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DOLABRANE-TYPE DITERPENOID AND LIGNAN CONSTITUENTS FROM THE STEM BARKS OF *CERIOPS DECANDRA* (GRIFF.) W. THEOB.

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Abstract. Three dolabrane-type diterpenoids (**1**–**3**) and a lignan (**4**) were isolated from a methanolic extract of *C. decandra* (Griff.) W. Theob. stem barks using various chromatographic separations. Their structures were elucidated to be tagalsine X (**1**), tagalsin P (**2**), *ent*-5α,2-oxodolabr-3-ene-3,15,16-triol (**3**), and pinoresinol (**4**) by detailed analysis via spectroscopic techniques (1D, 2D NMR, and ESI-MS data) as well as comparison with those reported.

Keywords: *Ceriops decandra*, Rhizophoraceae, diterpenoid, lignan.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Plants of the Rhizophoraceae family contain approximately 24 species in 4 genera including *Bruguiera* (7 species), *Ceriops* (5 species), *Kandelia* (2 species), and *Rhizophora* (10 species) [1-3]. The species have a wide distribution through both tropical and sub-tropical intertidal estuarine regions worldwide [1, 4]. Among them, the chemical compositions of the *Ceriops* genus have been investigated. Aurane, abietane, beyrane, dolabrane, and podocarpane-type diterpenoids are among the most frequently found secondary metabolites in this genus, which possess diverse structures due to many substituted moieties [2, 3]. Interestingly, dolabrane-type diterpenoids were found only in the *Ceriops* genus of the Rhizophoraceae family, making it a significant chemotaxonomic marker of that specific genus.

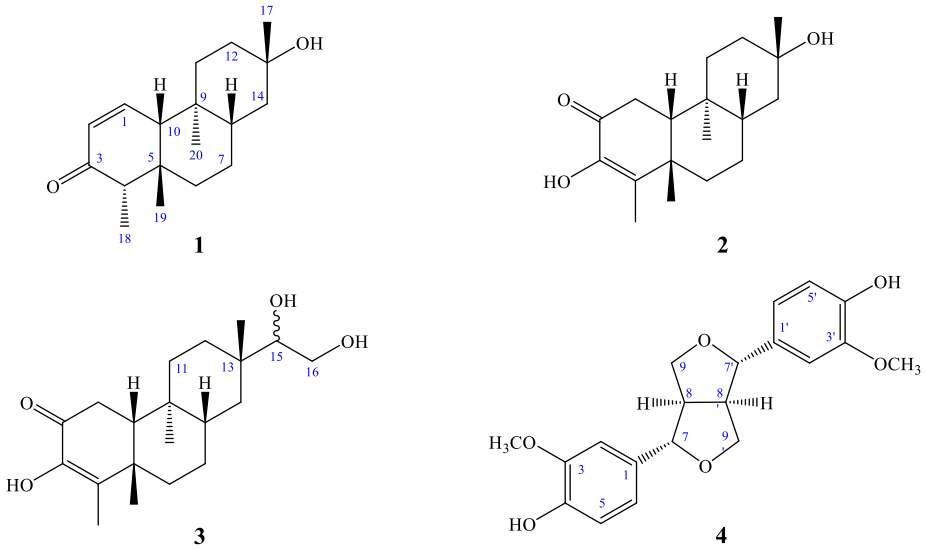

Figure 1. Structures of compounds **1**–**4** isolated from *C. decandra*.

Ceriops decandra (Griff.) W. Theob. is a mangrove species of ethnomedicinal significance having potent activity against a wide range of diseases like angina, boils, diabetes, diarrhea, dysentery, hepatitis, ulcers, and wounds [2, 3, 5 - 10]. Although the chemical constituents and pharmacological effects of the leaves and roots of *C. decandra* have been previously studied [2, 3, 5-8], however, the isolated metabolites (as abietane and podocarpane-type diterpenoids) from the stem barks of *C. decandra* have been limited [9, 10]. To date, a non-systematic phytochemical study that does not contain dolabrane-type diterpenoids has been described as the isolation from *C. decandra* distribution in Viet Nam. The current paper deals with detailed structure elucidation of four compounds (**1**–**4**, Figure 1) from this plant.

2. EXPERIMENTAL

2.1. General experimental procedures

The procedure and instruments used correspondingly to isolate compounds, measure optical rotation, and record IR, NMR, ESI-MS data collection, TLC and MPLC are similar to those described in a previous paper [11].

2.2. Plant material

The stem barks of *C. decandra* were collected at Ca Mau National Park, Ca Mau province, Viet Nam in May 2018, and taxonomically identified by Dr. Nguyen The Cuong (Institute of Ecology and Biological Resources, VAST). A voucher specimen (TĐPCCC-2018.01) was deposited at the Herbarium of Institute of Marine Biochemistry and Institute of Ecology and Biological Resources, VAST.

2.3. Extraction and isolation

The dried stem barks of *C. decandra* (2.0 kg) were cut into pieces and extracted with 95 % aqueous MeOH by percolation at room temperature to obtain 210 g of extract. The concentrated

95 % MeOH extract was suspended in H_2O and defatted with *n*-hexane and then was partitioned into an ethyl acetate-soluble fraction.

The EtOAc-soluble fraction (E, 20.5 g) was separated on silica gel MPLC (column: Biotage SNAP Cartridge, KP-SIL, 100 g) using a mobile phase of CH_2Cl_2-EtOAc (0 - 5 min 50 % EtOAc, 6-65 min 50 – 75 % EtOAc, 66 - 75 min 100 % EtOAc, 76 - 90 min 100 % MeOH, 15 mL/min, 90 min) to give twelve fractions (E-1 to E-12). Fractions E-7 (3.2 g) was further separated on a silica gel column, using CH_2Cl_2-acetone (40:1, v/v) as the mobile phase, to give five subfractions (E-7.1 to E-7.5). Subfraction E-7.5 (0.42 g) was chromatographed over an open ODS column eluted with acetone-H_2O (3:2, v/v) to yield three subfractions (E-7.5a to 7.5c). Subfraction E-7.5a was purified using preparative TLC with CH_2Cl_2-acetone (15:1, v/v), to give compound **2** (1.5 mg). Similarly, subfraction E-7.5b was chromatographed over a silica gel column with *n*-hexane-acetone (50:50 → 0:100) and *n*-hexane-EtOAc-MeOH (50:50:0.1 → 0:100:0) mixtures, and the resulting fraction was separated by a Sephadex LH-20 column using acetone-H_2O (1:1, v/v) to afford compound **1** (2.1 mg). Fraction E-11 (0.54 g) was subjected to a Sephadex LH-20 column eluted with MeOH to obtain three subfractions (E-11.1 to E-11.3). Compound **3** (2.5 mg) was purified from subfraction E-11.1 using silica gel CC eluting with *n*-hexane-CH_2Cl_2-acetone (2.5:1:1, v/v). Compound **4** (3.6 mg) was obtained by purifying subfraction E-12 on the YMC*GEL column and followed by separation on a Sephadex LH-20 column using a mixture of acetone-H_2O (1:1).

Tagalsine X (1): Pale yellow, amorphous powder; mp. 40 - 42 ^oC; $[\alpha]_D^{24}$+63.4 (*c* 0.2, MeOH); ESI-MS *m/z* 299 $[M + Na]^+$; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectroscopic data, see Table 1.

Tagalsin P (2): Colorless crystals; mp. 175 - 177 ^oC; $[\alpha]_D^{24}$ +50.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 288 (4.06) nm; ESI-MS *m/z* 315 $[M + Na]^+$; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectroscopic data, see Table 1.

***Ent*-5*α*,2-oxodolabr-3-ene-3,15,16-triol (3)**: White, needle-like solid; mp. 126 - 128 ^oC; $[\alpha]_D^{24}$ +28.9 (*c* 0.15, MeOH); ESI-MS *m/z* 337 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectroscopic data, see Table 1.

Pinoresinol (4): White, amorphous powder; mp. 120 - 121 ^oC; $[\alpha]_D^{24}$-16.6(*c* 0.05, MeOH); UV λ_{max}^{MeOH} (ε) 231 (14000), 280 (5800) nm; IR (KBr) ν_{max}: 3448, 1608, 1520, 1511, 1420, 1380, 1101, and 899 cm^{-1}; EI-MS *m/z* 341 $[M - H_2O + H]^+$; ^1H NMR (500 MHz, CD_3OD): δ_H 6.96 (2H, d, *J* = 1.5 Hz, H-2, H-2'), 6.79 (2H, d, *J* = 8.0 Hz, H-5, H-5'), 6.83 (2H, dd, *J* = 1.5, 8.0 Hz, H-6, H-6'), 4.72 (2H, d, *J* = 4.0 Hz, H-7, H-7'), 3.15 (2H, m, H-8, H-8'), 3.86 (2H, dd, *J* = 3.5, 9.0 Hz, H-9a, H-9'a), 4.24 (2H, dd, *J* = 7.5, 9.0 Hz, H-9b, H-9'b), 3.87 (6H, s, 3,3'-OC$\underline{H}_3$); ^{13}C NMR (125 MHz, CD_3OD): δ_C133.8 (C-1, C-1'), 111.0 (C-2, C-2'), 149.1 (C-3, C-3'), 147.3 (C-4, C-4'), 116.1 (C-5, C-5'), 120.1 (C-6, C-6'), 87.5 (C-7, C-7'), 55.4 (C-8, C-8'), 72.6 (C-9, C-9'), 56.5 (3,3'-O$\underline{C}H_3$).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a pale yellow, amorphous powder. Its molecular formula was found to be $C_{18}H_{28}O_2$ via the ^{13}C NMR spectroscopic data and a positive ESI-MS ion at *m/z* 299 $[M + Na]^+$. From this formula and its NMR data **1** was determined to have five degrees of unsaturation, two of which were due to a double bond and a ketone group. The ^1H NMR

spectroscopic data displayed resonances for three tertiary methyls [δ_H 1.31 (3H, s, H_3-17), 0.87 (3H, s, H_3-19), and 0.92 (3H, s, H_3-20)], a secondary methyl [δ_H 1.03 (3H, d, J = 6.5 Hz, H_3-18)], a pair of olefinic protons [δ_H 6.84 (1H, dd, J = 6.0, 10.5 Hz, H-1) and 6.12 (1H, d, J = 10.5 Hz, H-2)], a methine [δ_H 2.82 (1H, q, J = 6.5, 13.5 Hz, H-4)], along with a series of aliphatic protons [δ_H 1.26-1.96 ppm] (Table 1).

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for **1**–**3** (in $CDCl_3$).

Pos.	**1**		**2**		**3**	
	δ_C[a]	δ_H[b] (mult., *J* in Hz)	δ_C[a]	δ_H[b] (mult., *J* in Hz)	δ_C[a]	δ_H[b] (mult., *J* in Hz)
1	147.9	6.84 (dd, 6.0, 10.5)	33.5	2.72 (br d, 18.5) 2.85 (dd, 6.5, 18.5)	33.2	2.72 (br d, 18.5) 2.83 (dd, 6.5, 18.5)
2	130.2	6.12 (d, 10.5)	192.9	-	193.1	-
3	202.6	-	144.6	-	144.6	-
4	45.0	2.82 (q, 6.5, 13.5)	135.4	-	135.5	-
5	39.0	-	39.0	-	39.0	-
6	37.5	1.22 (m) 1.96 (m)	38.0	1.25 (m) 2.17 (dd, 3.0, 14.0)	38.0	1.26 (m) 2.16 (m)
7	25.3	1.21 (m)/1.33 (m)	26.6	1.19*	26.8	1.13 (m)/1.27 (m)
8	44.5	1.31 (m)	44.3	1.19 (m)	41.2	1.36 (m)
9	39.3	-	37.9	-	37.9	-
10	57.5	1.87 (br d, 6.0)	54.4	1.60 (br d, 6.0)	54.4	1.63 (dd, 2.0, 6.0)
11	37.5	1.26 (m) 1.72 (m)	36.4	0.97 (dt, 4.5, 13.5) 1.76 (ddd, 3.5, 4.5, 13.5)	33.8	1.06 (ddd, 3.5, 4.5, 13.5) 1.66 (m)
12	35.6	1.57 (m)/1.68 (m)	35.6	1.52 (m)/1.63 (m)	28.4	1.32 (m)/1.52 (m)
13	71.1	-	71.1	-	36.4	-
14	43.0	1.41 (d, 13.0) 1.52 (d, 13.0)	42.6	1.32 (m) 1.49 (m)	36.3	0.88 (m) 1.37 (m)
15	-	-	-	-	81.0	3.31 (br d, 9.0)
16	-	-	-	-	62.5	3.73 (br d, 10.0) 3.51 (dd, 9.0, 10.0)
17	27.0	1.31 (s)	26.9	1.26 (s)	19.1	0.93 (s)
18	7.9	1.03 (d, 6.5)	11.6	1.87 (s)	11.6	1.87 (s)
19	26.3	0.87 (s)	31.7	1.23 (s)	31.7	1.23 (s)
20	13.5	0.92 (s)	13.9	0.68 (s)	11.5	0.60 (s)
-OH						6.10 (s)

[a]125 MHz, [b]500MHz. *Overlapped signals assigned by HSQC and HMBC spectra without designating multiplicity.

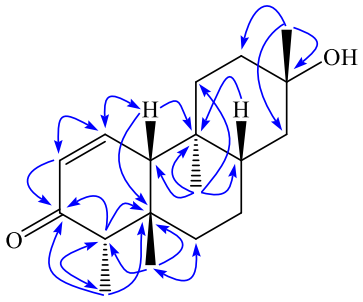

Figure 2. Key HMBC correlations of **1**.

Consistent with these observations, its ^{13}C NMR and HMQC spectrum denoted the presence of 18 resonances for the presence of four methyls, five sp^3 methylenes, five sp^3 methines, and five sp^2 quaternary carbons, of which the olefinic carbons [δ_C 147.9 (C-1) and 130.2 (C-2)] were attributed to a disubstituted double-bond and a carbon signal [δ_C 202.6 (C-3)] was assigned to a ketone group. Apart from a double-bond and a ketone group, the remaining elements of unsaturation were suggested to a tricyclic skeleton in the molecule of **1**. These spectroscopic data indicated that **1** was a dinordolabrane-type diterpenoid [12, 13]. On the other hand, the 6/6/6 tricyclic skeleton of the diterpenoid with 4,5,9,13-tetramet--hyl and 3-*α*,*β*-unsaturated ketone substitutions in the A-ring was established by 2D NMR experiments. This assignment was confirmed by the observation of HMBC correlations between δ_H 1.03 (H_3-18) to C-3 (δ_C 202.6), C-4 (δ_C 45.0), and C-5 (δ_C 39.0); between δ_H 0.87 (H_3-19) to C-4 (δ_C 45.0), C-5 (δ_C 39.0), C-6 (δ_C 37.5), and C-10 (δ_C 57.5); between δ_H 0.92 (H_3-20) to C-8 (δ_C 44.5) C-9 (δ_C 39.3), C-10(δ_C 57.5) and C-11 (δ_C 37.5); between δ_H 1.31 (H_3-17) to C-12 (δ_C 35.6), C-13 (δ_C 71.1), and C-14 (δ_C 43.0). A detailed 2D NMR spectral analysis, including HMQC and HMBC experiments, resulted in a gross structure of **1** (Figures 1-2).

The comparison of NMR spectroscopic data of **1** with reported literature found that they were similar suggesting that **1** was (4*S**,5*S**,8*S**,9*S**,10*R**)-13*S**-hydroxy-15,16-dinordolabr-1(2)-en-3-one and named tagalsine X (Table 1) [12]. From the above evidence, the structure of **1** was determined as tagalsine X. This compound was previously obtained from the leaves of *C. tagal* and had no cytotoxicity against CNE-2, A549, HepG2, and HCT-116 cell lines (IC_{50} > 50 μM), even with the concentration of 50 μM [12].

Compound **2** was isolated as a colorless crystal. Its molecular formula was determined to be $C_{18}H_{28}O_3$ based on a sodium adduct molecular ion peak at *m/z* 315 $[M + Na]^+$, consistent with five degrees of unsaturation. Analysis of the ^1H, ^{13}C NMR, and HSQC spectroscopic data of **2** displayed signals for all 18 carbons and 28 protons, including four tertiary methyls, six methylenes, two methines, and six non-protonated carbons. A detailed comparison of 1D and 2D NMR spectroscopic data showed that the structures of **2** and **1** [13] share the same B- and C-ring substitution patterns, with differences observed for the A-ring (Table 1). Further comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** showed that both compounds exhibit closely comparable data, except for the replacement of a disubstituted double-bond at C-2 in **1** by a methylene group [δ_H 2.72 (1H, br d, *J* = 18.5 Hz, H-1a), 2.85 (1H, dd, *J* = 6.5, 18.5 Hz, H-1b); δ_C 33.5 (C-1)] and a conjugated ketone group [δ_C 192.9 (C-2)] in **2**. In addition, *α*,*β*-unsaturated ketone group in **2** was a tetrasubstituted double-bond [δ_C 144.6 (C-3) and 135.4 (C-4)] with a hydroxygroup [δ_H 6.10 (1H, s], suggesting a 15,16-dinor-dolabrane containing a 3-hydroxy-4-methyl-2-enone cyclohexane moiety in its A-ring [13]. Furthermore, this was confirmed by the key HMBC correlations from δ_H 1.87 (H_3-18) to C-3 (δ_C 144.6), C-4 (δ_C 135.4), and C-5 (δ_C 39.0), as well as from δ_H 1.60 (H-10) to C-1 (δ_C 33.5), C-2 (δ_C 192.9), C-5 (δ_C 39.0), and C-9 (δ_C 37.9), respectively. NMR spectroscopic data of **2** were identical to those of tagalsin P[13]. Thus, the structure of compound **2** was determined as tagalsin P, named (5*S**,8*S**,9*S**,10*R**)-3,13*S**-dihydroxy-15,16-dinordolabr-3-en-2-one (Figure 1).

Compound **3** was obtained as a white, needle-like solid. The ESI-MS showed a protonated molecular ion peak at *m/z* 337 $[M + H]^+$, corresponding to a molecular formula of $C_{20}H_{32}O_4$, which is two carbons, four hydrogens, and one oxygen atom more than in **2**. The 1D NMR spectroscopic data of **3** were very similar to those of **2**, except for the presence of an additional dihydroxyethyl group [δ_H 3.31 (1H, br d, *J* = 9.0 Hz, H-15); δ_C 81.0 (C-15) and δ_H 3.51 (1H, dd, *J* = 9.0, 10.0 H-16a), 3.73 (1H, br d, *J* = 10.0 Hz, H-16b); δ_C 62.6 (C-16)] (Table 1). Furthermore, the location of the attached dihydroxyethyl group at C-13 was supported by a

downfield chemical shift of δ_C 36.4 (C-13) in the ^{13}C NMR spectra and a key HMBC correlation from δ_H 0.93 (s, H_3-17) to δ_C 81.0 (CH, C-15) of the dihydroxyethyl group. However, to date, the relative configuration at C-15 in **3** has been not yet reported. Comparisons of the NMR data of **3** with those of *ent*-5*α*,2-oxodolabr-3-ene-3,15,16-triol [14], as well as detailed analysis of HSQC and HMBC experiments led to identification of **3** as *ent*-5*α*,2-oxodolabr-3-ene-3,15,16-triol (Figure 1). Previously, compound **3** was obtained from the barks of *Endospermum diadenum* [14].

The remaining compound **4** was identified as pinoresinol based on our spectroscopic data and by comparison with those of reported data given in $CDCl_3$ [15]. This compound is widely distributed throughout the plants in Viet Nam, e.g, *Silybum marianum* [16], *Mallotus macrostachyus* [17], *Rhizophora stylosa* [18], *Trichosanthes kirilowii* [19], *Knema pachycarpa* [20], and *Balanophora laxiflora* [21].

4. CONCLUSIONS

In summary, we report here the isolation and structure elucidation of three dolabrane-type diterpenoids, agalsine X (**1**), tagalsin P (**2**), *ent*-5*α*,2-oxodolabr-3-ene-3,15,16-triol (**3**), and a lignan compound, pinoresinol (**4**) from a methanolic extract of *C. decandra* stem barks, using various chromatographic separations. The structures of these isolates were accomplished using comprehensive spectroscopic methods and comparison with those reported. The present work reports for the first time dolabrane-type diterpenoids study of this species distribution in Viet Nam. This work presents the discovery of dolabrane-type diterpenoid and lignan constituents and provides additional evidence to support mangrove plants as a promising source of chemical diversity.

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