CONFORMATIONAL STUDIES OF LIGNANS FROM *Durio oxleyanus* Griff. (*Bombacaceae*)

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ABSTRACT

*Searching for secondary metabolites from the wood bark of Durio oxleyanus has afforded two new lignans, namely threo-carolignan Y (**1**) and erythro-carolignan Y (**2**) together with three other known lignans erythro-carolignan X (**3**), boehmenan X (**4**) and boehmenan (**5**). The relative configurations of compounds **1** and **2** were established by J-based configurational analysis and 2D NOESY studies.*

Keywords: *carolignan, threo, erythro, Durio, Bombacaceae*

INTRODUCTION

The genus *Durio* contains 28 species, of which 19 are endemic species grown in Borneo, 13 species are found in peninsular Malaysia, 6 are known in Thailand and 7 species are found in Sumatra. Of the 28 species, at least eight are notable for producing edible fruit [1]. The species *D. oxleyanus* is one of an edible fruits, found growing wild in Borneo, Sumatra and Peninsular Malaysia. Heyne states that a concoction made from the bark of *D. oxleyanus* is used to treat malaria in Sumatra and the crushed seeds of *D. oxleyanus* are used to treat sores and wounds [2].

Previous investigation on secondary metabolites from other *Durio* species, *D. zibethinus*, *D. kutejensis* and *D. carinatus*, has yielded caffeoyl triterpenes, lignans and phenolics [3-4]. As part of our concern in chemistry of *Durio* plant, in this paper we report the isolation and characterisation of lignans from *D. oxleyanus* as well as the proposed biosynthesis of isolated lignans in relation with other lignans isolated from previously reported. The relative configurations of compounds **1** and **2** were established by measuring coupling values ($^{2,3}J_{HC}$) using the HSQC-HECADE method [5-6] together with 2D NOESY studies. Whereas, the known compounds **3**, **4** and **5** were characterized by comparison from the literature data [4,7-8].

EXPERIMENTAL SECTION

Materials

The bark of *D. oxleyanus* Griff. was collected in Bengkayang region, West Kalimantan in 2007, air dried, and powdered. The voucher specimens were identified and stored at the Bogoriense Herbarium in Bogor as 864/IPH.1.02/If.8/2007.

Apparatus

Optical rotations ($[\alpha]_D^{25}$) and CD spectra were measured on a Perkin-Elmer 241 MC Polarimeter and on a Jasco-J810 spectropolarimeter respectively. HRESIMS were measured using a Finnigan MAT 900 XL double focusing magnetic sector mass spectrometer in the positive ion mode. The ^{1}H, ^{13}C, HSQC, HMBC, DQF-COSY, NOESY and HSQC-HECADE spectra were recorded either on Bruker Avance 400, Bruker Avance 500, or Bruker Avance 750 MHz spectrometers. ^{1}H NMR spectra were recorded relative to $CDCl_3$ (δ = 7.24 ppm) and MeOH-d_4 (δ = 3.30 ppm) respectively, whereas ^{13}C NMR spectra were recorded relative to either $CDCl_3$ (δ = 77 ppm) or MeOH-d_4 (δ = 49 ppm). Vacuum liquid chromatography (VLC) was carried out on silica gel (Kieselgel 60 H) and flash column chromatography (FCC) was carried out on silica gel 60 (230-400 mesh). 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 ceric sulfate spray reagent. RP-HPLC was performed on an Agilent 1100 series instrument with a variable-wavelength UV detector. Semi-preparative separation used a μBondapak C_{18} (7.8 x 300mm) 10 mm column. All solvents used were distilled prior to use.

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Table 1. Complete NMR assignments for compound 1

#	$\delta^{13}C^{a}$	$\delta^{1}H$ $(J)^{b,c}$	HMBC[d,e]	COSY
1	127.2	-	3, 5, 7, 8	-
2/6	129.9	7.34, d, (8.6)	6, 2, 7	3/5
3/5	115.8	6.80, d, (8.6)	3, 5	2/6
4	157.6	-	2, 3, 5, 6	-
7	144.4	7.44, d, (15.9)	2, 6, 8	8
8	115.4	6.21, d, (15.9)	7	7
9	167.1	-	7, 8, 9 a, 9 b	-
1	130.1	-	2 , 5 , 6 , 7 , 8	-
2	109.9	6.91, d, (1.5)	6 , 7	-
3	146.5	-	2 , 5 , OMe-3	-
4	145.4	-	2 , 5 , 6	-
5	114.0	6.86, m	-	6
6	121.0	6.87, m	2 , 7	5
7	86.2	4.42, d, (6.1)	2 , 8 , 9 a, 9 b	8
8	82.5	4.47, m	7 , 9 a, 9 b	7 , 9 a, 9 b
9 a/9 b	63.7	4.51, m	7 , 8	8
1″	135.9	-	2″, 5″, 6″, 7″, 8″	-
2″	112.5	6.61, d, (1.8)	6″, 7″	-
3″	150.7	-	2″, 5″, OMe-3″	-
4″	146.1	-	8′, 2″, 5″, 6″	-
5″	118.9	6.63, d, (8.0)	-	6″
6″	120.4	6.59, dd, (1.8, 8.0)	2″, 7″	5″
7″	31.8	2.60, m	2″, 6″, 8″, 9″	8″
8″	30.3	1.93, m	7″, 9″	7″, 9″
9″	63.8	4.16, t, (6.6)	7″, 8″	8″
1‴	126.9	-	2‴, 5‴, 7‴, 8‴	-
2‴	109.4	7.01, d, (1.7)	6‴, 7‴	-
3‴	146.8	-	2‴, 5‴, OMe-3‴	-
4‴	148.0	-	2‴, 5‴, 6‴	-
5‴	114.7	6.90, d, (8.2)	-	6‴
6‴	123.0	7.05, dd, (1.7, 8.2)	2‴, 7‴	5‴
7‴	144.9	7.59, d, (15.9)	2‴, 6‴, 8‴	8‴
8‴	115.4	6.28, d, (15.9)	7‴	7‴
9‴	167.4	-	9″, 7‴, 8‴	-
OMe-3	55.9	3.82, s	-	-
OMe-3″	55.7	3.71, s	-	-
OMe-3‴	55.9	3.90, s	-	-
OMe-7	57.1	3.27, s	7	-

[a] 750 MHz, $CDCl_3$ referenced to ^{13}C at δ 77.0 ppm; [b] 750 MHz, $CDCl_3$ referenced to ^{1}H at δ 7.24 ppm; [c] Coupling constant in Hz; [d] HMBC connectivity from C to H; [e] Correlations observed for one bond $J_{C\text{-}H}$ of 145 Hz and long range $J_{C\text{-}H}$ of 8 Hz

Procedure

Powdered bark (7 kg) of *D. oxleyanus* Griff. was macerated with MeOH (3 x 20 L) to provide 700 g of residue (10%), which was subsequently dissolved in a mixture MeOH-H_2O (9:1) then partitioned using *n*-hexane (3 x 3 L), $CHCl_3$ (3 x 5 L) and EtOAc (3 x 5 L) respectively. The $CHCl_3$ extract (20.11 g) was fractionated by VLC using a gradient of *n*-hexane, $CHCl_3$ and MeOH (each collection was 250 mL) by increasing polarity to give twenty-three fractions (D1-D23) on the basis of TLC analyses. The combined fractions of D7 and D8 (1.23 g) were purified further by VLC using a gradient of *n*-hexane, EtOAc and MeOH (each collection was 100 mL) in order of increasing polarity to obtain nine fractions (D78_A - D78_I). Fraction D78_G (60 mg) was further subjected to Si gel FCC using a gradient of *n*-hexane, EtOAc and MeOH (each collection was 20 mL) in order of increasing polarity to yield three fractions (D78_GA - D78_GC). Fraction D78_GA (12 mg) was purified by C_{18}-HPLC [MeOH-H_2O (3:1, v/v) over 30 min, flow rate 1.5 mL/min, UV detection at 254 nm] to give compound **3** (5 mg). Compound **1** (3 mg) and compound **2** (5 mg) were also

Table 2. Complete NMR assignments for compound 2

#	$\delta^{13}C^{a}$	$\delta^{1}H$ $(J)^{b,c}$	HMBC[d,e]	COSY
1	127.2	-	3, 5, 7, 8	-
2/6	129.9	7.34, d, (8.6)	6, 2, 7	3/5
3/5	115.8	6.80, d, (8.6)	3, 5	2/6
4	157.6	-	2, 3, 5, 6	-
7	144.4	7.44, d, (15.9)	2, 6, 8	8
8	115.4	6.21, d, (15.9)	7	7
9	167.1	-	7, 8, 9 a, 9 b	-
1	130.1	-	2 , 5 , 6 , 7 , 8	-
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3	146.5	-	2 , 5 , OMe-3	-
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5	114.0	6.86, m	-	6
6	121.0	6.87, m	2 , 7	5
7	86.2	4.42, d, (6.1)	2 , 8 , 9 a, 9 b	8
8	82.5	4.47, m	7 , 9 a, 9 b	7 , 9 a, 9 b
9 a/9 b	63.7	4.51, m	7 , 8	8
1″	135.9	-	2″, 5″, 6″, 7″, 8″	-
2″	112.5	6.61, d, (1.8)	6″, 7″	-
3″	150.7	-	2″, 5″, OMe-3″	-
4″	146.1	-	8′, 2″, 5″, 6″	-
5″	118.9	6.63, d, (8.0)	-	6″
6″	120.4	6.59, dd, (1.8, 8.0)	2″, 7″	5″
7″	31.8	2.60, m	2″, 6″, 8″, 9″	8″
8″	30.3	1.93, m	7″, 9″	7″, 9″
9″	63.8	4.16, t, (6.6)	7″, 8″	8″
1‴	126.9	-	2‴, 5‴, 7‴, 8‴	-
2‴	109.4	7.01, d, (1.7)	6‴, 7‴	-
3‴	146.8	-	2‴, 5‴, OMe-3‴	-
4‴	148.0	-	2‴, 5‴, 6‴	-
5‴	114.7	6.90, d, (8.2)	-	6‴
6‴	123.0	7.05, dd, (1.7, 8.2)	2‴, 7‴	5‴
7‴	144.9	7.59, d, (15.9)	2‴, 6‴, 8‴	8‴
8‴	115.4	6.28, d, (15.9)	7‴	7‴
9‴	167.4	-	9″, 7‴, 8‴	-
OMe-3	55.9	3.82, s	-	-
OMe-3″	55.7	3.71, s	-	-
OMe-3‴	55.9	3.90, s	-	-
OMe-7	57.1	3.27, s	7	-

[a] 750 MHz, $CDCl_3$ referenced to ^{13}C at δ 77.0 ppm; [b] 750 MHz, $CDCl_3$ referenced to ^{1}H at δ 7.24 ppm; [c] Coupling constant in Hz; [d] HMBC connectivity from C to H; [e] Correlations observed for one bond $J_{C\text{-}H}$ of 145 Hz and long range $J_{C\text{-}H}$ of 8 Hz

purified from fraction D78_GB (10 mg) by C_{18}-HPLC [MeOH-H_2O (3:1, v/v) over 45 min, flow rate 1.5 mL/min, UV detection at 254 nm]. Fractions D78_D and D78_E were combined and subjected to FC using *n*-hexane, EtOAc and MeOH in order of increasing polarity to give six fractions (D78_DE1 - D78_DE6). Fraction D78_DE4 was compound **5** (64 mg), and fraction D78_DE2 was fractionated by FC following purification with C_{18}-HPLC [MeOH-H_2O (3:1, v/v) over 45 min, flow rate 1.5 mL/min, UV detection at 254 nm] to obtain **4** (9 mg).

RESULT AND DISCUSSION

Establishment of the relative and absolute configurations

Purification of fraction D78_GB using MeOH-H_2O (3:1, v/v) gave 2 major peaks at 38.4 and 42.3 min that corresponded to **1** and **2**, respectively. Both **1** and **2** were obtained as white amorphous solids each with molecular formula of $C_{40}H_{42}O_{12}$ by HRESIMS.

In $CDCl_3$, characteristic ^{1}H-NMR spectroscopic details for compound **1** included three methylenes at

δ 4.18 (2H, t, *J* = 6.5 Hz, H-9″), 2.63 (2H, m, H-7″) and 1.96 (2H, m, H-8″), two oxygenated methine protons at δ 4.49 (1H, m, H-8) and 4.46 (1H, d, *J* = 5.8 Hz, H-7), and geminal protons next to oxygen at δ 4.31 (1H, dd, *J* = 3.8, 11.7 Hz, H-9 a) and 4.13 (1H, dd, *J* = 6.0, 11.7 Hz, H-9 b). There were six methine aromatic protons at δ 6.86 (2H, m, each H-5 /H-6), 6.94 (1H, br s, H-2), 6.67 (2H, m, H-2″/H-6″), 6.91 (1H, d, *J* = 7.7 Hz, H-5″) and three methoxy protons at δ 3.30 (3H, s, OMe-7), 3.76 (3H, s, OMe-3″) and 3.83 (3H, s, OMe-3‴). DQFCOSY and HMBC data indicated the presence of a guaiacylglycerol 8 -*O*-4′ dihydroconiferyl alcohol ether derivative as the basic skeleton of the isolated compound [9-10].

The remaining proton signals were assigned by DQFCOSY and HMBC analysis to a *trans*-feruloyl group and a *trans*-*p*-coumaroyl group [11]. For the *trans*-feruloyl group, there were signals at δ 7.59 (1H, d, *J* = 15.9 Hz, H-7‴), 6.28 (1H, d, *J* = 15.9 Hz, H-8‴), 7.06 (1H, dd, *J* = 1.6, 8.1 Hz, H-6‴), 7.01 (1H, d, *J* = 1.6 Hz, H-2‴), 6.90 (1H, d, *J* = 8.1 Hz, H-5‴) and a methoxy at δ 3.91 (3H, s, OMe-3‴). For the *trans*-*p*-coumaroyl group, there were signals at δ 7.43 (1H, d, *J* = 15.9 Hz, H-7), 6.18 (1H, d, *J* = 15.9 Hz, H-8), 7.34 (2H, d, *J* = 8.6 Hz, H-2/H-6) and 6.81 (2H, d, *J* = 8.6 Hz, H-3/H-5).

The ^{13}C NMR and HSQC data confirmed the presence of four methylenes, nineteen methines, four methoxy carbons and thirteen quaternary carbons including two carbonyls at δ 167.3 (C, C-9‴) and 166.8 (C, C-9). The structure of the isolated compound was constructed by long-range correlations. In the HMBC spectrum, a geminal proton at δ 4.18 showed a correlation to the carbon at δ 167.3 assigned as C-9‴ of the *trans*-feruloyl group. Additionally, there were three bond correlations from the two protons at δ 4.31 and 4.13 to another carbonyl at δ 166.8 assigned as C-9 and part of the *trans*-*p*-coumaroyl group.

The second compound **2** had almost identical ^{1}H-NMR spectra to **1**, except for the replacement of two doublet-doublet signals at δ 4.31 and 4.13 in compound **2** by a multiplet signal at δ 4.51 that integrated as 2 protons. The coupling constant (*J* = 6.1 Hz) of H-7 in compound **2** was slightly larger than the coupling constant (*J* = 5.8 Hz) shown in **1**. Since the *J* values for compounds **1** and **2** were so similar, it was important to use $^{2,3}J_{HC}$ and NOESY data to attempt to distinguish between the two stereoisomers.

Matsumori et al. [12] has 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 compounds **1** and **2** with C_7C_8-dioxy substituents, the vicinal protons of coupling constants in an individual rotamer can be in the range 0-4 Hz (*gauche*) or 7-10 Hz (*anti*); these values are described

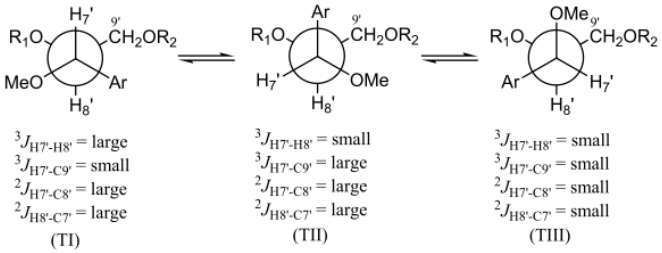

Fig 1. The three possible staggered conformers of compound **1**

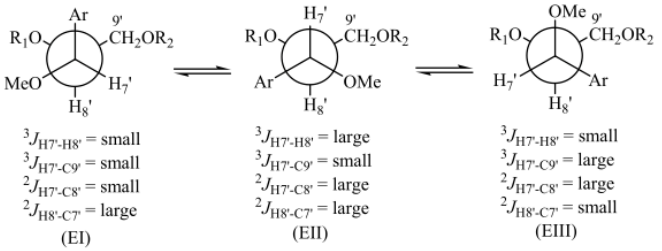

Fig 2. The three possible staggered conformers of compound **2**

as small or large, respectively. For heteronuclear coupling constants, the $^{3}J_{HC}$ value range from 1-3 Hz (*gauche*) or from 5-7 (*anti*), described as small or large, while $^{2}J_{HC}$ values can be 0-2 (small) or -4 to -6 Hz (large). The information from $^{2,3}J_{HC}$ can thus provide useful additional information about conformational preferences. In an individual compound, the coupling constant values are observed as a weighted average of the values of each rotamer that contributes to the conformational equilibrium [12]. Fig. 1 and 2 show three possible staggered conformers that can be drawn for each diastereoisomer and below each rotamer is listed the approximate magnitude of the $^{3}J_{HH}$ or $^{2,3}J_{HC}$ that would be expected for this conformation.

Since there is no hydrogen bonding involved, there will be a preference for conformer **TI** (Fig. 1) in compound **1** and conformer **EII** (Fig. 2) in compound **2**. In both of these rotamers, H-7 and H-8 are diaxial, leading to a prediction of the largest coupling constants, as was observed.

Next, NOESY data were used to attempt and provide additional support for the proposed stereochemistry. The NOESY data for compounds **1** and **2** are given in Tables 3 and 4, respectively. These tables describe the anticipated correlations for all the conformers shown in Fig. 1 and Fig. 2 then documents those that were observed. Both compounds showed similar NOESY correlations between H-2 /H-8 , H-6 /H-8 and H-7 /H-9 . In compound **1**, the geminal protons H-9 a and H-9 b showed correlations to the methine aromatic protons H-2 and H-6 , consistent with the major conformer **TI**. In contrast, the NOESY spectrum of compound **2** showed correlations between the

Table 3. NOESY data for three possible staggered conformers for compound 1 in $CDCl_3$

Conformer TI		**Conformer TII**		**Conformer TIII**	
Expected H – H correlations	Observed correlation	Expected H – H correlations	Observed correlation	Expected H – H correlations	Observed correlation
OMe - H-8	-	H-7 - H-8	-	H-2 - H-8	+
OMe - H-5″	+	H-7 - H-5″	-	H-2 - H-5″	-
OMe - OMe-3″	-	H-7 - OMe-3″	-	H-2 - OMe-3″	-
H-2 - H-9	+	OMe - H-8	-	H-6 - H-8	+
H-2 - H-8	+	OMe - H-9	-	H-6 - H-5″	-
H-6 - H-9	+	H-2 - H-9	+	H-6 - OMe-3″	-
H-6 - H-8	+	H-2 - OMe-3″	-	H-7 - H-9	+
H-7 - H-9	+	H-2 - H-5″	-	H-7 - H-8	-
H-7 - OMe-3″	-	H-6 - H-9	+	OMe - 9	-
H-7 - H-5″	-	H-6 - OMe-3″	-	OMe - OMe-3″	-
		H-6 - H-5″	-	OMe - H-5″	+

(**+**) = expected nOe correlation was observed; (**-**) = expected nOe correlation not observed.

Table 4. NOESY data for three possible staggered conformers for compound 2 in $CDCl_3$

Conformer E1		**Conformer EII**		**Conformer EIII**	
Expected H – H correlations	Observed correlation	Expected H – H correlations	Observed correlation	Expected H – H correlations	Observed correlation
OMe - H-8	+	H-2 - H-8	+	H-7 - H-8	-
OMe - H-5″	-	H-2 - H-5″	-	H-7 - H-5″	-
OMe - OMe-3″	-	H-2 - OMe-3″	+	H-7 - OMe-3″	+
H-2 - H-9	+	H-6 - H-8	+	H-2 - H-8	+
H-2 - OMe-3″	+	H-6 - H-5″	-	H-2 - H-9	+
H-2 - H-5″	-	H-6 - OMe-5″	-	H-6 - H-8	+
H-6 - H-9	+	OMe - H-8	+	H-6 - H-9	+
H-6 - OMe-3″	-	OMe - H-9	+	OMe - H-9	+
H-6 - H-5″	-	H-7 - H-9	+	OMe - OMe-3″	-
H-7 - H-8	-	H-7 - H-5″	-	OMe - H-5″	-
H-7 - H-9	+	H-7 - OMe-3″	+		

(**+**) = expected nOe correlation was observed; (**-**) = expected nOe correlation not observed.

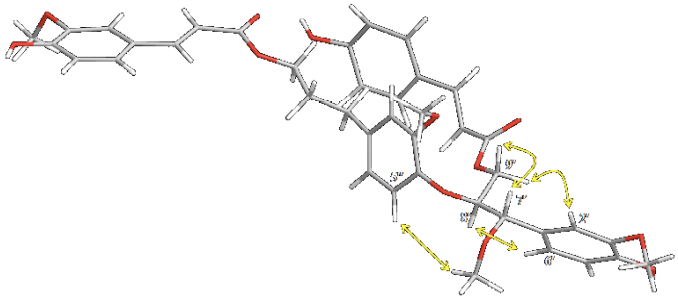

Fig 3. Three dimension model of conformer **TI** and some NOESY correlations for compound **1** in $CDCl_3$

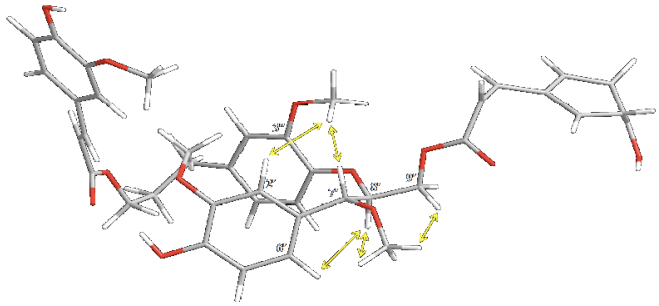

Fig 4. Three dimension model of conformer **EII** and some NOESY correlations for compound **2** in $CDCl_3$

geminal protons H-9 a and H-9 b to the -OMe protons, as expected for the major conformer **EII**. Even though NOESY correlations from the geminal protons H-9 to H-2 and H-6 were observed in compound **2** (Table 4), these were interpreted as contributions from minor conformers **EI** and **EIII**. Fig. 3 and 4 show the three dimension model of conformers **TI** and **EII** respectively.

Finally, the inspection of $^{2,3}J_{HC}$ values confirms the correctness of the configurational assignments. Analysis of the HSQC-HECADE [5-6] spectra in $CDCl_3$ indicated that compound **1** had a *gauche* relationship between H-7 and C-9 since the $^{3}J_{H7\text{-}C9}$ was +1.0 Hz, $^{2}J_{H7\text{-}C8}$ was -4.5 Hz and $^{2}J_{H8\text{-}C7}$ was -3.9 Hz, with each value conveniently described as small, large and large. Similarly compound **2** showed $^{3}J_{H7\text{-}C9}$ of +2.4, $^{2}J_{H7\text{-}C8}$ of -5.3 Hz and $^{2}J_{H8\text{-}C7}$ of -3.6 Hz, each value described as

small, large and large. These data confirmed that the conformer **TI** and the conformer **EII** were the major contributors to the conformational situation for compounds **1** and **2** respectively. With all of these data, it was apparent that the two methoxy compounds **1** and **2** had *threo* and *erythro* configurations, respectively.

The analysis of circular dichroism (CD) spectra allowed the absolute configuration for both **1** and **2** to be established. According to some literature [13-18], the lignan compounds all show a trend in which the Cotton effect in the 230 – 240 nm range is positive when the C-8 configuration is *S*. It was also apparent from the published CD data that the *erythro* isomers show a red shifted maxima compared to the *threo* isomers. Owing to solubility issues, the spectra of **1** and **2** were recorded in acetonitrile and as a result, compound **1** showed positive Cotton effects at 238.9 nm while compound **2** was red shifted to 240 nm. Hence, compound **1** may have *7S*,*8S* configuration while **2** may have *7R*,*8S* configuration. The $[\alpha]_D$ values of the two methoxy compounds **1** and **2** were -16.0 (*c* 0.2, MeOH) and -2.6 (*c* 0.2, MeOH) respectively.

CONCLUSION

Two new lignans **1** and **2** have been isolated and characterized by chemical studies. The intensive studies of conformational preferences, 2D NMR experiments including HSQC-HECADE and NOESY and the CD spectroscopy were very useful for assignment of the configuration of the lignans that showed multi-conformer equilibria. This study showed that NMR-based methods in combination with chiroptical approaches were able to confirm the relative and absolute configurations of complex lignans despite the presence of multiple conformers.

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REFERENCES

1. Subhadrabandhu, S., and Ketsa, S., 2001, *Durian King of Tropical Fruit*, CABI publisher, Wellington, 1-5.
2. Heyne, K., 1950. De Nuttige Planten van Indonesië. [the useful plants of Indonesia] [in Dutch]. 3rd ed., H. Veenman & Zonen, Wageningen (Volume 1, pages 389, 1056-1058 pertaining to the genus *Durio*).
3. Rudiyansyah, and Garson, M.J., 2006, *J. Nat. Prod.*, 69, 1218-1221.
4. Rudiyansyah, Suciati, Ross, B.P., and Garson, M.J., 2008, Proceeding of the IOCD International Symposium; Biology, Chemistry, Pharmacology and Clinical Studies of Asian Plants, Airlangga University Press, Surabaya, Indonesia, ISBN: 978-979-1330-35-0, 1-11.
5. Kozminski, W., and Nanz, D., 1997, *J. Magn. Reson.,* 124, 383-392.
6. Kozminski, W., and Nanz, D., 2000, *J. Magn. Reson.*, 142, 294-299.
7. Paula, V.F., Barbosa, L.C.A., Howarth, O.W., Demuner, A.J., Cass, Q.B., and Vieira, I.J.C., 1995, *Tetrahedron*, 51, 12453-12462.
8. Seca, A.M.L., Silva, A.M.S., Silvestre, A.J.D., Cavaleiro, J.A.S., Domingues, F.M.J., and Pascoal-Neto, C., 2001, *Phytochem.*, 56, 759-767.
9. Davin, L.B., and Lewis, N.G., 2003, *Phytochem. Rev.*, 2, 257-288.
10. Gang, D.R., Costa, M.A., Fujita, M., Dinkova-Kostoval, A.T., Wang, H.B., Burlat, V., Martin, W., Sarkanet, S., Davin, L.B., and Lewis, N.G., 1999, *Chem. Biol.,* 6, 143-151.
11. Chien, N.Q., Hung, N.V., Santarsiero, B.D., Mesecar, A.D., Cuong, N.M., Soejarto, D.D., Pezzuto, J.M., Fong, H.H.S., and Tan, G.T., 2004, *J. Nat. Prod.,* 67, 994-998.
12. Matsumori, N., Kaneno, D., Murata, M., Nakamura, H., and Tachibana, K., 1999, *J. Org. Chem.,* 64, 866-876.
13. Wu, P.L., Chuang, T.H., He, C.X., and Wu, T.S., 2004, *Bioorg. Med. Chem.*, 12, 2193-2197.
14. Arnoldi, A., and Merlini, L., 1985, *J. Chem. Soc., Perkin Trans. 1*, 2555-2557.
15. Greca, M.D., Molinaro, A., Monaco, P., and Previtera, L., 1994, *Phytochem.*, 35, 777-779.
16. Gellerstedt, G., Lundquist, K., Wallis, A.F.A. and Zhang, L., 1995, *Phytochem.*, 40, 263-265.
17. Matsuda, N., and Kikuchi, M., 1996, *Chem. Pharm. Bull.,* 44, 1676-1679.
18. Huo, C., Liang, H., Zhao, Y., Wang, B., and Zhang, Q., 2008, *Phytochemistry*, 69, No 3, 788-795.
19. Suzuki, S., and Umezawa, T., 2007, *J. Wood. Sci.*, 53, 273-284.