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Chemical composition of the essential oil from *Cunninghamia konishii* Hayata growing wild in Vietnam.

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Abstract

The chemical composition of one oil sample of *Cunninghamia konishii* Hayata isolated from wood harvested in Hà Giang province (Vietnam), was investigated by chromatographic (GC with retention indices) and spectroscopic techniques (MS, ^{13}C NMR). Forty six compounds accounting for 88.4% of the oil have been identified. *C. konishii* wood produced sesquiterpene-rich essential oil, whose composition was dominated by α -cedrene (18.4%), cedrol (11.2%) and γ -eudesmol (8.8%). α -Terpineol (9.6%) was the major monoterpene. ^{13}C NMR appeared really useful for the identification of β -funebrene which co-eluted with α -cedrene and sesquithuriferol which co-eluted with cedrol.

Keywords: *Cunninghamia konishii*, wood oil, chemical composition, ^{13}C NMR, Vietnam.

1. Introduction

Cunninghamia genus (Cupressaceae family) comprises two species native from East Asia (Taiwan and China): *Cunninghamia lanceolata* (Lamb.) and *Cunninghamia konishii* Hayata [syn. *Cunninghamia lanceolata* (Lamb.) Hook. var. *konishii* (Hay.) Fujita; *Cunninghamia kawakamii* Hayata].

Cunninghamia konishii is an endemic species to Taiwan, growing at altitudes from 1200 m to 2700 m. [1] recently, this species was discovered in the North of Laos. In Vietnam, specimens have been observed in Hà Giang, Thanh Hóa, Sơn La and Nghệ An provinces, in dense subtropical forest, mixed with *Fokienia hodginsii*, *Dacrydium pierrei*, and *Quercus bambusaefolia* [2]. In Vietnam, its vernacular name is Sa mộc dẫu.

C. konishii is a big tree, reaching 50 m height and 1.25 m in diameter with conical, dark green crown. Brown-red bark splits into thin scales. Leaves, 2-3 cm long and 0.5 cm wide, are densely concentrated at branch-tips. Male cones numerous, terminal, are enclosed by many green scales at the base. Female cones, 2-2.5 cm by 1.3 cm, globose or ovate, terminal, first green, later brown-reddish, consist of many scale-shaped carpels. Each carpel bears three winged seeds. This species differs from *C. lanceolata* by shorter leaves (2-3 cm), obtuse apex and smaller female cone (less than 3 cm in diameter).

C. konishii is considered as one of the five "precious" conifers in Taiwan. Its wood is used in the construction of houses. It is resistant to rot and termites. This species is considered as endangered due to over-exploitation and possible hybridization with *C. lanceolata*, introduced in plantations.

Phytochemicals belonging to various families of compounds have been isolated from solvent extracts of wood of *C. konishii*, including new compounds whose structures have been elucidated: various sesquiterpenes [3-5] such as konishiol, cadalenol, 3-cedranol, T-cadinol, cedrol, T-muurolol, and *epi*-cedrol among which cedrol, T-cadinol and T-muurolol may be considered as new natural antifungal agents [6]; numerous diterpenes bearing mostly the abietane or labdane skeleton [5-11]; sterols such as β -sitosterol and stigmasterol [3, 4], and various lignans [1, 5].

In comparison, only a few studies concerned the composition of essential oils. According to Cheng *et al.* [12], a pioneering work has been done as early as 1929 by Ikeda and Fujita who identified various monoterpenes and cedrol in wood oil of *C. konishii*. Recently, these

authors [12, 13] reported a composition dominated by cedrol (53.0%) and α -pinene (25.6%); α -cedrene (6.6%) and α -terpineol (4.4%) being present at substantial amounts. Wood oil of *C. konishii* and cedrol are effective as antifungal agents. Other results revealed that the same wood oil sample from *C. konishii* and its effective constituents served as a potential larvicide against the dengue mosquitoes. [13] A more or less similar composition has been reported for EO isolated from heart wood: cedrol (58.3%), α -cedrene (11.8%), α -terpineol (4.2%) and β -cedrene (3.5%) [14]. Antimicrobial activity and cytotoxic activity on human cancer cells have been evidenced. The active compound was determined to be cedrol. In contrast, monoterpenes were the main components of leaf oils. Indeed, α -pinene (36.4%) and α -thujene (11.4%) dominated the composition of Taiwanese leaf oil, accompanied by sesquiterpenes, α -eudesmol (8.1%), β -elemol (5.8%), β -elemene (3.5%), γ -eudesmol (2.8%) and γ -himachalene (2.7%). [15] In a second sample of Taiwanese origin, α -pinene (34.9%), *p*-cymene (16.7%) and γ -muurolene (11.4%) were the main components. [12] This leaf oil sample exhibited antifungal activities.

Finally, two studies concerned Vietnamese *C. konishii* wood oil. The composition of two oil samples isolated from plants harvested in Son La and Nghê An provinces, respectively, was dominated by cedrol (30.0% and 37.0%) while α -cedrene accounted for 4.5% and 5.3%, respectively. The two samples contained also a major component named β -penchyl alcohol (synonym, β -fenchyl alcohol, 16.1% and 27.5%, respectively) [16]. The third oil sample isolated from wood of *C. konishii* collected in Tay Con Linh Nature Reserve, Hà Giang province, differed drastically from the two former samples by the occurrence of α -terpineol (36.6%) and α -cedrol (29.8%) as major components and it was reported as a new chemotype. In contrast, β -fenchyl alcohol was not detected [17].

In continuation of our on-going work on the characterization of essential oil bearing plants from Vietnam, we report in the present study on the chemical composition of wood oil of *C. konishii*. Indeed, since previous works demonstrated that wood oil from Son La and Nghê An provinces exhibited similar composition which differed drastically from that of wood oil from Hà Giang province, we investigated the composition of wood oil from Thanh Hóa province, the fourth location where *C. konishii* grows wild in Vietnam. In order to avoid any misidentification of individual components which exhibit insufficiently resolved mass spectra (diastereoisomers for instance) or very close retention indices, qualitative and quantitative analysis of the oil sample has been carried out by combination of chromatographic (GC with retention indices) and spectroscopic techniques (MS, ^{13}C NMR).

2. Materials and methods

2.1 Plant Material and Essential Oil Production

Wood of *Cunninghamia konishii* was collected in April 2012 in Xuan Lien Nature Reserve, Thanh Hóa province (Vietnam). Wood (550g) was submitted to hydrodistillation in a Clevenger-type apparatus (4 h) and yielded 2.65 mL of EO. Plant material has been authenticated by one of the authors, Dr Nguyen Thi Ha, Vietnam National University. Voucher specimen has been deposited at the herbarium of IERB under the accession Thai N° 06 (HN).

2.2 Analytical GC

GC analysis was carried out using a Clarus 500 Perkin Elmer apparatus equipped with FID and two fused-silica capillary columns (50 m x 0.22 mm, film thickness 0.25 μm), BP-1 (polymethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min; injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: helium (0.8 mL/min); split: 1/60. The relative proportions of the oil constituents were expressed as percentage obtained by peak area normalization, without using correction factors. Retention indices (RI) were determined relative to the retention times of a series of *n*-alkanes with linear interpolation («Target Compounds» software from Perkin Elmer).

2.3 GC-MS Analysis

GC-MS analyses were carried out using a Agilent Technologie 7890A detector (quadrupole), directly coupled to a Agilent Technologie 5975C, equipped with a fused-silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm), HP-MS 5% phenylmethylsiloxane. Carrier gas, helium at 1 mL/min; split, 1:80; injection volume, 0.2 μL . The injection port was set at 250 °C; the oven temperature was programmed from 60 °C to 250 °C at 4 °C/min (52.5 min). Significant quadrupole MS operating parameters: Ion source temperature, 150 °C; electron impact ionization at 70 eV with scan mass range of 33-350 *m/z*.

2.4 ^{13}C NMR Analysis

NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.63 MHz for ^{13}C NMR, equipped with a 5 mm probe, in CDCl_3 , with all shifts referred to internal TMS. ^{13}C NMR spectra of the oil samples were recorded with the following parameters: pulse width = 4 μs (flip angle 45°); acquisition time = 2.7 s for 128K data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 3000 (50 mg of oil in 0.5 mL of CDCl_3).

2.5 Identification of Components

Identification of the individual components was based: (i) by comparison of their GC retention indices (RIs) on polar and apolar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation with those of reference compounds (ii) on computer search using digital libraries of mass spectral data and comparison with literature [18-23], (iii) on comparison of the signals in the ^{13}C NMR spectra of essential oils with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software. In the investigated samples individual components were identified by NMR at content as low as 0.3-0.4% [24-27].

3. Results & Discussion

Subjected to hydrodistillation, wood of *Cunninghamia konishii* furnished a pale yellow oil with a yield of 0.48% (v/w). The wood oil sample was analyzed by combination of chromatographic (GC/retention indices) and spectroscopic techniques, GC-MS and ^{13}C NMR following a computerized

method developed in our laboratory [24-27]. Forty six compounds, accounting for 88.4% of the whole composition have been identified (Table 1). All the main components have been identified by the three techniques. Most minor components have been identified by GC(RI) and GC-MS. A few minor components that co-eluted on the capillary column used to perform GC-MS analysis, have been identified by their RIs and by ^{13}C NMR.

The investigated sample of *C. konishii* wood oil may be considered quantitatively as a sesquiterpene-rich essential oil. Indeed, the identified monoterpene hydrocarbons accounted for only 3.3% of the whole composition. Oxygenated monoterpenes were present at higher content, 21.3%, with α -terpineol (9.6%) and borneol (4.4%) as main monoterpenes. Sixteen sesquiterpene hydrocarbons have been identified, identification of most of them being ensured by ^{13}C NMR. α -Cedrene (18.4%) was the major component of the oil sample, and it was accompanied by β -cedrene (5.2%) and their diastereoisomers α -funebrene (also known as 1,7-di-*epi*- α -cedrene, 1.4%) and β -funebrene (2.0%). Identification of cedrenes and funebrenes simultaneously present in an oil sample highlight perfectly the interest of using complementary analytical techniques (namely, SM and ^{13}C NMR) to perform the identification of individual components of sesquiterpene-rich essential oils. Indeed, cedrene and funebrene isomers differ only by the stereochemistry of the ring junction in the polycyclic tricyclo[5.3.1.0^{1,5}]undecane framework. They have super imposable mass spectra and their retention indices differ by a few units only [24]. For instance, α -funebrene was suggested by MS as α -cedrene. In parallel, β -funebrene was co-eluted with α -cedrene on the apolar capillary column used to perform GC-MS analysis. Therefore, its identification by MS was hazardous. Only six oxygenated sesquiterpenes have been identified. However, they accounted for 26.2% of the whole composition. Cedrol (11.2%) and γ -eudesmol (8.8%) were the main sesquiterpenols. Once again, it could be pointed

out that sesquithuriferol that co-eluted with cedrol on the capillary column used to carry out GC-MS experiment, was identified by ^{13}C NMR. Finally, ^{13}C NMR confirmed that β -elemene is a secondary metabolite present in the EO and not an artifact coming from the thermal rearrangement of germacrene A [28]. *C. konishii* wood oil contained also a phenylpropanoid, namely safrole (0.7%) whose identification was ensured by the observation of characteristic signals (particularly those of the protonated carbons of the aromatic ring) in the ^{13}C NMR spectrum.

Comparison of the chemical composition of the investigated sample in the present work with those reported in the literature suggested some comments:

- Cedrol is much more abundant in the Taiwanese oil samples (53.0 and 58.3%) than in the Vietnamese ones (29.8-37.0% in published samples and 11.2% in the present work). In contrast, the content of α -cedrene is higher in our sample (18.4%) than in Taiwanese samples (6.6 and 11.8%) and in other Vietnamese ones (3.4-5.3%).
- Camphor and borneol were present at appreciable contents in all the samples;
- α -Terpineol was the main component of a Vietnamese oil sample (36.6%, Hà Giang province);
- α -Pinene was abundant (25.6%) only in one Taiwanese wood oil sample although it was the major component of leaf oil samples;
- Fenchol was one of the major components of two Vietnamese oil samples (16.1 and 27.5%) while its content was very low in all other investigated samples (0.1-1.8%);
- The three eudesmol isomers accounted for 2.8-8.8% in the present oil sample. They were much more abundant than in all the other samples (0.0-1.9%);
- Safrole, β -funebrene, α -muurolene, β -bisabolene, β -elemol and sesquithuriferol were reported for the first time as components of *C. konishii* wood oil.

Table 1: Components of Vietnamese *Cunninghamia konishii* wood oil

N°	Components ^a	RI lit ^b	RIa ^c	RIp ^c	%	Identification
1	α -Thujene	932	924	1028	0.5	RI, MS
2	α -Pinene	936	932	1026	0.8	RI, MS, ^{13}C NMR
3	α -Fenchene	941	943	1062	0.1	RI, MS
4	Camphene	950	945	1072	0.3	RI, MS
5	Sabinene	973	966	1125	0.5	RI, MS, ^{13}C NMR
6	β -Pinene	978	972	1114	0.1	RI, MS
7	Myrcene	987	982	1163	0.1	RI, MS
8	1,4-Cineole	1012 ^d	1005	1180	0.1	RI, MS
9	<i>p</i> -Cymene	1015	1013	1273	0.6	RI, MS, ^{13}C NMR
10	Limonene *	1024	1022	1203	1.0	RI, MS, ^{13}C NMR
11	1,8-Cineole *	1025	1022	1211	0.4	RI, MS
12	β -Phellandrene *	1023	1022	1211		RI, MS
13	Fenchone	1069	1069	1398	1.0	RI, MS, ^{13}C NMR
14	Fenchol	1099	1100	1580	1.4	RI, MS, ^{13}C NMR
15	Terpin-3-en-1-ol	-	1118	nd	0.2	RI, MS
16	Camphor	1123	1122	1515	2.7	RI, MS, ^{13}C NMR
17	iso-Borneol	1142	1142	1665	1.0	RI, MS, ^{13}C NMR
18	Borneol	1150	1150	1697	4.4	RI, MS, ^{13}C NMR
19	Terpinen-4-ol	1164	1160	1603	tr	RI, MS
20	<i>p</i> -Cymen-8-ol	1169	1162	1843	0.2	RI, MS
21	α -Terpineol	1176	1174	1692	9.6	RI, MS, ^{13}C NMR
22	γ -Terpineol	1188	1180	1697	0.5	RI, MS, ^{13}C NMR
23	Piperitone	1226	1231	1734	tr	RI, MS

24	Safrole	1265	1263	1872	0.7	RI, MS, ¹³ C NMR
25	α -Copaene	1379	1381	1492	0.1	RI, MS
26	α -Funebrene	1385	1383	1506	1.4	RI, ¹³ C NMR
27	α -Duprezianene	1388	1386	1524	0.8	RI, MS, ¹³ C NMR
28	β -Elemene	1389	1388	1590	2.0	RI, MS, ¹³ C NMR
29	α -Cedrene*	1418	1415	1570	18.4	RI, MS, ¹³ C NMR
30	β -Funebrene*	1418	1415	1572	2.0	RI, ¹³ C NMR
31	β -Cedrene	1424	1420	1597	5.2	RI, MS, ¹³ C NMR
32	Thujopsene	1434	1430	1621	0.7	RI, MS, ¹³ C NMR
33	γ -Muurolene	1474	1470	1687	1.4	RI, MS, ¹³ C NMR
34	Selina-4,11-diene	1475	1474	1674	0.2	RI, MS
35	β -Selinene	1486	1482	1717	0.5	RI, MS, ¹³ C NMR
36	α -Muurolene	1496	1494	1722	0.5	RI, MS, ¹³ C NMR
37	Cuparene	1498	1500	1757	0.2	RI, MS
38	β -Bisabolene	1503	1507	1725	0.7	RI, MS, ¹³ C NMR
39	γ -Cadinene	1507	1514	1829	0.2	RI, MS
40	α -Calacorene	1527	1528	1912	0.2	RI, MS
41	β -Elemol	1541	1534	2073	1.5	RI, MS, ¹³ C NMR
43	Sesquithuriferol *	1592 ^e	1591	2107	0.4	RI, ¹³ C NMR
42	Cedrol*	1603	1591	2113	11.2	RI, MS, ¹³ C NMR
44	γ -Eudesmol	1618	1619	2177	8.8	RI, MS, ¹³ C NMR
45	β -Eudesmol	1641	1636	2242	3.0	RI, MS, ¹³ C NMR
46	α -Eudesmol	1653	1641	2233	2.8	RI, MS, ¹³ C NMR
	Total identified				88.4%	

^aOrder of elution and percentages of individual components are given on apolar (BP-1) capillary column except for compounds indicated by an asterisk, percentage on polar (BP-20) column. RIa, RIp: Retention indices measured on apolar and polar columns, respectively. RI lit: retention indices from reference ^[28] otherwise stated; ^d: reference ^[23]; ^e: reference ^[29]. Compounds 10, 11 and 12 were separated on the DB5 capillary column used to perform GC-MS analysis. nd = not determined.

4. Conclusions

In conclusion, according to our results and published data, it appears that *Cunninghamia konishii* essential oil isolated from wood harvested in four Vietnamese provinces exhibited various chemical compositions dominated either by monoterpenes (α -terpineol, β -fenchyl alcohol) or by sesquiterpenes (cedrol, α -cedrene). Therefore, it should be informative to investigate the chemical variability of Vietnamese *C. konishii* wood oil (chemical variability of genetic or pedo-climatic origin). This study could be carried out after normalization of the harvest procedure, essential oil isolation and analysis of oil samples by complementary analytical techniques.

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