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Abietane diterpenoids and neolignans from the roots of *Pinus kesiya*

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Abstract

The phytochemical investigation of the ethyl acetate extract of *Pinus kesiya* Royle ex Gordon roots led to the isolation of two abietane diterpenes, 7-oxo-15-hydroxy-dehydroabietic acid (**1**) and dehydroabietic acid (**2**) as well as two neolignans, cedrusin (**3**) and cedrusin-4-*O*-*β*-D-glucopyranoside (**4**). Their structures were determined by combination of spectral analysis and comparison with reported data. Among them, compound **1** was isolated from the genus *Pinus* for the first time.

Keywords. *Pinus kesiya*, abietane diterpenes, neolignans, dehydroabietic acid, cedrusin.

1. INTRODUCTION

Pinus is the largest genus in the pine family (Pinaceae), comprising 114 species [1]. There are eight *Pinus* species in Vietnam and *Pinus kesiya* Royle ex Gordon (local name known as *Thong ba la*, *Xa nu*, *Xa nui*, *Ngo* or *Tong thu*) mostly distributed among Langbiang plateau. This is a large tree, up to over 30 m tall with a bole free of branches for 15-20 m and up to 100 cm in diameter, with a thick, reticulately and deeply fissured bark [2]. In the folk medicine, the turpentine obtained from the resin of pine trees is antiseptic, diuretic. It is also a valuable remedy used internally in the treatment of kidney, bladder complaints, rheumatic affections, coughs, colds, influenza and tuberculosis [3]. So far, only one publication on the chemical composition of this species has been reported. From pinecone of *Pinus kesiya* R. collected in China, three diterpenoids named 15-hydroxydehydroabietic acid, 15-hydroxylabd- 8(17)-en-19-oic acid, junicedric acid and *β*-sitosterol, daucosterol have been isolated [4]. In our recent previous paper we described the isolation and structural elucidation of four phenolic compounds from the roots of *P. kesiya* [5]. In continuation of the study on this plant, two abietane diterpenoids and two lignans were isolated and identified from the same plant materials.

2. EXPERIMENTAL

2.1. General

NMR spectra were recorded on Bruker Avance 500 Ultrashield NMR Spectrometer. ESI-MS: Agilent LC-MSD-Trap SL. Optical rotation was measured with a JASCO P2000 Polarimeter at 25°C and D line of the sodium spectrum. TLC: Silica gel 60 F_{254} (0.25 mm, Merck); reversed phase RP_{18} F_{254S} (0.25 mm, Merck). CC: Silica gel 60 (230-400 mesh, Merck) for the first column, silica gel 60, 40-63 μm (Merck) and Sephadex LH-20 for the following columns.

2.2. Plant material

Roots of *P. kesiya* were collected in August 2015 in Dac Lac province of Vietnam and determined by Dr. Nguyen Tien Hiep, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST). A voucher specimen (VNMN.B000005007) is deposited at Vietnam National Museum of Nature, VAST.

2.3. Extraction and isolation

The dried roots of *P. kesiya* (2 kg) were ground, then extracted three times (each in 3 days) with 90 % MeOH. Evaporation of the solvent under reduced

pressure gave a crude methanolic extract (200 g). The extract was added to H_2O and partitioned in turn between *n*-hexane, EtOAc, *n*-butanol. The extracts were then concentrated under reduced pressure at 45 ^{0}C to give the corresponding residues. The EtOAc extract residue (19 g) was subjected to a silica gel column chromatography using a gradient mixture of CH_2Cl_2-MeOH-H_2O (100:5:0 to 25:10:1) as eluent to give seven fractions (F1-F7). Fraction F1 was purified on Sephadex LH-20 (MeOH) to give **2** (15 mg). Fraction F2 was chromatographed over silica gel column using the CH_2Cl_2-MeOH-H_2O mixtures (2:1:0 to 1:1:0.2) to yield five subfractions F2.1-F2.5. The subfraction F2.4 was purified on an RP_{18} column with MeOH:H_2O (2:3) as eluent to afford **1** (12 mg). Fraction F3 was subjected to CC over Sephadex LH-20 (MeOH) to give three subfractions (F.3.1-F.3.3). The subfraction F3.2 was rechromatographed on a silica gel column using CH_2Cl_2:EtOAc:MeOH (10:10:1) to yield **3** (15 mg). Fraction F6 was subjected to a Sephadex LH-20 column (MeOH) to afford four subfractions (F.6.1-F.6.4). The subfraction F6.1 was fractionated on RP_{18} column with MeOH:H_2O (2:3) as eluent, and then rechromatographed on a silica gel column with CH_2Cl_2-MeOH (5:1) to afford **4** (13 mg).

2.4. Spectral data of isolated compounds

7-Oxo-15-hydroxy-dehydroabietic acid (1): yellowish solid, mp. 119 ^{o}C; $[\alpha]^{25}_D$ +2.1 (*c* 0.5 in MeOH); ^{1}H- and ^{13}C-NMR, see table 1.

Dehydroabietic acid (2): yellowish solid, mp. 172 ^{o}C; ^{1}H- and ^{13}C-NMR, see table 1.

(+)-Cedrusin (3): white amorphous powder; $[\alpha]_D$ = +4.8 (*c* 0.6 in MeOH); ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 7.00 (1H, *d*, *J* = 1.5 Hz, H-2), 6.86 (1H, *dd*, *J* = 8.0 Hz, 1.5 Hz, H-6), 6.79 (1H, *d*, *J* = 8.5 Hz, H-5), 6.62 (1H, *s*, H-6′), 6.59 (1H, *s*, H-2′), 5.51 (1H, *d*, *J* = 6.0 Hz, H-7), 3.86 (1H, *dd*, *J* = 11.0 Hz and 5.0 Hz, H-9a), 3.80 (3H, *s*, 3-OCH_3), 3.58 (2H, *t*, *J* = 6.5 Hz, H-9′), 3.77 (1H, *dd*, 11.0 Hz and 7.5 Hz, H-9b), 3.47 (1H, *br q*, *J* = 6.0 Hz, H-8), 2.58 (2H, *t*, *J* = 7.5 Hz, H-7′), 1.81 (2H, *quintet*, *J* = 7.0 Hz, H-8′); ^{13}C-NMR (CD_3OD, 125 MHz): δ_C 135.1 (C-1), 110.6 (C-2), 149.0 (C-3), 147.3 (C-4), 116.1 (C-5), 119.7 (C-6), 88.7 (C-7), 55.7 (C-8), 65.1 (C-9), 136.7 (C-1′), 117.0 (C-2′), 141.8 (C-3′), 146.6 (C-4′), 129.8 (C-5′), 116.7 (C-6′), 32.7 (C-7′), 35.8 (C-8′), 62.3 (C-9′), 56.4 (3-OCH_3).

Cedrusin-4-*O*-*β*-D-glucopyranoside (4): white amorphous powder; ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 7.16 (1H, *d*, *J* = 8.5 Hz, H-5), 7.09 (1H, *d*, *J* = 1.5 Hz, H-2), 6.98 (1H, *dd*, *J* = 8.5 Hz, 1.5 Hz, H-6), 6.61 (1H, *s*, H-6′), 6.60 (1H, *s*, H-2′), 5.57 (1H, *d*, *J* = 5.5 Hz, H-7), 3.85 (3H, *s*, 3-OCH_3), 3.57 (2H, *t*, *J* = 6.5 Hz, H-9′), 2.58 (2H, *t*, *J* = 7.5 Hz, H-7′), 1.81 (2H, *quintet*, *J* = 6.5 Hz, H-8′); glucose: 4.98 (1H, *d*, *J* = 7.0 Hz, glc H-1), 3.36-3.83 (glc H-2 - H-6); ^{13}C-NMR (CD_3OD, 125 MHz): δ_C 138.7 (C-1), 111.1 (C-2), 150.8 (C-3), 147.4 (C-4), 118.0 (C-5), 119.3 (C-6), 88.1 (C-7), 55.9 (C-8), 62.5 (C-9), 136.9 (C-1′), 117.1 (C-2′), 142.0 (C-3′), 146.4 (C-4′), 129.4 (C-5′), 116.6 (C-6′), 32.7 (C-7′), 35.7 (C-8′), 62.3 (C-9′), 56.7 (3-OCH_3); glucose: 102.8 (glc C-1), 74.9 (glc C-2), 77.8 (glc C-3), 71.3 (glc C-4), 78.1 (glc C-5), 65.2 (glc C-6).

3. RESULTS AND DISCUSSION

Compound **1** was assigned a molecular formula of $C_{20}H_{26}O_4$ by combination of its NMR data and ESI-MS pseudo-molecular ion peak at *m*/z 331.2 $[M+H]^+$. The ^{1}H-NMR spectrum showed signals for four quaternary methyl groups [δ_H 1.28 (3H, *s*, H-20), 1.31 (3H, *s*, H-19) and 1.54 (6H, s, H-16 and H-17)], a 1,2,4-trisubstituted benzene ring at δ_H 8.07 (1H, *d*, 2.0 Hz, H-14), 7.73 (1H, *dd*, 8.5 Hz and 2.5 Hz, H-12) and 7.44 (1H, *d*, 8.5 Hz, H-11). The ^{13}C-NMR, DEPT and HSQC spectra indicated 20 carbons attributable to four methyls, four methylenes, four methines (including three aromatic protons) and eight quaternary carbons (containing two carbonyl groups). These spectroscopic data indicated **1** is an abietane diterpenoid. Besides, the sp^3quaternary carbon with a chemical shift at δ_C 72.6 helped to infer that **1** have a hydroxyl group at C-15. The position of each hydrogen to corresponding carbon in **1** were assigned by cross-peaks in the HSQC spectrum. The HMBC spectrum showed the long-range correlations between H-5 and C-19, C-20, C-10, C-6 and C-7; between H-11 and C-13, C-8 and C-10; between H-12 and C-14, C-9 and C-15; H-14 and C-7, C-9 and C-12; between H-16 and H-17 and C-13. Comparison of the NMR spectral data of **1** (Table 1) with those of 7-oxo-15-hydroxy-dehydroabietic acid [6], indicated that they were the same compound.

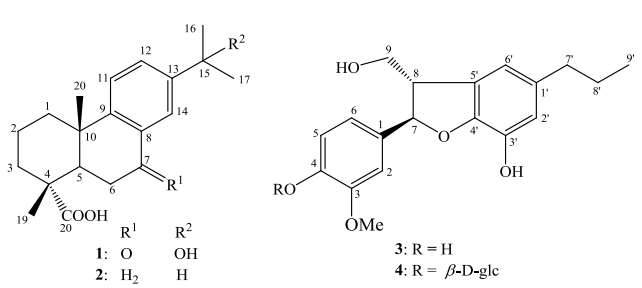

Figure 1: Structures of compounds **1-4** from the roots of *Pinus kesiya*

Compound **2** was assigned the molecular

formula $C_{20}H_{28}O_2$ by NMR data and ESI-MS with a pseudo-molecular ion peak at *m*/z 301.4 $[M+H]^+$ in ESI-MS spectrum. The ^{13}C-NMR and HSQC spectra showed the presence of four methyls, five methylenes, five methines (including three aromatic protons) and six quaternary carbons (containing a carbonyl group). The NMR data (table 1) indicated a structure closely similar to **1**. Nevertheless, there were some significant differences in the chemical shifts between **2** and **1**. Differences in the ^{1}H and ^{13}C NMR spectra were noted at C-7 and C-15. First, at C-7 of **2** a methylene group (δ_H 2.87 and δ_C 31.2) was present, instead of a carbonyl group (δ_C 202.1) in **1**. Second, the C-15 signals of **2** showed a methine group (δ_H 2.80, septet, J = 7.0 Hz and δ_C 34.8), while this signal indicated an oxygenated quaternary carbon (δ_C 72.6) in **1**. The above spectroscopic data resemble those of dehydroabietic acid [7, 8]. So, **2** was determined to be dehydroabietic acid, which is common in the resins.

Table 1: ^{13}C- and ^{1}H-NMR data of compounds **1** and **2** (125/500 MHz, CD_3OD, δ ppm)

C	1			2		
	DEPT	δC	δH (*J*, Hz)	DEPT	δC	δH (*J*, Hz)
1	CH_2	38.7	2.41, *d* (12.0) 1.62, *m*	CH_2	39.5	2.35, *br d* (12.5) 1.45, *m*
2	CH_2	19.9	1.87, *m* 1.75, *m*	CH_2	19.7	1.84, *m* 1.72, *m*
3	CH_2	38.5	1.66, *m*	CH_2	38.1	1.82, *m* 1.71, *m*
4	C	46.0	-	C	47.4*	-
5	CH	45.8*	2.79, *dd* (14.0, 2.0)	CH	46.6	2.18, *dd*, (12.5, 2.0)
6	CH_2	39.2	2.73, *dd* (16.5, 14.0) 2.52, *dd* (17.0, 1.5)	CH_2	39.7	1.87, *m* 1.17, *m*
7	C=O	202.1	-	CH_2	31.2	2.87, *m*
8	C	131.7	-	C	135.7	-
9	C	156.4	-	C	146.9	-
10	C	38.8	-	C	35.6	-
11	CH	124.9	7.44, *brd* (8.5)	CH	125.2	7.16, *d* (8.0)
12	CH	132.0	7.73, *dd* (8.5, 2.5)	CH	124.9	6.97, br *d* (8.5)
13	C	148.9	-	C	145.7	-
14	CH	124.0	8.07, *d* (2.0)	CH	127.8	6.86, *br s*
15	C	72.6	-	CH	34.8	2.80, septet (7.0)
16	CH_3	31.7	1.54, *brs*	CH_3	24.8	1.22, *d* (7.0)
17	CH_3	31.7	1.54, *brs*	CH_3	24.8	1.22, *d* (7.0)
18	C=O	186.0*	-	C=O	183.0*	-
19	CH_3	17.9	1.31, *brs*	CH_3	17.1	1.28, *brs*
20	CH_3	23.9	1.28, *brs*	CH_3	25.5	1.22, *brs*

The ESI-MS spectrum of **3** showed a pseudo-molecular ion peak at *m/z* 347.2 $[M+H]^+$, corresponding to the molecular formula of $C_{19}H_{22}O_6$. The ^{1}H-NMR spectrum exhibited the attendance of five aromatic protons [δ_H 7.00 (1H, *d*, 1.5 Hz, H-2), 6.86 (1H, *dd*, 8.0 Hz and 1.5 Hz, H-6), 6.79 (1H, *d*, 8.5 Hz, H-5), 6.62 (1H, *s*, H-6′) and 6.59 (1H, *s*, H-2′)], two methines [δ_H 5.51 (1H, *d*, 6.0 Hz, H-7) and 3.47 (1H, *br q*, 6.0 Hz, H-8)], a hydroxymethyl [δ_H 3.77 (1H, *dd*, 11.0 Hz and 7.5 Hz, H-9) and 3.86 (1H, *dd*, 11.0 Hz and 5.0 Hz, H-9) and a methoxy group [δ_H 3.80 (3H, *s*, 3-OCH_3)]. Other signals at δ_H 3.58 (2H, *t*, 6.5 Hz, H-9′), 1.81 (2H, *quintet*, J = 7.0 Hz, H-8′) and 2.58 (2H, *t*, J = 7.5 Hz, H-7′) indicated the presence of an *n*-propanol side-chain. In the ^{13}C-NMR and HSQC spectra, along with the methoxycarbon, 18 skeletal carbons were appeared. These spectral characteristics showed that **3** was a dihydro[*b*]benzofuran neolignan. In addition, these spectroscopic data were similar to those of cedrusin from *Cedrus deodara* [9]. Accordingly, compound **3** was confirmed as (+)-cedrusin.

The NMR spectral data of **4** were similar to those of **3** except the additional signals of a glucose moiety (δ_H 3.36-4.98 and δ_C 65.2-102.8 in the HSQC spectrum). The *β*-configuration of glucose was confirmed by the coupling constant of the anomeric proton (δ_H 4.98, *d*, J = 7.0 Hz). By means of the

analysis of NMR spectra and comparing with the data in reference [9], the structure of **4** was deduced as cedrusin-4-*O*-*β*-D-glucopyranoside.

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