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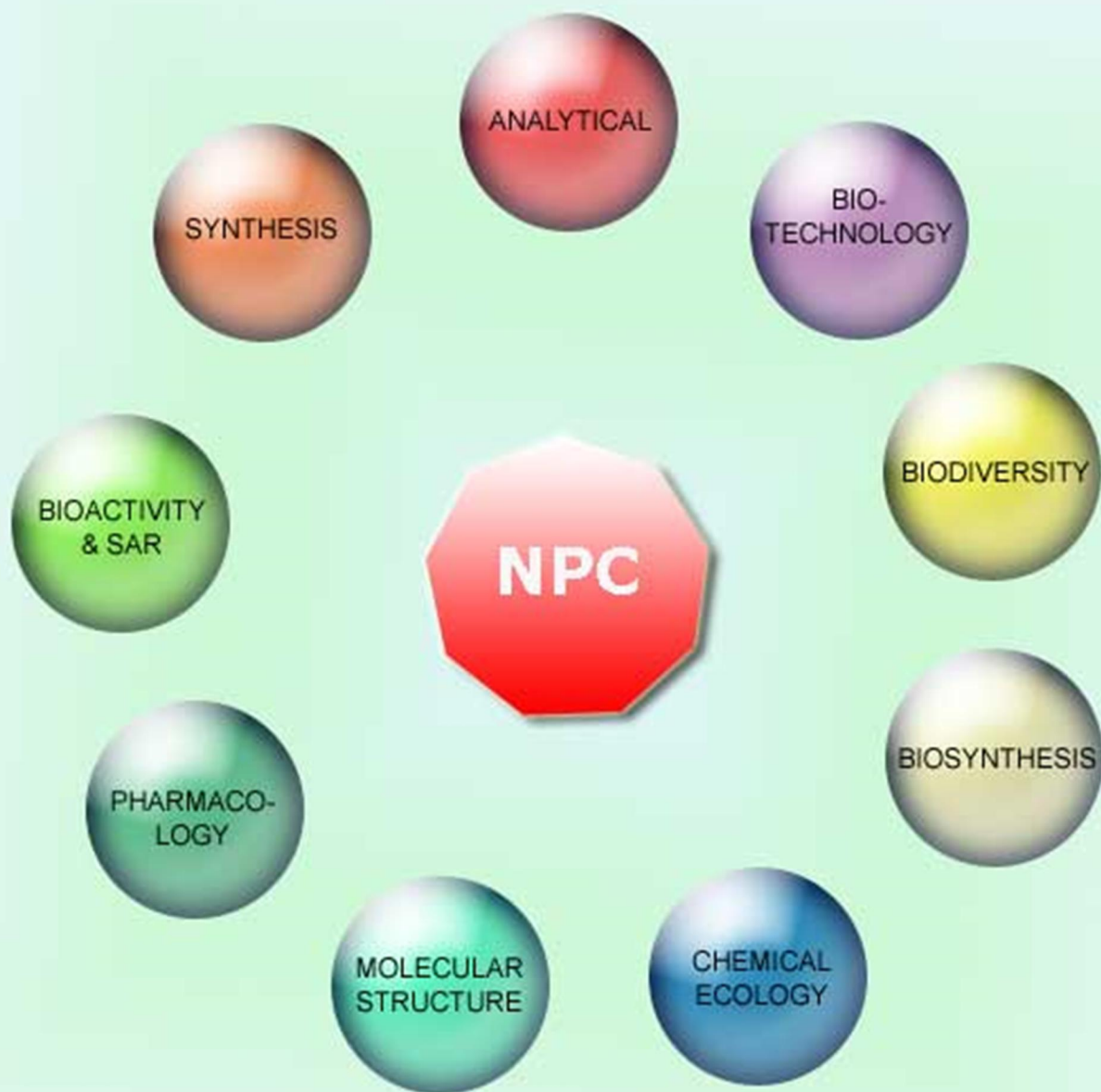
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## Volatile Organic Compounds of six French *Dryopteris* Species: Natural Odorous and Bioactive Resources

Didier Froissard<sup>a</sup>, Sylvie Rapior<sup>b</sup>, Jean-Marie Bessi re<sup>c</sup>, Alain Fruchier<sup>d</sup>, Bruno Buatois<sup>c</sup> and Fran oise Fons<sup>b\*</sup>

<sup>a</sup>Laboratoire de Botanique, Facult  de Pharmacie de Limoges, 2 rue du Docteur Raymond Marcland, F-87025 Limoges Cedex, France

<sup>b</sup>Laboratoire de Botanique, Phytochimie et Mycologie, Facult  de Pharmacie (Universit  Montpellier 1), UMR 5175 CEFE, B.P. 14 491, 15 avenue Charles Flahault, F-34093 Montpellier Cedex 5, France

<sup>c</sup>UMR 5175 CEFE, 1919 Route de Mende, F-34293 Montpellier Cedex 5, France

<sup>d</sup>ENSCM, UMR 5253, 8 Rue de l' cole Normale, F-34296 Montpellier Cedex 5, France

didier.froissard@unilim.fr

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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 D ll) 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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<b>Antiplatelet Aggregation Effects of Phenanthrenes from <i>Calanthe arisanensis</i></b> Chia-Lin Lee, Ming-Hon Yen, Fang-Rong Chang, Chin-Chung Wu and Yang-Chang Wu	83
<b><i>In Vivo</i> Anti-inflammatory Activity of Some Naturally Occurring <i>O</i>- and <i>N</i>-Prenyl Secondary Metabolites</b> Francesco Epifano, Salvatore Genovese, Serena Fiorito, Roberto della Loggia, Aurelia Tubaro and Silvio Sosa	85
<b>Phomopsolides and Related Compounds from the Alga-associated Fungus, <i>Penicillium clavigerum</i></b> Andrea A. Stierle, Donald B. Stierle, Grant G. Mitman, Shea Snyder, Christophe Antezak and Hakim Djaballah	87
<b>Qualitative Identification of Dibenzoylmethane in Licorice Root (<i>Glycyrrhiza glabra</i>) using Gas Chromatography-Triple Quadrupole Mass Spectrometry</b> Marisela D. Mancia, Michelle E. Reid, Evan S. DuBose, James A. Campbell and Kimberly M. Jackson	91
<b>Anti-<i>L. donovani</i> Activity in Macrophage/Amastigote Model of Palmarumycin CP<sub>18</sub> and its Large Scale Production</b> Humberto E. Ortega, Eliane de Morais Teixeira, Ana Rabello, Sarah Higginbotham and Luis Cubilla-Rios	95
<b>Medelamine C, A New <math>\omega</math>-Hydroxy Alkylamine Derivative from Endophytic <i>Streptomyces</i> sp. YIM 66142</b> Ju-Cheng Zhang, Ya-Bin Yang, Hao Zhou, Tian-Feng Peng, Fang-Fang Yang, Li-Hua Xu and Zhong-Tao Ding	99
<b>Enzyme-treated <i>Asparagus officinalis</i> Extract Shows Neuroprotective Effects and Attenuates Cognitive Impairment in Senescence-accelerated Mice</b> Takuya Sakurai, Tomohiro Ito, Koji Wakame, Kentaro Kitadate, Takashi Arai, Junetsu Ogasawara, Takako Kizaki, Shogo Sato, Yoshinaga Ishibashi, Tomonori Fujiwara, Kimio Akagawa, Hitoshi Ishida and Hideki Ohno	101
<b>Anticancer Activity of Binary Toxins from <i>Lysinibacillus sphaericus</i> IAB872 against Human Lung Cancer Cell Line A549</b> Wenjuan Luo, Cuicui Liu, Ruijuan Zhang, Jianwei He and Bei Han	107
<b>The Use of Cycleave PCR for the Differentiation of the Rejuvenating Herb Species <i>Pueraria candollei</i> (White Kwao Khruea), <i>Butea superba</i> (Red Kwao Khruea), and <i>Mucuna macrocarpa</i> (Black Kwao Khruea), and the Simultaneous Detection of Multiple DNA Targets in a DNA Admixture</b> Suchaya Wiriyakarun, Shu Zhu, Katsuko Komatsu and Suchada Sukrong	111
<b>Chemical Compositions and Antimicrobial Activity of the Essential Oils of <i>Hornstedtia havilandii</i> (Zingiberaceae)</b> Siti Erneyanti Hashim, Hasnah Mohd Sirat and Khong Heng Yen	119
<b>Chemical Composition, Antioxidant and Antimicrobial Activity of Essential Oil and Extracts of <i>Tragopogon graminifolius</i>, a Medicinal Herb from Iran</b> Mohammad Hosein Farzaei, Roja Rahimi, Farideh Attar, Farideh Siavoshi, Parastoo Saniee, Mannan Hajimahmoodi, Tahmineh Mirnezami and Mahnaz Khanavi	121
<b>Antinociceptive and Anti-edematous Activities of the Essential Oils of Two Balkan Endemic <i>Laserpitium</i> Species</b> Višnja Popović, Silvana Petrović, Maja Tomić, Radica Stepanović-Petrović, Ana Micov, Milica Pavlović-Drobac, Maria Couladis and Marjan Niketić	125
<b>Chemical Composition of the Essential Oil from <i>Croton kimosorum</i>, an Endemic Species to Madagascar</b> Delphin J. R. Rabehaja, Harilala Ihandriharison, Panja A. R. Ramanolaina, Rakotonirina Benja, Suzanne Ratsimamanga-Urverg, Ange Bighelli, Joseph Casanova and Félix Tomi	129
<b>Intraspecific Variability of the Essential Oil of <i>Cladanthus mixtus</i> from Morocco</b> Anass Elouaddari, Abdelaziz El Amrani, Jamal JamalEddine, José G. Barroso, Luis G. Pedro and Ana Cristina Figueiredo	133
<b>Volatile Organic Compounds of six French <i>Dryopteris</i> Species: Natural Odorous and Bioactive Resources</b> Didier Froissard, Sylvie Rapior, Jean-Marie Bessière, Alain Fruchier, Bruno Buatois and Françoise Fons	137
<b>Essential Oil Compositions of Two Populations of <i>Salvia samuelssonii</i> Growing in Different Biogeographical Regions of Jordan</b> Ammar Bader, Pier Luigi Cioni, Nunziatina De Tommasi and Guido Flamini	141



# Natural Product Communications

## 2014

Volume 9, Number 1

### Contents

<u>Original Paper</u>	<u>Page</u>
<b>New Guaian-type Sesquiterpene from <i>Wikstroemia indica</i></b> Mamoru Kato, Yu-Min He, Dya Fita Dibwe, Feng Li, Suresh Awale, Shigetoshi Kadota and Yasuhiro Tezuka	1
<b>Differences in the Chemical Composition of <i>Arnica montana</i> Flowers from Wild Populations of North Italy</b> Maria Clauser, Nicola Aiello, Fabrizio Scartezzini, Gabriella Innocenti and Stefano Dall'Acqua	3
<b>A New Dolabellane Diterpenoid and a Sesquignan from <i>Aglaia odorata</i> var. <i>microphyllina</i></b> Shuai Liu, Wei Yang, Shou-Bai Liu, Hui Wang, Zhi-Kai Guo, Yan-Bo Zeng, Wen-Hua Dong, Wen-Li Mei and Hao-Fu Dai	7
<b>New Diterpenes from <i>Azorella spinosa</i></b> Luis Astudillo, Margarita Gutiérrez, Luisa Quesada, Aurelio San-Martín, Luis Espinoza and Patricio Peñailillo	9
<b>A New Diterpenoid from the Aerial Parts of <i>Andrographis paniculata</i></b> Chun-Hua Wang, Wen Li, Rui-Xia Qiu, Miao-Miao Jiang and Guo-Qiang Li	13
<b>Isolation of a New Anti-inflammatory 20, 21, 22, 23, 24, 25, 26, 27-Octanorecurbitacin-type Triterpene from <i>Ibervillea sonorae</i></b> Angel Jardón-Delgado, Gil Alfonso Magos-Guerrero and Mariano Martínez-Vázquez	15
<b>Determination of Triterpenic Acids and Screening for Valuable Secondary Metabolites in <i>Salvia</i> sp. Suspension Cultures</b> Sibylle Kümmitz, Christiane Haas, Atanas I. Pavlov, Doris Geib, Roland Ulber, Thomas Bley and Juliane Steingroewer	17
<b>Inhibitory Effect of the Plant <i>Clusia fluminensis</i> against Biological Activities of <i>Bothrops jararaca</i> Snake Venom</b> Eduardo Coriolano de Oliveira, Maria Carolina Anholeti, Thaisa Francielle Domingos, Camila Nunes Faioli, Eladio Flores Sanchez, Selma Ribeiro de Paiva and André Lopes Fuly	21
<b>Chiral Resolution and Absolute Configuration of 3<math>\alpha</math>,6<math>\beta</math>-Dicinnamoyloxytropene and 3<math>\alpha</math>,6<math>\beta</math>-Di(1-ethyl-1<i>H</i>-pyrrol-2-ylcarbonyloxy)tropene, Constituents of <i>Erythroxylum</i> Species</b> Marcelo A. Muñoz, Solange Arriagada and Pedro Joseph-Nathan	27
<b>Aporphine Alkaloids of <i>Cinnamomum mollissimum</i> and their Bioactivities</b> Fatin Fasahah Masnon, Najmah PS Hassan and Farediah Ahmad	31
<b>Antifungal Activity of Metabolites from the Marine Sponges <i>Amphimedon</i> sp. and <i>Monanchora arbuscula</i> against <i>Aspergillus flavus</i> Strains Isolated from Peanuts (<i>Arachis hypogaea</i>)</b> Cynthia Arevabini, Yasmin D. Crivellini, Mariana H. de Abreu, Tamires A. Bitencourt, Mário F. C. Santos, Roberto G. S. Berlinck, Eduardo Hajdu, René O. Belebóni, Ana L. Fachin and Mozart Marins	33
<b>Synthesis of Sepiapterin-C via Hydrolysis of 6-Ethynylpteridine</b> Winston Nxumalo and Andrew Dinsmore	37
<b>Flavonoids Produced by Tissue Culture of <i>Dracaena cambodiana</i></b> Hui Wang, Guanyong Luo, Jiayuan Wang, Haiyan Shen, Ying Luo, Haofu Dai and Wenli Mei	39
<b>Determination of Catechins from <i>Elephantorrhiza elephantina</i> and <i>Pentanisia prunelloides</i> using Voltammetry and UV spectroscopy</b> Smart J. Mpofo, Omotayo A. Arotiba, Lerato Hlekelele, Derek T. Ndinteh and Rui W.M. Krause	41
<b>In vitro Antioxidant Activity, Phenolic Compounds and Protective Effect against DNA Damage Provided by Leaves, Stems and Flowers of <i>Portulaca oleracea</i> (Purslane)</b> Rúben Silva and Isabel S. Carvalho	45
<b>In Vitro Antiviral Activity of a Series of Wild Berry Fruit Extracts against Representatives of <i>Picornia</i>-, <i>Orthomyxo</i>- and <i>Paramyxoviridae</i></b> Lubomira Nikolaeva-Glomb, Luchia Mukova, Nadya Nikolova, Ilian Badjakov, Ivayla Dincheva, Violeta Kondakova, Lyuba Doumanova and Angel S. Galabov	51
<b>Induction of Apoptosis and Cell Cycle Arrest in Human Colon Carcinoma Cells by <i>Corema album</i> Leaves</b> Antonio J. León-González, Margaret M. Manson, Miguel López-Lázaro, Inmaculada Navarro and Carmen Martín-Cordero	55
<b>How to Deal with Nomenclatorial Ambiguities of Trivial Names for Natural Products? – A Clarifying Case Study Exemplified for "Corymbosin"</b> Vatsavaya Ramabharathi and Wolfgang Schuehly	57
<b>Chromatographic Analysis and Antioxidant Capacity of <i>Tabernaemontana catharinensis</i></b> Aline A. Boligon, Mariana Piana, Thiago G. Schawnz, Romaiana P. Pereira, João B. T. Rocha and Margareth L. Athayde	61
<b>Simultaneous Determination of 13 Chemical Marker Compounds in Gwakhyangjeonggi-san, a Herbal Formula, with Validated Analytical Methods</b> Jung-Hoon Kim, Hyeun-Kyoo Shin and Chang-Seob Seo	65
<b>Single Crystal X-ray Diffraction, Spectroscopic and Mass Spectrometric Studies of Furanocoumarin Peucedanin</b> Magdalena Bartnik, Marta Arczewska, Anna A. Hoser, Tomasz Mroczek, Daniel M. Kamiński, Kazimierz Głowniak, Mariusz Gagoś and Krzysztof Woźniak	71
<b>8-Hydroxycudraxanthone G Suppresses IL-8 Production in SP-C1 Tongue Cancer Cells</b> Arlette S. Setiawan, Roosje R. Owen, Supriatno, Willyanti Soewondo, Sidik and Unang Supratman	75
<b>Antiausterity Activity of Arctigenin Enantiomers: Importance of (2<i>R</i>,3<i>R</i>)-Absolute Configuration</b> Suresh Awale, Mamoru Kato, Dya Fita Dibwe, Feng Li, Chika Miyoshi, Hiroyasu Esumi, Shigetoshi Kadota, and Yasuhiro Tezuka	79

Continued inside backcover