SHORT REPORT

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Rec. Nat. Prod. 8:2 (2014) 195-198

records of natural products

Isolation and Absolute Configuration of Boehmenan from *Durio affinis* Becc.

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(Received March 14, 2013; Revised October 01, 2013, Accepted November 14, 2013)

Abstract: Boehmenan (**1**) is a lignan which has two feruloyl moieties at C-9 and C-9"' respectively. The structural characterization of (+)-boehmenan was confirmed by ^{1}H-NMR, ^{13}C NMR and HRESIMS as well as by direct comparison with the literature. CD measurements established a *7S'*, *8R'* configuration. Cytotoxicity evaluation showed that boehmenan is moderately active against the T47D cell line with IC_{50} 13.7 µg/mL and shows weak activity against the HeLa cancer cell line with IC_{50} 93.5 µg/mL. Boehmenan is also non-cytotoxic to the Vero cell line. From this study, boehmenan X and *erythro*-carolignan E were also obtained.

Keywords: Bombacaceae; Boehmenan; *Durio affinis*; T47D cell.

1. Plant Source

This paper discusses the isolation, structure elucidation and cytotoxicity evaluation of boehmenan (**1**) from an ethanolic extract of the bark of *Durio affinis* Becc.

Bark samples of *D. affinis* Becc. were collected from Arus Deras village, 50 km South of Pontianak, West Kalimantan, Indonesia, in March 2009. The plant was identified by staff of the Herbarium Bogoriense, Biology-LIPI Research Centre, Cibinong, Indonesia, where the voucher specimens are stored. The voucher specimen has an accession number 460/IPH.1.02/If.8/V/2009 for further reference.

2. Previous Studies

Boehmenan X, *threo*- and *erythro*-carolignan E, *threo*- and *erythro*-carolignan X, *threo*- and *erythro*-carolignan Y as well as boehmenan (**1**) have been isolated from *Durio* plants [1,2]. However, secondary metabolites profile of *D. affinis* have not been explored until now.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/17/2014 EISSN: 1307-6167

3. Present Study

The bark (5.5 kg) of the plant was powdered, macerated in ethanol for three days in a closed glass chamber, then filtered. The ethanol extract was evaporated using a rotary evaporator to give a dark brown residue (341.5 g, 6.2 %). This residue was solubilized in a mixture of EtOH:H_2O (9:1, v/v) and partitioned between *n*-hexane and $CHCl_3$. The chloroform extract (33.2 g) was dried under vacuum then stored in the refrigerator.

The $CHCl_3$ fraction was purified by vacuum column chromatography (VLC) on silica gel and eluted by stepwise gradient of *n*-hexane:EtOAc (8:2 – 1:9, v/v) to give seven subfractions (AO_1-AO_7). Fractions AO_5 and AO_6 were combined (AOC, 1.07 g) and further subjected to flash column chromatography (FCC) using *n*-hexane:$CHCl_3$ (1:1 – 0:10, v/v) and $CHCl_3$:MeOH (99:1, v/v) to yield seven fractions (AOC_1-AOC_7). Fraction AOC_6 (143 mg) was purified by preparative C_{18}-HPLC (65% MeCN-H_2O isocratic over 30 min, flow rate 1.5 mL/min, UV detection at λ_{254} nm) to afford a white amorphous solid compound **1** (58 mg, Figure 1). By the same method, fraction AO_7 (14.8 g) produced boehmenan X (9 mg) and *erythro*-carolignan E (7 mg). This is the first report of the secondary metabolites associated with *D. affinis* Becc.

*Boehmenan (**1**)*: White amorphous solid: $[\alpha]^{25}_D$ +10.2 (*c* 0.5, MeOH), (Lit.[1] $[\alpha]^{22}_D$ +7.8 (*c* 0.32, MeOH); Lit.[3] $[\alpha]_D$ -14.3 (*c* 0.03, $CHCl_3$)); ^{1}H NMR (MeOH-d_4, 500 MHz) δ (ppm) = 7.48 (1H, d, *J* = 16.0 Hz, H-7'''), 7.40 (1H, d, *J* = 16 Hz, H-7), 7.10 (1H, d, *J* = 2.3 Hz, H-2'''), 7.04 (1H, d, *J* = 2.3 Hz, H-2), 7.00 (1H, dd, *J* = 2.3, 8.5 Hz, H-6'''), 6.95 (1H, dd, *J* = 2.3, 8.5 Hz, H-6), 6.91 (1H, d, *J* = 2.3 Hz, H-2'), 6.80 (1H, dd, *J* = 2.3, 8.5 Hz, H-6'), 6.78 (1H, d, *J* = 8.5 Hz, H-5 and H-5'''), 6.76 (1H, d, *J* = 8.5 Hz, H-5'), 6.72 (1H, br s, H-2''), 6.70 (1H, br s, H-6''), 6.26 (1H, d, *J* = 16.0 Hz, H-8'''), 6.21 (1H, d, *J* = 16 Hz, H-8), 5.34 (1H, d, *J* = 9.0 Hz, H-7'), 4.51 (1H, dd, *J* = 6.3, 13.8 Hz, H-9'a), 4.36 (1H, dd, *J* = 9.8, 13.8 Hz, H-9'b), 4.13 (2H, t, *J* = 8.5 Hz, H-9'), 3.83 (3H, s, OCH_3, C-3'''), 3.82 (3H, s, OCH_3, C-3), 3.80 (3H, s, OCH_3, C-3''), 3.76 (1H, m, H-8'), 3.73 (3H, s, OCH_3, C-3'), 2.65 (2H, t, *J* = 8.5 Hz, H-7''), 1.95 (2H, t, *J* = 8.5 Hz, H-8''); ^{13}C NMR (MeOH-d_4, 125 MHz) δ (ppm) = 169.2 (C, C-9'''), 168.8 (C, C-9), 150.8 (C, C-4), 150.6 (C, C-4'''), 149.3 (C, C-3 and C-3'''), 149.0 (C, C-3'), 147.8 (C, C-4'), 147.6 (C, C-4''), 147.2 (CH, C-7), 146.6 (CH, C-7'''), 145.4 (C, C-3''), 136.5 (C, C-1''), 133.7 (C, C-1'), 129.0 (C, C-5''), 127.7 (C, C-1'''), 127.5 (C, C-1), 124.2 (CH, C-6), 124.0 (CH, C-6'''), 120.3 (CH, C-6'), 117.7 (CH, C-6''), 116.5 (each CH, C-5, C-5'''), 116.2 (CH, C-5'), 115.5 (CH, C-8'''), 115.0 (CH, C-8), 114.3 (CH, C-2''), 111.8 (CH, C-2'''), 111.7 (CH, C-2), 111.0 (CH, C-2'), 90.2 (CH, C-7'), 66.6 (CH_2, C-9'), 65.0 (CH_2, C-9''), 56.7 (OCH_3, C-3''), 56.4 (OCH_3, C-3''' and C-3), 56.3 (OCH_3, C-3'), 51.8 (CH, C-8'), 33.2 (CH_2, C-7''), 31.7 (CH_2, C-8''); HRESIMS *m/z* $[M + Na]^+$ 735.2422 (calculated for $C_{40}H_{40}O_{12}Na$, 735.2418).

Bioactivity test: Cytotoxic assays of ethanolic crude extract and boehmenan (**1**) against two cancerous human-cell lines, T47D (human breast cancer) and HeLa (human cervical cancer), as well as a normal Vero cell line were conducted by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method as described by Mosmann [4]. The anthracycline antibiotics doxorubicin was used as a positive control in all assays. In brief, 100 mL aliquots of cells cultured at a concentration of 10^4 cell/mL were placed into 96 well microplates, and incubated for 24 hours. Next, 100 mL of each concentration from a test solution was poured into the well and incubated for 24 hours. The cells were washed by phosphate buffer saline (PBS) and incubated for 24 hours then 100 mL of MTT solution was added in each well. The cells were incubated overnight at room temperature and protected from light. At the end of the incubation process, the plates were shaken for 10 minutes and analyzed using an ELISA reader at λ_{595} nm.

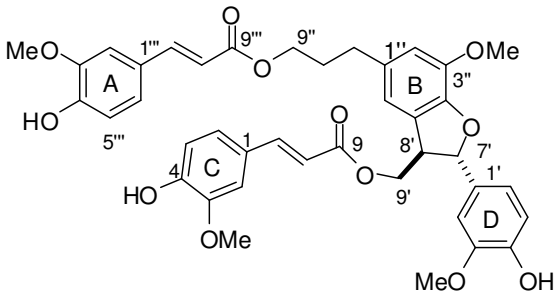

Figure 1. Structure of Boehmenan (**1**) isolated from the bark of *D. affinis* Becc.

Compound **1** was obtained as an amorphous white solid. The positive-ion HRESIMS of **1** gave an adduct $[M+Na]^+$ ion at *m/z* 735.2422, corresponding to the molecular formula $C_{40}H_{40}O_{12}$. The ^{1}H NMR spectrum established the presence of three contiguous methylene groups at δ = 2.65 (2H, t, *J* = 8.5 Hz, H-7"), 1.95 (2H, t, *J* = 8.5 Hz, H-8") and 4.13 (2H, t, *J* = 8.5 Hz, H-9"). Three methine signals at δ = 5.34 (1H, d, *J* = 9.0 Hz, H-7') and at δ = 3.76 (1H, m, H-8') together with resonances for a pair of geminal protons at δ = 4.51 (1H, dd, *J* = 6.3, 13.8 Hz, H-9'a) and 4.36 (1H, dd, *J* = 9.8, 13.8 Hz, H-9'b) all indicated the presence of oxygenated protons. The ^{1}H NMR spectrum also showed signals at δ = 7.40 (1H, d, *J* = 16.0 Hz, H-7), 6.21 (1H, d, *J* = 16.0 Hz, H-8), 7.48 (1H, d, *J* = 16.0 Hz, H-7"') and 6.26 (1H, d, *J* = 16.0 Hz, H-8"'), which indicated the presence of two *trans* alkenes. Analysis of the ^{1}H NMR data also established two different types of aromatic rings. The 1,3,4-trisubstituted A, C and D rings gave resonances at δ = 6.91 (1H, d, *J* = 2.3 Hz, H-2'), 6.76 (1H, d, *J* = 8.5 Hz, H-5'), 6.80 (1H, dd, *J* = 2.3, 8.5 Hz, H-6') for ring D, at δ = 7.04 (1H, d, *J* = 2.3 Hz, H-2), 6.95 (1H, dd, *J* = 2.3, 8.5 Hz, H-6) for ring C, and at δ = 7.10 (1H, d, *J* = 2.3 Hz, H-2"'), 6.78 (1H, d, *J* = 8.5 Hz, H-5/H-5"'), 7.00 (1H, dd, *J* = 2.3, 8.5 Hz, H-6"') for ring A. The B ring was assigned as a 1,3,4,5-tetrasubstituted aromatic unit by the signals at δ = 6.72 (1H, br s, H-2") and 6.70 (1H, br s, H-6"). There were four methoxy signals at δ = 3.83 (3H, s, OCH_3-3"'), 3.82 (3H, s, OCH_3-3), 3.80 (3H, s, OCH_3-3"), and, at δ = 3.73 (3H, s, OCH_3-3').

The ^{13}C NMR data at 125 MHz exhibited four methylenes, seventeen methines, fifteen quaternary carbons including two carbonyl resonances at δ = 168.7 (C-9) and 169.2 (C-9"') and, four methoxy carbons at δ = 56.7 (OCH_3-3"), 56.4 (OCH_3-3/3"), and at δ = 56.3 (OCH_3-3'). By comparison of ^{1}H and ^{13}C NMR data with literature [1,2,5,6] it was suggested that compound **1** was boehmenan. It is noteworthy that boehmenan has been isolated from several species of *Durio* [1,2]. The presence of lignans and boehmenan in the genus *Durio* may be of chemotaxonomic value.

The relative configuration of compound **1** was determined as *trans* from the 9 Hz coupling constant between H-7' and H-8'. Wallis *et al*. have provided substantial NMR evidence that the relative configuration of natural neolignans is always *trans* in accordance with their biosynthesis by oxidative coupling of free radical intermediates [7]. The absolute configuration of (+)-boehmenan (**1**) was identified as *7S',8R'* from the positive Cotton effect at 290 nm (Δε + 4.18) [8]. As with other lignan compounds isolated from *Durio* plants [2], the sign and magnitude of the $[\alpha]_D$ measurement for boehmenan may vary according to the solvent chosen for the chiroptic measurements.

^{1}H and ^{13}C NMR data for boehmenan X and *erythro*-carolignan E were comparable with literature [2]. Additionally, the $[\alpha]^{28}_D$ value for boehmenan X was +20 (*c* 0.2, $CHCl_3$), (Lit^2 $[\alpha]^{22}_D$ +11.2 (*c* 0.41, $CHCl_3$)), whereas the $[\alpha]^{28}_D$ value measured for *erythro*-carolignan E was +15.1 (*c* 0.12, $CHCl_3$), (Lit^2 $[\alpha]^{22}_D$ +23.4 (*c* 0.32, $CHCl_3$)).

The ethanol extract of *D. affinis* was inactive against all cancer cell lines used in this study. Likewise, boehmenan demonstrated weak cytotoxic activity against the cancer cell lines T47D and HeLa with IC_{50} 13.7 µg/mL and IC_{50} 93.5 µg/mL, respectively but was non-toxic toward a normal VERO cell line with IC_{50} 790.9 µg/mL. Similarly, Chin *et al* have also reported that boehmenan exhibited weak activity against the cell lines Lu1, LNCaP, MCF-7 and HUVEC with ED_{50} values of 10.4, 9.5, 10.0 and 9.0 µg/mL, respectively [3]. Moreover, Wu *et al* mentioned that boehmenan was demonstrated to be weakly cytotoxic against the A549 and MCF-7 cell lines with EC_{50} values of 18.4

and 10.9 µg/mL, respectively [9]. All of those data consistently proved that boehmenan showed weakly cytotoxic activity against various cancer lines.

On the other hand, doxorubicin was used as a positive control [10]. Biological activity of doxorubicin against cell lines T47D, HeLa and Vero showed values of IC_{50} 9.5, 9.4 and 611.3 µg/mL respectively. The IC_{50} values for boehmenan, ethanol extract and doxorubicin were calculated as shown in Table 1. In summary, although boehmenan showed weaker cytotoxicity against T47D cell line than doxorubicin, the latter was found to be more toxic against a normal Vero cell line than boehmenan. Hence, boehmenan might still have capability to be developed as a cytotoxic agent for other cell lines.

Table 1. Cytotoxic activities of boehmenan, *D. affinis* ethanol extract, and doxorubicin against twocancer cell lines (T47D, HeLa) and a normal Vero cell line

Cell lines	IC_{50} (µg/mL)		
	boehmenan	ethanol extract	doxorubicin
T47D	13.7 ± 0.4	828.3 ± 43.5	9.5 ± 0.5
HeLa	93.5 ± 9.2	300.5 ± 12.2	9.4 ± 0.2
Vero	790.9 ±12.5	4103.4 ± 33.2	611.3 ± 16.1

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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