STUDY OF CONFORMATIONAL PREFERENCES OF ERYTHRO-CAROLIGNAN E ASSESSED BY THE COUPLING BETWEEN H7'-H8' IN DIFFERENT NMR SOLVENTS

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ABSTRACT

Erythro-carolignan E (**1**) has been obtained from the ethanol extract of the wood bark of *Durio affinis* Becc. This research was conducted in order to prove that conformational preferences of compound **1** were solvent dependent. On the basis of ^{1}H-NMR data, the relative configuration of compound **1** was characterized by a coupling constant ($^{3}J_{HH}$) value of 3.3 Hz at H-7' in $CDCl_3$. The coupling constant ($^{3}J_{HH}$) values of H-7' in compound **1** has changed to 4.1 Hz and 5.3 Hz in pyridine-d_5 and acetonitrile-d_3 respectively. As a result, the conformation of compound **1** at C7'-C8' has changed in different NMR solvents. In conclusion, structure of *erythro*-carolignan E that contains a dihydroxy group at C7'-C8' is able to change in different NMR solvent.

Key words: Durio, *erythro*-Carolignan E, Newman Projection

INTRODUCTION

Durio or durian, well known as the *King of Fruits*, has an economic value as a source of timber and fruits. This plant grows only in tropical region of Southeast Asia. Indonesia has 28 species of *Durio*, of which 19 are endemic species grown in Borneo Island, hence Borneo is known as thecentre of *Durio* in the world (Subhadrabandhu *and* Ketsa, 2001; Morton, 1987). *Durio affinis* is anon-edible fruit species and endemic to Kalimantan. This plant is also known as bird Durio. The wood of *D. affinis* can be used for making furniture (Uji, 2005).

In previous studies, six carolignan compounds, namely *erythro*-carolignan E (**1**), *threo*-carolignan E, *erythro*-carolignan X, *threo*-carolignan X, *erythro*-carolignan Y and *threo*-carolignan Y, have been isolated from the wood bark of *D. zibethinus*, *D. carinatus* and *D. oxleyanus*(Rudiyansyah *et al*., 2006, 2010; Lim, 2012). Rudiyansyah *et al*. (2010) have reported the conformational preferences for *erythro*- and *threo*-carolignan X in the NMR solvents $CDCl_3$ and CD_3OD. The conformation for *erythro*-carolignan X changed

from conformer **EI** to **EII** (Figure 2) since coupling constant ($^3J_{HH}$) for proton H-7' changed from 2.6 Hz in $CDCl_3$ to 6.1 Hz in CD_3OD.

This research was conducted in order to prove that conformational preferences of compound **1**were solvent dependent, which it has been reported previously in literature (Rudiyansyah *et al*., 2010). As part of our concern in the structures and conformations of carolignans, in this paper, we report the conformation changing for *erythro*-carolignan E (**1**) by studying the coupling constants ($^3J_{HH}$) of proton H-7' in $CDCl_3$ and other NMR solvents, pyridine-d_5 and acetonitrile-d_3. Thus, the conformational preferences for compound **1** will be characterized. In this study, we also isolated other known compounds boehmenan and boehmenan X (Rudiyansyah *et al*., 2006, 2010, 2014).

METHODS

Materials

The wood bark of *D. affinis* Becc.was collected in Arus Deras village, sub-district of Teluk Pakedai, district of Kubu Raya, Province of West Kalimantan in March 2009, air dried, and powdered. The voucher specimens were identified and stored at the Bogoriense Herbarium in Cibinong as 460/IPH.1.02/If.8/V/2009. All solvents were distilled prior to use.

Procedures

Extraction

Powdered bark (5.5 kg) of *D. affinis* was macerated in EtOH (3 x 24 hours) to provide 341.5 g of residue (6.21%), which was subsequently dissolved in a mixture of MeOH-H_2O (9:1) then partitioned using *n*-hexane (3 x 500 mL) and $CHCl_3$ (3 x 1500 mL) respectively.

Chromatography

Thin-layer chromatography (TLC) analysis was performed on pre-coated silica gel plates (Kieselgel 60 F_{254} or RP-18 F_{254s}, 20 x 20 cm, 0.25 mm thick, Merck). Spots were detected under UV light at λ_{254} and λ_{366} nm or by using 15% ceric sulfate spray. The $CHCl_3$ extract (33.2 g) was fractionated by VLC (silica gel Kieselgel 60 H) using a gradient of *n*-hexane-EtOAc (8:2 – 0:10, MeOH 100%, each collection was 300mL) to give seven fractions (DA1 - DA7) on the basis of TLC analyses. Fraction DA7 (14.8 g) was chromatographed further by VLC using a gradient of *n*-hexane-EtOAc (5:5 – 0:10, MeOH 100%, each collectionwas 100 mL) to obtain eight fractions (DA7a - DA7h). The combined fractions of DA7c and DA7d (505 mg) were fractionated by reverse phase (RP)

FCC using a gradient of MeOH-H_2O (3:1, 9:1, 10:0, each collection was 200 mL) to yield three fractions(DA7cd1 - DA7cd3). Fraction DA7cd1 (176 mg) was fractionated further by normal phase (NP) FCC (silica gel 60; 230-400 mesh) using gradient of *n*-hexane-EtOAc (6:4 – 0:10, each collection was 150 mL) to obtain twenty-seven fractions. Fraction DA7cd1-26 (25 mg) was purified by C_{18}-HPLC (Agilent 1100 series instrument with a variable-wavelength UV detector, semi-preparative separation used a μBondapak C_{18} (7.8 x 300 mm) 10 μm column), solvent [MeCN-H_2O (6:4, v/v) over 20 minutes, flow rate 1.5 mL/min, UV detection at 254 nm] to give compound **1** (2 mg).

Structural Elucidation

The ^{1}H and ^{13}C NMR spectra were recorded either on a Bruker Avance 400 or Bruker Avance 500 spectrometers. ^{1}H NMR spectra were recorded relative to $CDCl_3$ (δ = 7.24 ppm), pyridine-d_5 (δ = 7.22 ppm) and acetonitrile-d_3 (δ = 1.94 ppm) respectively, whereas ^{13}C NMR spectra were recorded relative to $CDCl_3$ (δ = 77 ppm). HRESIMS data was measured using a Finnigan MAT 900 XL double focusing magnetic sector mass spectrometer in the positive ion mode. Samples were prepared at a concentration of 10 μM/ml. Specific rotations $[\alpha]_D$ were measured on a Jasco-P2000 spectropolarimeter.

DISCUSSION

Establishment of structure of *erythro*-carolignan E

Compound **1** was obtained as a white amorphous solid. The positive-ion HRESIMS of **1** gave an adduct $[M+Na]^+$ ion at *m/z* 753.2524, corresponding to a molecular formula $C_{40}H_{42}O_{13}$. Compound **1** has a specific rotation $[\alpha]^{25}_D$ +25.2 (*c* 0.15, $CHCl_3$). The ^{1}H NMR spectrum (in $CDCl_3$) of **1** showed the characteristic ^{1}H NMR signals for two *trans*-feruloyl groups at δ 7.59 (1H, d, *J* = 15.9 Hz, H-7'''), 6.28 (1H, d, *J* = 15.9 Hz, H-8'''), 7.01 (1H, m, *J* = 2.0 Hz, H-2'''), 7.01 (1H, m, H-2'''), 6.88 (1H, d, *J* = 8.2, H-5''') 7.05 (1H, dd, *J* = 8.2, 1.9 Hz, C-6''') and at δ 7.49 (1H, d, *J* = 15.9 Hz, H-7), 6.21 (1H, d, *J* =15.9 Hz, H-8), 7.00 (1H, m, H-2), 6.90 (1H, d, *J* = 8.1 Hz, H-5), 7.02 (1H, m, H-6). Furthermore, there were 1,2,3-trioxygenated propanoid signals at δ 4.89 (1H, d, *J* = 3.3 Hz, H-7'), 4.46 (1H, m, H-8'), 4.25 (1H, m,H-9'a), and 4.44 (1H, m,H-9'b). The ^{1}H NMR spectrum also established the presence of three contiguous methylene groups at δ 2.68 (2H, t, *J* = 7.3 Hz, H-7''), 1.99 (2H, m, H-8'') and 4.19 (2H, t, *J* = 6.5 Hz, H-9''). Two 1,3,4-trisubstituted rings gave resonances at δ 6.98 (1H, d, *J* = 1.8 Hz, H-2'), 6.95 (1H, d, *J* = 8.0 Hz, H-5'), 6.81 (1H, dd, *J* = 8.0, 1.8 Hz, H-6') for ring A and at δ 6.74 (1H, m,H-2''), 6.85 (1H, d, *J* = 8.1 Hz, H-5''),

6.72 (1H, m, H-6") for ring B. There were four methoxy signals at δ 3.91 (3H, s, OCH_3-3"'), 3.90 (3H, s, OCH_3-3), 3.86 (3H, s, OCH_3-3") and 3.85 (3H, s, OCH_3-3').

The ^{13}C NMR data at 100 MHz exhibited four methylenes, eighteen methines, fourteen quaternary carbons including two carbonyl resonances at δ 167.1 (C-9) and 167.3 (C-9"') and four methoxy carbons at δ 55.9 (OCH_3-3"'/OCH_3-3'/OCH_3-3) and at δ 56.8 (OMe-3"). All of these data were consistent with *erythro*-carolignan E (Figure 1) in which this compound has been previously reported by Rudiyansyah *etal*., (2010), Huang *et al*., (2012),Paula *et al.*,(1995) and by Wu *et al.*, (2005). The relative configuration of **1** was elucidated by a triplet signal for H-7' with a coupling constant of 3.3 Hz (in $CDCl_3$) which is diagnostic of an *erythro* diastereomer (Braga *et al*., 1984).

Conformational Preferences of *erythro*-carolignan E

Braga *et al*., (1984) stated that the *erythro* isomer would have 3.2 Hz coupling since intramolecular hydrogen bonding would favour conformation **EI** (Figure 2). Braga also noted that conformer **EIII**, in which the hydrogens are also *gauche*, might contribute to the conformational equilibria for the *erythro* compound. Moreover, Karplus(1963) mentioned that the coupling constant between H-7' and H-8' could be used to determine the conformation around the C-7'/C-8' bond. Furthermore, Bifulco *et al.*,(2007) stated that the conformational relationship in acyclic systems between adjacent stereocentres is explained by staggered conformers. Additionally, Riccio *et al.*, (2003) and Matsumori*et al.*, (1999) have developed a method to determine the relative configuration of acyclic compounds on the basis of proton-proton coupling constants together with proton-carbon coupling constants. For *erythro* compounds with C_7C_8-dioxy substituents, the vicinal coupling constants in an individual rotamer can be in the range 0-4 Hz (*gauche*) or 7-10 Hz (*anti*); these values are described as small or large, respectively.

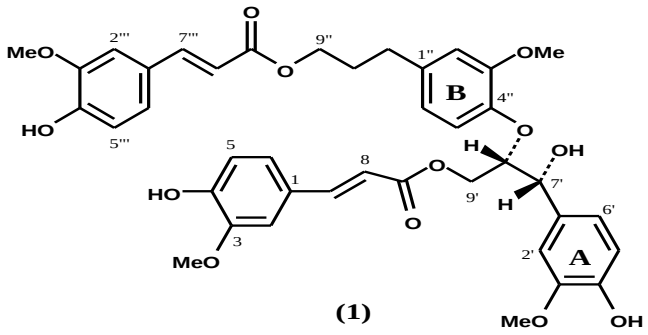

Figure 1. Structure of compound **1**

Consequently, in compound **1**, the hydrogen bonding effect forces the *erythro* isomer to adopt conformation **EI** and **EIII**in which the hydrogens are *gauche*, hence the 3.3 Hz

coupling that was observed. Moreover, conformer **EII** is reasonable since the bulky substituents are separated and dipole effects are minimized. Based on a $^{3}J_{HH}$value, it is clear that the hydrogen bonding effect dictates the conformational preferences, thus **EI** and **EIII** are the predominant conformers for the *erythro* compound **1**.

From our previous study, the NMR data for *erythro*-carolignan X were solvent dependent. When the NMR spectrum of the *erythro* compound was re-run in MeOH-d_4, a coupling constant of proton H-7' changed from 2.6 Hz to 6.1 Hz (Rudiyansyah *et al*., 2010). These data suggested a change in conformation for the *erythro* compound.

Recent study also proved that the conformation of compound **1** changed when the spectral data were run in either pyridine-$d_5$or acetonitrile-d_3. In acetonitrile-d_3, with a coupling constant of 5.3 Hz, the hydroxyl group is now hydrogen-bonded to the solvent in preference to the adjacent OAryl group.

Conformational preferences will be decided by steric effects and by dipole repulsion effects. Consequently conformer **EII** in which the H-7'/H-8' is diaxial will be important. On the other hand, in pyridine-d_5, a coupling constant of 4.1 Hz was found, indicating that the Newman projection has changed insignificantly. Compound **1** both in $CDCl_3$ and pyridine-d_5 showed similar coupling constants that were 3.3 Hz and 4.1 Hz respectively.

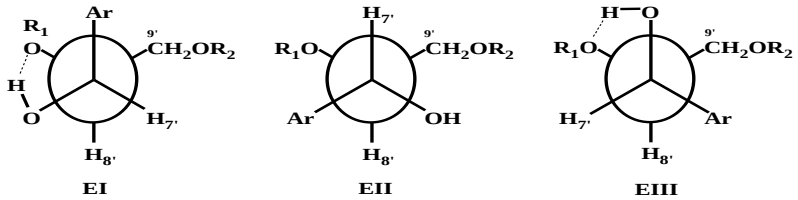

Figure 2. Three possible conformers for compound **1**

This study showed that it was important to consider solvent effects on conformational preferences when determining relative configuration. The value of the coupling constant ($^{3}J_{H7'-H8}$) for *erythro*-carolignan E (**1**) differed in different NMR solvents. In order to study more about preferred conformation for *erythro* compounds, ^{1}H-NMR experiment in cool condition should be conducted. Recently, Ardá *et al.*, (2010) have measured the $^{3}J_{HH}$ coupling by doing low-temperature NMR analysis of flexible acyclic systems in order to get the existence of multiple conformer. Other chemical structures from this plant, boehmenan and boehmenan X, were also characterized by comparison between their spectroscopic data with data from the literature [Rudiyansyah *et al*., 2014; Paula *et al*., 1995; Seca*et al*., 2001).

CONCLUSIONS

A lignan, namely *erythro*-carolignan E (**1)** have been isolated and characterized by ^{1}H and ^{13}C NMR including HRESIMS. The relative configuration of compound **1** was determined from the coupling constant of H-7' and H-8' that was 3.3 Hz. A structure of *erythro*-carolignan E that contains a dihydroxy group at C7'-C8' is able to interchangeable in different NMR solvent.

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