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Lignans isolated from the ethyl acetate extract of *Knema pachycarpa* fruit

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Abstract

Knema is a genus of tropical evergreen trees of the family Myristicaceae found in South East Asian countries such as Vietnam, Thailand, and Malaysia. In this paper, four lignans, (+)-pinoresinol (**1**),(+) epi-pinoresinol (**2**), piperitol (**3**), and pluviatilol (**4**), were isolated from the ethyl acetate extract of the fruit of *Knema pachycarpa*, an indigenus tree in Vietnam. The chemical structures were determined by spectroscopic data and comparison with the reported literature. These compounds were isolated from *Knema* genus for the first time.

Keywords. *Knema pachycarpa* de Wilde, (+)-Pinoresinol, (+)-Epi-pinoresinol, Piperitol, Pluviatilol.

1. INTRODUCTION

Knema is a genus of tropical evergreen trees of the family Myristicaceae found in South East Asian countries such as Vietnam, Thailand, and Malaysia. At least 13 species are found in Vietnam, where they are commonly known as "mau cho" referring to the red resin secreted in the bark [1]. Traditionally, *Knema* species have been used to treat sore, pimples, cancers and skin diseases. The genus *Knema* contains variety of natural compounds including cardanols, flavonoids, acetophenones, lignans, acylphloroglucinols, acylresorcinols, and anacardic acids [2-4].

Knema pachycarpa de Wilde "Mau cho trai day" is an indigenus tree in Vietnam and the chemical study of this *Knema* species has not been reported. In this paper, we report the isolation of 4 lignan compounds from the ethyl acetate extract of *K. pachycarpa* fruit including (+)-pinoresinol (**1**), epi-pinoresinol (**2**), piperitol (**3**), and pluviatilol (**4**). Their chemical structures were determined by spectroscopic data and comparison with the reported literature.

2. EXPERIMENTAL

2.1. General Experimental Procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded by a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh) or Sephadex LH-20. Thin layer chromatography used precoated silica gel plates (Merck 60 F_{254}). Compounds were visualized by spraying with Ce-Mo stain.

2.2. Plant material

The fruit of *Knema pachycarpa* de Wilde was collected at A-Luoi, Hue city, Viet Nam, in 2015 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (VN-1527) was deposited at the Institute of Marine Biochemistry, VAST.

2.3. Extraction and Isolation

The fruits of *K. pachycarpa* were sliced into small pieces and dried. The material (380 g) was extracted with MeOH at room temperature (3 times, 1 day/time). The extracts were combined and evaporated *in vacuo* and the residue was suspended in H_2O. The suspension was successively partitioned with *n*-hexane and ethyl acetate to give *n*-hexane

residue (105 g) and ethyl acetate residue (2.3 g).

The ethyl acetate residue (2.28 g) was subjected to column chromatography on silica gel, eluted using gradient solvents with *n*-hexane-ethyl acetate (50:1 to 0:1, v/v) to afford 5 fractions (E1-E5**)**.

The E2 fraction (702 mg) was separated into 3 sub-fractions (E2.1-E2.3) using CC on Sephadex eluted with MeOH. The E2.2 sub-fraction (618 mg) was chromatographed on silica gel column eluted with CH_2Cl_2/MeOH 98/2 (v/v) to give 3 sub-fractions E2.2.1-E2.2.3. Purification of fraction E2.2.1 (230 mg) with silica gel CC eluted with *n*-hexane-ethyl acetate 85:15 (v/v) furnished compound **1** (24 mg) and compound **2** (11 mg). The E1 fraction (400 mg) was also fractionated by column chromatography on Sephadex eluted with MeOH to give 3 sub-fractions (E1.1-E1.3). The E1.2 sub-fraction (68.5 mg) was purified on silica gel column using *n*-hexane-ethyl acetate 85:15 (v/v) to yield compound **3** (16.4 mg) and compound **4** (2.6 mg).

(+)-Pinoresinol (1): white solid, $[\alpha]^{25}_D$ = +75.0º ($CHCl_3$, *c* = 0.06), mp: 115-116 ºC. ESI-MS: *m/z* 359 $[M+H]^+$, molecular formula $C_{20}H_{22}O_6$ (M = 358). ^{1}H-NMR and ^{13}C-NMR data, see table 1.

(+)-Epipinoresinol (**2**): white solid, $[\alpha]^{25}_D$ = +113.2º ($CHCl_3$, *c* = 0.30), mp: 133-135 ºC. ESI-MS: *m/z* 359 $[M+H]^+$, molecular formula $C_{20}H_{22}O_6$ (M = 358). ^{1}H-NMR and ^{13}C-NMR data, see table 1.

Piperitol (**3**): clear oil, $[\alpha]^{25}_D$ = -63.6º ($CHCl_3$, *c* = 0.25). ESI-MS: *m/z* 357 $[M+H]^+$, molecular formula $C_{20}H_{20}O_6$ (M = 356). ^{1}H-NMR and ^{13}C-NMR data, see table 2.

Pluviatilol (**4**): white solid, $[\alpha]^{25}_D$ = +36.6º ($CHCl_3$, *c* = 0.3), mp: 160-161 ºC. ESI-MS: *m/z* 357 $[M+H]^+$, molecular formula $C_{20}H_{20}O_6$ (M = 356). ^{1}H-NMR and ^{13}C-NMR data, see table 2.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white solid. The ESI-MS showed a molecular ion peak *m/z* 359 $[M+H]^+$, indicating that a molecular formula of **1** is $C_{20}H_{22}O_6$. In the ^{13}C-NMR spectra, there were 10 carbon signals suggesting that structure of **1** is symmetric. The ^{1}H NMR spectrum revealed the signals ABX spin systems in the phenyl ring [δ_H: 6.90 (1H, d, *J* = 2.5 Hz), 6.87 (1H, d, *J* = 8.5 Hz), 6.81 (1H, dd, *J* = 2.0 Hz, *J* = 8.0 Hz)] with a methoxy and hydroxyl group signals at δ_H 3.89 (s, 3H) and 5.68 (br s, 1H), respectively. In addition, the signals of *bis*-lignan furan ring were found at δ_H 4.73 (1H, d, 4.5 Hz), 4.26 (1H, dd, *J* = 9 Hz; *J* = 7 Hz), 3.86 (1H, dd, *J* = 9 Hz; *J* = 3.5 Hz) and 3.09 (1H, m). The ^{13}C-NMR showed the signals of aromatic carbons at δ_C 146.7 (C-4'), 145.2 (C-3'), 132.9 (C-1'), 118.9 (C-6'), 114.2 (C-5'), 108.6 (C-2'); a methoxy group at δ_C 55.96 and *bis*-lignan furan ring at δ_C 85.8 (C-7,7'), 54.1 (C-8,8') and 71.6 (C-9,9'). Analytical NMR, MS and optical data indicated that the structure of compound **1** is (+)-pinoresinol. The NMR data is in good agreement with those in the reported literature [5].

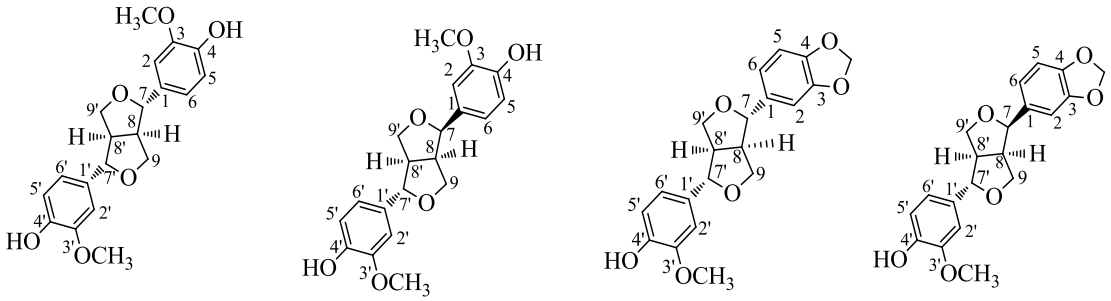

Fig. 1: Chemical structures of isolated lignans **1-4**

Compound **2** was isolated as a white powder, mp 133-135 ºC. The ESI-MS (molecular ion peak *m/z* 359) and NMR (20 carbon signals, 22 protons) data indicated that a molecular formula of **2** is $C_{20}H_{22}O_6$, the same as compound **1**, pinoresinol. The ^{1}H NMR spectrum showed typical signals of two ABX systems [δ_H 6.95 (d, 1H, *J* = 1.0 Hz), 6.91 (d, 1H, *J* = 1.5 Hz), 6.88 (d, 1H, *J* = 8.0 Hz), 6.90 (d, 1H, *J* = 8.5 Hz), 6.83 (dd, 1H, *J* = 8.5 Hz, 2.0 Hz), 6.77 (dd, 1H, *J* = 8.0 Hz, 1.0 Hz)] with two methoxy groups at 3.91 (s, 3H), 3.90 (s, 3H). The remaining protons signals [δ_H 4.86 (d, 1H, *J* = 5.5 Hz), 4.43 (d, 1H, *J* = 7.0 Hz), 4.12 (d, 1H, *J* = 9.5 Hz), 3.86-3.83 (m, 2H), 3.35-3.30 (m, 2H), 2.92-2.88 (m, 1H)] were

analysed and assigned as epi-furofuran ring using COSY spectrum. The ^{13}C-NMR and DEPT spectra of **2** showed the signals of 20 carbons including a 12 aromatic carbon signals [δ_C 146.7, 146.4, 145.3, 144.4, 133.0, 130.3, 119.1, 118.4, 114.2, 114.2, 108.5, and 108.4], 2 methoxy group signals [δ_C 56.01 and 55.96] and six carbon signals of epi-furofuran ring at δ_C 87.75, 82.13, 71.01, 69.69, 56.0, 55.9, 54.4 and 50.1. Therefore, compound **2** was identified as (+) epi-pinoresinol, an isomer of pinoresinol. The NMR data are nearly identical to those of reported (+) epi-pinoresinol [6].

Table 1: ^{1}H and ^{13}C-NMR data of lignans **1**-**2** and reference compounds

1					**2**			
C	$^{@}\delta_C$	$^{a,b}\delta_C$	$^{@}\delta_H$ (mult., *J* = Hz)	$^{b,c}\delta_H$ (mult., *J* = Hz)	$^{d}\delta_C$	$^{a,b}\delta_C$	$^{b,c}\delta_H$ (mult., *J* = Hz)	$^{d}\delta_H$ (mult., *J* = Hz)
1	132.8	132.9	-	-	130.8	130.3	-	-
2	108.6	108.6	6.87, d (1.6)	6.90, d (2.0)	108.9	108.4	6.95, d (1.0)	6.97-6.76, m
3	146.5	146.7	-	-	146.9	146.4	-	-
4	145.1	145.2	-	-	145.1	144.4	-	-
5	114.2	114.2	6.86, d (8.0)	6.87, d (8.5)	114.7	114.2	6.88, d (8.0)	6.97-6.76, m
6	118.9	118.9	6.79, dd (8.0, 1.6)	6.81, dd (8.0, 2.0)	118.9	118.4	6.83, dd (8.5, 2.0)	6.97-6.76, m
7	85.8	85.8	4.72, d (4.4)	4.73, d (4.5)	82.6	82.1	4.86, d (5.5)	4.86, d (5.0)
8	54.2	54.1	3.08, m	3.09, m	55.0	54.4	2.92-288, m	2.94-287, m
9a	71.6	71.6	4.23 dd (8.8, 6.8)	4.26, dd (9.0, 7.0)	70.2	69.6	3.86-3.83, m	3.89-3.80, m
9b			3.88, dd (8.8, 3.6)	3.86, dd (9.0, 3.5)			3.35-3.30, m	3.37-3.23, m
1’	132.8	132.9	-	-	133.5	133.0	-	-
2’	108.6	108.6	6.87, d (1.6)	6.90, s	109.0	108.5	6.91, d (1.5)	6.97-6.76, m
3’	146.5	146.7	-	-	147.2	146.7	-	-
4’	145.1	145.2	-	-	145.8	145.3	-	-
5’	114.2	114.2	6.86, d (8.0)	6.87, d (8.5)	114.7	114.2	6.90, d (8.5)	6.97-6.76, m
6’	118.9	118.9	6.79, dd (8.0, 1.6)	6.81, dd (8.0, 2.0)	119.6	119.1	6.77, dd (8.0, 1.0)	6.97-6.76, m
7’	85.8	85.8	4.72, d (4.4)	4.73, d (4.5)	88.2	87.7	4.43, d (7.0)	4.44, d (7.0)
8’	54.2	54.1	3.08, m	3.09, m	50.6	50.1	3.35-3.30, m	3.37-3.23, m
9a’	71.6	71.6	4.23 dd (8.8, 6.8)	4.26, dd (9.0, 7.0)	71.5	71.0	4.12, d (9.5)	4.12, d (9.3)
9b’			3.88, dd (8.8, 3.6)	3.86, dd (9.0, 3.5)			3.86-3.83, m	3.89-3.80, m
3-OCH_3	55.9	55.9	3.83	3.89	56.5	56.0	3.91, s	3.91, s
3’-OCH_3	55.9	55.9	3.83	3.89	56.4	55.9	3.90, s	3.89, s

[a]125 MHz, [b] $CDCl_3$, [c]500 MHz, @: (+)-Pinoresinol [5], d: (+)-Epi-pinoresinol [6].

Compound **3** was isolated as an oil. The NMR features indicate that the structure of **3** is also a lignan. The ^{1}H NMR spectrum showed 6 signals of two ABX spin systems in the aromatic region [δ_H: 6.87-6.89 (2H, m), 6.85 (1H, d, *J* = 1.5 Hz), 6.79-6.82 (2H, m) and 6.77 (1H, s)], with a methylene dioxide, hydroxyl and methoxy group signals at δ_H 5.94 (2H, s), 5.62 (1H, s) and 3.90 (3H, s), respectively. The signals of *bis*-lignan furan ring [δ_H 4.73 (dd, 4.5, 2.0, 2H), 4.26-4.21 (dd, 9.0, 6.5, 2H), 3.89-3.85 (dd, 9.0, 4.0, 2H) and 3.11-3.03 (2H, m)] are similar to those of pinoresinol. The ^{13}C-NMR

showed 20 carbon including 12 signals of aromatic carbons, methylene dioxide group at δ_C 101.07, a methoxy group at δ_C 55.96 and *bis*-lignan furan ring at δ_C 85.87, 85.83, 71.72, 71.68, 54.33 and 54.18. The ESI-MS showed a molecular ion peak *m/z* 357 $[M+H]^+$, indicating that a molecular formula of **3** is $C_{20}H_{20}O_6$. On the basis of the above spectral evidences, compound **3** is determined as piperitol. The analytical NMR data of **3** are in accordance with those published [7, 8].

Table 2: ^{1}H and ^{13}C-NMR data of lignans **3**-**4** and reference compounds

	3				**4**	
C	$^{\#}\delta_C$	$^{a,b}\delta_C$	$^{b,c}\delta_H$ (mult., *J* = Hz)	$^{*}\delta_H$ (mult., *J* = Hz)	$^{b,c}\delta_H$ (mult., *J* = Hz)	$^{\&}\delta_H$ (mult., *J* = Hz)
1	135.1	135.1	-	-	-	-
2	106.5	106.5	6.77-6.89, m	6.76-6.93, m	6.77-6.94, m	6.81-6.89, m
3	148.0	147.9	-	-	-	-
4	146.8	146.7	-	-	-	-
5	108.2	108.1	6.77-6.89, m	6.76-6.93, m	6.77-6.94, m	6.81-6.89, m
6	119.3	119.3	6.77-6.89, m	6.76-6.93, m	6.77-6.94, m	6.81-6.89, m
7	85.9	85.8	4.73, dd (4.5, 2.0)	4.72, d (4.5)	4.85, d (4.5)	4.86, d (6.0)
8	54.3	54.3	3.03-3.11, m	2.85-3.25, m	3.31, m	3.32, m
9a	71.7	71.7	4.26, dd (9.0, 6.5)	4.26, dd (9.0, 6.5)	3.84, m	3.85, m
9b			3.89, dd (9.0, 4.0)	3.85, dd (9.0, 3.5)	3.30, m	3.32, m
1’	132.9	132.9	-	-	-	-
2’	108.7	108.6	6.77-6.89, m	6.76-6.93, m	6.77-6.94, m	6.81-6.89, m
3’	147.1	147.1	-	-	-	-
4’	145.3	145.2	-	-	-	-
5’	114.4	114.3	6.77-6.89, m	6.76-6.93, m	6.77-6.94, m	6.81-6.89, m
6’	119.0	119.0	6.77-6.89, m	6.76-6.93, m	6.77-6.94, m	6.81-6.89, m
7’	85.9	85.8	4.73, dd (4.5, 2.0)	4.72, d (4.5)	4.43, d (7.0)	4.42, d (7.5)
8’	54.2	54.1	3.03-3.11, m	2.85-3.25, m	2.88, m	2.91, m
9a’	71.7	71.6	4.26, dd (9.0, 6.5)	4.26, dd (9.0, 6.5)	4.11, d (9.5)	4.13, dd (9.5, 1.0)
9b’			3.89, dd (9.0, 4.0)	3.85, dd (9.0, 3.5)	3.86, m	3.85, dd (9.5, 6.5)
3’-OCH_3	56.0	55.9	3.90, s	3.90, s	3.91, s	3.91, s
-OH			5.62, br s	5.75, br s	5.76, br s	
-OCH_2O-	101.1	101.0	5.94, s	5.96, s	5.94, s	5.97, s

[a]125 MHz, [b] $CDCl_3$, [c]500 MHz, $^{*}\delta_H$: Piperitol [7], $^{\#}\delta_C$: Piperitol [8], $^{\&}\delta_H$: Pluviatilol [9].

Compound **4** was obtained as a white solid, mp 160-161 ^oC. In the NMR spectrum, the proton signals of aromatic ring are similar to those of compound **3** (piperitol) with 6 proton signals in the aromatic region, a methylene dioxide, a hydroxyl and a methoxy group at δ_H 5.94 (2H, s), 5.76 (1H, s) and 3.91 (3H, s). However, the remaining protons signals are similar to those of compound **2**, epi-pinoresinol with 8 protons at δ_H 4.85 (1H, d, *J* = 4.5 Hz), 4.43 (1H, d, *J* = 7.0 Hz), 4.11 (1H, d, *J* = 9.5 Hz), 3.86-3.84 (2H, m), 3.31-3.30 (2H, m), 2.88 (1H, m). Therefore, compound **4** was identified as pluviatilol, an isomer of piperitol. The ^{1}H-NMR data are in good agreement with the reported literature [9].

4. CONCLUSION

A phytochemical investigation of the ethyl acetate extract of the fruit of *K. pachycarpa* led to the

isolation of four lignans including (+)-pinoresinol (**1**), (+) epi-pinoresinol (**2**), piperitol (**3**), and pluviatilol (**4**). Their chemical structures were elucidated by spectroscopic NMR and MS data. These lignans were isolated from *Knema* genus for the first time.

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