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The Leaf Essential Oils of the New Conifer Genus, *Xanthocyparis*: *Xanthocyparis vietnamensis* and *X. nootkatensis*

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Abstract

A new conifer genus was recently discovered in northern Vietnam and named *Xanthocyparis* with two species: *X. vietnamensis* in Vietnam and *X. nootkatensis* (= *Chamaecyparis nootkatensis*) in western North America. This is the first analysis of the leaf essential oil of *X. vietnamensis*. The oil of *X. vietnamensis* is dominated by sesquiterpenes and diterpenes. The major components are hedycaryol (1.4–34.4%), phyllocladene (0.9–37.8%), sandaracopimara-8(14),15-diene (0.7–8.9%), germacrene D (3.7–7.9%), and α-pinene (0.8–14.9%). The oil of *Xanthocyparis nootkatensis* (*Chamaecyparis nootkatensis*) is dominated by monoterpenes. The major constituents of *X. nootkatensis* are limonene (35.4–42.4%), δ-3-carene (11.5–23.4%), α-pinene (8.7–16.3%) and nezukol (3.3–4.8%). The oils differ in many components but share many of the unusual diterpenes. This seems to indicate a rather distant relationship and a long period of divergence. The oil samples of *X. vietnamensis* were extremely variable (e.g. hedycaryol 1.4–34.4%; phyllocladene 0.9–37.8%) suggesting chemical races or botanical varieties may be present.

Key Word Index

Xanthocyparis vietnamensis, *Xanthocyparis nootkatensis*, *Chamaecyparis nootkatensis*, essential oil composition, hedycaryol, phyllocladene, α-pinene, δ-3-carene, limonene.

Introduction

In 2002, a new conifer genus, *Xanthocyparis*, was described from northern Vietnam. The new Vietnamese species, *Xanthocyparis vietnamensis* Farjon et Hiep, is restricted to a small area of karst limestone in the northern part of Hagiang Province (1). The morphological features of this new conifer strongly suggested affinity to *Chamaecyparis nootkatensis* (D. Don) Spach in the *Cupressaceae*, leading Farjon et al. (1) to move *Chamaecyparis nootkatensis* (D. Don) Spach to the new genus as *Xanthocyparis nootkatensis* (D. Don) Farjon & Harder. *Chamaecyparis nootkatensis* has had a troubled taxonomic history having been placed in four different genera: *Cupressus*, *Chamaecyparis*, *Callitropsis* and *Xanthocyparis* (see Little et al. (2) for a review of the taxonomic history).

Recently, Little et al. (2), using ITS (nrDNA), matK and rbcL sequence data showed that *Xanthocyparis* is a distinct clade (Figure 1, 93% bootstrap) but the *Xanthocyparis* clade is closely related to the clade of *Cupressus* species from the western hemisphere (Figure 1, 92% bootstrap). Notice that a larger clade exists that contains *Cupressus* from both the eastern hemisphere (EH) and western hemisphere (WH), along with *Juniperus* and *Xanthocyparis* nested between the *Cupressus* (EH, WH) clades. Due to nomenclatural priority, Little et al. (2) resurrected the genus *Callitropsis* with two species: *Callitropsis nootkatensis* (D. Don) Orest. and *C. vietnamensis* (Farjon et Hiep) D.P. Little.

There are no analyses of the leaf oil of *Xanthocyparis vietnamensis*. The most recent complete analysis of the leaf oil of *Ch. nootkatensis* was by Cheng and von Rudloff in 1970 (3). Subsequently, totarol (*trans*-) was isolated from *Ch. nootkatensis* (4). Cool (5) found new ent-daucane and acoranes from *x Cupressocyparis leylandii*, but the compounds were not found in either of the putative parents (*Ch. nootkatensis*, *Cup. macrocarpa*).

In this work, we make the first report on the composition of the leaf oil of *X. vietnamensis* and compare it with detailed analyses of *X. nootkatensis* (*Ch. nootkatensis*) leaf oils.

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Experimental

For *X. nootkatensis* (*Ch. nootkatensis*), leaf material was obtained by field sampling. Materials from *X. vietnamensis* were obtained from plants growing at the Royal Botanical Garden Edinburgh from young trees with mature foliage from known trees in Vietnam. *Xanthocyparis nootkatensis*: *Adams 9086*, Seattle, WA, USA, *Adams 9274, 9276*, Vancouver Island, BC, Canada; *X. vietnamensis*, *To Quang Thao 16, 22, 26* at 1230 m, Hang Tong Trong mountain, Vietnam (cloned and cultivated at Royal Bot. Gard. Edinburgh as accessions 20030528, 20030532 and 20030536, respectively). Voucher specimens for Adams collections have been deposited at Baylor University Herbarium (BAYLU) and Thao collections at Royal Bot. Gard. Edinburgh (E).

Fresh leaves (200 g fresh wt) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (6). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (48 h, 100°C) for determination of oil yields.

The essential oils were analyzed on a HP5971 MSD mass spectrometer, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 µm coating thickness, fused silica capillary column (see 7 for operating details). Identifications were made by library searches of our volatile oil library (7), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using the HP Chemstation software.

Results and Discussion

The oils of the two species were very different (Table I). The oil of *X. vietnamensis* was dominated by sesquiterpenes and diterpenes such as hedycaryol (1.4–34.4%), phyllocladene (0.9–37.8%), sandaracopimara-8(14),15-diene (0.7–8.9%), germacrene D (3.7–7.9%). The monoterpenes were low in the common components normally found in conifers, except for α-pinene (0.8–14.9%), which was very variable. In contrast, the oil of *X. nootkatensis* (*Ch. nootkatensis*) is dominated by monoterpenes. The major constituents of *X. nootkatensis* are limonene (35.4–42.4%), δ-3-carene (11.5–23.4%), α-pinene (8.7–16.3%) and nezukol (3.3–4.8%). The oils differed in many components (Table I) and this seems to indicate a rather distant relationship and a long period of divergence. This distant

Table I. Comparisons of the percent total oil for leaf oils for *Xanthocyparis vietnamensis* and *X. nootkatensis* (= *Chamaecyparis nootkatensis*)

KI	Compound	*X. vietnamensis*			*X. nootkatensis*		
802	hexanal	0.1	t	1.1	0.1	t	t
855	(E)-2-hexenal	0.9	1.2	5.6	0.5	t	-
902	heptanal	t	-	t	-	-	-
926	tricyclene	-	-	-	t	t	t
931	α-thujene	-	-	-	0.1	0.4	0.4
939	α-pinene	9.6	0.8	14.9	16.3	8.7	9.8
953	α-fenchene	-	-	-	1.6	1.0	0.9
976	sabinene	0.1	-	-	0.2	8.7	10.5
979	1-octen-3-ol	0.8	1.9	4.2	1.3	t	t
980	β-pinene	t	-	-	1.9	0.5	t
991	myrcene	0.3	6.4	7.9	2.6	3.2	3.0
1011	δ-3-carene	-	-	-	23.4	12.0	11.5
1018	α-terpinene	-	-	-	0.1	2.3	2.3
1026	p-cymene	t	t	t	t	t	t
1031	limonene	0.6	0.2	0.4	35.4	42.4	36.5
1062	γ-terpinene	0.1	0.4	t	0.2	2.3	2.6
1068	*cis*-sabinene hydrate	-	-	-	0.1	1.0	1.4
1081	2-methoxy ethyl benzene	-	-	-	0.2	-	t
1088	terpinolene	0.3	0.9	0.9	3.3	3.1	3.1
1097	*trans*-sabinene hydrate	-	-	-	0.1	0.3	0.4
1098	linalool	t	0.3	t	-	-	-
1101	nonanal	0.5	0.3	1.9	t	-	-
1117	α-fenchol (endo-fenchol)	-	-	-	0.2	t	0.1
1121	*cis*-p-menth-2-en-1-ol	-	-	-	0.3	0.4	0.4
1140	*trans*-p-menth-2-en-1-ol	-	-	-	0.1	0.2	0.2
1143	camphor	-	-	-	0.1	-	t
1147	3-methyl-2-butenyl isovalerate	-	-	-	0.1	-	-
1149	citronellal	-	-	-	t	t	0.1
1165	borneol	-	-	-	0.1	t	0.1
1177	terpinen-4-ol	-	-	-	0.2	4.0	6.2
1189	α-terpineol	-	-	0.3	0.1	0.1	0.4
1220	α-fenchyl acetate (endo-fenchyl acetate)	-	-	-	0.2	0.2	0.3

Table I. continued

KI	Compound	*X. vietnamensis*			*X. nootkatensis*		
1235	methyl thymol	0.1	1.5	-	-	-	-
1252	piperitone	-	-	-	0.1	t	0.1
1285	bornyl acetate	-	-	-	0.2	0.1	0.2
1293	(E,Z)-2,4-decadienal	-	-	-	0.4	-	-
1314	(E,E)-2,4-decadienal	-	-	-	0.3	-	-
1350	α-terpinyl acetate	-	-	-	0.6	1.8	2.3
1359	neryl acetate	0.2	t	t	-	-	-
1379	geranyl acetate	0.8	1.5	t	-	-	-
1418	β-caryophyllene	0.5	1.6	1.0	-	-	-
1448	*cis*-muurola-3,5-diene	1.2	0.5	1.0	-	-	-
1454	α-humulene	t	0.3	0.6	-	-	-
1461	*cis*-muurola-4(14),5-diene	2.8	1.5	2.3	-	-	-
1471	dauca-5,8-diene	-	-	-	0.1	0.1	0.1
1480	germacrene D	6.9	3.7	7.9	-	-	-
1482	γ-himachalene	2.9	3.7	7.1	-	-	-
1491	drim-8(12)-ene	0.2	t	0.5	-	-	-
1669	epi-zonarene	0.8	0.9	1.1	-	-	-
1499	α-muurolene	0.1	-	-	-	-	-
1524	δ-cadinene	0.5	0.5	0.6	0.1	-	-
1534	10-epi-cubebol	0.4	0.3	0.3	-	-	-
1547	*cis*-muurola-5-en-4β-ol	t	0.2	t	-	-	-
1547	hedycaryol	34.4	1.4	14.0	t	0.1	0.4
1559	*cis*-muurola-5-en-4α-ol	0.3	0.5	0.5	-	-	-
1561	(E)-nerolidol	-	-	-	0.2	0.1	0.1
1562	geranyl butyrate	0.2	t	t	-	-	-
1581	caryophyllene oxide	-	t	t	-	-	-
1600	hexadecane	-	-	0.4	-	-	-
1630	eremoligenol	0.3	-	-	-	-	-
1627	benzophenone	t	1.0	0.6	-	-	-
1631	γ-eudesmol	3.3	1.0	2.4	-	-	-
1640	epi-α-cadinol	-	-	-	t	-	-
1640	epi-α-muurolol	-	-	-	t	-	-
1649	β-eudesmol	2.6	0.7	1.5	-	-	-
1652	α-eudesmol	1.8	0.5	1.2	-	-	-
1653	α-cadinol	1.8	0.5	1.2	0.1	t	t
1670	bulnesol	-	-	0.2	-	-	-
1684	epi-α-bisabolol	-	-	-	0.2	0.1	0.2
1685	germacra-4(15),5,10(14)-trien-1α-ol	0.1	t	t	-	-	-
1700	heptadecane	t	0.4	0.3	-	-	-
1734	isobicyclogermacrenal	t	t	0.7	-	-	-
1800	octadecane	0.1	0.2	0.3	-	-	-
1906	isopimara-9(11),15-diene	0.2	1.0	t	0.2	0.2	0.1
1933	isohibaene	0.4	2.4	0.2	-	-	-
1949	pimaradiene	0.5	2.5	0.9	0.1	0.1	0.1
1960	sandaracopimara-8(14),15-diene	1.6	8.9	0.7	0.1	0.2	0.1
1966	isophyllocladene	t	t	t	0.1	0.4	0.3
1998	epi-13-manoyloxide	-	-	-	1.2	0.6	0.6
2017	phyllocladene	7.1	37.8	0.9	0.1	t	t
2017	manoyloxide	-	-	-	0.4	0.2	0.2
2054	abietatriene	0.2	1.1	5.0	0.1	t	t
2088	abietadiene	-	-	-	0.1	t	0.1
2116	diterpene,191,43,69,81,95,(290)	1.3	1.1	0.3	-	-	-
2133	nezukol	0.2	1.1	t	3.6	4.8	3.3
2210	phyllocladanol	0.1	0.5	0.2	1.4	0.4	0.2
2283	sempervirol	0.3	1.5	0.7	-	-	-
2314	*trans*-totarol	-	-	-	t	t	t
2500	pentacosane	t	t	t	-	-	-
2700	heptacosane	0.2	0.5	0.7	-	-	-

KI = Kovat's Indexon DB-5 (=SE54) column; *tentatively identified; compositional values less than 0.1% are denoted as traces (t); unidentified components less than 0.5% are not reported

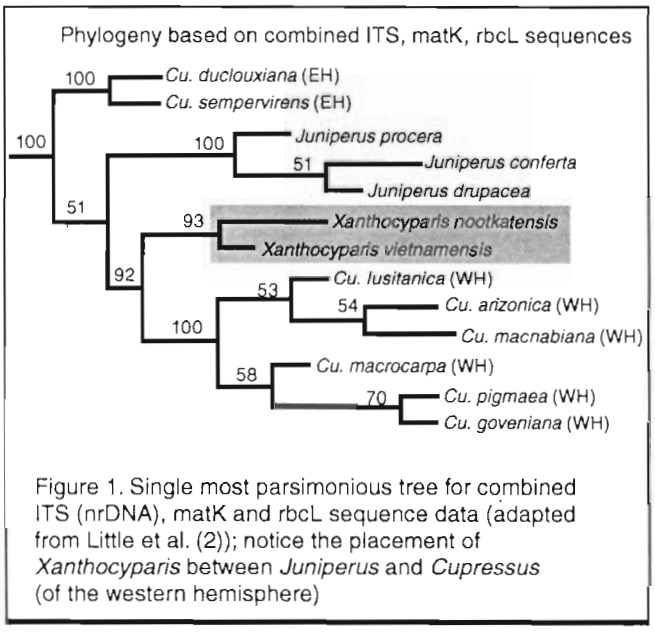

Figure 1. Single most parsimonious tree for combined ITS (nrDNA), matK and rbcL sequence data (adapted from Little et al. (2)); notice the placement of *Xanthocyparis* between *Juniperus* and *Cupressus* (of the western hemisphere)

relationship is depicted in their DNA sequence data (Figure 1). Notice that *X. vietnamensis* and *X. nootkatensis* form a clade supported at the 93% level, but they are closely associated with *Cupressus* from the western hemisphere.

However, *X. vietnamensis* and *X. nootkatensis* do share several diterpenes (isoprimara-9(11),15-diene, pimaradiene, sadaracopimara-8(14),15-diene, isophyllocladene, phyllocladene, nezukol, and phyllocladanol) that are unusual in conifers from the northern hemisphere (8,9), so the leaf oils do support the DNA sequencing data (2).

The oil samples of *X. vietnamensis* were extremely variable (e.g. hedycaryol 1.4–34.4%, phyllocladene 0.9–37.8%, α-pinene 0.8–14.9%) suggesting chemical races or botanical varieties may be present. It appears from field notes that the three individuals analyzed came from different populations. Additional samples from these populations are needed to determine if there are botanical varieties or merely chemical polymorphisms within the populations.

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