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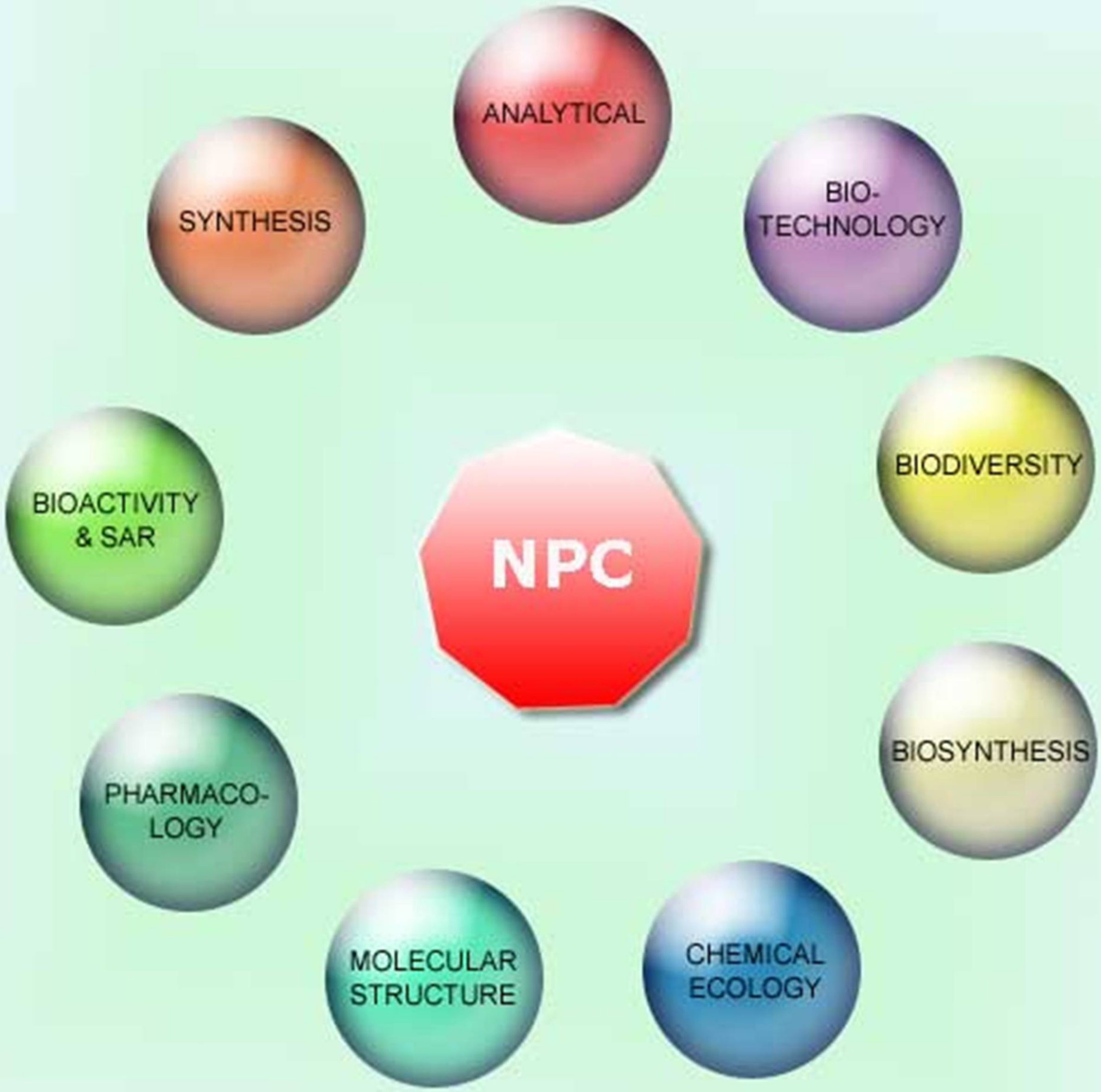

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Biodiversity of Volatile Organic Compounds from Five French Ferns

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Five French ferns belonging to different families were investigated for volatile organic compounds (VOC) by GC-MS using organic solvent extraction. Fifty-five VOC biosynthesized from the shikimic, lipidic and terpenic pathways including monoterpenes, sesquiterpenes and carotenoid-type compounds were identified. The main volatile compound of *Adiantum capillus-veneris* L. (Pteridaceae) was (*E*)-2-decenal with a plastic or "stink bug" odor. The volatile profiles of *Athyrium filix-femina* (L.) Roth (Woodsiaceae) and *Blechnum spicant* (L.) Roth (Blechnaceae) showed similarities, with small amounts of isoprenoids and the same main volatile compounds, i.e., 2-phenylethanal (odor of lilac and hyacinth) and 1-octen-3-ol (mushroom-like odor). The main volatile compound of *Dryopteris filix-mas* (L.) Schott (Dryopteridaceae) was (*E*)-nerolidol with a woody or fresh bark note. Polyketides, as acylfilicinic acids, were mainly identified in this fern. *Oreopteris limbosperma* (Bellardi ex. All.) J. Holub (Thelypteridaceae), well-known for its lemon smell, contained the highest biodiversity of VOC. Eighty percent of the volatiles was issued from the terpenic pathway. The main volatiles were (*E*)-nerolidol, α-terpineol, β-caryophyllene and other minor monoterpenes (for example, linalool, pinenes, limonene, and γ-terpinen-7-al). It was also the fern with the highest number of carotenoid-type derivatives, which were identified in large amounts. Our results were of great interest underlying new industrial valorisation for ferns based on their broad spectrum of volatiles.

Keywords: ferns, Pteridophytes, volatile organic compounds (VOC), filicinic acid, 2-phenylethanal, (*E*)-nerolidol, (*E*)-2-decenal, 1-octen-3-ol.

Pteridophytes, including ferns and fern allies, prevailing since the Carboniferous era, are still distributed worldwide [1a,1b]. Some are used as food and others in traditional medicine for their anthelmintic, schistosomicidal, antiviral, diuretic, antihemorrhagic, cholagogue, anti-rheumatism, anti-inflammatory, and remineralizing properties, and for the treatment of Meniere's syndrome [2a-2e]. Ferns have been widely investigated for their non-volatile organic components, i.e., phenolics, amino acids, di- or sesqui-terpene glycosides, and alkaloids [3a-3g]. However, fewer studies have focused on the volatile organic compound (VOC) profile [2c,4a-4f]. The objective of this project was to identify and compare the VOC contents of ferns from France [5] that have never been studied with this end. As a result, *Adiantum capillus-veneris* L. (Pteridaceae), *Athyrium filix-femina* (L.) Roth (Woodsiaceae), *Blechnum spicant* (L.) Roth (Blechnaceae), *Dryopteris filix-mas* (L.) Schott (Dryopteridaceae) and *Oreopteris limbosperma* (Bellardi ex. All.) J. Holub (Thelypteridaceae) were chosen to determine their volatile chemical profiles.

Fresh plant material was utilized. Fifty-five components biosynthesized from the shikimic, lipidic and terpenic pathways were identified from the concentrated diethyl ether extracts of the five ferns (Table 1). Seventeen constituents of *A. capillus-veneris* extract were identified, including mainly polyketide derivatives (87.9% of the volatile fraction), along with small amounts of aromatics (9.4%), and only one monoterpene (myrcene: 2.5%).

Table 1: Percentage of volatile organic compounds[a] in fresh aerial parts of ferns.

Compounds	RI[b]	*Adiantum capillus-veneris*	*Athyrium filix-femina*	*Blechnum spicant SF*[c]	*Blechnum spicant FF*[d]	*Dryopteris filix-mas*	*Oreopteris limbosperma*
Aromatic compounds		**9.4**	**29.9**	**47.5**	**51.2**	**1.5**	**10.1**
Benzaldehyde	962		1.2	1.5	4.4	1.5	3.6
2-Phenylethanal	1046	2.5	24.8	45.1	45.6		0.7
2-Phenylethanol	1117	1.9	1.2	0.2			0.7
Benzoic acid	1187	5					1.4
Coumarin	1431		2.7				
Cinnamic acid	1453						0.8
Ethyl vanillate	1554			0.7	1.2		1.5
Vanillic acid	1589						1.4
Polyketide compounds		**87.9**	**61.5**	**48.8**	**45.1**	**30.8**	**10.4**
1.3-Octadiene	835		5.7				
(*E*)-2-Hexenal	851		7.1				
(*Z*)-3-Hexenol	855		6.9			3.1	
(*E*)-2-Hexenol	866		1.9				
(*E*)-2-Heptenal	956	7.4		3.5			
1-Octen-3-ol	983	2.5	24.8	43.1	37.5	9.1	3.6
3-Octanone	985						1.1
3-Octanol	995		1.4	1.1	0.9	1.2	1.2
(*Z*)-3-Hexenoic acid	1017		2.6			0.8	0.9
(*E*)-2-hexenoic acid	1035		6				
(*E*)-2-Octenol	1072		1.2		1.5		
(*E*)-2-Nonenal	1161	2.5	2.8				
(*Z*)-2-Decenal	1250	3.7					
(*E*)-2-Decenal	1263	32.1		0.6	1.5		
Nonanoic acid	1283	3.1	0.7	0.5			0.9
(2*E*,4*Z*)-Decadienal	1296	3.7					
(2*E*,4*E*)-Decadienal	1321	5					
Octanoic amide	1357	3.7					
Acetylfilicinic acid	1445					1.2	
Nonanoic amide	1463	5.6					
NI[e]	1474				3.7		
Propionylfilicinic acid	1529					8.1	
Decanoic amide	1564	2.5					
Butyryl filicinate	1612					7.3	
(*Z*)-6-Dodecen-4-olide	1665		0.4				2.6
Undecanoic amide	1670	3.7					
Lauric amide	1770	12.4					
Monoterpenic compounds		**2.5**	**2.7**	**0.8**	**2.2**	**4.3**	**40.3**
α-Pinene	933						5.3
β-Pinene	978						1.1
Myrcene	996	2.5				2.0	
Limonene	1030						2.5
3.7-Dimethyloctan-3-ol	1079		2.7	0.8	2.2		
Linalool	1102					2.3	4.2
Terpinen-4-ol	1184						1.5
α-Terpineol	1202						13.5
cis-Dihydrocarvone	1206						1.2
trans-Dihydrocarvone	1209						1.4
8.9-Dihydrocarveol	1215						0.7
γ-Terpinen-7-al	1298						2.3
Perillic acid	1306						3.1
Carvone hydrate	1416						0.9
NI (oxygenated monoterpene)	1483						2.6
Sesquiterpenic compounds		**0.0**	**0.0**	**0.8**	**0.0**	**59.8**	**19.7**
β-Caryophyllene	1421						5.0
Selinadiene derivative	1467					21.1	
(*E*)-Nerolidol	1557			0.8		38.7	14.7
Carotenoid derivatives		**0.0**	**3.8**	**1.0**	**0.0**	**3.3**	**18.1**
NI	1601		3.0				7.3
Epoxy-α-ionone	1640		0.5	0.5		0.5	1.2
4-Hydroxyepoxy-β-ionol	1685		0.3	0.5		0.9	4.6
Tetrahydro-β-ionol acetate	1688						3.0
Blumenol C	1700					1.9	2.0
Extraction process[f]		1.26 / 40	53.5 / 300	21.0 / 150	11.7 / 140	58.7 / 370	5.64 / 90

[a] Relative percentage of the VOC based on the GC-MS chromatographic area.
[b] RI = Retention Indices on SLB™-5MS column (Supelco).
[c] SF = Sterile Fronds.
[d] FF = Fertile Fronds.
[e] NI = non identified.
[f] Fresh Weight of fern and volume of diethyl ether used for the extraction (g/mL).

The main volatiles were (*E*)-2-decenal (32.1%), lauric amide (12.4%) and (*E*)-2-heptenal (7.4%). (*E*)-2-decenal, a natural plant and mushroom VOC with a plastic [6a] or oxidized mutton fat [6b] odor is also responsible for the unpleasant scent of "stink bug". (*E*)-2-decenal is the major compound produced by these insects, with (2*E,*4*Z*)-decadienal and (2*E,*4*E*)-decadienal [6c,6d] also detected in *A. capillus-veneris*.

Nineteen compounds were identified for *A. filix-femina* with essentially polyketides (61.5%) and aromatics (29.9%), as well as small amounts of carotenoids (3.8%) and monoterpenes (2.7%) (Table 1). The main volatiles were 2-phenylethanal (24.8%) and 1-octen-3-ol (24.8%). This C_8-derivative, also dominant in *B. spicant*, is well-known to be responsible for the mushroom aroma and usually found in large amounts in various fungi [7a-7g] and plants [7h].

Thirteen and eight compounds were identified from the sterile and fertile fronds of *B. spicant*, respectively (Table 1). Both volatile profiles showed 95% of polyketides and aromatics in quasi equal proportions and very small or negligible amounts of monoterpenes, sesquiterpenes and carotenoids (2-3%). The main volatiles were also 2-phenylethanal (about 45%), with an odor of lilac and hyacinth [7b], and 1-octen-3-ol (43.1 and 37.5%, respectively). Low amounts of benzaldehyde [7a,7f,7g] were detected, mainly in the fertile fronds (4.4%), with only 1.5% in the sterile ones.

Fifteen compounds were identified for *D. filix-mas*, mostly sesquiterpenoids (almost 60%), and polyketides (30.8%); the other groups of volatiles were minor (Table 1). The major volatiles were (*E*)-nerolidol (38.7%) with a floral, woody or fresh bark odor [7a,7c], acylfilicinic acids (16.6%) and 1-octen-3-ol (9.1%). Filicinic acid derivatives, well-known in *Dryopteris* and related genera of Dryopteridaceae, possess various biological activities (antioxidant, antibacterial, antitumor promoting activities), which may lead to therapeutic applications [2d,3b].

The volatile components of *O. limbosperma*, well-known for its lemon smell, were distributed in the five types of VOC (Table 1). The volatile profile of this odorous fern showed logically the highest biodiversity: thirty-one compounds were identified by GC-MS. Eighty percent of the volatiles were issued from the terpenic pathway (monoterpenes 40.3%; sesquiterpenes 19.7%; carotenoids 18.1%), and ten percent from the shikimic or lipidic pathways. The main volatiles were (*E*)-nerolidol (14.7%) and α-terpineol (13.5%), with a lemon odor, β-caryophyllene (5%), with a spicy odor, and other monoterpenes (for example, linalool, pinenes, limonene, and γ-terpinen-7-al), which contribute to the fern's pleasant fragrance [7a]. It was also this fern that had the highest amount of carotenoid-type derivatives (18.1%).

More and more industries are searching for novel resources for natural compounds. This paper demonstrates that ferns can generate a broad spectrum of volatile organic components (VOC). Obviously, these kinds of natural volatiles are promising for various industrial formulations since they may be bioproduced, if required. Indeed ferns are attractive natural sources for research programs and for aroma applications in various industries.

Experimental

Plant material: Fresh aerial parts of ferns were collected from metropolitan France as follows: *Adiantum capillus-veneris* L. (Pteridaceae): 15/11/2009, grown in a pot. *Athyrium filix-femina* (L.) Roth (Woodsiaceae), *Blechnum spicant* (L.) Roth (Blechnaceae) and *Dryopteris filix-mas* (L.) Schott (Dryopteridaceae): 15/07/2009, Gimel-les-Cascades, Corrèze. Sterile fronds (SF) with narrow pinnae and fertile fronds (FF) of *B. spicant* with large pinnae were collected from the same plant at the same time. *Oreopteris limbosperma* (Bellardi ex. All.) J. Holub (Thelypteridaceae): 20/09/2009, Ambrugeat, Corrèze.
The ferns were identified by Dr. Didier Froissard and voucher specimens are deposited at the Laboratory of Botany (Faculty of Pharmacy, Limoges, France).

Extraction: Aerial parts of ferns were cubed and extracted with diethyl ether (Carlo Erba, 6 ppm BHT; Table 1). After maceration for one week, the concentrated organic extracts were directly used for Gas Chromatography-Mass Spectrometry (GC-MS) analysis, as previously described [7g,7h].

GC-MS analyses: GC-MS analyses were carried out using a gas chromatograph-mass spectrometer Shimadzu QP2010plus (Kyoto, Japan) with a potential of 70 eV for ionization by electron impact. The temperature of the transfer line and the ion source were programmed to 250°C and 200°C, respectively. The spectrometer was used in scan mode, from 40 to 300 m/z ratio. Solvent extract analyses were performed using a 30 m x 0.25 mm x 0.25 µm SLB™-5MS (Supelco) fused silica capillary column. The injector temperature was 250°C. The column was temperature programmed as follows: 50°C (hold 2 min.), from 50 to 100°C at 3.3°C/min., from 100 to 140°C at 2.90°C/min., from 140 to 180°C at 2.70°C/min., and finally to 270°C at 10°C/min. The carrier gas was helium with a constant flow rate set close to 1.0 mL/min. VOC were identified by comparison with National Institute of

Standards and Technology Mass Spectral Library [8a], and retention indices as reported in the literature [8b].

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