Me	β-H	Me
18	Me	β-OH	Me
19	phytyl	β-H	Me
20	phytyl	β-H	CHO
21	phytyl	β-OH	Me
22	phytyl	α-OH	Me

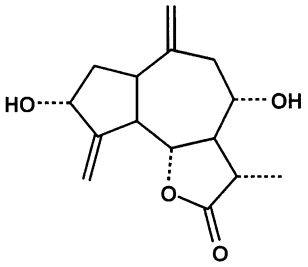

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Fig. 1. The structure of compounds **1–24**.

manner (HMBC and H–H COSY spectrum), the hydroxyl group was proposed to be attached to C-3. Acetylation of **5** afforded the monoacetylate (**5**a), and the proton signal of H-3 (δ_H 4.90, in **5**a) was downfield related to that of **5** (δ_H 3.91, in **5**). Thus, the hydroxyl group was assigned to the C-3 position. In the NOESY spectrum, the proton signal at δ_H 7.03 (H-7) correlated with the signal at δ_H 1.20 (H_3-12), while the signal at δ_H 3.91 (H-3) correlated with the signal at δ_H 0.98 (H_3-11). Therefore, the structure of **5** was 3β-hydroxy-5α,6α-epoxy-7-megastimen-9-one (Fig. 1).

Known compounds were identified by comparison of their spectroscopic data with literature values as follow: lirioresinol B (**6**) (Briggs et al., 1968), (+)-pinoresinol (**7**) and (+)-medioresinol (**8**) (Tsukamoto et al., 1984), matairesinol (**9**), arctigenin (**10**) and arctiin (**11**) (Rahman et al., 1990), (−)-berchemol (**12**) (Sakurai et al., 1989), lariciresinol (**13**) (Fonseca et al., 1978), epipinoresinol (**14**) (Rahman et al., 1990), secoisolariciresinol (**15**) (Fang et al., 1989), phaeophorbide-a (**16**) (Kobayashi et al., 1991), methyl phaeophorbide-a (**17**) (Wray et al., 1979; Nakatani et al., 1981), methyl-13^{2}β-hydroxy phaeophorbide-a (**18**) pheophytin a (**19**), pheophytin b (**20**), 13^{2}β-hydroxy pheophytin a (**21**) and 13^{2}α-hydroxy pheophytin a (**22**) (Nakatani et al., 1981), loliolide (**23**) (Hodges and Porte, 1964) and 3α,8α-dihydroxy-11βH-11,13-dihydrodehydrocostuslactone (**24**) (Li and Jia, 1989).

In a screen for immunosuppressive activity (Duan et al., 2000) for isolated compounds, we examined the inhibitory effect on cytokine production and show the bioactivity data for isolated compounds in Table 1. Two lignans (**6** and **7**) showed a significant inhibitory effect on cytokine production from lipopolysaccharide (or phytohemagglutinin)-stimulated human peripheral mononuclear cells compared with the reference compound (prednisolone; Kita et al., 1992). Two chlorophyll derivatives showed weak inhibitory effects, compound **3** inhibited IL-2 and IFN-γ production, and **4** inhibited IL-1β and IL-4 production.

3. Experimental

NMR experiments were run on a Bruker ARX-400 instrument. ^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz, using TMS as int. stand. MS were obtained on a JEOL JMSD-300 instrument. Chromatography column: silica gel 60 (Merck), Sephadex LH-20 (pharmacia), and Toyopearl HW-40 (TOSOH); HPLC: GPC (Shodex H-2001, 2002, $CHCl_3$), silica gel HPLC (Si_1: YMC-Park SIL-06 SH-043-5-06, 250×20 mm; Si_2: Hibar RT 250-25, LiChrosorb Si 60). IR spectra were recorded on a 1720 Infrared Fourier Transform spectrometer (PERKIN-ELMER), UV spectra were run on a UV 2100 UV-vis recording spectrometer (Shimadzu). Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

Table 1
The inhibition effect on cytokines of isolated compounds[a]

Compound	Inhibition (%)					
	TNF-α	IL-1β	IL-8	IL-2	IL-4	IFN-γ
1	8	27	−33	60	37	10
2	6	7	−10	35	24	33
3	32	48	−121	90	36	65
4	−4	63	−43	−20	66	3
6	93	80	99	100	100	98
7	99	98	94	100	100	99
8	1	34	−63	82	59	80
10	59	44	−43	46	59	84
11	−15	−36	68	−8	40	16
12	−27	39	25	49	17	17
13	46	62	85	100	100	67
14	13	18	−43	35	28	58
17	−1	44	1	−8	56	4
19	−14	3	68	31	25	31
21	−19	41	38	46	−107	8
22	−24	24	−21	−23	12	10
23	−12	−12	25	−16	9	19
24	−25	14	25	19	43	11
Prednisolone	52	68	15	65	76	75

[a] Concentration: isolated compounds, 10 μg/ml; prednisolone, 0.3 μg/ml; TNF-α: tumor necrosis factor alpha; IL-1β, 2, 4, and 8: interleukin-1β, 2, 4, and 8; IFN-γ: interferon gamma.

3.1. Isolation of compounds 1–24

The aerial part of *S. medusa* Maxim was purchased in 1998 from Tibet, People's Republic of China, and identified by Professor Dr. Guo-Liang Zhang (Lanzhou University, China). A voucher specimen is deposited in the Faculty of Pharmaceutical Sciences, University of Tokushima, Japan.

The aerial part (5.1 kg) of *S. medusa* was crushed and extracted ×3 with MeOH (20 l each) at 60 °C for 6 h. The MeOH extracts were conc. in vacuo to give a residue (540 g), which was partitioned between EtOAc and H_2O. The EtOAc layer was concd. to give a residue (140 g), which was applied to a silica gel (1.2 kg) column (90 x 850 mm, 500 ml each part). The column was eluted with solvent of increasing polarity [hexane–EtOAc (3:1, 3:2, 1:1, 1:2, and 1:4), EtOAc, EtOAc–MeOH (19:1, 9:1, 4:1) and MeOH] to give 17 frs (fr. 1–17). Fr. 11 + 12 (11.3 g) was applied to a silica gel column (800 g, 9×90 cm) and eluted with solvents of increasing polarity [$CHCl_3$–MeOH (95:5, 9:1, MeOH)] to give seven frs (fr. 11.1–11.7). Fr. 11.4 (3.5 g) was subjected to Sephadex LH-20 chromatography (MeOH) to give four frs (fr. 11.4.1–11.4.4). Fr. 11.4.2 was separated by GPC ($CHCl_3$) and then Si HPLC (Si_1) to obtain **5** (3 mg), **10** (12 mg) and **23** (12 mg). Fr. 11.4.3 was applied to a GPC ($CHCl_3$) to give six frs (fr. 11.4.3.1–11.4.3.6). Fr. 11.4.3.2 was separated by Si HPLC (Si_1 and then Si_2) to give **6** (6.5 mg) and **15** (3 mg). Fr. 11.4.3.3 was separated by Si HPLC (Si_1, hexane–EtOAc, 2:3) to give **7** (42 mg),

8 (5 mg), **9** (3 mg) and **14** (5 mg). Fr. 11.5 (3.1 g) was subjected to Sephadex LH-20 chromatography (MeOH) to give five frs (fr. 11.5.1–11.5.5). Fr. 11.5.4 was separated by Si HPLC (Si_2 and then Si_1) to give **2** (18 mg) and **13** (33 mg). Fr. 11.6 (2 g) was applied to a Sephadex LH-20 column (MeOH) to give six frs (fr. 11.6.1–11.6.6). Fr. 11.6.3 was separated by Si HPLC (Si_1, hexane–EtOAc–MeOH, 9:11:1) to give **11** (5 mg) and **24** (8 mg). Fr. 11.6.4 was separated by Si HPLC (Si_1, hexane-EtOAc-MeOH, 9:11:1) to give **1** (9 mg) and **12** (3 mg).

Fr. 5 (7.7 g) was subjected to silica gel column chromatography ($CHCl_3$-MeOH, 98:2, 95:5) to give eight frs (fr. 5.1–5.8). Combined frs. 5.4 and 5.5 (3 g) were applied to a Toyopearl HW-40 column ($CHCl_3$–MeOH, 2:1) to give five frs (fr. 5.4.1–5.4.5). Fr. 5.4.1 was separated using Si HPLC and then preparative TLC (PTLC) to give **19** (35 mg). Fr. 5.4.2 was separated by GPC and PTLC ($CHCl_3$–EtOAc, (9:1) to give **21** (8 mg) and **22** (6 mg). Combined frs. 7 and 8 were subjected to Sephadex LH-20 chromatography to give five frs (fr. 7.1–7.5). Fr. 7.4 was separated by GPC and Si HPLC (Si_1) to give **4** (39 mg), **17** (6 mg) and **18** (12 mg). Combined frs. 13 and 14 were applied to a Sephadex LH-20 column to give five frs (fr. 13.1–13.5). Fr. 13.5 was separated by Si HPLC (Si_1 and Si_2) to give **3** (20 mg), **16** (8 mg) and **20** (7 mg).

3.2. 2α-Guaicyl-4-oxo-6α-catechyl-3,7-dioxabicyclo[3.3.0] octane (1)

Amorphous powder, $[\alpha]_D^{25}$ −16.0° (MeOH, *c* 0.7). UV λ_{max}^{MeOH} nm (log ε: 280 (3.73), 232 (4.08). IR ν_{max}^{KBr} cm^{-1}: 3470, 2927, 1757, 1616, 1521, 1463, 1224, 1115, 816. ^1H NMR (CD_3OD): δ 6.83 (1H, *d*, *J* = 1.8 Hz, H-2″), 6.76 (1H, *d*, *J* = 8.1 Hz, H-5″), 6.72 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6″), 6.65 (2H, *s*, H-2′ and 6′), 5.37 (1H, *d*, *J* = 3.9 Hz, H-2), 5.18 (1H, *d*, *J* = 3.6 Hz, H-6), 4.26 (1H, *dd*, *J* = 9.4, 6.9 Hz, H-8α), 4.03 (1H, *dd*, *J* = 9.4, 4.1 Hz, H-8β), 3.62 (1H, *dd*, *J* = 9.1, 3.6 Hz, H-5), 3.32 (1H, *m*, H-1), 3.85 (6H, *s*,-OMe). ^{13}C NMR (CD_3OD): δ 182.4 (*s*, C-4), 152.3 (*s*, C-3′ and 5′), 149.2 (*s*, C-4″), 148.9 (*s*, C-3″), 139.8 (*s*, C-4′), 136.0 (*s*, C-1″), 143.4 (*s*, C-1′), 121.0 (*d*, C-6″), 119.0 (*d*, C-5″), 107.0 (*d*, C-2′ and 6′), 89.9 (*d*, C-2), 87.8 (*d*, C-6), 76.4 (*t*, C-8), 57.0 (*d*, C-5), 53.9 (*d*, C-1), 59.6 (*q*,-OMe). EI MS: *m*/*z* 388 $[M]^+$ (12), 310 (12), 268 (15), 182 (23), 167 (29), 163 (20), 151 (29), 137 (58), 121 (35), 115 (16), 107 (16), 95 (19), 83 (29), 69 (33), 55 (50), 44 (100), 36 (69). HR EIMS: *m*/*z* 388.1176 $[M]^+$, $C_{20}H_{20}O_8$ requires 388.1158.

3.3. 1α-Hydroxy-2α,4α-guaicyl-3,7-dioxabicyclo[3.3.0] octane (2)

Amorphous powder, $[\alpha]_D^{25}$ +20.7° (MeOH, *c* 1.4). UV λ_{max}^{MeOH} nm (log ε: 280 (3.71), 231 (4.14). IR ν_{max}^{KBr} cm^{-1}: 3425, 2930, 1608, 1517, 1461, 1368, 1276, 1038, 800. ^1H NMR (CD_3OD): δ7.05 (2H, *br s*, H-2′ and H-2″), 6.87 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-6′), 6.85 (1H, *dd*, *J* = 7.8, 1.5 Hz, H-6″), 6.79 (1H, *d*, *J* = 7.8 Hz, H-5″), 6.78 (1H, *d*, *J* = 8.0 Hz, H-5′), 4.84 (1H, *d*, *J* = 5.2 Hz, H-4), 4.67 (1H, *s*, H-2), 4.46 (1H, *dd*, *J* = 9.1 8.6 Hz, H-6α), 4.03 (1H, *d*, *J* = 9.3 Hz, H-8β), 3.85 (1H, *d*, *J* = 9.3 Hz, H-8α), 3.76 (1H, *dd*, *J* = 9.1, 6.3 Hz, H-6β), 3.05 (1H, *m*, H-5), 3.86 and 3.87 (each 3H, *s*,-OMe). ^{13}C NMR (CD_3OD): δ 151.8 (*s*, C-3′), 151.4 (*s*, C-3″), 150.2 (*s*, C-4′), 150.1 (*s*, C-4″), 136.3 (*s*, C-1′), 131.8 (*s*, C-1″), 124.3 (*d*, C-6″), 123.2 (*d*, C-6′), 118.8 (*d*, C-5′), 118.4 (*d*, C-5″), 95.5 (*s*, C-1), 92.0 (*d*, C-2), 90.5 (*d*, C-4), 78.8 (*t*, C-8), 74.7 (*t*, C-6), 65.1 (*d*, C-5), 59.1 (*q*,-OMe). EI MS: *m*/*z* 374 $[M]^+$ (100), 237 (8), 222 (20), 207 (55), 193 (15), 165 (34), 151 (57), 137 (49), 131 (37), 103 (24), 93 (23), 77 (12), 65 (18). HR EIMS: *m*/*z* 374.1393 $[M]^+$, $C_{20}H_{22}O_7$ requires 374.1366.

3.4. 13-epi-Phaeophorbide-a (3)

Black-green powder, UV λ_{max}^{MeOH} nm (log ε): 657 (4.52), 605 (3.52), 566 (3.32), 533 (3.50), 421 (5.10), 318 (3.97). IR ν_{max}^{KBr} cm^{-1}: 3525, 2960, 2318, 1736, 1698, 1618, 1498, 1223, 1035, 987, 739. ^1H NMR ($CDCl_3$): δ 9.40 (1H, *s*, H-10), 9.23 (1H, *s*, H-5), 8.53 (1H, *s*, H-20), 7.88 (1H, *dd*, *J* = 17.8, 11.5 Hz, H-3^1), 6.27 (1H, *s*, H-13^2), 6.22 (1H, *d*, *J* = 17.8 Hz, H-3^{2a}), 6.12 (1H, *d*, *J* = 11.5 Hz, H-3^{2b}), 4.45 (1H, *q*, *J* = 7.2 Hz, H-18), 4.19 (1H, *br d*, *J* = 8.5 Hz, H-17), 3.87 (3H, *s*,-OMe), 3.63 (3H, *s*, H_3-12^1), 3.57 (2H, *q*, *J* = 7.2 Hz, H-8^1), 3.34 (3H, *s*, H_3-2^1), 3.12 (3H, *s*, H_3-7^1), 2.62 (1H, *m*, H-17^{1a}), 2.59 (1H, *m*, H-17^{2a}), 2.34 (1H, *m*, H-17^{2b}), 2.26 (1H, *m*, H-17^{1b}), 1.81 (3H, *d*, *J* = 7.2 Hz, H_3-18^1), 1.64 (3H, *t*, *J* = 7.2 Hz, H_3-8^2). ^{13}C NMR ($CDCl_3$): δ 189.8 (*s*, C-13^1), 176.1 (*s*, C-17^3), 172.3 (*s*, C-19), 169.8 (*s*, C-13^3), 161.5 (*s*, C-16), 155.7 (*s*, C-6), 151.0 (*s*, C-9), 149.7 (*s*, C-14), 145.2 (*s*, C-8), 142.1 (*s*, C-1), 137.8 (*s*, C-11), 136.2 (*s*, C-4), 136.2 (*s*, C-7), 136.1 (*s*, C-3), 131.9 (*s*, C-2), 129.0 (*s*, C-12), 129.0 (*d*, C-3^1), 128.8 (*s*, C-13), 122.7 (*t*, C-3^2), 105.1 (*s*, C-15), 104.4 (*d*, C-10), 97.5 (*d*, C-5), 93.2 (*d*, C-20), 64.8 (*d*, C-13^2), 52.9 (*q*,-OMe), 51.2 (*d*, C-17), 50.2 (*d*, C-18), 31.1 (*t*, C-17^2), 30.0 (*t*, C-17^1), 23.1 (*q*, C-18^1), 19.4 (*t*, C-8^1), 17.4 (*q*, C-8^2), 12.1 (*q*, C-2^1), 12.1 (*q*, C-12^1), 11.2 (*q*, C-7^1). FAB MS: *m*/*z* 593 $[M+H]^+$; HR FABMS: *m*/*z* 593.2760 $[M+H]^+$, $C_{35}H_{37}O_5N_4$, requires 593.2764.

3.5. 13-epi-Phaeophorbide-a methyl ester (4)

Black-green powder, UV λ_{max}^{MeOH} nm (log ε): 669 (4.59), 610 (3.83), 539 (3.89), 508 (3.90), 414 (4.93), 329 (4.27), 276 (4.05). IR ν_{max}^{KBr} cm^{-1}: 3446, 2954, 2868, 1737, 1697, 1617, 1497, 1435, 1263, 1222, 1163, 1095, 1032, 895, 728. ^1H NMR ($CDCl_3$): δ9.43 (1H, *s*, H-10), 9.26 (1H, *s*, H-5), 8.56 (1H, *s*, H-20), 7.91 (1H, *dd*, *J* = 17.1, 11.7 Hz, H-3^1), 6.28 (1H, *s*, H-13^2), 6.25 (1H, *d*, *J* = 17.1

Hz, H-3^{2a}), 6.14 (1H, *d*, *J* = 11.7 Hz, H-3^{2b}), 4.48 (1H, *q*, *J* = 7.1 Hz, H-18), 4.23 (1H, *br d*, *J* = 7.7 Hz, H-17), 3.91 (3H, *s*, C-13^2,-OMe), 3.60 (3H, *s*, C-17^3,-OMe), 3.68 (3H, *s*, H$_3$-12^1), 3.56 (2H, *q*, *J* = 7.2 Hz, H-8^1), 3.38 (3H, *s*, H$_3$-2^1), 3.14 (3H, *s*, H$_3$-7^1), 2.67 (1H, *m*, H-17^{1a}), 2.55 (1H, *m*, H-17^{2a}), 2.36 (1H, *m*, H-17^{1b}), 2.29 (1H, *m*, H-17^{2b}), 1.86 (3H, *d*, *J* = 7.2 Hz, H$_3$-18^1), 1.67 (3H, *t*, *J* = 7.2 Hz, H$_3$-8^2). ^{13}C NMR ($CDCl_3$): δ 189.7 (*s*, C-13^1), 173.4 (*s*, C-17^3), 172.2 (*s*, C-19), 169.7 (*s*, C-13^3), 161.3 (*s*, C-16), 155.6 (*s*, C-6), 151.0 (*s*, C-9), 149.7 (*s*, C-14), 145.2 (*s*, C-8), 142.1 (*s*, C-1), 138.0 (*s*, C-11), 136.2 (*s*, C-4), 136.3 (*s*, C-7), 136.2 (*s*, C-3), 131.9 (*s*, C-2), 129.1 (*s*, C-12), 129.1 (*d*, C-3^1), 128.9 (*s*, C-13), 122.8 (t, C-3^2), 105.2 (*s*, C-15), 104.4 (*d*, C-10), 97.5 (*d*, C-5), 93.2 (*d*, C-20), 64.8 (*d*, C-13^2), 52.9 (*q*, C-13^2,-OMe), 51.8 (*q*, C-17^3,-OMe), 51.2 (*d*, C-17), 50.2 (*d*, C-18), 31.1 (*t*, C-17^2), 29.9 (*t*, C-17^1), 23.2 (*q*, C-18^1), 19.4 (*t*, C-8^1), 17.4 (*q*, C-8^2), 12.1 (*q*, C-2^1), 12.1 (*q*, C-12^1), 11.2 (*q*, C-7^1). FAB MS: *m*/*z* 607 $[M+H]^+$, HR FABMS: *m*/*z* 607.2936 $[M+H]^+$, $C_{36}H_{39}O_5N_4$, requires 607,2920.

3.6. 3β-Hydroxy-5α,6α-epoxy-7-megastigmen-9-one (5)

Amorphous powder, $[\alpha]_D^{25}$ −74.3° (MeOH, *c* 0.3). UV λ_{max}^{MeOH} nm (log ε: 312 (2.57), 292 (2.77), 231 (3.92). IR ν_{max}^{KBr} cm^{-1}: 3420, 2927, 1677, 1364, 1260, 1181, 1033, 987, 698. ^{1}H NMR ($CDCl_3$): δ7.03 (1H, *d*, *J* = 15.6 Hz, H-7), 6.29 (1H, *d*, *J* = 15.6 Hz, H-8), 3.91 (1H, *m*, H-3), 2.39 (1H, *dd*, *J* = 14.5, 5.0 Hz, H-4β), 2.28 (3H, *s*, H$_3$-10), 1.67 (1H, *m*, H-4α), 1.64 (1H, *m*, H-2α), 1.26 (1H, *m*, H-2β), 1.20 (6H, *s*, H$_3$-12 and 13), 0.98 (3H, *s*, H$_3$-11). ^{13}C NMR ($CDCl_3$): δ 197.5 (*s*, C-9), 142.4 (*d*, C-7), 132.7 (*d*, C-8), 69.6 (*s*, C-6), 67.3 (*s*, C-5), 64.1 (*d*, C-3), 46.7 (*t*, C-2), 40.7 (*t*, C-4), 35.2 (*s*, C-1), 29.4 (*q*, C-12), 28.4 (*q*, C-10), 25.1 (*q*, C-11), 19.9 (*q*, C-13). EI MS: *m*/*z* 224 $[M]^+$ (10), 167 (4), 151 (4), 123 (100), 109 (8), 95 (8), 83 (6), 79 (5), 69 (4), 55 (7), 43 (57). FAB MS: *m*/*z* 247 $[M+Na]^+$, HR FABMS: *m*/*z* 247.1286 $[M+Na]^+$, $C_{13}H_{20}O_3Na$, requires 247.1310.

Acetylation of **5**: Compound **5** (1.2 mg) was treated with Ac_2O (0.3 ml) and C_5D_5N (0.5 ml) at room temperature over night. The reaction mixture was worked up in the usual way to give monoacetate **5**a (1 mg). Compound **5**a: ^{1}H NMR ($CDCl_3$): δ 7.01, 6.29 (each 1H, *d*, *J* = 15.6 Hz); 4.90 (1H, *m*), 2.39 (1H, *dd*, *J* = 14.5, 5.1 Hz), 2.26, 2.00, 1.19, 1.17 and 0.97 (each 3H, *s*); 1.78 (1H, *dd*, *J* = 14.5, 6.8 Hz), 1.65 (1H, *dd*, *J* = 13.7, 3.0 Hz), 1.37 (1H, *m*).

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