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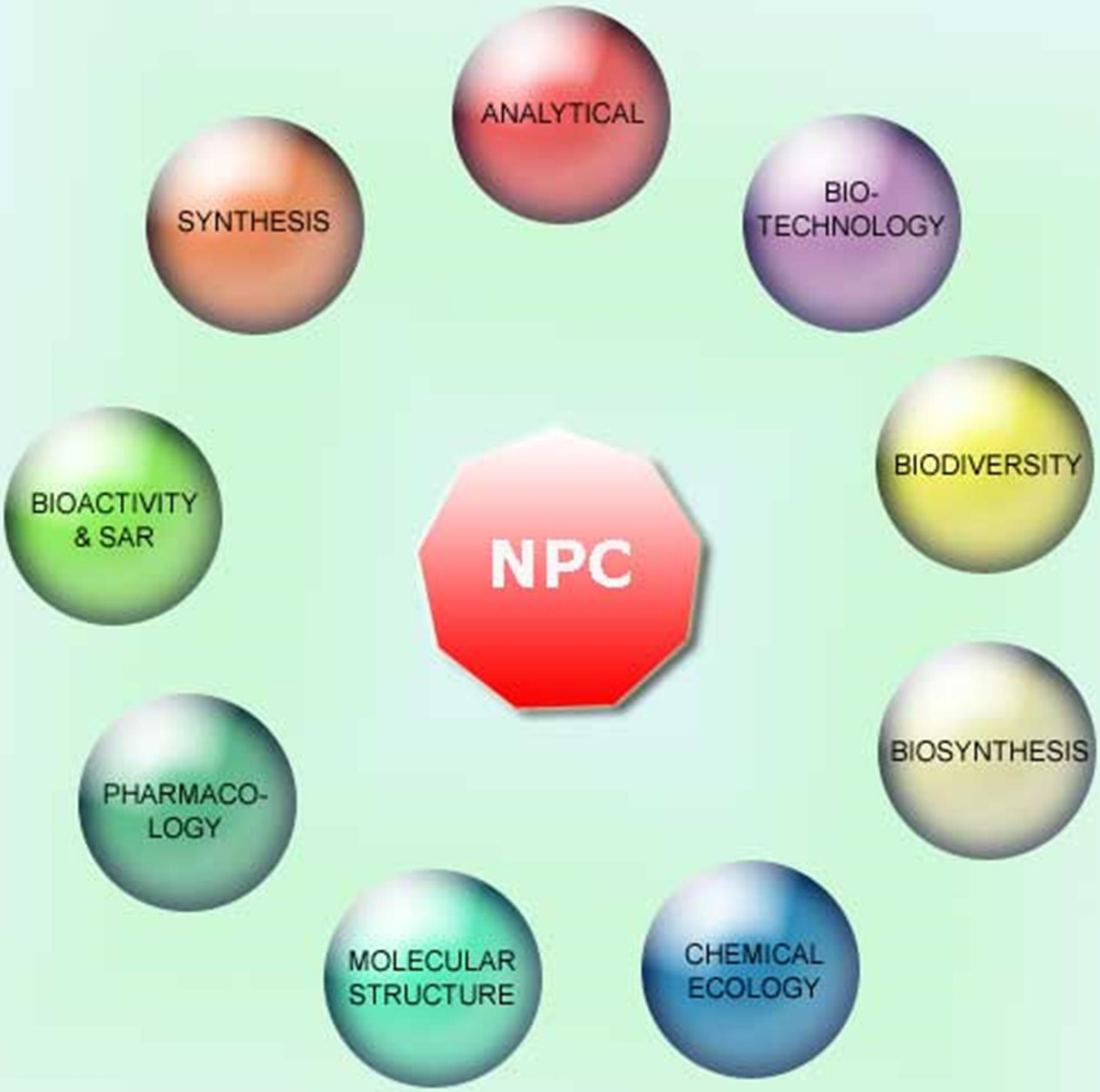

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On the Occasion of his 60th Birthday

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Asplenioideae Species as a Reservoir of Volatile Organic Compounds with Potential Therapeutic Properties

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Twelve French *Asplenioideae* ferns (genera *Asplenium* and subgenera *Ceterach* and *Phyllitis*) were investigated for the first time for volatile organic compounds (VOC) using GC-MS. Sixty-two VOC biosynthesized from the lipidic, shikimic, terpenic and carotenoid pathways were identified. Several VOC profiles can be highlighted from *Asplenium jahandiezii* and *A.* ×*alternifolium* with exclusively lipidic derivatives to *A. onopteris* with an equal ratio of lipidic/shikimic compounds. Very few terpenes as caryophyllene derivatives were identified, but only in *A. obovatum* subsp. *bilotii*. The main odorous lipidic derivatives were (*E*)-2-decenal (waxy and fatty odor), nonanal (aldehydic and waxy odor with a fresh green nuance), (*E*)-2-heptenal (green odor with a fatty note) and 1-octen-3-ol (mushroom-like odor), reported for all species. A few VOC are present in several species in high content, i.e., 9-oxononanoic acid used as a precursor for biopolymers (19% in *A. jahandiezii*), 4-hydroxyacetophenone with a sweet and heavy floral odor (17.1% in *A. onopteris*), and 4-hydroxybenzoic acid used as a precursor in the synthesis of parabens (11.3% in *A. foreziense*). Most of the identified compounds have pharmacological activities, i.e., octanoic acid as antimicrobial, in particular against *Salmonellas,* with fatty and waxy odor (41.1% in *A. petrarchae*), tetradecanoic acid with trypanocidal activity (13.3% in *A. obovatum* subsp. *bilotii*), 4-hydroxybenzoic acid (8.7% in *A. onopteris*) with antimicrobial and anti-aging effects, 3,4-dihydroxybenzaldehyde as an inhibitor of growth of human cancer cells (6.7% in *Ceterach officinarum*), and phenylacetic acid with antifungal and antibacterial activities (5.8% in *A. onopteris*). Propionylfilicinic acid was identified in the twelve species. The broad spectrum of odorous and bioactive VOC identified from the *Asplenium*, *Ceterach* and *Phyllitis* species are indeed of great interest to the cosmetic and food industries.

Keywords: *Asplenium*, *Ceterach*, *Phyllitis*, Volatile Organic Compounds, 9-Oxononanoic acid, 3,4-Dihydroxybenzaldehyde, Antimicrobial, Anti-aging.

Asplenioideae Link is a great and homogeneous subfamily of *Aspleniaceae* Newman. *Asplenium* L. is the major genus with approximately seven hundred subcosmopolitan ferns distributed worldwide, and seventeen species in France. Several subgenera have been separated, such as *Ceterach* (Willd) Vida ex Bir, Fraser-Jenkins & Lovis and *Phyllitis* (Hill) Jermy & Viane [1a-1e].

The scientific name *Asplenium* was given by Pedanius Dioscorides (Roman physician, pharmacologist and botanist of Greek origin) to these plants that are well-known for their medicinal properties to cure the spleen; their common name "spleenwort" derives from the doctrine of signatures. *Aspleniaceae* includes many species reported for various traditional medicinal uses. Leaves and/or rhizomes of *Asplenium adiantum-nigrum* L., *Ceterach officinarum* Willd. (= *A. ceterach* L.), *A. cuneatum* Lam., *A. falcatum* Lam., *A. marinum* L., *A. monanthes* L., *A. nidus* L., *A. ruta-muraria* L., *Phyllitis scolopendrium* (L.) Newman (= *A. scolopendrium* L.) and *A. trichomanes* L. are used against worms, lung afflictions, cough inflammation, hypertension, jaundice, enlarged spleen, intestinal disorder, kidney stones, burns, elephantiasis and ulcers, and as an emetic, depurative, diaphoretic and sedative in traditional medicine [2a-2g]. Recently, antioxidant, antimicrobial and antibacterial properties of *A. ceterach* and *A. nidus* were demonstrated [2h-2i]. Regarding the chemical composition of *Aspleniaceae*, *A. adiantum nigrum, A. fontanum*, *A. foresiense*, *A. incisum*, *A. normale*, *A. obovatum*, *A. ruta-muraria, A. trichomanes* and *Ceterach officinarum* were investigated for their phenolic derivatives [3a-3e].

Very few *Asplenium* are known to have an odor: *A. auritum* Sw. has pleasantly fragrant fronds and *A. lamprophyllum* Carse smells of wintergreen [4a]. Consequently, little is known about the volatile organic compounds (VOC) of these ferns. The terpenoid constituents of *A. scolopendrium* were studied [4b]. In addition, *A. trichomanes* subsp. *trichomanes* was investigated for its volatile profile [4c], which showed mainly polyketides, for example octanoic acid (= caprylic acid; fatty and waxy odor), nonanoic acid (waxy, dairy note), (*E*)-2-decenol (waxy note), (*E*)-2-heptenal (green odor with a fatty note) with globally an oily or waxy odor.

In this new work, fresh aerial parts of twelve French species of *Aspleniaceae* were investigated for their volatile profiles using GC-MS, as reported in the literature for the twenty-three monilophytes previously studied [4c-4f]. Sixty-two components biosynthesized from the shikimic, lipidic, terpenic and carotenoid pathways were identified from the concentrated diethyl ether extracts of the twelve *Aspleniaceae* (Table 1).

The VOC profile of *Ceterach officinarum* is widely dominated by lipidic derivatives (77.4%), in particular (*E*)-2-decenal (10.5%), a natural plant and mushroom VOC with waxy and fatty odor type

Table 1: Percentage of volatile organic compounds[a] in fresh aerial part of *Asplenium*, *Ceterach* and *Phyllitis* species.

Compounds	RI[b]	*Ceterach officinarum*	*Phyllitis scolopendrium*	*Asplenium onopteris*	*A. petrarchae*	*A. jahandiezii*	*A.obovatum ssp. bilotii*	*A. septentrionale*	*A. foreziense*	*A. balearicum*	*A. ruta-muraria*	*A. fontanum*	*A. ×alternifolium*
Lipidic derivatives		**77.4**	**79.3**	**46.9**	**71.3**	**98.0**	**59.4**	**79.9**	**66.0**	**89.9**	**74.5**	**83.6**	**99.3**
Heptanal	**905**					1.2	0.2			1.5	0.6	0.8	3.3
(*E*)-2-Heptenal	**957**	8.6	3.9	5.8	2.8	10.5	3.9	3.1	6.7	13.0	5.6	8.3	8.8
1-Octen-3-ol	**982**	5.7	8.8	5.8	2.0	3.7	4.5	2.7	1.8	7.4	1.7	3.9	6.1
2,3-Octanedione	**986**	0.5	1.9			2.9		0.7		1.5		1.2	3.2
2-Pentylfuran	**991**	1.9	1.7	0.9	0.6	1.9	0.9	1.5	1.8	2.7	1.9	2.6	4.3
Octanal	**1005**	0.2		2.1	0.1	2.2		0.3		1.6	1.3	1.2	1.8
Hexanoic acid	**1008**		1.8		0.4	2.0		3.7			1.3	1.3	0.4
(*Z*)-3-Hexenoic acid	**1017**			1.1	0.1	0.3	0.5	0.7				0.2	1.1
3-Octen-2-one	**1029**	3.1		1.0	0.7	0.5	1.4	2.1		2.2		0.4	4.2
(*E*)-2-Hexenoic acid	**1035**			1.3	0.1	1.8	1.1	2.3			0.9	0.5	2.0
(*E*)-2-Octenal	**1056**	1.1		0.3	0.6	1.8		2.3		1.2	1.5	1.0	1.4
(*E*)-2-Octenol	**1069**	0.5		1.0	0.2	0.5	0.5	1.5		1.7		1.0	1.9
Octanol	**1067**		2.1	0.8	0.4	2.4	0.1			4.0	2.0	1.5	4.1
N I	**1098**	2.3	0.5					3.1					
Nonanal	**1104**	10.0	9.3	2.8	2.8	7.5	7.2	5.0	11.2	13.3	14.0	8.5	4.4
N-Acetylpyrrolidone	**1155**	2.9			0.5	0.7		3.1					
(*E*)-2-Nonenal	**1162**	1.0	1.2		0.4	0.8	0.5	0.7	0.9	0.9	1.3	1.4	1.6
Octanoic acid (Caprylic acid)	**1185**	1.1	2.2	2.8	41.1	5.4	1.8	3.2	7.2	1.2	3.3	3.9	3.2
(*E,E*)-2,4-Nonadienal	**1215**	1.0		0.5	0.2	1.7	0.4	0.7	1.2	0.4	1.1	0.8	0.9
N I	**1228**	0.5				0.4							
N I	**1256**	0.3			0.3				3.3				
(*E*)-2-Decenal	**1261**	10.5	6.5	6.2	4.2	13.1	6.1	10.9	13.0	20.2	13.1	11.5	12.8
2-Decenol	**1265**	0.3	0.5		0.8	2.1	0.9	1.9		2.2	0.6	1.5	1.3
Nonanoic acid	**1285**	3.1	7.3	2.0	0.9	1.4	2.7			6.4	7.8	0.6	2.1
(*E,Z*)-2,4-Decadienal	**1294**		0.8	2.0	1.0	2.3	0.6		5.3	1.0		2.2	1.8
(*E,E*)-2,4-Decadienal	**1319**	2.7	2.0	3.1	1.5	2.2	1.1	3.1		2.0	1.3	3.2	4.1
N I	**1361**				0.5	2.5				1.6	2.6	0.9	0.7
Decanoic acid	**1384**	0.4	0.5		0.2	1.5		1.5	1.5	1.0	2.8	3.9	3.2
9-Oxononanoic acid	**1502**		6.2		2.1	19.0	2.2	3.7	3.8		4.4	4.1	7.0
N I	**1510**		7.9		0.5			4.6				1.5	0.2
Methyl laurate	**1524**						0.7						
Propionylfilicinic acid	**1552**	3.6	5.4	0.7	0.4	0.4	1.2	0.3	1.8	2.8	2.1	1.8	0.4
Dodecanoic acid (Lauric acid)	**1582**		2.0			0.6	0.9	0.2				0.5	
(*E*)-2-Tridecenoic acid	**1687**	5.6						4.8					6.4
N I	**1693**	1.1	2.9	1.1		1.5	0.8	1.5				2.4	0.6
Methyl myristate	**1726**						1.8					1.0	
Tetradecanoic acid	**1778**	4.8	2.8	1.8	1.4	3.0	13.3	3.7	6.5		3.3	7.3	4.2
Hexahydrofarnesylacetone	**1840**	4.8	1.1	2.5	1.2	0.3	2.7	5.6				2.2	
Pentadecanoic acid	**1885**			1.0			1.7	1.5				0.5	1.8
N I	**1917**				2.3								
N I	**1931**				1.0								
Shikimic compounds		**21.3**	**14.1**	**48.0**	**13.5**	**1.8**	**24.4**	**5.8**	**23.4**	**1.6**	**13.5**	**11.6**	**0**
Benzaldehyde	**961**		2.3	1.0	0.4		0.6					1.2	
Benzyl alcohol	**1043**		1.4	1.1		0.8	1.6			1.6	1.3	1.4	
2-Phenylethanal	**1046**				0.7		0.2		1.5				
Benzoic acid	**1195**	1.0	3.6	2.1	4.6		1.7				1.1	0.5	
2,3-Dihydrobenzofuran	**1235**	0.2		2.0			1.1						
2-Amino-4-methoxyphenol	**1246**	2.3		0.7	1.5	1.0	2.7		0.9			1.2	
Phenylacetic acid	**1265**	0.4		5.8			0.7	1.9			5.6		
3,4-Dihydroxystyrene	**1293**	0.2		1.3	0.3		1.8		0.9				
Isovanillin	**1397**				0.1		0.8	0.4	1.8			1.5	
N I	**1415**			2.5			0.4				5.6	3.3	
Coumarin	**1433**							0.1					
4-Hydroxyacetophenone	**1478**			17.1									
Methyl 3-methoxy-4-hydroxybenzoate	**1517**		2.5	2.1	0.2		0.7		2.6				
4-Hydroxybenzoic acid	**1560**	5.8	3.5	8.7	4.2		7.8	0.3	11.3				
3-Methoxy-4-hydroxybenzoic acid	**1568**	4.8	0.8	0.7	1.5		4.4		4.4			2.5	
3,4-Dihydroxybenzaldehyde	**1628**	6.7						3.1					
3- or 4-Chlorocinnamic acid	**1638**			0.9									
3-Phenylpropionic acid	**1757**			1.8									
Terpenic compounds		**0.0**	**0.0**	**0.0**	**5.8**	**0.0**	**2.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0**
Caryophyllene oxide	**1586**						0.8						
β-Caryophyllene alcohol	**1645**						1.6						
N I	**1683**				1.6								
N I	**1706**				1.8								
N I	**1760**				2.4								
Carotenoid derivatives		**0.8**	**5.9**	**4.3**	**9.0**	**0.0**	**13.1**	**13.3**	**9.7**	**7.6**	**11.4**	**4.0**	**0**
α-Ionone	**1435**		2.1		0.2		1.3			2.0			
β-Ionone	**1480**			0.4	0.3		0.2						
Dihydroactinidiolide	**1505**		3.8	0.7	0.2		1.2	2.3	2.6			0.6	
4-Hydroxy-7,8-dihydro-β-ionone	**1633**						1.6			1.2			
4-Hydroxy-β-ionone	**1639**	0.3			4.6		1.9	1.8	1.5	0.6		1.8	
N I	**1642**				0.5		1.8						
3-Oxo-α-ionol	**1647**			1.2	0.7		1.3	0.2	1.8	2.0	2.1		
4-Hydroxy-5,6-epoxyionol	**1675**				2.3		1.7			1.8	7.8		
7,8-Epoxy-β-ionone	**1688**			1.4			1.3	0.5	3.8			0.7	
3-Hydroxy-5,6-epoxy-β-ionone	**1696**	0.2		0.5	0.2		1.1	3.1			1.5	0.9	
3-Oxo-7,8-dihydro-α-ionol	**1710**							3.1					
N I	**1798**	0.3						2.3					

[a] Relative percentage of the VOC based on the GC-MS chromatographic area; [b] RI = Retention Indices on SLB^{TM}-5MS column (Supelco) ; [c] NI = Not identified.

[5a-5c], nonanal (10%), with an aldehydic and waxy odor type and a fresh green nuance [5d] and recently reported to attract *Culex* mosquitoes [5e], (*E*)-2-heptenal (8.6%), with a green vegetable-like odor with fatty undertone, 1-octen-3-ol (5.7%), previously reported for mushrooms [5f, 5g], ferns [4c, 4d, 4f], horsetails [4e] and Angiosperms [5h], (*E*)-2-tridecenoic acid (5.6%) and hexahydrofarnesyl acetone (4.8%), which are used as flavor and fragrance agents. *C. officinarum* also contains several aromatic compounds (24.9%), i.e., 3,4-dihydroxybenzaldehyde (= protocatechuic aldehyde; 6.7%), a precursor of vanillin using biotechnology and recently reported to inhibit the growth of human cancer cells [5i], and 4-hydroxybenzoic acid (5.8%).

Phyllitis scolopendrium demonstrated a broad spectrum of VOC from the lipidic pathway (79.3%) including nonanal (9.3%), 1-octen-3-ol (8.8%), a C_8-derivative responsible for the mushroom-like aroma [6a], nonanoic acid (7.3%), a C_9-derivative with waxy dairy note, (*E*)-2-decenal (6.5%), 9-oxononanoic acid (6.2%), (*E*)-2-heptenal (3.9%), (*E*,*Z*)-dodecadienal (2.9%), tetradecanoic acid (myristic acid; 2.8%), octanoic acid (2.2%) and octanol (2.1%). It should be noted that propionylfilicinic acid (5.4%), a filicinic derivative of biological interest to the pharmaceutical, cosmetic and hygiene industries [4f], was identified in *P. scolopendrium*, as well as in the other ferns (Table 1). Benzoic acid, 4-hydroxybenzoic acid, methyl 3-methoxy-4-hydroxybenzoate (= methyl vanillate), with a warm spicy vanilla odor, and benzaldehyde, with a bitter almond odor [6b-6e], complete the aromatic profile (14.1%). Dihydroactinidiolide (3.8%), with a fruity odor [5d], and α-ionone (2.1%), with a floral smell, represent the major VOC of the carotenoid profile.

The VOC fraction of *Asplenium onopteris* is based on equal contents of shikimic and lipidic derivatives (48% and 46.9%, respectively). Twenty-one lipidic derivatives were identified, i.e., (*E*)-2-decenal (6.2%), (*E*)-2-heptenal (5.8%), 1-octen-3-ol (5.8%), (*E*,*E*)-2,4-decadienal (3.1%) with aldehyde, oily and fatty odor, and which is a potent plant nematicidal agent [7e]. The major aromatic compounds are 4-hydroxyacetophenone (17.1%), an aromatic ketone with a sweet, and heavy floral odor used for the synthesis of pharmaceuticals, agrochemicals, flavor and fragrances, and 4-hydroxybenzoic acid (8.7%), used in cosmetic and ophthalmologic industries as a precursor in the synthesis of parabens, and which also shows antimicrobial activity and an anti-aging effect [7a, 7b], as well as a hypoglycemic property in rats [7c]. Phenylacetic acid (5.8%), used in the flavor industry for its honey-like odor, is a powerful antifungal and antibacterial agent, which is also produced by ants [7d]. Benzoic acid (2.1%), methyl 3-methoxy-4-hydroxybenzoate (2.1%), 2,3-dihydrobenzofuran (2%), and 3-phenylpropionic acid (= dihydrocinnamic acid) with a sweet, floral scent (1.8%), complete the shikimic derivatives. 7,8-Epoxy-β-ionone (1.4%) and 3-oxo-α-ionol (1.2%), with spicy odor, represent the main carotenoid derivatives (4.3%).

Asplenium petrarchae is a small and thermophile fern growing only on the sunny calcareous rocks of the Mediterranean coast. Its VOC profile is dominated by twenty-seven identified lipidic compounds (71.3%), with a very high level of octanoic acid (41.1%) with a fatty, soapy odor. This fatty acid is an antimicrobial ingredient used in particular against *Salmonella* species in cosmetics and foods; it presents the advantage of being less toxic than most other antimicrobial agents and does not affect beneficial organisms [8a-8c]. (*E*)-2-Decenal, (*E*)-2-heptenal, nonanal, 9-oxononanoic acid and 1-octen-3-ol were also identified as lipidic derivatives from *A. petrarchae*. The volatile fraction contains 13.5% of aromatic compounds, i.e., benzoic acid (4.6%), used as a food additive for its preservative property, as well as 9% of carotenoid derivatives, i.e., 4-hydroxy-β-ionone (4.6%) and 4-hydroxy-5,6-epoxyionol (2.3%).

Asplenium jahandiezii is a small and protected fern only located in the canyon of Verdon (France) [1a]. Its VOC spectrum is almost exclusively dominated by twenty-nine identified lipidic compounds (98%). The major volatile is 9-oxononanoic acid (19%), an interesting VOC as a renewable resource of a precursor for biopolymers [9a]. It was recently discovered that 9-oxononanoic acid stimulates the activity of phospholipase A_2, the key enzyme of the arachidonate cascade [9b]. The other lipidic derivatives with various odorous or pharmacological properties were (*E*)-2-decenal (13.1%), (*E*)-2-heptenal (10.5%), nonanal (7.2%), octanoic acid (5.4%), 1-octen-3-ol (3.7%) and tetradecanoic acid (3%). The minor odorous and bioactive lipidic derivatives were 2,3-octanedione (2.9%), with a dill cooked broccoli buttery odor [9c], and octanol (2.4%), with a sweetish odor and toxic to *Colletotrichum gloeosporioides, an endophytic plant pathogen* [9d], as well as (*E*,*Z*)-2,4-decadienal (2.3%), with a fatty, green and waxy odor, (*E*,*E*)-2,4-decadienal (2.2%), octanal (2.2%), with an aldehyde, fatty, orange peel, pungent and soapy flavor, (*E*)-2-decenol (2.1%), with a waxy, citrus and fresh note, and hexanoic acid (2%).

Asplenium obovatum subsp. *bilotii* has a broad spectrum of VOC with a high content of lipidic derivatives (59.4%) including 13.3% of tetradecanoic acid. This saturated fatty acid is one of the lipidic constituents of the cellular membrane of Eucaryotes. It is used as a lubricant and in the manufacture of flavors, pharmaceuticals, soaps and cosmetics, and has a trypanocidal activity, which was highlighted against *Trypanosoma evansi* two decades ago [10a]. This acid was identified in eleven of the studied ferns (Table 1). Nonanal (7.2%), (*E*)-2-decenal (6.1%), 1-octen-3-ol (4.5%), (*E*)-2-heptenal (3.9%), nonanoic acid (2.7%), hexahydrofarnesylacetone (2.7%), 9-oxononanoic acid (2.2%), and octanoic acid (1.8%) were also identified from the organic extract. Aromatic compounds represent 25.5% of the VOC content of *A. obovatum* subsp. *bilotii* with 4-hydroxybenzoic acid (7.8%), 3-methoxy-4-hydroxybenzoic acid (4.4%), 2-amino-4-methoxyphenol (2.7%) as well as 3,4-dihydroxystyrene (1.8%), an inhibitor of phenylalanine hydroxylase used for the production of experimental phenylketonuria [10b]. Finally, the carotenoids (13.1%) are dominated by 4-hydroxy-β-ionone (1.9%), 4-hydroxy-5,6-epoxyionol (1.7%) and 4-hydroxy-7,8-dihydro-β-ionone (1.6%), which is well-known as a key odorant in yellow wines [10c]. Two sesquiterpenes, i.e., caryophyllene alcohol and caryophyllene oxide, complete the VOC composition of *A. obovatum* subsp. *bilotii*. This oxygenated terpenoid, which is a flavoring agent used in cosmetics and food, also displays biological activities (anti-inflammatory, antifungal, skin enhancing and anti-carcinogenic) [10d].

The six others *Asplenium* species also investigated for their VOC content for the first time were *A. septentrionale, A. foreziense*, *A. balearicum*, *A. ruta-muraria, A. fontanum* and *Asplenium ×alternifolium*.

Most of their volatile constituents were mentioned above for the six first detailed VOC fern profiles. The main VOCs identified for *A. septentrionale* were two aldehydes, i.e., (*E*)-2-decenal (10.9%) and nonanal (5%). In addition *A. septentrionale* contained the highest concentration of hexahydrofarnesylacetone (5.6%), hexanoic acid (3.7%), a fatty acid found in animal oils with a fatty, waxy or cheesy flavor, *N*-acetylpyrrolidone (3.1%) and 3-hydroxy-5,6-epoxy-β-ionone (3.1%). *A. foreziense* also produced a high level of aldehydes (nonanal, (*E*)-2-decenal...) and the highest proportions of 4-hydroxybenzoic acid (11.3%), (*E*,*Z*)-2,4-decadienal (5.3%), with a fatty, green and waxy odor, and 7,8-epoxy-β-ionone (3,8%) when compared with the other *Asplenioideae* (Table 1).

The highest percentages of (*E*)-2-decenal (20.2%), with a waxy fatty odor, and (*E*)-2-heptenal (13%), with a green fatty note, were found in *A*. *balearicum*, which also produced a high level of nonanal (13.3%), with an aldehydic and green scent. The volatile fraction of *A*. *ruta-muraria* contained 13.5% of shikimic compounds, i.e., phenylacetic acid (5.6%), as well as 11.4% of carotenoid derivatives, i.e., 4-hydroxy-5,6-epoxyionol (7.8%). Its major lipid derivatives (74.5%) were nonanal (14%), (*E*)-2-decenal (13.1%), nonanoic acid (7.8%) and (*E*)-2-heptenal (5.6%), already found in the other *Asplenium* species. The global VOC profile of this species (lipidic derivatives / shikimic derivatives ratio) can be compared with those of *P*. *scolopendrium* and *A*. *petrarchae*.

The major VOC of *A*. *fontanum* were three aldehydes, i.e., (*E*)-2-decenal, nonanal and (*E*)-2-heptenal, as well as tetradecanoic acid, already found in most of the *Asplenium* species. The *Asplenium* ×*alternifolium* VOC profile was close to that of *A*. *jahandiezii*, with uniquely lipidic derivatives (99.3%) and no shikimic compounds. The major lipidic compounds of *A*. ×*alternifolium* were (*E*)-2-decenal (12.8%), (*E*)-2-heptenal (8.8%), 9-oxononanoic acid (7%), (*E*)-2-tridecenoic acid (6.4%) and 1-octen-3-ol (6.1%), also produced by *C*. *officinarum* and *A*. *septentrionale*. Compared with the eleven other species investigated (Table 1), *A*. ×*alternifolium* contained the highest amount of 2-pentylfuran (4.3%), 3-octen-2-one (4.2%; earthy spicy herbal with mushroom nuances), octanol (4.1%), (*E*,*E*)-2,4-decadienal (4.1%), heptanal (3.3%), and 2,3-octanedione (3.2%).

Conclusion: The twelve French ferns from the family *Aspleniaceae* investigated for VOC mainly contain derivatives of lipidic origin. Several VOC profiles can be highlighted from *A*. ×*alternifolium*, with exclusively lipidic derivatives, to *A*. *onopteris*, with an equal ratio of lipidic / shikimic compounds. Minor volatile components were identified from the shikimic pathway and very few terpenes, as caryophyllene derivatives, were found. Aldehydes, i.e., (*E*)-2-heptenal, nonanal and (*E*)-2-decenal, were often identified from the species, as well as acids (octanoic, nonanoic, 9-oxononanoic and tetradecanoic acids). 1-Octen-3-ol was found in all samples, as in most of the previously reported ferns [4c-4f]. It should be mentioned that propionylfilicinic acid (from 0.3% to 5.4% of the volatile fraction) was identified in the twelve studied *Aspleniaceae*. This chemical trait must be noticed because many other ferns previously studied do not produce these volatile phloroglucinol derivatives; filicinic acids were mainly found in large amounts in *Dryopteris* [4f]. The broad spectrum of odorous and bioactive VOC identified from the twelve *Asplenium*, *Ceterach* and *Phyllitis* species from France are indeed of great interest for their various odorous and pharmacological properties that could be of interest to the cosmetics and food industries. Further investigations should be carried out through plant tissue cultures.

Experimental

Plant material: Fresh aerial parts of the ferns were collected as follows: *C*. *officinarum*: 13/07/2010, Limoges; *P*. *scolopendrium*: 13/07/2010, Limoges; *A*. *onopteris*: 05/04/2010, Le Pradet (Var); *A*. *petrarchae*: 15/04/2013, Toulon; *A*. *jahandiezii*: 26/07/2013, Aiguine (Var); *A*. *obovatum* subsp. *bilotii*: 14/04/2010, Le Lavandou (Var); *A*. *septentrionale*: 30/05/2011: Saint-Etienne-Vallée-Française (Lozère); *A*. *foreziense*: 20/09/2009, Meymac (Corrèze); *A*. *balearicum*: 16/07/2013, Porquerolle Island (Var); *A*. *ruta-muraria*: 13/07/2010, Limoges; *A*. *fontanum*: 20/07/2010 Plan d'Aups Sainte Baume (Var); *Asplenium* ×alternifolium: 30/05/2011: Saint-Etienne-Vallée-Française (Lozère). Authorization of harvest of the protected *Aspleniaceae*: 80-2013/06. Voucher specimens are deposited at the Laboratory of Botany (Faculty of Pharmacy, Limoges, France).

Plant part and GC-MS analyses: Fresh aerial parts of *Asplenium, Ceterach* and *Phyllitis* species were cubed and extracted with diethyl ether (Carlo Erba, 6 ppm BHT). After 1 week of maceration at room temperature, the concentrated organic extracts were used for gas chromatography mass spectrometry (GC-MS) analyses, as reported in the literature [4c-4f]. The main volatile components were identified by comparison with the National Institute of Standards and Technology Mass Spectral Library [10a-10b]. Internal standards (*n*-alkanes) were used as reference points in the calculation of relative retention indices. The analyses were performed at the Platform for Chemical Analyses in Ecology of the "SFR 119 Montpellier Environnement Biodiversité".

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Accounts/Reivews

Natural Product Communications
2015
Volume 10, Number 6

Contents

Continued inside backcover