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Megastigmane, aliphatic alcohol and benzoxazinoid glycosides from *Acanthus ebracteatus*

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Abstract

From the aerial part of *Acanthus ebracteatus*, a megastigmane glycoside (ebracteatoside A), three aliphatic alcohol glycosides (ebracteatosides B–D), as well as 7-chloro-(2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (7-Cl-DIBOA-Glc) were isolated together with 22 known compounds. Structural elucidations were based on analyses of spectroscopic data.

Keywords: *Acanthus ebracteatus*; Acanthaceae; Megastigmane glycoside; Aliphatic alcohol glycoside; Benzoxazinoid glucoside; Ebracteatosides A–D

1. Introduction

As part of our ongoing study on Thai medicinal plants in the genus *Acanthus* (Kanchanapoom et al., 2001a,b), we investigated the constituents of *Acanthus ebracteatus* Vahl (Acanthaceae, Thai name: Ngueak-Pla-Mo, Nam-Mo) collected from Pattani province. *A. ebracteatus* is a spiny herb distributed in the mangroves of southern Thailand. In Thai traditional medicine, the plant is widely used as a purgative and an anti-inflammatory, as well as the leaves dispensed with pepper (*Piper nigrum* L.) as tonic pills for longevity. In the preliminary studies, anti-mutagenicities of the organic extracts have been reported (Rojanapo et al., 1990). The present study deals with the isolation and structural elucidation of one new megastigmane glycoside (**3**), three new aliphatic alcohol glycosides (**5**–**7**) and one new benzoxazinoid glucoside (**10**), along with 22 known compounds from the aerial part of this plant. Also, the usage in Thai traditional medicines is discussed.

2. Results and discussion

The methanolic extract of the aerial part of *A. ebracteatus* was suspended in H_2O and defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer resin of styrene and divinylbenzene, using H_2O, 30% MeOH, 60% MeOH, MeOH and Me_2CO, successively. The fractions eluted with 30% and 60% MeOH were repeatedly subjected to silica gel column chromatography, as well as other RP-18 or prep. HPLC to afford 27 compounds. Twenty two were identified as known compounds; plucheoside B (**1**), alangionoside C (**2**) (Otsuka et al., 1995), premnaionoside (**4**) (Sudo et al., 2000), (2*R*)-2-*O*-β-D-glucopyranosyl-2*H*-1,4-benzoxazin-3(4*H*)-one (HBOA-Glc, **8**) (Tietze et al., 1991), (2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc, **9**) (Hartenstein and Sicker, 1994), phenethyl alcohol 8-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**11**) (Ono et al., 1999), benzyl alcohol 7-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside (zizybeoside I, **12**) (Okamura et al., 1981), adenosine (**13**) (Otsuka et al., 1989), verbascoside (**14**), isoverbascoside (**15**), leucosceptoside A (**16**) (Miyase et al., 1982), martynoside (**17**) (Sasaki et al., 1978), β-hydroxyacteoside (**18**) (Kitakawa et al., 1984), vecenin-2 (**19**), schaftoside (**20**) (Markham and Chari, 1982), luteolin-7-*O*-β-D-glucuronide (**21**), apigenin-7-*O*-β-D-glucuronide (**22**) (Hase et al., 1995), (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**23**), (−)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**24**) (Achenbach et al., 1992), (8*R*, 7′*S*, 8′*R*)-5,5′-dimethoxylariciresinol 4′-*O*-β-D-glucopyranoside (**25**) (Ida et al., 1994), magnolenin C (**26**) (Rao and Wu, 1978), and (+)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-*O*-β-D-glucopyranoside (**27**) (Kinjo et al., 1991)

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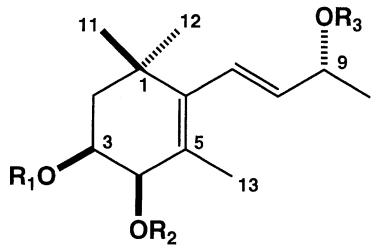
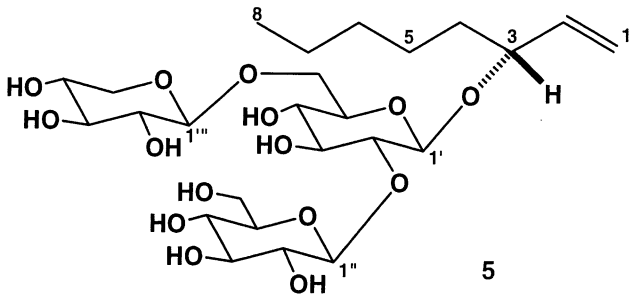


	R_1	R_2	R_3
1	Glc	H	H
2	H	Glc	H
3	H	H	Glc (2'-1") Api
1a	H	H	H



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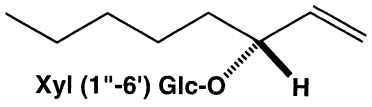
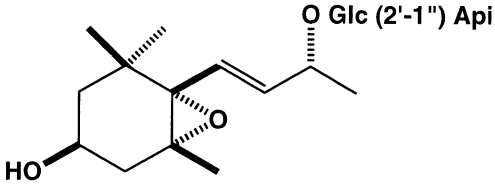


5a



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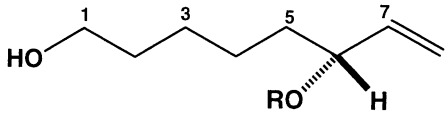
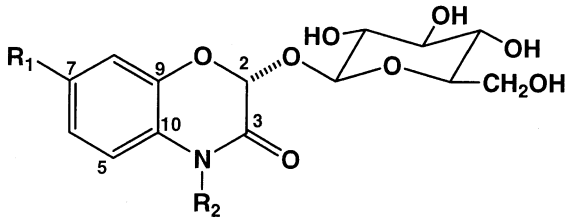


R

6 Glc (2'-1") Glc

7 Glc (6'-1") Xyl



	R_1	R_2
8	H	H
9	H	OH
10	Cl	OH

by comparison of physical data with literature values and from spectroscopic evidence.

The molecular formula of compound **3** was determined as $C_{24}H_{40}O_{12}$ by HR-FAB mass spectrometry. The ^1H NMR spectrum revealed the presence of three singlet methyls, one methyl doublet, two olefinic protons, and two anomeric protons of sugar moieties. The ^{13}C NMR spectrum indicated the presence of the β-D-apiofuranosyl-(1→2)-*O*-β-D-glucopyranose unit, compared to compound **4**. The stereochemistry of an apiose could assign as D-form by the coupling of H-5″ at δ 3.61, which showed the magnetically equivalent (Snyder and Serianni, 1987). The remaining 13 carbon signals were suggested to present a megastigmane skeleton. The aglycone moiety of compound **3** was expected to be the same as that of plucheoside B (**1**) and alangionoside C (**2**). On enzymatic hydrolysis of **1**, **2** and **3** gave the same compound **1a**, identified by spectral analyses and comparing the HPLC retention time (R_f 3.38). Comparison of the ^{13}C NMR spectrum of **3** with those of **1a** showed

a significant downfield shift of C-9 (+7.9 ppm) together with the upfield shifts of C-8 (−2.1 ppm) and C-10 (−2.8 ppm), indicating that the sugar moiety is located at C-9. The absolute configuration at C-9 of **3** was assigned to be *R* from the chemical shifts at C-9 and C-10 (77.4 and 21.0, respectively) (Pabst et al., 1992; Takeda et al., 1997). Also, the absolute configurations at C-9 of **1** and **2**, which could not be determined from the previous reports (Uchiyama et al., 1989; Otsuka et al., 1995), were concluded to be *R*. Consequently, the structure of compound **3** was elucidated as shown, named ebracteatoside A.

The molecular formula of compound **5** was determined as $C_{25}H_{44}O_{15}$ by HR-FAB mass spectrometry. The ^{13}C NMR spectrum showed the presence of three sugar moities, which were identified to be a terminal β-D-glucopyranose, a terminal β-D-xylopyranose connected to a core β-D-glucopyranose unit, in addition to eight carbon signals for the aglycone. Acid hydrolysis gave D-xylose and D-glucose which were identified by TLC and comparison of the optical rotation with authentic samples. DEPT experiments indicated the presence of one methyl (δ 14.5), five methylenes (δ 23.6, 25.5, 32.9, 35.7 and 116.9) as well as two methines (δ 82.3 and 140.5) in the aglycone moiety. The chemical shifts of **5** were almost the same as those of (3*R*)-1-octen-3-ol-3-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranoside (**5a**), previously isolated from *Mentha spicata* (Yamamura et al., 1998), except for the additional signals of a β-D-glucopyranosyl unit. The downfield shift of C-2′ (+8.5 ppm) together with the upfield shift of C-1′ (-1.7) and C-3′ (−0.7 ppm) of the substituted sugar established the attachment of an additional unit to C-2′. Also, the HMBC correlations confirmed the positions of the sugar moieties as shown in Fig 1. Moreover, the EI–MS of its trimethylsilyl ethers exhibited the fragment ion characteristic of the 1,6-linked biose unit at *m*/*z* 481, due to TMSi-hexose-(6→1)-TMSi-pentose (Kasai et al., 1977), indicating the position of β-D-xylopyranose to C-6′. Therefore, the structure of compound **5** was assigned as (3*R*)-1-octen-3-ol-3-*O*-β-D-xylopyranosyl-(1‴→6′)-*O*-[β-D-glucopyranosyl-(1″→2′)]-*O*-β-D-glucopyranoside, named ebracteatoside B.

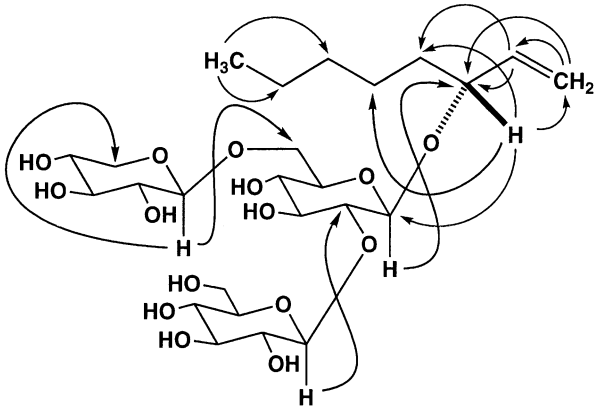


Fig. 1. The HMBC correlations of compound **5**.

Compound **6** had the molecular formula $C_{20}H_{36}O_{12}$, determined from HR-FAB mass spectrometry. The ^{13}C NMR spectrum revealed the presence of two β-D-glucopyranosyl units, one of which was attached at C-2′ due to the downfield shift (δ 83.6) of this carbon signal, in addition to eight signals for the aglycone moiety. Acid hydrolysis afforded D-glucose, identical by TLC and comparison of the optical rotation with authentic sample. The chemical shifts of the aglycone carbons were similar to those of **5**. However, the absence of the methyl signal at δ 14.5 together with the presence of one more methylene carbon at δ 62.9 were observed in **6**, indicating that the terminal methyl was substituted by a hydroxyl group. The absolute configuration at C-6 was suggested to be in the *R*-form by comparing the ^{13}C NMR spectral data with those of **5**. From these results, the structure of compound **6** was identified as (6*R*)-7-octene-1,6-diol 6-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside, named ebracteatoside C.

Compound **7** had the molecular formula $C_{19}H_{34}O_{11}$, deduced from HR-FAB mass spectrometry. The chemical shifts were very similar to those of **6**, except for the difference signals of the terminal sugar which was identified as a β-D-xylopyranose. The terminal sugar was assigned to attach at C-6′ (δ 69.5) of β-D-glucopyranose, due to the downfield shift of this signal. Consequently, the structure of compound **7** was elucidated to be (6*R*)-7-octene-1,6-diol 6-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranoside, named ebracteatoside D.

Compound **10** showed the characteristic pattern due to have a chlorine atom from negative FAB–MS. The molecular formula was determined by HR–FAB mass spectrometry as $C_{14}H_{16}ClNO_9$. The ^1H and ^{13}C NMR spectral data were similar to those of DIBOA-Glc (**9**). However, the ^1H NMR spectrum revealed the presence of an ABX system at δ 7.16 (1H, $J=8.5$, 2.2 Hz), δ 7.24 (1H, $J=8.5$ Hz) and δ 7.30 (1H, $J=2.2$ Hz), indicating that the chlorine atom was attached to an aromatic ring. The position of the chlorine atom was assigned to C-7 by comparing the calculation of the chemical shifts effect from the Cl atom and the observed chemical shifts from ^{13}C NMR spectrum. The chemical shift differences of the aromatic ring between **9** and **10** as to the *ipso* (C-7), *ortho* (C-6), *ortho* (C-8), *meta* (C-5), *meta* (C-9) and *para* (C-10) were +4.6, −1.0, +0.5, +1.3, +0.9 and −0.5, respectively, which agreed with those for the chlorine substituted at C-7 of the aromatic ring (Silverstein et al., 1981). The configuration at C-2 was suggested to be *R* since the optical rotation measured has a positive value as **9**. Therefore, the structure of compound **10** was identified as 7-chloro-(2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (7-Cl-DIBOA-Glc).

The biological activities of the isolated compounds have not been investigated. The presence of benzoxazinoids (**8**–

9) and phenylpropanoids (**14**–**15**) as the major constituents are in agreement with Thai traditional usage as an anti-inflammatory preparation (Otsuka et al., 1988; Cometa et al., 1993). Furthermore, the toxicological and pharmacological properties of benzoxazinoids have been reported as the chemical resistance factors against insects, fungi, bacteria and viruses in many crop plants of the family Gramineae (Niemeyer, 1988; Sicker et al., 2000), as well as having mutagenic activities (Hashimoto and Shudo, 1996). Also, the aglycone of **9** (DIBOA) was shown to be mutagenic in a test with *Salmonella typhimurium* TA 100 and TA 98 (Hashimoto and Shudo, 1996). The results on the mutagenicities of benzoxazinoids contrasted with the reported as anti-mutagenicities of the organic extracts of this plants by Rojanapo and co-workers (1990), which could be explained by the structural requirements for biological activities of benzoxazinoids. The 2-hydroxyl group of benzoxazinoids has been considered to be important for biological activities more than glucoside forms (Niemeyer, 1988). From these reports, the usage of this species for internal treatments in Thai traditional medicine should be considered.

3. Experimental

3.1. General

NMR spectra were recorded in methanol-d_4 or DMSO-d_6 using a JEOL JNM A-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as internal standard. MS were recorded on a Jeol JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (150×20 mm i.d., YMC) and polyamine II (250×20 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. Analytical HPLC was carried out on a column of ODS (100×4.6 mm i.d., YMC). For CC, silica gel G 60 (Merck), YMC-gel ODS (50 μm, YMC), and highly porous copolymer resin of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) EtOAc–EtOH–H_2O (4:1:0.1), (II) EtOAc–EtOH–H_2O (7:3:0.3), (III) EtOAc–EtOH–H_2O (6:4:1), (IV) 10% MeCN, (V) 80% MeCN, (VI) 75% MeCN, (VII) EtOAc–MeOH-H_2O (4:1:0.1), (VIII) EtOAc–MeOH–H_2O (7:3:0.3), (IX) EtOAc–EtOH–H_2O (6:4:1), (X) 15% MeCN, (XI) 20% MeCN, (XII) 25% MeCN and (XIII) 40% MeOH. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2. Plant material

A. ebracteatus Vahl was collected in May 1997 from Pattani Province, Southern Thailand. The identification of the plant was confirmed by Professor Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (KKU-0002) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried aerial part (2.8 kg) of *A. ebracteatus* was extracted with hot MeOH. After removal of the solvent by evaporation, the residue (513.0 g) was defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer resin of styrene and divinylbenzene and eluted with H_2O, 30% MeOH, 60% MeOH, MeOH and Me_2CO, successively. The fraction eluted with 30% MeOH (21.0 g) was subjected to a column of silica gel (systems I, II and III, respectively) affording six fractions. Fraction 2 (4.0 g) was separated on RP-18 (system IV) to provide compound **8** (2.2 g). Fraction 3 (5.6 g) was purified by using RP-18 (system IV) and HPLC-polyamine II (system V) to give compounds **1** (30 mg), **3** (30 mg), **4** (36 mg), **9** (3.8 g) and **11** (42 mg). Fraction 4 (2.1 g) was subjected to RP-18 (system IV), then followed by HPLC-polyamine II (system VI) to afford compounds **6** (78 mg), **7** (5 mg) **12** (18 mg) and **13** (22 mg). Fraction 6 (2.5 g) was further purified by using RP-18 (system V) to provide compound **19** (190 mg).

The fraction eluted with 60% MeOH (48.0 g) was subjected to a column of silica gel (systems VII, VIII and IX, respectively) to give seven fractions. Fraction 2 (10.0 g) was subjected to a column of RP-18 (system X), the followed by HPLC-ODS (system XI or XII) to afford compounds **14** (3.0 g), **15** (325 mg), **16** (38 mg) and **17** (30 mg). Fraction 4 (3.7 g) was purified by using RP-18 (system X) and HPLC-ODS (system XIII) to give compounds **2** (11 mg), **18** (20 mg), **23** (112 mg) and **24** (50 mg). Fraction 5 (3.9 g) was separated on RP-18 (system X) to give nine fractions. Fractions 5-5 and 5-6 were purified by HPLC-ODS (system XIII) to provide compounds **10** (12 mg), **25** (33 mg) and **26** (36 mg). Fractions 5-8 and 5-9 were similarly purified by HPLC-ODS (system XII) to afford compounds **5** (142 mg) and **27** (34 mg). Fraction 6 (2.5 g) was subjected to RP-18 (system X) to provide six fractions. Compound **20** (75 mg) was crystallized from fraction 6-3. Finally, fraction 7 (9.9 g) was purified by RP-18 (system X), then followed by HPLC-ODS (system X) to give compounds **21** (98 mg) and **22** (66 mg).

3.4. Ebracteatoside A (*3*)

Amorphous powder, $[\alpha]_D^{17}$ −52.2° (MeOH, *c* 2.0); ^{1}H NMR (methanol-d_4): δ 1.77 (1H, *dd*, J = 12.7, 12.5 Hz, H-2ax), δ 1.43 (1H, *ddd*, J = 12.2, 3.4, 1.2 Hz, H-2eq), δ 3.74 (1H, *dt*, J = 12.0, 3.9 Hz, H-3), δ 6.07 (1H, *d*, J = 16.1 Hz, H-7), δ 5.56 (1H, *dd*, J = 16.1, 7.0 Hz, H-8),

δ 4.42 (1H, *qd*, *J*=6.6, 1.0 Hz, H-9), δ 3.82 (1H, *brd*, *J*=3.4 Hz, H-4), δ 1.30 (3H, *d*, *J*=6.4 Hz, H-10), δ 1.07 (3H, *s*, H-11), δ 1.03 (3H, *s*, H-12), δ 1.83 (3H, *s*, H-13), δ 4.44 (1H, *d*, *J*=7.8 Hz, H-1′ Glc), δ 3.51 (1H, *dd*, *J*=9.3, 7.8 Hz, H-2′ Glc), δ 3.46 (1H, *dd*, *J*=9.0, 9.0 Hz, H-3′ Glc), δ 3.33 (1H, *dd*, *J*=9.5, 8.5 Hz, H-4′ Glc), δ 3.18 (1H, *ddd*, *J*=9.5, 4.6, 2.2 Hz, H-5′ Glc), δ 3.78 (1H, *dd*, *J*=11.7, 2.4 Hz, H-6′ Glc), δ 3.66 (1H, *dd*, *J*=11.9, 4.9 Hz, H-6′ Glc), δ 5.38 (1H, *d*, *J*=1.7 Hz, H-1″ Api), δ 3.94 (1H, *d*, *J*=1.7 Hz, H-2″ Api), δ 4.06 (1H, *d*, *J*=9.5 Hz, H-4″ Api), δ 3.71 (1H, *d*, *J*=9.5 Hz, H-4″ Api), δ 3.61 (2H, *s*, H-5″ Api); ^{13}C NMR (methanol-d_4): Table 1; negative HR–FAB–MS, *m*/*z* 519.2449 ($C_{24}H_{39}O_{12}$ requires 519.2441)

3.5. Enzymatic hydrolysis of plucheoside B (*1*), alangionoside C (*2*), and ebracteatoside A (*3*)

Each sample of plucheoside B (**1**) (17 mg), alangionoside C (**2**) (9 mg) and ebracteatoside A (**3**) (15 mg) was dissolved in 0.5 ml of MeOH. A solution of crude hesperidinase (100 mg in 20 ml of H_2O) was added in each experiment. After stirring at 37 °C for 5 days, the mixtures were extracted with EtOAc, and concentrated to dryness, affording **1a** (7, 2 and 4 mg, respectively). The structure was identified by spectral analyses and comparing the HPLC retention time (R_f 3.38, solvent system: 50% MeOH)

Table 1
^{13}C NMR spectral data of compounds **1**, **1a**, **2** and **3** (methanol-d_4, 100 MHz)

C	1	1a	2	3
1	37.8	37.7	37.9	37.7
2	40.0	41.7	42.9	41.6
3	76.1	67.9	67.4	67.9
4	70.1	72.6	84.3	72.5
5	127.9	128.9	127.6	129.1
6	143.0	142.4	140.8	142.2
7	126.7	126.8	126.5	128.6
8	140.6	140.5	140.7	138.4
9	69.4	69.5	69.4	77.4
10	23.8	23.8	23.8	21.0
11	27.7	27.7	27.4	27.8
12	30.3	30.3	30.2	30.3
13	19.9	19.8	19.8	19.9
Glc-1′	102.6		106.1	100.8
2′	75.3		75.1	79.3
3′	78.1		78.0	77.9
4′	71.6		71.4	71.3
5′	78.1		78.2	77.7
6′	62.7		62.5	62.4
Api-1″				110.8
2″				78.6
3″				80.6
4″				75.3
5″				66.0

3.6. Aglycone of plucheoside B, alangionoside C and ebracteatoside A (*1a*)

Amorphous powder, $[\alpha]_D^{22}$ −65.4° (MeOH, *c* 0.52); ^{1}H NMR (methanol-d_4): δ 1.78 (1H, *dd*, *J*=12.7, 12.5 Hz, H-2ax), δ 1.42 (1H, *ddd*, *J*=12.0, 3.4, 1.2 Hz, H-2eq), δ 3.74 (1H, *dt*, *J*=12.9, 3.9 Hz, H-3), δ 3.82 (1H, *brd*, *J*=3.7 Hz, H-4), δ 6.02 (1H, *d*, *J*=16.1 Hz, H-7), δ 5.50 (1H, *dd*, *J*=16.1, 6.1 Hz, H-8), δ (1H, *qd*, *J*=6.3, 1.0 Hz, H-9), δ 1.27 (3H, *d*, *J*=6.4 Hz, H-10), δ 1.06 (3H, *s*, H-11), δ 1.03 (3H, *s*, H-12), δ 1.82 (3H, *s*, H-13); ^{13}C NMR (methanol-d_4): Table 1; negative HR–FAB–MS, *m*/*z* 225.1456 ($C_{13}H_{21}O_3$ requires 225.1490).

3.7. Ebracteatoside B(*5*)

Amorphous powder, $[\alpha]_D^{22}$ −45.0° (MeOH, *c* 2.11); ^{1}H NMR (methanol-d_4): δ 5.21 (1H, *brd*, *J*=17.3 Hz, H-1), δ 5.13 (1H, *brd*, *J*=10.5 Hz, H-1), δ 5.85 (1H, *ddd*, *J*=17.3, 10.3, 7.3 Hz, H-2), δ 4.12 (1H, *dd*, *J*=12.9, 6.6 Hz, H-3), δ 1.66 (1H, *m*, H-4), δ 1.49 (1H, *m*, H-4), δ 1.24–1.38 (6H, *m*, H-5,6,7), δ 0.89 (3H, *t*, *J*=6.8 Hz, H-8), δ 4.63 (1H, *d*, *J*=7.6 Hz, H-1″ Xyl), δ 4.42 (1H, *d*, *J*=7.6 Hz, H-1′ Glc), δ 4.32 (1H, *d*, *J*=7.3 Hz, H-1″ Glc); ^{13}C NMR (methanol-d_4): Table 2; negative HR–FAB–MS, *m*/*z* 583.2555 ($C_{25}H_{43}O_{15}$ requires 583.2601).

Table 2
^{13}C NMR spectral data of compounds **5**–**7** (methanol-d_4, 100 MHz)

C	5	C	6	7
1	116.9	8	116.6	116.2
2	140.5	7	140.8	140.8
3	83.8	6	83.6	82.5
4	35.7	5	35.7	35.7
5	25.5	4	25.7	25.8
6	32.9	3	33.6	33.6
7	23.6	2	26.9	26.9
8	14.5	1	62.9	62.9
Glc-1′	101.7	Glc-1′	101.7	103.3
2′	82.3	2′	82.6	75.3
3′	77.5[a]	3′	77.7[a]	78.1[a]
4′	71.0	4′	71.3	71.2
5′	77.5[a]	5′	78.0[a]	77.6[a]
6′	69.3	6′	62.8	69.5
Glc-1″	104.8	Glc-1″	105.0	
2″	75.9	2″	76.1	
3″	77.8[a]	3″	77.7[a]	
4″	71.4	4″	71.5	
5″	78.0[a]	5″	78.2[a]	
6″	62.7	6″	62.6	
Xyl-1‴	105.1	Xyl-1″		105.3
2‴	74.7	2″		74.9
3‴	77.5[a]	3″		77.0
4‴	71.4	4″		71.5
5‴	66.7	5″		66.9

[a] Assignments may be interchanged in each column.

3.8. Trimethylsilylation of ebracteatoside B (**5**)

Ebracteatoside B (ca. 2 mg) was heated with trimethylsilylimidazole (three drops) in a stoppered microtube at 90 °C for 1 h. After dilution with H_2O, the reaction mixture was extracted with *n*-hexane. The *n*-hexane layer was concentrated to dryness by blowing N_2 gas at room temperature. The residue was subjected to determination of EI–MS.

3.9. Ebracteatoside C (**6**)

Amorphous powder, $[\alpha]_D^{22}$ −19.8° (MeOH, *c* 2.62); ^{1}H NMR (methanol-d_4): δ 3.54 (2H, *t*, *J* = 6.1 Hz, H-1), δ 1.35–1.44 (4H, *m*, H-2, 4), δ 1.54 (2H, *m*, H-3), δ 1.72 (1H, *m*, H-5), δ 1.56 (1H, *m*, H-5), δ 4.15 (1H, *dd*, *J* = 12.9, 6.6 Hz, H-6), δ 5.89 (1H, *ddd*, *J* = 17.8, 10.0, 7.6 Hz, H-7), δ 5.22 (1H, *brd*, *J* = 17.3 Hz, H-8), δ 5.12 (1H, *brd*, *J* = 10.5 Hz, H-8), δ 4.44 (1H, *d*, *J* = 7.6 Hz, H-1′ Glc), δ 4.63 (1H, *d*, *J* = 7.8 Hz, H-1″ Glc); ^{13}C NMR (methanol-d_4): Table 2; negative HR–FAB–MS, *m*/*z* 467.2093 ($C_{20}H_{35}O_{12}$ requires 467.2128)

3.10. Acid hydrolysis of ebracteatoside B (**5**) and ebractestoside C (**6**)

Ebracteatoside B (50 mg) was dissolved in 5% HCl and heated at 90 °C for 2 h. After cooling, the reaction mixture was extracted with Et_2O. The aqueous layer was neutralized with $NaHCO_3$, concentrated to dryness. The residue was then applied to a silica gel column (system VII), affording D-xylose (6 mg, R_f 0.29, $[\alpha]_D^{24}$ +20.0°) and D-glucose (8 mg, R_f 0.16. $[\alpha]_D^{24}$ +49.5°), comparing with authentic samples. By the same method, ebracteatoside C (30 mg) provided D-glucose (5 mg, $[\alpha]_D^{24}$ +47.4°).

3.11. Ebracteatoside D (**7**)

Amorphous powder, $[\alpha]_D^{22}$ −57.7° (MeOH, *c* 0.35); ^{1}H NMR (methanol-d_4): δ 3.53 (2H, *t*, *J* = 6.6 Hz, H-1), δ 1.35–1.44 (4H, *m*, H-2, 4), δ 1.55 (2H, *m*, H-3), δ 1.68 (1H, *m*, H-5), δ 1.56 (1H, *m*, H-5), δ 4.14 (1H, *dd*, *J* = 12.9, 6.6 Hz, H-6), δ 5.87 (1H, *ddd*, *J* = 17.3, 10.5, 6.8 Hz, H-7), δ 5.22 (1H, *brd*, *J* = 17.3 Hz, H-8), δ 5.10 (1H, *brd*, *J* = 10.3 Hz, H-8), δ 4.31 (1H, *d*, *J* = 8.0 Hz, H-1′ Glc), δ 4.33 (1H, *d*, *J* = 7.3 Hz, H-1″ Xyl); ^{13}C NMR (methanol-d_4): Table 2; negative HR–FAB–MS, *m*/*z* 437.2015 ($C_{19}H_{33}O_{11}$ requires 437.2022).

3.12. 7-Chloro-(2R)-2-O-β-D-glucopyranosyl-4-hydroxy-2H-1,4-benzoxazin-3(4H)-one(7-Cl-DIBOA-Glc, **10**)

Amorphous powder, $[\alpha]_D^{22}$ +18.6° (DMSO, *c* 0.86); ^{1}H NMR (DMSO-d_6): δ 5.94 (1H, *s*, H-2), δ 7.24 (1H, *d*, *J* = 8.5 Hz, H-5),δ 7.16 (1H, *dd*, *J* = 8.5, 2.2 Hz, H-6), δ 7.30 (1H, *d*, *J* = 2.2 Hz, H-8), δ 4.56 (1H, *d*, *J* = 7.8 Hz, H-1 Glc), δ 2.91 (1H, *dd*, *J* = 8.6, 8.0 Hz, H-2 Glc), δ 3.13 (1H, *dd*, *J* = 9.3, 9.0 Hz, H-3 Glc), δ 2.99 (1H, *dd*, *J* = 9.5, 9.3 Hz, H-4 Glc), δ 3.17 (1H, *m*, H-5 Glc), δ 3.40 (1H, *dd*, *J* = 12.0, 5.9 Hz, H-6 Glc), δ 3.70 (1H, *brd*, *J* = 12.0 Hz, H-6 Glc), δ 11.10; ^{13}C NMR (DMSO-d_6): Table 3; negative HR–FAB–MS, *m*/*z*: 376.0403 ($C_{14}H_{15}O_9NCl$ requires 376.0435).

Table 3
^{13}C NMR spectral data of compounds **9** and **10** (DMSO-d_6, 100 MHz)

C	9	10
2	96.2	96.4
3	155.4	155.3
5	112.8	114.1
6	123.9	122.9
7	122.9	127.5
8	117.5	118.0
9	140.3	141.2
10	128.3	127.8
1′	102.5	102.8
2′	73.2	73.1
3′	77.3	77.6
4′	69.6	69.7
5′	76.6	76.7
6′	60.9	61.1

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