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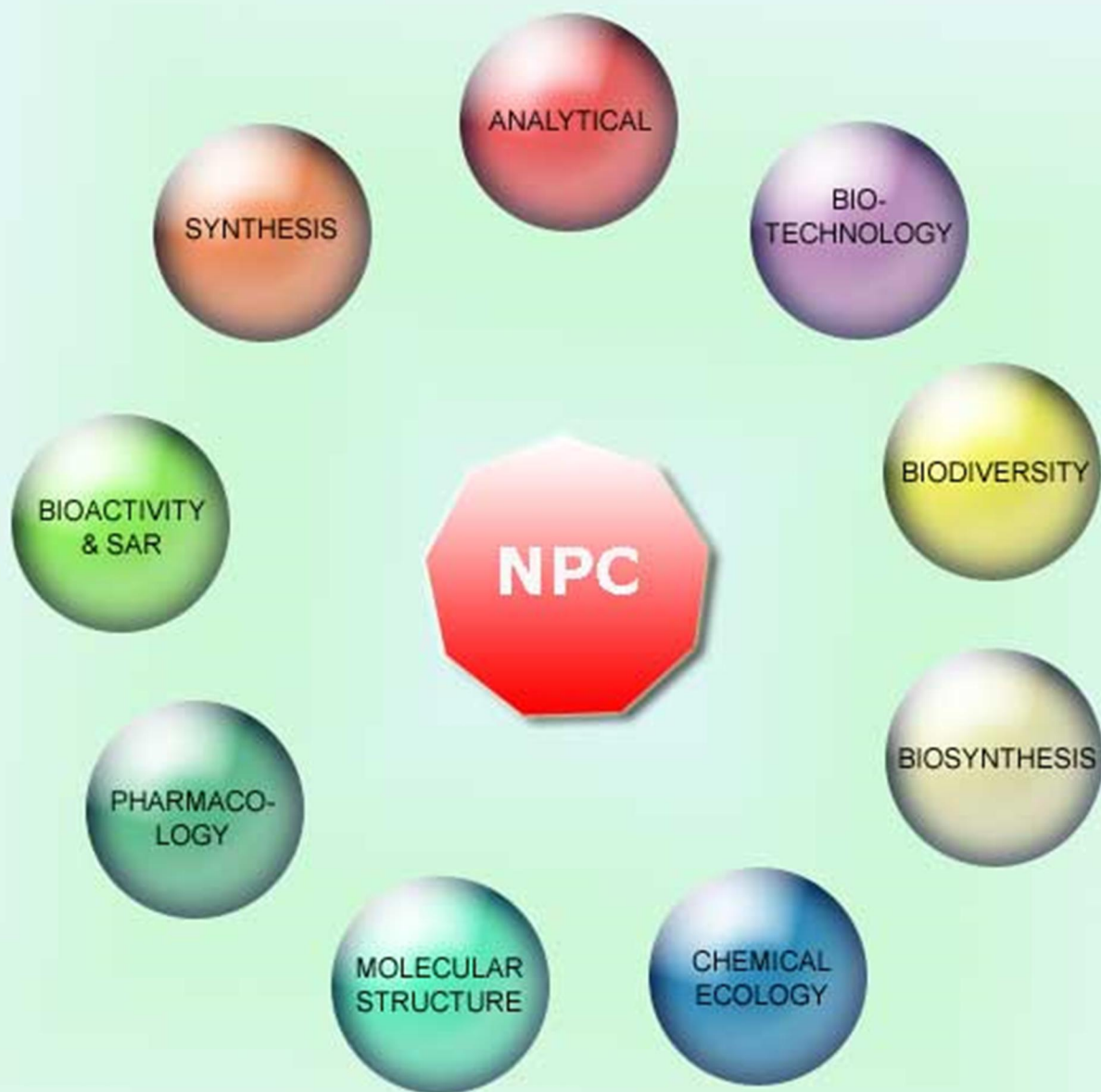
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## Volatile Organic Compounds of six French *Dryopteris* Species: Natural Odorous and Bioactive Resources

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Aerial parts of six *Dryopteris* species collected in France were investigated for volatile organic compounds (VOC) for the first time. Fifty-three biosynthesized VOC from the shikimic, lipidic and terpenic pathways were identified using gas chromatography/mass spectrometry. Many bioactive polyketide compounds as filicinic derivatives (from 8.5 to 23.5%) and phloroglucinol derivatives (from 8.2 to 53.8%) with various pharmacological activities were detected in high amount from five analysed *Dryopteris* species, in particular *D. oreades* and *D. borrieri*, i.e., propionylfilicinic acid (> 10% in *D. affinis* and *D. ardechensis*) and 2,6-dihydroxy-4-methoxy-3-methylbutyrophenone (aspidinol) (19.1% and 14.6% in *D. oreades* and *D. borrieri*, respectively). Several terpenic derivatives with a low odor threshold were identified, i.e., carota-5,8-diene (from 2.5 to 18.4%: floral, woody or fresh bark note), (*E*)-nerolidol (> 10% for *D. borrieri* and *D. cambrensis*; floral or woody odor),  $\alpha$ -selinene (> 7% for *D. ardechensis*; woody-spicy odor), and aristolene (12.8% in *D. affinis*; flower, sweet odor). The main isoprenoid derivatives were 4-hydroxy-5,6-epoxyionol, 3-oxo- $\alpha$ -ionol and 4-oxo-7,8-dihydro- $\beta$ -ionone (essentially in *D. remota*), whereas the main aromatic compound was 4-hydroxy-3-methoxyacetophenone (20.6% and 12.6% in *D. cambrensis* and *D. borrieri*, respectively) and the main lipid derivative was 1-octen-3-ol with a mushroom-like odor (from 0.4 to 8.3%). *Dryopteris* species resources are of great interest as a reservoir of odorous and bioactive compounds.

**Keywords:** Fern, Volatile Organic Compounds, Carota-5,8-diene, (*E*)-Nerolidol, Phloroglucinols, Filicinic derivatives, 1-Octen-3-ol, Biological activities.

*Dryopteridaceae* is a subcosmopolitan family including approximately one thousand species and with only two native genera in Western Europe: *Dryopteris* Adans and *Polystichum* Roth [1a]. *Dryopteris affinis* group is constituted in Western Europe by *D. affinis* (R. Lowe) Fras.-Jenk., *D. borrieri* (Newm.) Newm. ex Oberh. & Tavel, *D. cambrensis* (Fras.-Jenk.) Beitel & W.R. Buck, the uncommon *D. pseudodisjuncta* (Tavel ex Fras.-Jenk.) Fras.-Jenk. and a very recently reported species, i.e., the rare *D. lacunosa* S. Jess., Zenner, Ch. Stark & Bujnoch [1b,1c].

*Dryopteris* species have been mainly investigated for phloroglucinol derivatives due to their various pharmacological activities for humans and many ethnoveterinary uses, i.e., schistosomicidal, antimicrobial, antitumor, anti-inflammatory and antioxidant properties [2a-2m].

Few *Dryopteris* species are known having an odor. *D. villarii* (Bellardi) Schinz & Thell. and *D. mindshelkensis* Pavlov have a balsamic odor when crushed [3a]. *D. aemula* (Aiton) O. Kuntze and *D. crispifolia* Rasbach, Reichstein & Vida smell like newly mown hay, whereas *D. fragrans* (L.) Schott has a spicy odor when dry [3b]. Very few data have been reported previously on the volatile organic compounds (VOC) of *Dryopteris* species and none from the *D. affinis* group. *D. filix-mas* (L.) Schott contains in particular (*E*)-nerolidol (38.7%) with floral, woody or fresh bark odor [4a], acylfilicinic acids and 1-octen-3-ol [4b]. *D. dilatata* (Hoffm.) A. Gray odor is characterized by (*E*)-2-hexenal and (*Z*)-3-hexenal, responsible for the "green odor" and 1-octen-3-ol [4c], whereas the

odor of *D. fragrans* is based on terpenic derivatives such as 10-hydroxy-15-oxo- $\alpha$ -cadinol, albicanyl acetate,  $\alpha$ -cadinene and albicanol [4d].

Fresh aerial parts of three main species from the *D. affinis* group, i.e., *D. affinis*, *D. borrieri*, and *D. cambrensis*, and three related species, *D. oreades* Fomin, *D. ardechensis* Fraser-Jenkins and *D. remota* (A. Braun ex Döll) Druce were investigated for their volatile profiles using GC-MS. The VOC were identified as previously reported [4b, 4c, 4e, 4f, 5a].

Fifty-three components biosynthesized from the shikimic, lipidic and terpenic pathways were identified from the concentrated diethyl ether extracts of the six *Dryopteris* species (Table 1).

*D. affinis* was dominated by a large number of terpenic derivatives (49.5%), i.e., carota-5,8-diene (14.7%; floral, woody or fresh bark note), aristolene (12.8%; flower, sweet odor) [5b], (*E*)-nerolidol (5.9%; with floral odor), aristola-1(10),8-diene (3.4%),  $\alpha$ -selinene (3%; amber type odor or sweet-woody, slightly peppery odor [5c]),  $\beta$ -selinene (2.5%; mild, woody, warm, herbaceous peppery odor) and eremophilene (2.4%). It also contained polyketide derivatives (36.4%) as filicinic compounds (19.7%; [5d]), i.e., propionylfilicinic acid (11%) and acetylfilicinic acid (6.7%), and phloroglucinol compounds (16.7%). The main lipid derivative was 1-octen-3-ol (6.9%; mushroom-like odor), well-known for many mushrooms [5e-5g], and previously reported for ferns, including *D. filix-mas* and *D. dilatata*, horsetails and Angiosperms [4b, 4c, 5a, 5h].

**Table 1:** Percentage of volatile organic compounds<sup>a</sup> in fresh aerial parts of *Dryopteris* species.

Compounds	RI <sup>b</sup>	<i>D. affinis</i>	<i>D. borrieri</i>	<i>D. cambrensis</i>	<i>D. oreades</i>	<i>D. ardechensis</i>	<i>D. remota</i>
<b>Lipid derivatives</b>		<b>9.1</b>	<b>5.7</b>	<b>4.4</b>	<b>3.4</b>	<b>3.2</b>	<b>20.7</b>
1-Octen-3-ol	982	6.9	4.8	4.4	3.0	0.4	8.3
3-Octanol	1000	1.6	0.9		0.3	0.9	2.5
3-Hexenoic acid	1017					0.3	3.1
2-Hexenoic acid	1025					0.5	2.1
Nonanal	1106	0.7			0.1	0.3	2.6
Decanal	1205					0.8	2.0
<b>Polyketide compounds</b>		<b>36.4</b>	<b>45.5</b>	<b>22.3</b>	<b>62.3</b>	<b>31.6</b>	<b>0.0</b>
Acetylfilicinic acid	1443	6.7	2.9	4.9			
Propionylfilicinic acid	1546	11.0	7.7	4.4	0.5	10.1	
Butyrylfilicinic acid	1615		1.9	3.1	4.3	4.4	
Valeroylfilicinic acid	1622	2.1	1.6	1.1	2.5	5.2	
NI <sub>c</sub>	1686		2.7		1.2	3.8	
= <b>Sub-total filicinic compounds</b>		<b>(19.8)</b>	<b>(16.8)</b>	<b>(13.5)</b>	<b>(8.5)</b>	<b>(23.5)</b>	<b>(0.0)</b>
2,4,6-Trimethoxy-propiofenone	1855				6.0		
2,6-Dihydroxy-4-methoxypropiofenone	1882		0.3		3.3		
2,6-Dihydroxy-3-methyl-4-methoxypropiofenone	1942		6.7		3.1		
2,6-Dihydroxy-4-methoxybutyrophenone (desaspidinol)	1984				2.7		
NI	2028				6.7		
2,6-Dihydroxy-4-methoxy-3-methylbutyrophenone (aspidinol)	2038		14.6	1.8	19.1		
2,6-Dihydroxy-4-methoxyvalerophenone	2091	2.9	7.2	7.0	12.9	8.2	
NI	2140	13.8					
= <b>Sub-total phloroglucinol compounds</b>		<b>(16.7)</b>	<b>(28.8)</b>	<b>(8.8)</b>	<b>(53.8)</b>	<b>(8.2)</b>	<b>(0.0)</b>
<b>Aromatic compounds</b>		<b>1.4</b>	<b>18.4</b>	<b>24.8</b>	<b>0.2</b>	<b>5.7</b>	<b>6.6</b>
Benzaldehyde	962		1.1			0.8	1.8
Benzyl alcohol	1043		0.7			2.5	2.9
Phenylethanal	1046	1.0	0.7		0.1		
Cinnamaldehyde	1164		0.7				
4-Hydroxy-3-methoxystyrene	1293		0.5	1.3		2.5	1.8
4-Hydroxy-3-methoxybenzaldehyde (vanilline)	1385		1.2	1.3			
2,4,6-Trihydroxybutyrophenone (phlorobutyrophenone)	1552				0.1		
4-Hydroxy-3-methoxyacetophenone	1560	0.4	12.6	20.6			
4-Hydroxy-3-methoxybenzoic acid (vanillic acid)	1576		0.6	1.7			
4-Hydroxy-3-methoxycinnamaldehyde	1694		0.4				
<b>Terpenic compounds</b>		<b>49.4</b>	<b>21.2</b>	<b>32.8</b>	<b>26.3</b>	<b>48.5</b>	<b>0.6</b>
Linalool	1104	1.3				2.7	0.6
Thymoquinone	1240		2.0	1.2			
Daucene	1380	0.4				0.8	
β-Elemene	1391		1.0	0.8		2.3	
Aristolene	1419	12.8				0.4	
γ-Elemene	1427	2.6				2.3	
Aristola-1(10),8-diene	1458	3.4			2.1	6.6	
Carota-5,8-diene	1473	14.7	2.5	12.7	18.4	9.3	
β-Selinene	1487	2.5					
Eremophilene	1489	2.4	3.1	4.4	0.9	4.4	
Valencene	1494	0.3	0.6			1.1	
α-Selinene	1496	3.0			0.5	7.8	
Germacrene A	1501		0.6	1.5			
7-Epi-α-selinene	1519					3.1	
(E)-Nerolidol	1556	5.9	11.5	12.2	4.3	7.9	
<b>Isoprenoid derivatives</b>		<b>3.7</b>	<b>9.2</b>	<b>15.7</b>	<b>7.7</b>	<b>11.0</b>	<b>72.1</b>
NI	1335						1.4
2,3-Dehydro-α-ionol	1384						3.1
3,4-Dehydro-7,8-dihydro-β-ionone	1413						1.3
2,3-Dehydro-α-ionone	1428						1.0
β-Ionone	1480		0.2			1.1	0.5
Dihydroactinidiolide	1505		1.1				
NI	1599			0.2			10.4
NI	1608						5.4
NI	1615						3.4
4-Hydroxy-7,8-dihydro-β-ionone	1632		1.1	3.1			3.6
4-Hydroxy-β-ionone	1638				1.6	1.4	3.6
3-Oxo-α-ionol	1645				2.0		5.8
3-Oxo-α-ionone	1652		1.1	0.9		1.2	0.8
4-Oxo-β-ionone	1663		0.4				0.8
4-Hydroxy-5,6-epoxyionol	1672						19.1
4-Oxo-7,8-dihydro-β-ionone	1675		1.8	2.5			8.1
NI	1692	1.1	3.5	9.0	4.1	7.3	2.1
NI	1708	0.5					
NI	1717	1.0					
NI	1746	1.2					1.8

<sup>a</sup> Relative percentage of the VOC based on the GC-MS chromatographic area.<sup>b</sup> RI = Retention Indices on SLB<sup>TM</sup>-5MS column (Supelco).<sup>c</sup> NI = Not identified.

The volatile pattern of *D. borrieri* was based on polyketide derivatives (45.5%), mainly phloroglucinols (28.8%), i.e., 2,6-dihydroxy-4-methoxy-3-methylbutyrophenone, also named aspidinol (14.6%), 2,6-dihydroxy-4-methoxyvalerophenone (7.2%)

and 2,6-dihydroxy-3-methyl-4-methoxypropiofenone (6.7%), as well as filicinic compounds (16.8%), in particular propionylfilicinic acid (7.7%). The investigation indicated a lower amount of terpenic derivatives (21.2%), i.e., (*E*)-nerolidol (11.5%) and eremophilene

(3.1%), aromatic components (18.4%), i.e., 4-hydroxy-3-methoxyacetophenone (12.6%), and 1-octen-3-ol (4.8%). It should be noted that low amounts of strong odorous components were detected as benzaldehyde (bitter almond odor; [5i]), cinnamaldehyde (cinnamon odor), vanillin, vanillic acid (sweet cream with vanilla aroma) and dihydroactinidiolide (fruity odor [5j]), also found in *Equisetum arvense* L. [5a].

The broad spectrum of volatile components identified in *D. cambrensis* contributed to its complex smell, including isoprenoid, polyketide, aromatic and terpenic derivatives and also an odorous lipid derivative, 1-octen-3-ol. The terpenic profile (32.8%) was based on carota-5,8-diene (12.7%), (*E*)-nerolidol (12.2%) and eremophilene (4.4%). The fern also contained a high level of aromatic components (24.8%), mainly 4-hydroxy-3-methoxyacetophenone (20.6%). Table 1 lists the polyketide derivatives (22.3%) divided into filicinic compounds (13.5%), i.e., acetylfilicinic acid (4.9%), propionylfilicinic acid (4.4%) and butyrylfilicinic acid (3.1%), as well as phloroglucinols (8%), mainly 2,6-dihydroxy-4-methoxyvalerophenone (7%) and aspidinol (1.8%). The main isoprenoids (15.7%) were  $\beta$ -ionone derivatives, i.e., 4-hydroxy-7,8-dihydro- $\beta$ -ionone (3.1%) and 4-oxo-7,8-dihydro- $\beta$ -ionone (2.5%), already found in *Dryopteris dilatata* and *Phegopteris connectilis* (Michx) Watt, and in *Gymnocarpium dryopteris* (L.) Newman, respectively [4c].

The volatile content of *D. oreades* was mainly dominated by polyketide derivatives (62.3%), essentially phloroglucinols (53.8%) as 2,6-dihydroxy-4-methoxy-3-methylbutyrophenone (aspidinol; 19.1%), 2,6-dihydroxy-4-methoxyvalerophenone (12.9%), 2,4,6-trimethoxypropiophenone (6%) and 2,5-dihydroxy-4-methoxybutyrophenone (desaspidinol; 2.7%), as well as filicinic components (8.8%), in particular butyrylfilicinic acid (4.3%). It should be noted that aspidinol or desaspidinol are well-known for their anthelmintic, anti-tumor and antibacterial properties at very low concentration [6a,6b,2e]. The main terpenes were carota-5,8-diene (18.4%) and (*E*)-nerolidol (4.3%), and the major lipid derivative was still 1-octen-3-ol (3%).

The major VOCs from *D. ardechensis* were terpenic derivatives (48.5%), i.e., carota-5,8-diene (9.3%), (*E*)-nerolidol (7.9%),  $\alpha$ -selinene (7.8%), aristola-1(10),8-diene (6.6%) and linalool (2.7%; orange flower odor [6c]). It should be noted that eremophilene, valencene,  $\alpha$ - and  $\beta$ -selinene and 7-epi- $\alpha$ -selinene have the same biosynthetic pathway; several of them have antifungal or insecticidal properties [6d-6g]. The polyketide pathway (31.6%) produced three filicinic compounds (23.5%), namely propionylfilicinic acid (10.1%), valeroylfilicinic acid (5.2%) and butyrylfilicinic acid (4.4%), and only one phloroglucinol compound, i.e., 2,6-dihydroxy-4-methoxyvalerophenone (8.2%). Several isoprenoid derivatives, such as 4-hydroxy- $\beta$ -ionone (1.4%), 3-oxo- $\alpha$ -ionone (1.2%) and  $\beta$ -ionone (1.1%) with a violet flower odor [6c] were also identified. Six lipid derivatives (3.2%), such as 3-octanol, decanal and 1-octen-3-ol completed the VOC content of *D. ardechensis*.

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*D. remota* showed the highest amount of isoprenoid derivatives (72%), including eleven identified compounds. The VOC profile was widely dominated by 4-hydroxy-5,6-epoxyionol (19.1%), 4-oxo-7,8-dihydro- $\beta$ -ionone (8.1%) and 3-oxo- $\alpha$ -ionol (5.8%; spicy odor). It also contained several odorous C<sub>8</sub> to C<sub>10</sub> derivatives (20.7%), such as 1-octen-3-ol (8.3%), 3-hexenoic acid (3.1%; honey odor somewhat waxy fruity and herbal [6h]), nonanal (2.6%; orange and green scent [5j]), 3-octanol (2.5%; mushroom-like odor and buttery) used in the food, flavor, cosmetic, pharmaceutical, tobacco and perfume industries [6i], and 2-hexenoic acid (2.1%), with a powerful fruity odor. Benzyl alcohol (2.9%; walnut flavor [6j]) and benzaldehyde (1.8%; bitter almond odor [4f]) emphasized the great interest of *D. remota* for aroma and flavor companies. Regarding the eight *Dryopteris* species investigated for VOC (fresh aerial part) in Table 1 and previously by the authors [4b,4c], *D. remota* and *D. dilatata* do not contain any filicinic derivatives, phloroglucinols or (*E*)-nerolidol.

This paper emphasizes that ferns are novel resources for natural compounds. Table 1 demonstrates that *Dryopteris* species can generate a broad spectrum of VOC for both odorous and bioactive ingredients. Within the former, terpenic compounds with floral, fruity or spicy notes, i.e., linalool, (*E*)-nerolidol, and ionone derivatives are the main fragrant components required for aroma applications. Within the last, phloroglucinol and filicinic derivatives are of various biological interests for the pharmaceutical as well as the cosmetic and hygiene products industries. Because only aerial parts have been used, intensive culture of ferns may be developed to extract biomolecules from the natural resources without any plant destruction. Natural components can also be used for hemisynthesis of di- to polymeric bioactive derivatives. *Dryopteris* species resources are indeed of great interest as candidates for bioactive aroma ingredients and for the discovery of new drugs with various therapeutic applications due to their potential antioxidant, antibacterial and antitumor promoting properties [7a-7d].

**Plant material:** Fresh aerial parts of ferns were collected as follows: *Dryopteris affinis*: 14/07/2009, Gimel les Cascades (Corrèze), *D. borrieri* and *D. cambrensis* 30/05/2010, Saint Etienne Vallée Française, (Ardèche). *D. oreades* 13/07/2011, Murat-le-Quaire, (Puy de Dôme), *D. ardechensis* and *D. remota*: 31/08/2010, Botanical Garden of Strasbourg. Voucher specimens are deposited at the Laboratory of Botany (Faculty of Pharmacy, Limoges, France).

**Plant part and GC-MS analyses:** Fresh aerial parts of 6 *Dryopteris* species were treated and investigated for volatile organic compounds as previously reported [4b,4c,5a].

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