



Benzoxazinoid glucosides from *Acanthus ilicifolius*

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Abstract

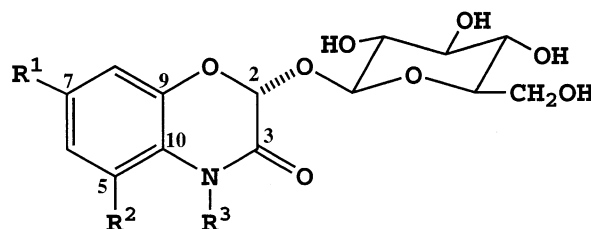
From the aerial parts of *Acanthus ilicifolius*, two benzoxazinoid glucosides, 7-chloro-(2*R*)-2-*O*-β-D-glucopyranosyl-2*H*-1,4-benzoxazin-3(4*H*)-one and (2*R*)-2-*O*-β-D-glucopyranosyl-5-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one have been isolated, together with six known compounds. The structural elucidations were based on the analyses of spectroscopic data. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Acanthus ilicifolius*; Acanthaceae; Aerial parts; Benzoxazinoid glucoside

1. Introduction

Acanthus ilicifolius L. (Acanthaceae, Thai name: Ngueak-Plaa-Moo) is a spiny herb distributed in the mangroves of southern Thailand. In Thai traditional medicine, the plant is 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 a previous paper (Kanchanapoom et al., 2001), we reported the isolation and structural elucidation of lignan glucosides. Further investigation of the aerial part of the same plant affords two new benzoxazinoid glucosides (**4**, **5**) together with six known compounds (**1–3**, **6–8**). Benzoxazinoids have been found to occur as allochemicals from monocotyledonous plants in the family Gramineae (Hofman and Hofmanova, 1969; Nagao et al., 1985), as well as reported from dicotyledonous plants in the family Acanthaceae (Wolf et al., 1985; Pratt et al., 1995), Ranunculaceae (Ozden et al., 1992) and Scrophulariaceae (Pratt et al., 1995). The present paper deals with the structural determination of these compounds. Also

the usage of this plant in Thai traditional medicines are discussed.



	R ¹	R ²	R ³
1	H	H	H
2	H	H	OH
3	OH	H	H
4	Cl	H	H
5	H	OH	H

2. Results and discussion

From the methanolic extract of the aerial part of *A. ilicifolius*, eight compounds (**1–8**) were isolated. Six were identified as known compounds; (2*R*)-2-*O*-β-D-gluco-

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pyranosyl-2*H*-1,4-benzoxazin-3(4*H*)-one (HBOA-Glc, blepharin) (**1**) (Tietze et al., 1991), (2*R*)-2-*O*- β -D-glucopyranosyl-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) (**2**) (Hartenstein and Sicker, 1994), (2*R*)-2-*O*- β -D-glucopyranosyl-7-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DHBOA-Glc) (**3**) (Nagao et al., 1985), adenosine (**6**) (Otsuka et al., 1989), syringic acid β -glucopyranosyl ester (**7**) (Inoshiri et al., 1987) and 2,6-dimethoxy-*p*-hydroquinone 1-*O*- β -glucopyranoside (**8**) (Otsuka et al., 1989) by comparison of physical data with literature values and from spectroscopic evidence.

Compound **4** (Fig. 1) exhibited the characteristic pattern of a substance having a chlorine atom from negative FAB-MS. The molecular formula was determined by HR-FAB mass spectrometry as C₁₄H₁₆O₈NCl. The ¹H and ¹³C NMR spectral data were similar to those of **1**. However, the ¹H NMR spectrum showed the presence of an ABX system at δ 6.91 (1H, *d*, *J* = 8.4 Hz), δ 7.05 (1H, *dd*, *J* = 8.4, 2.5 Hz) and δ 7.23 (1H, *d*, *J* = 2.5 Hz), indicating that the chlorine atom was attached to the aromatic ring. The position of the chlorine atom was assigned to C-7 by irradiation of N-H at δ 11.10 which induced a 7% NOE at δ 6.91 (*J* = 8.4 Hz, H-5). Moreover, all carbon signals were established by using COSY, HSQC and HMBC. The (2*R*)-stereochemistry of the linkage at the anomeric carbon of the aglycone part could be deduced from the positive values, $[\alpha]_D^{26} + 198.0^\circ$, of the optical rotation. Such positive values are a typical feature for (2*R*)-configuration. On the basis of these spectral data, the structure of compound **4** was elucidated to be 7-chloro-(2*R*)-2-*O*- β -D-glucopyranosyl-2*H*-1,4-benzoxazin-3(4*H*)-one. This chlorinated compound is a natural product, and was not found as an artifact since the process of extraction and isolation did not involve acidic media. Furthermore, since this plant was collected from the mangrove forest, the source of chlorine atom might come from a mineral source (NaCl).

The molecular formula of compound **5** was determined as C₁₄H₁₇O₉N by HR-FAB mass spectrometry. The ¹H and ¹³C NMR spectra suggested it to be a benzoxazinoid glucoside. Inspection of the FAB mass spectrometry revealed a molecular ion 16 mass units larger than **1**, and the ABB' system at δ 6.53 (2H, *brd*, *J* = 8.3 Hz) and δ 6.75 (1H, *dd*, *J* = 8.3, 8.1 Hz) was observed from the ¹H NMR

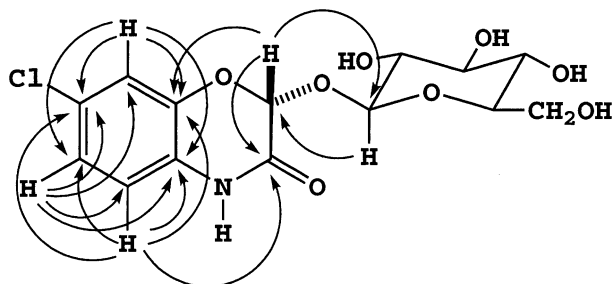


Fig. 1. HMBC correlations of compound **4**.

spectrum, indicating the presence of a hydroxyl group on the aromatic ring. The hydroxyl group was assigned to attach at C-5 due to the downfield shift of C-5 (+29.5 ppm) together with the upfield shift of C-6 (-14.7 ppm), C-8 (-8.2 ppm) and C-10 (-11.5 ppm), compared to **1**. Besides, irradiation of the N-H signal at δ 12.20, the NOE enhancement was not observed from the spectrum. The (2*R*)-stereochemistry could be determined from the positive values, $[\alpha]_D^{26} + 95.0^\circ$, of the optical rotation. Consequently, the structure of compound **5** was concluded as (2*R*)-2-*O*- β -D-glucopyranosyl-5-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one.

The biological activities of the isolated benzoxazinoids have not been investigated. The presence of benzoxazinoids (**1**, **2**) as the major constituents are in agreement with Thai traditional usage as an anti-inflammatory (Otsuka et al., 1988). However, the toxicological and pharmacological properties of benzoxazinoids have been reported as the chemical resistance factors against insects, fungi, bacteria and virus in many crop plants of the family Gramineae (Niemeyer, 1988; Sicker et al., 2000), as well as mutagenic activities (Hashimoto and Shudo, 1996). In addition, the aglycone of **2** (DIBOA) was shown to be mutagenic in a test with *Salmonella typhimurium* TA 100 and TA 98 (Hashimoto and Shudo, 1996). On the basis of these results, the internal use of this species for treatments in Thai traditional medicine should be considered.

3. Experimental

3.1. General

NMR spectra were recorded in DMSO-*d*₆ or methanol-*d*₄ using a JEOL JNM A-400 spectrometer (400 MHz for ¹H NMR and 100 MHz for ¹³C NMR) and JNM-ECP 500 (500 MHz for ¹H NMR and 125 MHz for ¹³C 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) with a Tosoh refraction index (RI-8) detector. For CC, silica gel G 60 (Merck), NH-silica gel (Fuji Silysia Chemical, Ltd.), YMC-gel ODS (50 μ m, YMC), polyamide C-200 (Wako) and highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co., Ltd.) were used. The solvent systems were: (I) EtOAc-EtOH-H₂O (4:1:0.1), (II) EtOAc-EtOH-H₂O (7:3:0.3), (III) EtOAc-EtOH-H₂O (6:4:1), (IV) 14% MeCN, (V) 90–50% MeCN, (VI) 10% MeCN, (VII) 90% MeCN, (VIII) EtOAc-MeOH-H₂O (4:1:0.1), (IX) EtOAc-MeOH-H₂O (7:3:0.3), (X) EtOAc-EtOH-H₂O (6:4:1) and 15% MeCN. The spray reagent used for TLC was 10% H₂SO₄ in 50% EtOH.

3.2. Plant material

Acanthus ilicifolius L. was collected in April 1997 from Satun Province, Southern of 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-0001) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried aerial part (2.5 kg) of *A. ilicifolius* was extracted with hot MeOH. After removal of the solvent by evaporation, the residue (380.0 g) was defatted with Et₂O. The aqueous layer was subjected to a column of highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind.) and eluted with H₂O, 30% MeOH, 60% MeOH, MeOH and Me₂CO, successively. The fraction eluted with 30% MeOH (9.2 g) was applied to a column of silica gel (system I, II and III, respectively) affording four fractions. Fraction 2 (3.3 g) was further separated on columns of RP-18 (system IV) and NH-silica gel (system V) to provide compounds **1** (1.0 g), **3** (15 mg), **5** (7 mg), **7** (15 mg) and **8** (10 mg). Fraction 2 (2.2 g) was purified by using RP-18 (system VI) and polyamide (system VII) columns to give compounds **2** (840 mg) and **6** (49 mg). The fraction eluted with 60% MeOH was subjected to a column of silica gel (system VIII, IX and X, respectively) to give five fractions. A portion of fraction 2 was purified by ODS-HPLC (system XI) to provide compound **4** (15 mg).

3.4. 7-Chloro-(2R)-2-O-β-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one

White amorphous powder, $[\alpha]_D^{26} + 198.0^\circ$ (DMSO, *c* 0.33); ¹H NMR (DMSO-*d*₆): δ 2.91 (1H, *dd*, *J* = 8.5, 8.3 Hz, H-2 Glc), δ 3.00 (1H, *dd*, *J* = 9.3, 9.0 Hz, H-4 Glc), δ 3.13 (1H, *dd*, *J* = 8.9, 8.8 Hz, H-3 Glc), δ 3.17 (1H, *m*, H-5 Glc), δ 3.41 (1H, *dd*, *J* = 11.7, 5.7 Hz, H-6 Glc), δ 3.69 (1H, *brd*, *J* = 11.7 Hz, H-6 Glc), δ 4.54 (1H, *d*, *J* = 8.1 Hz, H-1 Glc), δ 5.70 (1H, *s*, H-2), δ 6.91 (1H, *d*, *J* = 8.4 Hz, H-5), δ 7.05 (1H, *dd*, *J* = 8.4, 2.5 Hz, H-6), δ 7.23 (1H, *d*, *J* = 2.5 Hz, H-8), δ 11.10 (1H, *brs*, N-H); ¹³C NMR (DMSO-*d*₆): Table 1; negative HR-FAB-MS, *m/z*: 360.0486 (C₁₄H₁₅O₈NCl requires 360.0486).

3.5. (2R)-2-O-β-D-Glucopyranosyl-5-hydroxy-2H-1,4-benzoxazin-3(4H)-one

Amorphous powder, $[\alpha]_D^{26} + 95.0^\circ$ (DMSO, *c* 0.40); ¹H NMR (DMSO-*d*₆): δ 2.89 (1H, *dd*, *J* = 8.3, 8.1 Hz, H-2 Glc), δ 3.02 (1H, *dd*, *J* = 9.3, 9.0 Hz, H-4 Glc), δ 3.13 (1H, *dd*, *J* = 9.3, 8.8 Hz, H-3 Glc), δ 3.14 (1H, *m*, H-5

Table 1
¹³C NMR spectral data of compounds 1–5 (DMSO-*d*₆, 100 MHz)

C	1	2	3	4	5
2	94.5	96.2	94.6	94.5	94.6
3	160.4	155.4	159.7	160.0	159.9
5	115.5	112.8	115.9	117.1	145.0
6	123.2	123.9	109.5	123.4	108.5 ^a
7	122.8	122.9	153.5	126.1	122.6
8	117.7	117.5	104.8	118.7	109.5 ^a
9	140.2	140.3	141.0	141.6	141.3
10	126.2	128.3	118.2	127.1	114.7
G-1	102.5	102.5	102.5	102.7	102.4
G-2	73.2	73.2	73.3	73.2	73.3
G-3	77.4	77.3	77.3	77.5	77.3
G-4	69.7	69.6	69.7	69.7	69.6
G-5	76.6	76.6	76.7	76.7	76.7
G-6	61.0	60.9	61.0	61.0	61.0

^a Assignments may be interchanged.

Glc), δ 3.43 (1H, *dd*, *J* = 11.0, 4.6 Hz, H-6 Glc), δ 3.66 (1H, *brd*, *J* = 11.5 Hz, H-6 Glc), δ 4.53 (1H, *d*, *J* = 7.8 Hz, H-1 Glc), δ 5.62 (1H, *s*, H-2), δ 6.53 (2H, *brd*, *J* = 8.3 Hz, H-6,8), δ 6.75 (1H, *dd*, *J* = 8.3, 8.1 Hz, H-7), δ 12.20 (1H, *brs*, N-H); ¹³C NMR ((DMSO-*d*₆): Table 1; negative HR-FAB-MS, *m/z*: 342.0820 (C₁₄H₁₆O₉N requires 342.0825).

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References

- Hartenstein, H., Sicker, D., 1994. (2R)-2-β-Glucopyranosyloxy-4-hydroxy-2H-1,4-benzoxazin-3(4H)-one from *Secale cereale*. Phytochemistry 35, 827–828.
- Hashimoto, Y., Shudo, K., 1996. Chemistry of biologically active benzoxazinoids. Phytochemistry 43, 551–559.
- Hofman, J., Hofmanova, O., 1969. 1,4-Benzoxazine derivatives in plants. European Journal of Biochemistry 8, 109–112.
- Inoshiri, S., Sasaki, M., Kohda, H., Otsuka, H., Yamasaki, K., 1987. Aromatic glycosides from *Berchemia racemosa*. Phytochemistry 26, 2811–2814.
- Kanchanapoom, T., Kamel, M.S., Kasai, K., Yamasaki, K., Picheansoonthon, C., Hiraga, Y., 2001. Lignan glucosides from *Acanthus ilicifolius*. Phytochemistry 56, 369–372.
- Nagao, T., Otsuka, H., Kohda, H., Sato, T., Yamasaki, K., 1985. Benzoxazinones from *Coix lachryma-jobi* var. *ma-yuen*. Phytochemistry 24, 2959–2962.
- Niemeyer, H.M., 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. Phytochemistry 27, 3349–3358.
- Otsuka, H., Hirai, Y., Nagao, T., Yamasaki, K., 1988. Anti-inflammatory activity of benzoxazinoids from roots of *Coix lachryma-jobi* var. *ma-yuen*. Journal of Natural Products 51, 74–79.
- Otsuka, H., Takeuchi, M., Inoshiri, S., Sato, T., Yamasaki, K., 1989. Phenolic compounds from *Coix lachryma-jobi* var. *ma-yuen*. Phytochemistry 28, 883–886.

- Ozden, S., Ozden, T., Attila, I., Kucukislamoglu, M., Okatan, A., 1992. Isolation and identification via high-performance liquid chromatography and thin-layer chromatography of benzoxazolinone precursors from *Consolida orientalis* flowers. *Journal of Chromatography* 609, 402–406.
- Pratt, K., Kumar, P., Chilton, W.S., 1995. Cyclic hydroxamic acids in dicotyledonous plants. *Biochemical Systematics and Ecology* 23, 781–785.
- Sicker, D., Frey, M., Schulz, M., Gierl, A., 2000. Role of natural benzoxazinones in the survival strategy of plants. In: Jeon, K.W. (Ed.), *International Review of Cytology: A Survey of Cell Biology*. Academic, NY, 1998, pp. 319–346.
- Tietze, L., Beller, M., Terfort, A., Dolle, A., 1991. First synthesis and structural determination of blepharin and 1'-epiblepharin. *Synthesis* 1118–1120.
- Wolf, R.B., Spencer, G., Plattner, R.D., 1985. Benzoxazolinone, 2,4-dihydroxy-1,4-benzoxazin-3-one, and its glucoside from *Acanthus mollis* seeds inhibit velvetleaf germination and growth. *Journal of Natural Products* 48, 59–63.