VIETNAM JOURNAL OF CHEMISTRY 54(2) 209-212 APRIL 2016
DOI: 10.15625/0866-7144.2016-00262

THE NON-ALKALOIDAL CONSTITUENTS OF *CEPHALOTAXUS MANNII*, COLLECTED IN LAM DONG PROVINCE

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Received 10 March 2016; Accepted for publication 25 April 2016

Abstract

Three non-alkaloidal constituents: harringtonolide (nor-diterpene lactone), epicatechin and epigallocatechin have been isolated from *Cephalotaxus mannii*, collected in Lam Dong province, Vietnam in the frame work of the Tay Nguyen program III (TN III-15). Their structures were elucidated by the spectroscopic methods as the IR, ESI-MS, HR ESI-MS and NMR (1D, 2D) spectroscopy and comparison with published data. This is the first report of these compounds from *Cephalotaxus mannii.*

Keywords. *Cephalotaxus mannii*, harringtonolide, epicatechin, epigallocatechin.

1. INTRODUCTION

Cephalotaxus mannii Hook. f. is a big tree, belonging to the family Cephalotaxaceae. This rare and endangered plant is growing in China, India and South- East Asia including Vietnam. In Vietnam *C. mannii* (Đỉnh Tùng) is distributed in the Middle and the Highland (Tây Nguyên) in the altitude of 600-2000 m. This plant is used for treatment of tumours in Vietnamese folk medicine [1, 2]. In the family Cephalotaxaceae, there are few reports on the chemical studies of *C. mannii*. Richard G. Powell et al. have reported the isolation of cephalomannine, a new anti-tumour alkaloid with a taxane skeleton, which is structurally unrelated to the harringtonine series of alkaloids characteristic of most *Cephalotaxus* species [3]. Xuan Lu et al. isolated six aromatic compounds from endophytic fungus *Colletotrichum sp*. L10 from *C. hainaniensis* Li [*C. mannii* Hook.f.] which is an indigenous tree to Hainan and Guangxi provinces of China and has been used in Chinese folk medicine as anti-cancer agents [4]. Heng Xue et al. isolated eleven compounds belonging to eight structure types from *Aspergillus* sp. CM9a, an endophytic fungus of *C. mannii*; three among them are new compounds [5, 6]. Besides these chemical studies, there were some studies to enhance the cephalotaxine alkaloid production in *Cephalotaxus mannii* suspension cultures [7, 8].

2. EXPERIMENTAL

2.1. Methods

The IR spectra were recorded on an IMPACT 410 Nicolet machine (KBr), ESI-MS and HR-ESI-MS spectra were measured on a 100 Agilent LC/MS ion Trap and a FT-ICR-MS Varian 7 Tesla, respectively. NMR spectra (^{1}H, ^{13}C, DEPT, HSQC, HMBC) were measured on a Bruker Avance 500 MHz with TMS as internal standard. TLC was carried out on plates precoated with silica gel F254 (Merck). Column chromatography was performed on silica gel 300-400 mesh (Merck).

2.2. Plant material

The leaves, twigs and barks of *C. mannii* Hook. f. were collected in August 2012 in Lam Dong province. A voucher specimen (no. CPC 4718) was identified by Dr. Nguyen Tien Hiep, Vietnam Museum of Nature and is deposited in the Institute of Chemistry, VAST, 18 Hoang Quoc Viet, Cau Giay district, Hanoi, Vietnam.

2.3. Extraction and isolation of compounds

The ground dried bark of *C. mannii* (500 g) was extracted with 80 % MeOH three times (3x3 L) at 65 ^{o}C for 10 hours. The filtrate was concentrated under reduced pressure to yield a residue (80 g), which was suspended with H_2O (0.5 L), acidified with 3 % citric acid and partitioned by EtOAc (3x1 L) to give a residue (30 gram). The aqueous phase was then neutralized with NH_3 to pH ≈ 9 and extracted with dichloromethane (3x1 L), evaporated to produce a residue (7.9 g).

The EtOAc soluble portion (30 g) was subjected to column chromatography over silica gel eluted with a gradient mixture of methanol in dichloromethane (from 5 to 30 % in volume) to yield five major fractions: F1 (2.5 g), F2 (4.2 g), F3 (4.8 g), F4 (3.6 g) and F5 (8.2 g). Fraction F3 was re-purified on silica gel column chromatography eluted with CH_2Cl_2/MeOH (9/1→3/1) to yield compounds **1** (50 mg) and **2** (48 mg). Fraction F5 was first subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 9/1→3/1), followed by reverse phase RP-18 column (MeOH/H_2O, 2/1) to afford compound **3** (250 mg).

*2.3.1. Epicatechin (**1**)*

^{1}H-NMR (CD_3OD, δ ppm): 2.76 (1H, dd, 2.1, 16.8 Hz, H-4a), 2.90 (1H, dd, 3.5, 16.8 Hz, H-4b), 4.18 (1H, brs, H-3β), 4.82 (1H, s, H-2β), 5.96 (1H, s, H-6), 5.98 (1H, s, H-8), 6.79 (1H, d, 7.6 Hz, H-5'), 6.82 (1H, d, 7.6 Hz, H-6'), 7.01 (1H, s, H-1').

*2.3.2. Epigallocatechin (**2**)*

^{1}H-NMR (CD_3OD, δ ppm): 2.75 (1H, dd, 2.8, 16.7 Hz, H-4a), 2.87 (1H, dd, 4.6, 16.7 Hz, H-4b), 4.19 (1H, brs, H-3), 4.77 (1H, s, H-2), 5.93 (1H, d, 2.2 Hz, H-6), 5.96 (1H, d, 2.2 Hz, H-8), 6.54 (2H, s, H-1', H-6').

*2.3.3. Harringtonolide (**3**)*

FTIR (KBr, cm^{-1}): 2965, 2830, 1748, 1636, 1572, 1533, 1445, 1076, 1010. ESI-MS *m/z*: 311.06 (100) $[M+1]^+$, HR-ESI-MS *m/z*: 311.12835 $[M+1]^+$ (calculated for $C_{19}H_{19}O_4$ 311.12779).
^{1}H- and ^{13}C-NMR, see table 1.

3. RESULTS AND DISCUSSION

The ^{1}H-NMR spectrum of **1** indicated the presence of a flavonol derivative with the signals of five aromatic protons, two oxymethines and two protons of a methylene group. Important fact for the structure elucidation of compound **1** is the appearance of two one-proton singlets at 4.18 and 4.82 ppm, which is characteristic for a 2,3-*cis*-configuration of a flavonol derivative. By comparison of **1** on TLC with an authentic sample of epicatechin from our lab, compound **1** showed very good identity. The ^{1}H-NMR spectral data of **1** was also identical with the published data for epicatechin [9]. So **1** is epicatechin.

The ^{1}H-NMR spectrum of compound **2** was similar to the spectrum of compound **1** indicating, that **2** is a derivative of **1**.

The ^{1}H-NMR spectrum of **2** showed one aromatic proton less than that of **1**. The singlet of two protons at δ 6.54 ppm indicated a symmetry of the β-ring of compound **2**. By comparison of ^{1}H-NMR spectrum of compound **2** with that of epigallocatechin [10] it can be concluded that **2** is epigalloctechin.

The FTIR spectrum of compound **3** showed a strong absorption bands at 1748 and 1076 cm^{-1} (lactone) as well as 1626 and 1572 cm^{-1} (aromatic -C=C-)

The ^{13}C-NMR spectrum of **3** exhibited signals for 19 carbon atoms, which indicated of a norditerpene derivative. Among the carbon atom signals there were two carbonyls at δ 173.4 ppm (lactone or ester) and 186.33 ppm (conjugated carbonyl), and six olefinic carbons at 139.10, 141.44 ppm (both CH), 143.47, 144.92, 145.58, 145.78 ppm (all quaternary carbons).

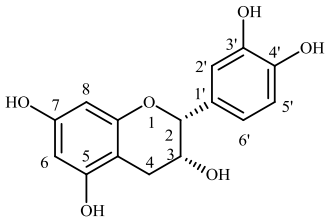
The ^{1}H-NMR spectrum of **3** contained two methyl groups [δ 0.88 ppm (d, 7.6 Hz), 2.37 ppm (s)], three oxymethine [3.98 ppm (d, 5.6 Hz), 5.19-5.21 ppm (m), 5.35-5.36 ppm (m)] and two aromatic protons [6.87 ppm (t-like, 1.8 Hz), 6.95 (brs)].

The ESI-MS spectrum of **3** exhibited a pseudo-molecular ion peak at *m/z* 311.06 (100) $[M+H]^+$. Its HR-ESI-MS spectrum showed a base peak at *m/z* 311.12835 (calculated for $C_{19}H_{19}O_4$ is 311.12779). Thus, the formula of compound **3** is $C_{19}H_{18}O_4$. The ^{1}H and ^{13}C-NMR spectral data of **3** are totally identical with the published data for harringtonolide [11, 12].

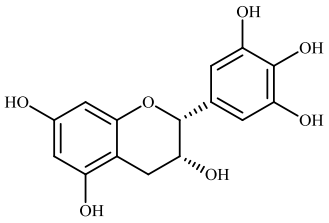
Harringtonolide was isolated and structurally elucidated first time in 1978 from the seed of *Cephalotaxus harringtonia* var. *drupacea* [11]. Its absolute configuration was determined later by the X-ray anomalous scattering of its bromination product [12]. Harringtonolide showed the plant growth inhibitory and strong cytotoxic and antifungal activities. Its IC_{50} value on KB cells is 0.043 µM, being more active than 5-fluorouracil (IC_{50} = 0.47 µM) and harringtonine (IC_{50} = 0.071

μM) [12].

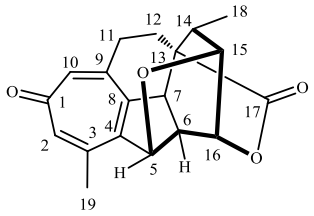
Conclusion: Epicatechin, epigallocatechin and harringtonolide were isolated and determined from *Cephalotaxus mannii* for the first time. The very strong cytotoxic activity of harringtonolide against the cancer cell lines: KB, HT29, 3T3EF [12] suggested a further investigation on *Cephalotaxus mannii* and harringtonolide.



1: Epicatechin



2: Epigallocatechin



3: Harringtonolide

Table 1: ^{1}H and ^{13}C-NMR spectral data of **3*** and harringtonolide

Position	Compound **3** ($CDCl_3$)		Harringtonolide [11] $CDCl_3$	
	δ_C	δ_H	δ_C	δ_H
1	186.332		186.39	
2	139.10	6.87 (t-like, 1.8 Hz)	139.14	6.92 (s)
3	143.47		143.58	
4	144.92		145.02	
5	79.91	5.35-5.36 (m)	79.95	5.47 (m)
6	41.70	1.28 (m)	41.73	1.25 (m)
7	49.85	2.65 (m)	49.88	2.70 (m)
8	145.58		145.65	
9	145.78		145.86	
10	141.44	6.95 (brs)	141.50	6.98 (s)
11	32.24	3.41 (m)	32.28	3.51 (m)
12	22.31	2.81 (m)	22.33	2.70 (m)
13	45.72		43.75	
14	39.92	1.75 (q,7.6 Hz)	39.95	1.75 (q)
15	79.62	5.19 (m)	79.95	5.32 (m)
16	85.96	3.98 (d,5.6 Hz)	85.49	4.00 (m)
17	173.40		173.46	
18	14.65	0.88 (d,7.6 Hz)	14.70	0.90 (d)
19	23.76	2.37 (s)	23.84	2.36 (s)

*The assignments were made based on the analysis of the DEPT, HSQC and HMBC spectra.

Acknowledgements. *This work has been financially supported by the Tay Nguyen III program, project TN-15. We thank M.Sc. Dang Vu Luong for the NMR, M.Sc. Ngo Van Quang for the MS measurement and Dr. Nguyen Tien Hiep for the collection and identification of C. mannii.*

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