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SECONDARY METABOLITES FROM *DIPTEROCARPUS OBTUSIFOLIUS* TEIJSM. ex MIQ.

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Abstract

Six known compounds daphneresinol (**1**), (+)-neo-olivil (**2**), methyl gallate (**3**), bergenin (**4**), asiatic acid (**5**) and blumenol A (**6**) were obtained from the organic extract of the leaves of *Dipterocarpus obtusifolius* Teijsm. Ex Miq. by various chromatographic techniques. Structural elucidation of the metabolites was carried out by analysis of their spectroscopic data and by comparison with those reported in the literature. Compounds **1**–**4** and **6** were isolated from this plant for the first time.

Keywords. *Dipterocarpus obtusifolius*, Dipterocarpaceae, secondary metabolite.

1. INTRODUCTION

Dipterocarpus obtusifolius (Dipterocarpaceae) is a woody plant that grows in areas of Western Highlands and Southern Vietnam. In traditional medicine, the oil of *D. obtusifolius* is used for the treatment of gonorrhea, pimples, and skin diseases [1]. Diterpenes, sesquiterpenes and triterpenes have been separated from the leaves of this plant and some of them were found to be cytotoxic against one or more human cancer cell lines [2]. In this paper, we reports the isolation and structure elucidation of six known compounds **1**–**6** from *D. obtusifolius.* These compounds have never been reported before from this plant except for the compound **5**.

2. EXPERIMENTAL

2.1. General experimental procedures

The ESI-MS was measured on Agilent 1260 series single quadrupole LC/MS systems. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker, Billerica, MA, U.S.A.) using TMS as an internal standard. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 70–230 mesh and 230-400 mesh, Merck, Darmstadt, Germany) or YMC RP-18 resins (30-50 μm, Fuji Silysia Chemical Ltd, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (1.15685.0001, Merck, Darmstadt, Germany) and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3-5 minutes.

2.2. Plant material

The samples of the plant *Dipterocarpus obtusifolius* Teijsm. ex Miq. were collected in May 2013 at Bao Lam, Lam Dong and identified by Dr. Nong Van Duy from the Tay Nguyen Institute of Scientific Research, VAST. A voucher specimen (No. TN3/242) was deposited at the Tay Nguyen Institute of Scientific Research, VAST.

2.3. Extraction and isolation

The air dried and powdered leaves of *D. obtusifolius* (4.5 kg) were extracted with methanol at 40 ^oC three times. Methanolic extracts were combined and evaporated under vacuum. This extract (500 g) was suspended in water and partitioned in turn with *n*-hexane, CH_2Cl_2, and EtOAc to provide the corresponding extracts: *n*-hexane (H, 37 g), CH_2Cl_2 (C, 26 g), EtOAc (E, 95 g) and a water layer.

Extracts E were crudely separated by silica gel CC using a gradient concentration of MeOH in CH_2Cl_2 (0-100 %) to obtain nine fractions (E1–E9). Fraction E4 (2.3 g) was further separated by YMC RP-18 CC and eluted with MeOH–water (1:2, v/v), followed by silica gel CC with *n*-hexane–EtOAc (1:3, v/v) to give compound **6** (7.0 mg) and subfraction E4A. Further purification of fraction E4A, using silica gel CC and *n*-hexane–EtOAc-methanol (1:1:0.1, v/v/v), produced compound **2** (40 mg), compound **3** (10 mg) and compound **5** (12 mg). Fraction E6 (25 g) was subjected to a silica gel CC and eluted with CH_2Cl_2/MeOH (from 20:1 to 0:1, v/v) to afford six subfractions E6A-E6F. Subfraction E6D was separated by YMC using MeOH/H_2O (1:5, v/v) as eluent to give compound **4** (12 mg) and compound **1** (4.0 mg).

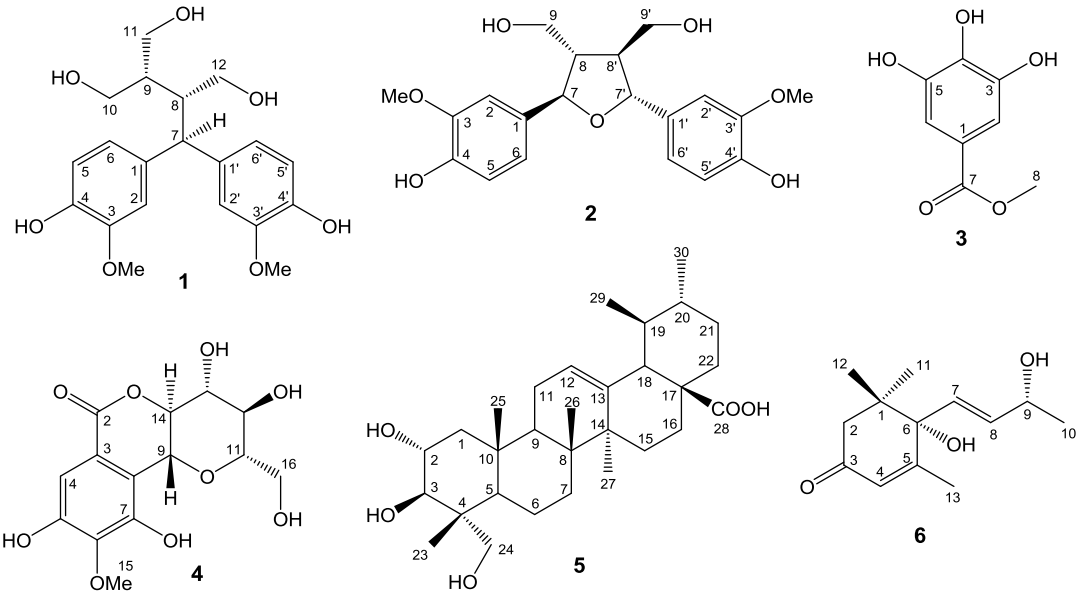

Figure 1: Structures of compounds **1**–**6**

Daphneresinol (**1**): Yellow oil; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 1; Positive ESI-MS *m/z* 379 $[M+H]^+$.

(+)-neo-olivil (**2**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2. Positive ESI-MS *m/z* 377 $[M+H]^+$.

Methyl gallate (**3**): White solid; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 3.83 (3H, s, H-8), 7.07 (2H, s, H-2, 6).

Bergenin (**4**): White needles; Positive ESI-MS *m/z* 429 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ 7.11 (1H, s, H-4), 4.98 (1H, d, *J* = 10.5 Hz, H-9), 3.68 (overlap signal, H-11), 3.45 (1H, t, *J* = 9.0 Hz, H-12), 3.83 (1H, t, *J* = 9.0 Hz, H-13), 4.08 (1H, dd, *J* = 9.5 Hz, *J* = 10.5 Hz, H-14), 3.93 (3H, s, H-15), 3.70 (overlap signal, H-16a), 4.06 (1H, dd, *J* = 4.0 Hz, *J* = 11.0 Hz, H-16b); ^{13}C-NMR (125 MHz, CD_3OD) δ 165.75 (C-2), 152.35 (C-3), 111.05 (C-4), 142.30 (C-5), 149.45 (C-6), 117.31 (C-7), 119.44 (C-8), 74.26 (C-9), 83.06 (C-11), 71.92 (C-12), 75.63 (C-13), 81.44 (C-14), 60.88 (C-15), 62.67 (C-16).

Asiatic acid (**5**): White powder; Positive ESI-MS *m/z* 511 $[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ 1.04 (1H, m, H-1a), 1.97 (1H, m, H-1b), 3.72 (1H, m, H-2), 3.38 (1H, d, *J* = 9.5 Hz, H-3), 1.31 (1H, m, H-5), 1.42 (1H, m, H-6a), 1.47 (1H, m, H-6b), 1.33 (1H, m, H-7a), 1.68 (1H, m, H-7b), 1.68 (1H, m, H-9), 1.02 (1H, m, H-10), 2.0 (2H, m, H-11), 5.26 (1H, t, *J* = 3.3 Hz, H-12), 1.11 (1H, m, H-15a), 1.95 (1H, m, H-15b), 1.66 (1H, m, H-16a), 2.06 (1H, m, H-16b), 2.23 (1H, d, *J* = 11.5 Hz, H-18), 1.00 (1H, m, H-19), 1.40 (1H, m, H-20), 1.37 (1H, m, H-21a), 1.52 (1H, m, H-21b), 1.65 (1H, m, H-22a), 1.72 (1H, m, H-22b), 3.29 (1H, d, *J* = 11.3 Hz, H-23a), 3.53 (1H, d, *J* = 11.3 Hz, H-23b), 0.72 (3H, s, H-24), 1.07 (3H, s, H-25), 0.88 (3H, s, H-26), 1.16 (3H, s, H-27), 0.91 (3H, d, *J* = 6.5 Hz, H-29), 0.99 (3H, s, H-30); ^{13}C-NMR (125 MHz, CD_3OD) δ 48.05 (C-1), 69.70 (C-2), 78.24 (C-3), 43.40 (C-4), 48.04 (C-5), 19.08 (C-6), 33.66 (C-7), 40.80 (C-8), 48.82 (C-9), 38.99 (C-10), 24.46 (C-11), 126.61 (C-12), 139.89 (C-13), 43.40 (C-14), 29.19 (C-15), 25.35 (C-16), 48.50 (C-17), 54.41 (C-18), 40.42 (C-19), 40.44 (C-20), 31.80 (C-21), 38.14 (C-22), 66.38 (C-23), 13.91 (C-24), 17.67 (C-25), 17.88 (C-26), 24.14 (C-27), 180.52 (C-28), 17.67 (C-29), 21.57 (C-30).

Blumenol A (**6**): White amorphous powder; Positive ESI-MS *m/z* 225 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ 2.18 (1H, d, *J* = 17.0 Hz, H-2a), 2.53 (1H, d, *J* = 17.0 Hz, H-2b), 5.90 (1H, t, *J* = 1.3 Hz, H-4), 5.81 (1H, overlap signal, H-7), 5.82 (1H, overlap signal, H-8), 4.34 (1H, dd, *J* = 4.5 Hz; *J* = 6.5 Hz, H-9), 1.26 (3H, d, *J* = 6.5 Hz, H-10), 1.06 (3H, s, H-11), 1.03 (3H, s, H-12), 1.94 (3H, s, H-13); ^{13}C-NMR (125 MHz, CD_3OD) δ 42.42 (C-1), 50.73 (C-2), 201.27 (C-3), 127.10 (C-4), 167.48 (C-5), 79.95 (C-6), 130.10 (C-7), 136.92 (C-8), 68.72 (C-9), 23.82 (C-10), 23.46 (C-11), 24.47 (C-12), 19.56 (C-13).

3. RESULTS AND DISCUSSION

Compound **1**, a yellow oil, exhibited a molecular ion peak $[M + H]^+$ at *m/z* 379 in ESI-MS, suggesting a molecular formula of $C_{20}H_{26}O_7$. The ^{13}C NMR spectrum exhibited 20 carbon resonances, consisting of six sp^2 methines, six sp^2 quaternary signals, two methoxy groups, three sp^3 methines and three oxymethylene carbon signals. The ^{1}H-NMR spectrum showed signals for two methoxy group at δ 3.85 (3H, s), 3.85 (3H, s) and two ABX coupling system at δ 6.97 (1H, d, *J* = 2.0 Hz, H-2), 6.73 (1H, d, *J* = 8.0 Hz, H-5), 6.85 (1H, dd, *J* = 2.0, *J* = 8.0 Hz, H-6) and δ 6.96 (1H, d, *J* = 2.0 Hz, H-2'), 6.71 (1H, d, *J* = 8.0 Hz, H-5'), 6.84 (1H, dd, *J* = 2.0, *J* = 8.0 Hz, H-6'), that indicated the existence of two 1,3,4-trisubstituted benzene rings. In the aliphatic region, three methine proton signals at δ 4.01 (1H, d, *J* = 12.0 Hz, H-7), 2.65 (1H, m, H-8), 1.98 (1H, m, H-9), together three oxymethylene proton signals observed at δ 3.74 (2H, m, H-10), δ 3.64 (1H, dd, *J* = 4.5, *J* = 11.5 Hz, H_a-11)/3.72 (1H, m, H_b-11) and δ 3.41 (1H, dd, *J* = 6.0, *J* = 11.5 Hz, H_a-12)/3.56 (1H, dd, *J* = 2.0, *J* = 11.5, H_b-12) suggesting the presence of 2,3-dihydroxymethylbutanol fragment. This fragment was linked to two benzene ring base on the long-range correlations (Fig. 2) from H-7 to C-1, C-2, C-6, C-1', C-2', C-6', C-8, C-9, C-12, from H-12 to C-9, C-7, and from H-11 to C-8, C-10. The HMBC correlations from proton 3-OMe to C-3 and from proton 3′-OMe to C-3′ confirmed the positions of two methoxy group. The absolute configuration of **1** was determined on the basis of the good agreement of NMR spectral data with those of daphneresinol. Therefore, compound **1** was identified to be daphneresinol [3].

Table 1: The ^{1}H (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) data of compound **1**

C	$^a\delta_C$	δ_C	δ_H (mult., *J* = Hz)	**C**	$^a\delta_C$	δ_C	δ_H (mult., *J* = Hz)
1	137.8	137.17	-	11	59.9	59.71	3.64 (1H, dd, 4.5, 11.5) 3.72 (1H, m)
2	113.3	113.03	6.97 (1H, d, 2.0)	12	60.4	60.15	3.41 (1H, dd, 6.0, 11.5) 3.56 (1H, dd, 2.0,11.5)
3	149.1	149.04	-	1'	137.2	137.76	-
4	145.9	145.80	-	2'	113.1	112.82	6.96 (1H, d, 2.0)
5	116.4	116.28	6.73 (1H, d, 8.0)	3'	148.9	148.83	-
6	121.9	121.74	6.85 (1H, dd, 2.0, 8.0)	4'	145.8	145.69	-
7	52.2	52.1	4.01 (1H, d, 12.0)	5'	116.3	116.14	6.71 (1H, d, 8.0)
8	45.1	44.99	2.65 (1H, m)	6'	121.5	121.33	6.84 (1H, dd, 2.0, 8.0)
9	44.0	43.91	1.98 (1H, m)	3-OMe	56.6	56.46	3.86 (3H, s)
10	63.8	63.65	3.74 (2H, m)	3'-OMe	56.6	56.40	3.85 (3H, s)

$^a\delta_C$ of daphneresinol recorded in CD_3OD [3].

Compound **2** was obtained as a white powder. The ESI-MS spectra of **2** exhibited a ion peak $[M + H]^+$ at *m/z* 377, which is in agreement with the molecular formula $C_{20}H_{24}O_8$. In the ^{13}C NMR, there were only ten carbon signals, suggesting that **2** might be a symmetrical lignan structure. The ^{1}H NMR spectrum revealed the presence of two 1,3,4-trisubstituted symmetrical aromatic rings at δ 7.05 (2H, d, *J* = 2.0 Hz, H-2, H-2'), 6.81 (1H, d, *J* = 8.0 Hz, H-5, H-5'), 6.90 (1H, dd, *J* = 2.0, *J* = 8.0 Hz, H-6, H-6'), together two oxymethylene groups at δ 3.62 (2H, dd, *J* = 5.0, *J* = 11.5 Hz, H_a-9, H_a-9') and 3.72 (2H, dd, *J* = 3.5, *J* = 11.5 Hz, H_b-9, H_b-9'), two oxygenated methine groups at δ 4.95 (2H, d, *J* = 8.5 Hz, H-7, H-7'), two methine groups at δ 2.34 (2H, m, H-8, H-8') and two methoxy groups at δ 3.90 (6H, s). From these functionalities, compound **2** was suggested to be a 2,5-diaryl tetrahydrofuranoid-type

lignan. It was also confirmed by the cross peak between H-7 and C-2, C-6, C-8, C-9, C-8', as well as between H-7' and C-2', C-6', C-8', C-9', C-8. The relative configurations between H-7 and H-8, and H-7' and H-8' were established as *trans*-configurations due to the large coupling constant (J = 8.5 Hz) between H-7 (or H-7') and H-8 (or H-8'). On the basis of the above evidence, the structure of **2** was identified as (+)-neo-olivil by comparison of spectral data with those reported in the literature [4].

Table 2: The ^{1}H (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) data of compound **2**

C	$^{a}\delta_C$	δ_C	δ_H (mult., J = Hz)	C	$^{a}\delta_C$	δ_C	δ_H (mult., J = Hz)
1, 1'	134.9	134.97	-	6, 6'	120.5	120.52	6.90 (1H, dd, 2.0, 8.0)
2, 2'	111.1	111.23	7.05 (1H, d, 2.0)	7, 7'	84.4	84.43	4.95 (1H, d, 8.5)
3, 3'	149.1	149.12	-	8, 8'	55.4	55.43	2.34 (1H, m)
4, 4'	147.6	147.36	-	9, 9'	61.7	61.86	3.62 (1H, dd, 5.0, 11.5) 3.72 (1H, dd, 3.5, 11.5)
5, 5'	116.0	116.05	6.81 (1H, d, 8.0)	3, 3'-OMe	56.4	56.45	3.90 (3H, s)

$^{a}\delta_C$ of (+)-neo-olivil recorded in CD_3OD [4].

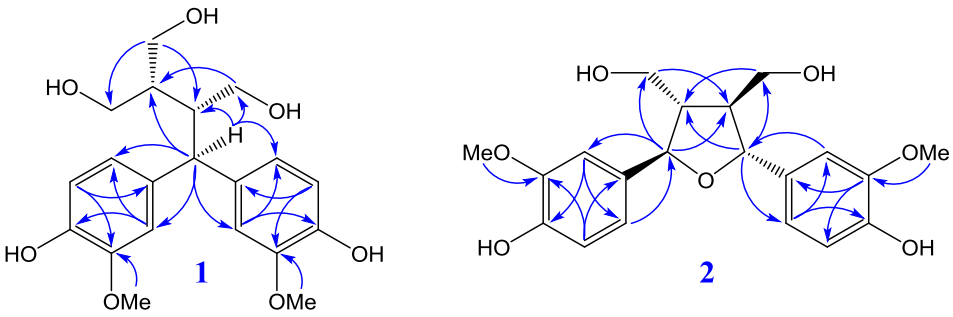

Figure 2: Key HMBC correlations of compounds **1** and **2**

Compound **3-6** were identified as methyl gallate (**3**) [5], bergenin (**4**) [6], asiatic acid (**5**) [7], and blumenol A (**6**) [8] by comparing the NMR spectral data with those reported in literature.

4. CONCLUSION

From the MeOH extract of the leaves of *Dipterocarpus obtusifolius*, using column chromatography, six known compounds daphneresinol (**1**), (+)-neo-olivil (**2**), methyl gallate (**3**), bergenin (**4**), asiatic acid (**5**), and blumenol A (**6**) were isolated. Based on 1D NMR and 2D NMR as well as comparison with published data, their chemical structures were elucidated. Compounds **1–4** and **6** have not been previously isolated from *D. obtusifolius*.

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