Chemical Diversity of Podocarpaceae in New Caledonia: Essential Oils from Leaves of *Dacrydium*, *Falcatifolium*, and *Acmopyle* Species

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Plant secondary metabolites can be useful chemosystematic markers to distinguish species at different taxonomy levels. For example, sesquiterpenes and diterpenes show specific distribution patterns within conifers and so provide especially precious information about the diversity and evolutionary relationships of this group. The aim of the present study was to provide a first insight into the terpene diversity of endemic Podocarpaceae from New Caledonia. The leaf essential oils of *Dacrydium araucarioides* Brongn. & Gris, *Dacrydium balansae* Brongn. & Gris, *Dacrydium guillauminii* J.Buchholz, *Dacrydium lycopodioides* Brongn. & Gris, *Falcatifolium taxoides* (Brongn. & Gris) de Laub., and *Acmopyle pancheri* (Brongn. & Gris) Pilg. from New Caledonia were characterized by GC/FID and GC/MS analyses, and the chemotaxonomic relationships of these species were determined by comparison of their terpene compositions. Cluster analysis based on the biosynthetic origin of their volatile terpenes led to the description of three distinct groups of essential oils and showed close relationships between those of *D. araucarioides* and *D. balansae* as well as between those of *A. pancheri* and *F. taxoides*.

Introduction. – The Podocarpaceae are an ancient family of conifers of Gondwanan origin, dating to the Triassic – Jurassic period. The family comprises 18 genera and 173 species, essentially distributed in tropical and subtropical regions of the southern hemisphere [1]. Its genus richness is higher in the Malesian region and in New Caledonia, where a distinctive conifer flora has evolved on account of its geological history and its isolation in the southwestern Pacific Ocean. The 20 endemic species of Podocarpaceae in New Caledonia belong to eight genera: *Podocarpus*, *Dacrydium*, *Retrophyllum*, *Falcatifolium*, *Dacrycarpus*, *Acmopyle*, *Prumnopitys*, and *Parasitaxus* [2][3].

Recent attention has been paid to the Podocarpaceae family, which has been studied at the species level on the basis of molecular, biogeographical, morphological, and anatomical data [4][5]. Analyses of the anatomy, embryology, and chemistry allowed the classification of the Podocarpaceae by proposing two groups for this family: *Podocarpus sensu latissimo* (*sl*) and *Dacrydium sl*, each including several subsections [6]. An analysis of the family based on *rbc*L (ribulose bisphosphate carboxylase large chain) sequences helped to identify three major clades: Podocarpoid, Dacrydioid, and Prumnopityoid [7]. More recently, the study of *Knopf et al.* [5], also based on the analysis of *rbc*L sequences, classified the species of the *Dacrydium* group in two subclades: the Melanesian subclade and the New Caledonian subclade,

themselves included in the Dacrydioid clade. Species of the genus *Falcatifolium* form a monophyletic group and appear very close to *Dacrydium* in a phylogenetic perspective. However, assumptions obtained from these phylogenetic studies were not consistent with those based on morphology and geographical distribution.

Secondary metabolites synthetized by plants can be useful chemosystematic markers to distinguish species at different taxonomy levels. Terpene compounds are derived from precursors that can follow many biosynthetic pathways; a first classification can be achieved according to the predominance of sesquiterpenes or diterpenes in the essential oils; a second, more accurate classification may be proposed by comparing the structures of these major terpenes and their biosynthetic pathways [8]. Sesquiterpenes are the most important class of terpene compounds, with more than 2500 compounds and 120 structures [9]. Diterpenes are the second largest class with over 2200 compounds and 130 types of skeletons [10]. These terpenoid compounds show specific distribution patterns within conifers and so provide especially precious information about the diversity and evolutionary relationships of this group [9]. Furthermore, terpenes keep the molecular structure of their biochemical predecessor during diagenesis and, thus, can be used to identify the origin of fossil samples [11].

Regarding the conifer families, Pinaceae and Cupressaceae have been well investigated for their terpene composition and distribution, but Podocarpaceae remain less documented. The chemical compositions of the essential oils from a number of species of *Podocarpus sl* were characterized using GC/MS analysis [12].

Early reports on the foliar volatile terpenes of *Dacrydium* species from New Zealand and Fiji have been previously published. *D. cupressinum*, the New Zealand rimu tree, contained diterpenes, mainly rimuene (**1**), phyllocladene (**2**), and lauren-1-ene [13]. Several sesquiterpenes, as longifolene, β-caryophyllene (**3**), and caryophyllene oxide, were also identified in this species [14]. The major foliar volatiles of the two Fijian endemic *Dacrydium* species were α- and β-pinene, β-caryophyllene, and rimuene for *D. nidulum* var. *nidulum* and α-pinene, camphene, and rimuene for *D. nausoriense* [15]. Other phytochemical studies of *D. araucarioides* and *D. balansae*, focusing on the isolation of biflavonoids, sterols, and stilbene derivatives, have been previously performed [16–18].

The aim of the present study was to provide a first insight into the terpene diversity of endemic Podocarpaceae from New Caledonia belonging to the genera *Dacrydium*, *Falcatifolium*, and *Acmopyle*. The genus *Dacrydium*, which belongs to the Dacrydioid clade, contains 16 species distributed from New Zealand to southern China. Four endemic species to New Caledonia were studied here: *D. araucarioides* Brongn. & Gris, *D. balansae* Brongn. & Gris, *D. guillauminii* J. Buchholz, and *D. lycopodioides* Brongn. & Gris.

In addition, two other species, belonging to the genera *Acmopyle* and *Falcatifolium*, were also investigated. *Falcatifolium*, which is close to *Dacrydium*, is a small genus (five species) of the Dacrydioid clade and has a distribution area comprised between New Caledonia and the Malay Peninsula. The only New Caledonian endemic *Falcatifolium* species, *F. taxoides* (Brongn. & Gris) de Laub., is well known for being the host of the only parasitic gymnosperm, *i.e.*, *Parasitaxus usta*. There is only one report about the identification of podocarpic acid and sitosterol in the wood of *F. taxoides* [19]. The

Acmopyle genus is sister of the Dacrydioid clade and is present in Fiji and New Caledonia (*A. pancheri* (Brongn. & Gris) Pilg.), with one species in each island. The phytochemistry of this genus has never been studied.

The leaf essential oils of these six species were characterized by GC/FID and GC/MS analyses, to understand their chemotaxonomic relevance by comparison of their terpene compositions.

Results and Discussion. – *Chemistry.* Leaves of *D. araucarioides*, *D. balansae*, *D. guillauminii*, *D. lycopodioides*, *F. taxoides*, and *A. pancheri* were collected in the main island of New Caledonia. The collection sites and oil yields are listed in *Table 1*. The characterization of the leaf essential oils by GC-FID and GC/MS analyses allowed the identification of 104 components, representing 88.2–96.8% of the total oil compositions. The chemical composition of the oils obtained from the six studied species as well as the linear retention indices (*LRI*s) and relative contents, expressed as percentages, of the identified compounds are given in *Table 2*, where they are listed according to their elution order from a non-polar *HP-5* column. The chemical structures of the main components are represented in *Fig. 1*.

The essential oils of two *Dacrydium* species, *i.e.*, *D. araucarioides* and *D. balansae*, were distinguished by a similar chemical composition rich in sesquiterpenes. The *D. araucarioides* oil was mainly composed by sesquiterpenes (92.7%), of which germacrene D (**7**, 34.6%), β-caryophyllene (**3**, 32.5%), α-humulene (**6**, 5.7%), and γ-amorphene (**8**, 5.5%) were the most abundant. In addition, small amounts of monoterpenes (2.8%) were identified, and no diterpenes could be detected. As mentioned above, a similar terpenoid profile to that of *D. araucarioides* was observed for the oil of *D. balansae*, which was also rich in sesquiterpenes (96%), weak in monoterpenes (0.8%), and devoid of diterpenoid compounds. The major constituents of this oil were compound **3** (37.5%), β-elemene (**5**, 12.3%), compound **6** (9.7%), and α-copaene (**4**, 9.3%).

In the *D. guillauminii* oil, the amount of sesquiterpenes was less important (67.0%) than in the two oils described above, including bicyclogermacrene (**9**, 38.7%) as principal component. On the other hand, this oil contained also significant amounts of monoterpenes (19.1%), with α-pinene (16.0%) as the principal monoterpene, and diterpenes (9.5%), with rosa-5,15-diene (**10**, 7.0%) as the major diterpene.

The oil of *D. lycopodioides* was marked by high contents of diterpenes (50.8%), represented mainly by rimuene (**1**, 41.6%) and phyllocladene (**2**, 8.0%), and

Table 1. *Plant Collection and Leaf-Oil Yield of Endemic* Dacrydium, Falcatifolium, *and* Acmopyle *Species from New Caledonia*

Plant species	Abbreviation	Voucher number	Collection site	Oil yield [% (*w*/*w*)]
Dacrydium araucarioides	*Da*	NL07	Lac de Yaté	0.020
Dacrydium balansae	*Db*	NL37	Rivière Confiance	0.035
Dacrydium guillauminii	*Dg*	NL26	Plaine des lacs	0.719
Dacrydium lycopodioides	*Dl*	RF 01	Mont Humboldt	0.240
Falcatifolium taxoides	*Ft*	NL51	Paéoua	0.088
Acmopyle pancheri	*Ap*	NL52	Paéoua	0.387

Table 2. *Chemical Composition of the Leaf Essential Oils of Endemic* Dacrydium, Falcatifolium, *and* Acmopyle *Species from New Caledonia*

Entry	Compound[a])	Terpene group	LRI_n[b])	LRI_p[c])	LRI_{lit}[d])	Relative content [%][e])					
						Ap[f])	*Ft*	*Da*	*Db*	*Dg*	*Dl*
1	α-Pinene	Monoterpene	937	1019	932	tr	0.2	1.0	–	16.0	0.5
2	β-Pinene	Monoterpene	971	1116	979	tr	tr	0.1	–	0.7	–
3	Myrcene	Monoterpene	982	–	990	–	–	0.2	–	0.8	–
4	ϱ-Cymene	Monoterpene	1025	1273	1020	tr	–	–	–	0.1	–
5	Limonene	Monoterpene	1028	1202	1030	1.6	–	–	0.8	–	–
6	β-Phellandrene	Monoterpene	1038	1214	1025	–	tr	0.2	–	0.6	–
7	Terpinolene	Monoterpene	1078	1287	1088	–	tr	tr	–	–	–
8	*endo*-Fenchol	Monoterpene	1113	1581	1116	–	tr	–	–	–	–
9	*cis*-*p*-Menth-2-en-1-ol	Monoterpene	1122	1559	1122	–	tr	–	–	–	–
10	Camphene hydrate	Monoterpene	1147	–	1149	–	tr	–	–	–	–
11	Borneol	Monoterpene	1167	–	1169	tr	tr	–	–	–	–
12	Terpinen-4-ol	Monoterpene	1178	1601	1177	0.5	0.3	0.4	–	0.2	–
13	α-Terpineol	Monoterpene	1194	1693	1188	0.9	1.1	0.9	–	0.7	–
14	Myrtenol	Monoterpene	1204	1787	1194	–	tr	–	–	–	–
15	Verbenone	Monoterpene	1214	1703	1205	–	tr	–	–	–	–
16	*trans*-Carveol	Monoterpene	1223	1861	1215	–	tr	–	–	–	–
17	Thujanol acetate	Monoterpene	1276	1843	1276	tr	–	–	–	–	–
18	Terpinyl acetate	Monoterpene	1305	1631	1300	tr	–	–	–	–	–
		Total monoterpenoids				3.0	1.6	2.8	0.8	19.1	0.5
19	δ-Elemene	Germacrane	1320	1785	1338	–	tr	0.1	–	–	–
20	α-Cubebene	Cadalane	1353	1451	1348	0.3	tr	0.1	1.2	–	–
21	Cyclostavinene	Cadalane	1366	1468	1369	–	tr	–	–	–	–
22	α-Ylangene	Cadalane	1370	1482	1375	tr	tr	–	–	0.2	–
23	α-Copaene (**4**)	Cadalane	1376	1481	1376	tr	0.2	0.3	9.3	–	–
24	Isoledene	Germacrane	1382	–	–	–	–	–	–	0.1	–
25	β-Cubebene	Cadalane	1378	1538	1387	–	tr	–	–	–	–
26	β-Bourbonene	–	1384	1581	1388	–	tr	–	–	–	–
27	β-Elemene (**5**)	Germacrane	1397	1586	1390	2.9	0.3	0.7	12.3	0.8	8.0
28	Sandvicene	–	1400	1611	1399	tr	–	–	–	–	–
29	Sibirene	Germacrane	1404	1552	1400	–	0.3	–	–	–	–
30	Acora-3,7(14)-diene	Bisabolane	1410	1560	1408	tr	–	–	–	–	–
31	β-Caryophyllene (**3**)	Humulane	1427	1598	1419	0.8	0.3	32.5	37.5	–	–
32	Isocaryophyllene	Humulane	1423	1613	–	–	0.2	–	–	–	–
33	Unidentified (MW = 204)	–	1424	1537	–	0.9	–	–	–	–	–
34	β-Barbatene	Bisabolane	1440	1646	1440	tr	–	–	–	–	–
35	α-Guaiene	Germacrane	1441	1719	1439	0.7	–	–	–	0.3	–
36	β-Cedrene	Bisabolane	1445	–	–	–	–	0.3	–	–	–
37	Aromadendrene	Germacrane	1452	–	–	–	–	0.7	–	4.7	–
38	α-Humulene (**6**)	Humulane	1459	1661	1454	tr	0.1	5.7	9.7	–	–
39	β-Farnesene	Farnesane	1460	1621	1456	0.5	–	–	0.8	–	–
40	Unidentified (MW = 204)	–	1466	1714	–	0.8	–	–	–	–	–
41	β-Acoradiene	Bisabolane	1471	1678	1470	–	0.4	–	–	–	–
42	Cadinadiene	Cadalane	1474	1714	1476	–	0.2	–	–	–	–
43	γ-Muurolene	Cadalane	1480	1687	1479	0.4	0.8	2.0	–	–	–
44	Widdra-2,4(14)-diene	Bisabolane	1482	1725	1482	–	0.3	–	–	–	–
45	9-*epi*-(*E*)-Caryophyllene	Humulane	1484	1659	–	–	–	–	1.1	–	–

Table 2 (cont.)

Entry	Compound[a])	Terpene group	LRI_n[b])	LRI_p[c])	LRI_{lit}[d])	Relative content [%][e])					
						Ap[f])	*Ft*	*Da*	*Db*	*Dg*	*Dl*
46	Germacrene D (**7**)	Germacrane	1487	1687	–	–	–	34.6	2.0	–	20.8
47	γ-Amorphene (**8**)	Cadalane	1490	–	–	–	–	5.5	–	–	–
48	β-Selinene	Germacrane	1493	1685	1490	2.4	–	–	2.7	–	4.3
49	Valencene	Germacrane	1495	–	–	–	–	–	–	0.3	–
50	β-Alaskene	Bisabolane	1498	1696	1498	2.5	–	–	–	–	–
51	α-Muurolene	Cadalane	1500	1723	1500	tr	0.1	–	–	–	–
52	Bicyclogermacrene (**9**)	Germacrane	1503	1735	1500	–	–	0.2	–	38.7	–
53	α-Selinene	Germacrane	1503	1703	–	–	–	–	4.2	–	3.6
55	*cis*-β-Guaiene	Germacrane	1505	1693	–	–	–	–	2.0	–	–
55	γ-Patchoulene	Germacrane	1519	–	–	–	–	0.7	–	–	–
56	γ-Cadinene	Cadalane	1522	1759	–	–	0.2	2.7	–	–	1.0
57	δ-Cadinene	Cadalane	1528	1751	1523	1.4	0.6	0.1	4.2	–	1.5
58	α-Cadinene	Cadalane	1551	1693	–	–	–	0.1	–	–	–
59	Germacrene B	Germacrane	1561	1749	1561	–	0.3	–	–	–	0.4
60	Maaliol	Germacrane	1565	1994	1567	–	0.3	–	–	–	–
61	Spathulenol	Germacrane	1586	2108	1578	2.5	1.1	0.7	0.7	3.7	–
62	Caryophyllene oxide	Humulane	1596	1963	1583	–	–	1.0	2.4	–	–
63	Globulol	Germacrane	1603	2065	1590	2.4	1.3	0.5	0.7	4.2	–
64	Viridiflorol	Germacrane	1597	2060	1592	0.4	0.9	–	–	3.4	–
65	Rosifoliol	Germacrane	1610	2100	1601	–	0.2	0.5	–	1.1	–
66	Humulene epoxide II	Humulane	1612	2040	1608	0.5	–	–	–	–	–
67	1,10-di-*epi*-Cubenol	Cadalane	1612	2098	1619	–	0.6	–	–	–	–
68	Unidentified (MW = 200)	–	1622	2101	–	–	0.2	–	–	–	–
69	Eudesmol	Germacrane	1624	–	–	–	–	–	–	0.8	–
70	1-*epi*-Cubenol	Cadalane	1620	2059	1628	0.7	0.5	–	0.9	–	–
71	Unidentified (MW = 220)	–	1634	–	–	–	–	–	–	0.6	–
72	τ-Muurolol	Cadalane	1650	2105	1642	0.4	–	1.7	1.6	–	0.7
73	Cubenol	Cadalane	1642	–	1646	–	0.6	–	–	–	–
74	α-Muurolol	Cadalane	1652	–	–	–	–	–	–	–	0.5
75	α-Cadinol	Cadalane	1657	2106	1654	1.5	1.1	2.0	–	–	1.4
76	Selina-3,11-diene-6α-ol	Germacrane	1655	–	–	–	–	–	–	0.2	–
77	Selin-11-en-4α-ol	Germacrane	1666	2242	1667	–	–	–	3.4	0.5	1.1
78	Unidentified (MW = 220)	–	1675	2303	–	0.5	–	–	–	–	–
79	Isolongifolol	Himachalane	1728	2421	1728	0.5	–	–	–	–	–
80	Xanthorrizol	Bisabolane	1741	2706	1751	0.5	–	–	–	–	–
81	γ-Costol	Germacrane	1757	–	–	–	–	–	–	3.0	–
82	β-Costol	Germacrane	1779	–	–	–	–	–	–	3.0	–
83	α-Costol	Germacrane	1784	–	–	–	–	–	–	2.0	–
84	Unidentified (MW = 250)	–	1852	–	–	0.5	–	–	–	–	–
		Total sesquiterpenoids				21.3	10.9	92.7	96.0	67.0	43.3
85	Isopimara-9(11),15-diene	Isopimarane	1912	–	1905	–	0.3	–	–	–	0.1
86	Rimuene (**1**)	Rimuane	1920	–	–	–	–	–	–	–	41.6
87	Unidentified (MW = 272)	–	1911	–	–	0.4	0.7	–	–	–	–
88	Rosa-5,15-diene (**10**)	Rosane	1935	2239	1934	0.5	–	–	–	7.0	–
89	Pimaradiene (**11**)	Pimarane	1951	2226	1949	4.0	5.3	–	–	–	0.4
90	13-Isopimaradiene	Isopimarane	1973	2223	1969	1.4	1.1	–	–	–	0.5
91	Unidentified (MW = 272)	–	1973	2735	–	0.6	–	–	–	–	–

Table 2 (cont.)

Entry	Compound[a])	Terpene group	LRI_n[b])	LRI_p[c])	LRI_{lit}[d])	Relative content [%][e])					
						Ap[f])	*Ft*	*Da*	*Db*	*Dg*	*Dl*
92	Sclarene	Labdane	1982	–	–	–	–	–	–	2.0	–
93	Unidentified (MW = 256)	–	1988	3179	–	–	0.2	–	–	–	–
94	Manool oxide	Labdane	2007	–	–	–	–	–	–	0.1	–
95	Phyllocladene (**2**)	Phyllocladane	2026	2377	2017	29.4	65.5	–	–	0.2	8.0
96	Kaurene (**12**)	Kaurane	2055	2424	2043	8.3	0.2	–	–	–	–
97	Abietatriene	Abietane	2060	2498	2056	1.5	0.4	–	–	0.2	0.2
98	Abietadiene	Abietane	2092	–	–	–	–	–	–	tr	–
99	Phyllocladanol	Phyllocladane	2209	2687	2210	3.8	0.3	–	–	–	–
100	Sandaracopimarinol	Isopimarane	2271	3989	2269	–	2.1	–	–	–	–
101	Unidentified (MW = 288)	–	2275	3344	–	0.5	–	–	–	–	–
102	Unidentified (MW = 288)	–	2281	2922	–	1.7	–	–	–	–	–
103	Sempervirol	Abietane	2306	3480	2283	0.7	–	–	–	–	–
104	*trans*-Ferruginol (**13**)	Abietane	2335	3782	2332	14.3	4.4	–	–	–	–
		Total diterpenoids				63.9	79.3	0.0	0.0	9.5	50.8
		Total identified				88.2	91.8	95.5	96.8	95.6	94.6

[a]) Compound identification based on the comparison of linear retention indices and mass spectra with literature data [24–26] and mass spectral libraries, *cf. Exper Part.* [b]) LRI_n: Linear retention indices determined on the non-polar *HP-5* column. [c]) LRI_p: Linear retention indices determined on the polar *DB-Wax* column. [d]) LRI_{lit}: Linear retention indices from the literature (non-polar column) [20–22]. [e]) Relative content determined by assuming that all response factors are equal to 1 and expressed as percentage; tr, traces;–, not detected. [f]) Species abbreviation: *Ap* = *Acmopyle pancheri*, *Ft* = *Falcatifolium taxoides*, *Da* = *Dacrydium araucarioides*, *Db* = *Dacrydium balansae*, *Dg* = *Dacrydium guillauminii*, *Dl* = *Dacrydium lycopodioides*.

sesquiterpenes (43.3%), predominantly constituted of germacrene D (**7**, 20.8%) and β-elemene (**5**, 8.0%).

The *F. taxoides* oil was characterized by a high amount of diterpenes (79.3%), dominated mainly by compound **2** (65.5%) and pimaradiene (**11**, 5.3%). Phyllocladane-type diterpenoids have been found to be well represented in several genera of Podocarpaceae, while pimarane-type compounds have only been observed in few taxa, as *Prumnopitys taxifolia* [23] and *Lagarostrobos franklinii* [10].

Finally, a high amount of diterpenes (63.9%) was also identified in the *A. pancheri* oil, represented mainly by compound **2** (29.4%) and *trans*-ferruginol (**13**, 14.3%), an abietane-related diterpenoid. Kaurene (**12**) and compound **11** were present in both the *F. taxoides* and *A. pancheri* essential oils.

Biosynthetic Classification. Because monoterpenes are largely distributed among all conifers, they were considered as only one group, while sesquiterpenes and diterpenes were classified according to their biosynthetic pathways given in *Schemes 1* and *2* for sesquiterpenoids and diterpenoids, respectively. Hence, all identified compounds were classified according to their chemical classes (monoterpenes, sesquiterpenes, and diterpenes) and, furthermore, the most abundant components (sesquiterpenes and diterpenes) were distinguished on the basis of their biosynthetic pathways [24][25] (*Table 2*).

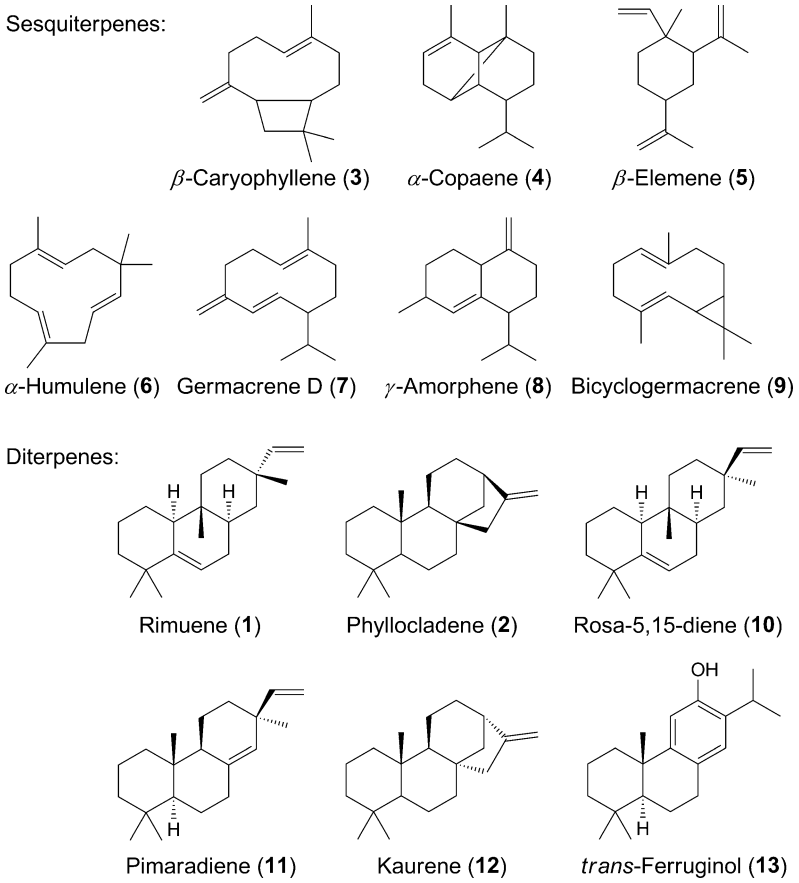


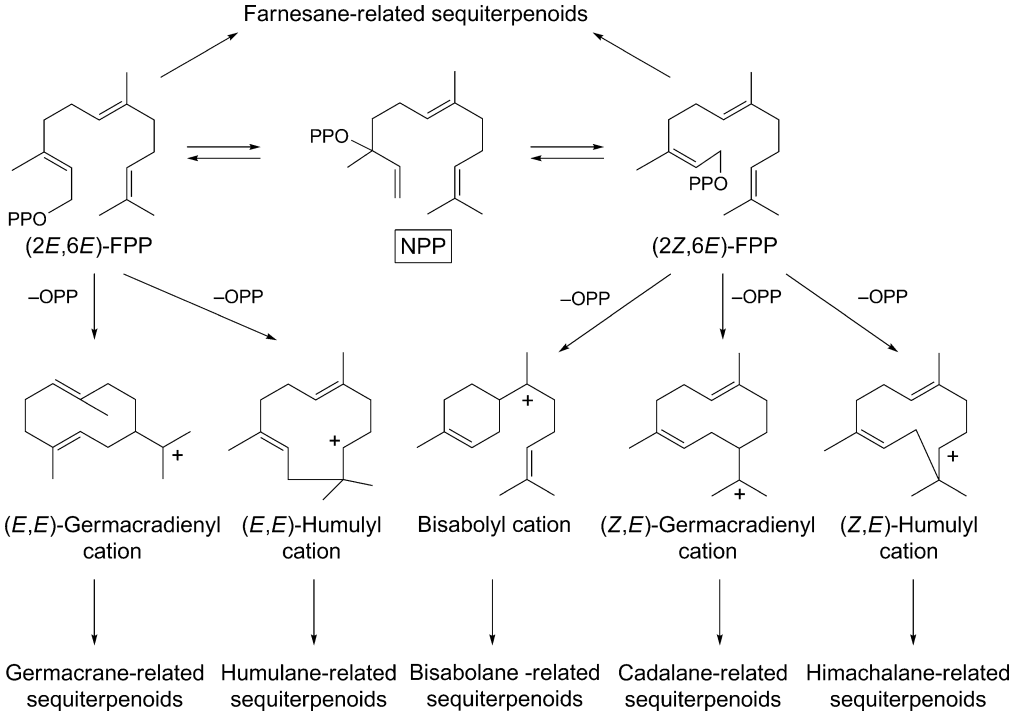
Fig. 1. *Chemical structures of the main constituents of the studied leaf essential oils of* Dacrydium, Falcatifolium, *and* Acmopyle *species endemic to New Caledonia*

The precursors (2*Z*,6*E*)- and (2*E*,6*E*)-farnesyl pyrophosphate (FPP) and their isomer nerolidyl pyrophosphate (NPP) are the starting points for the formation of sesquiterpene compounds. After a series of rearrangements, many structural classes can be obtained (*Scheme 1*).

Diterpenes are obtained from the precursor geranylgeranyl pyrophosphate (GGPP), leading to different cyclic structures (*Scheme 2*), *i.e.*, bicyclic, tricyclic, and tetracyclic diterpenes. For the biosynthesis of bicyclic diterpenes, the precursors of the labdane and *ent*-labdane series are obtained by cyclisation of GGPP by two different stereochemical routes. The tricyclic pimarenyl and isopimarenyl cations derived from GGPP can give other tricyclic structures (pimaranes, abietanes, rimuanes, and isopimaranes). Tetracyclic diterpenes are derived from the cyclization of the pimarenyl or isopimarenyl cations to afford kauranes, and phyllocladanes (*Scheme 2*).

This biosynthetic classification of the compounds identified in the oils of the six New Caledonian species presented in this study (*Table 3*) allowed the evaluation of

Scheme 1. *Biosynthetic Pathways of Sesquiterpenoids* [24]

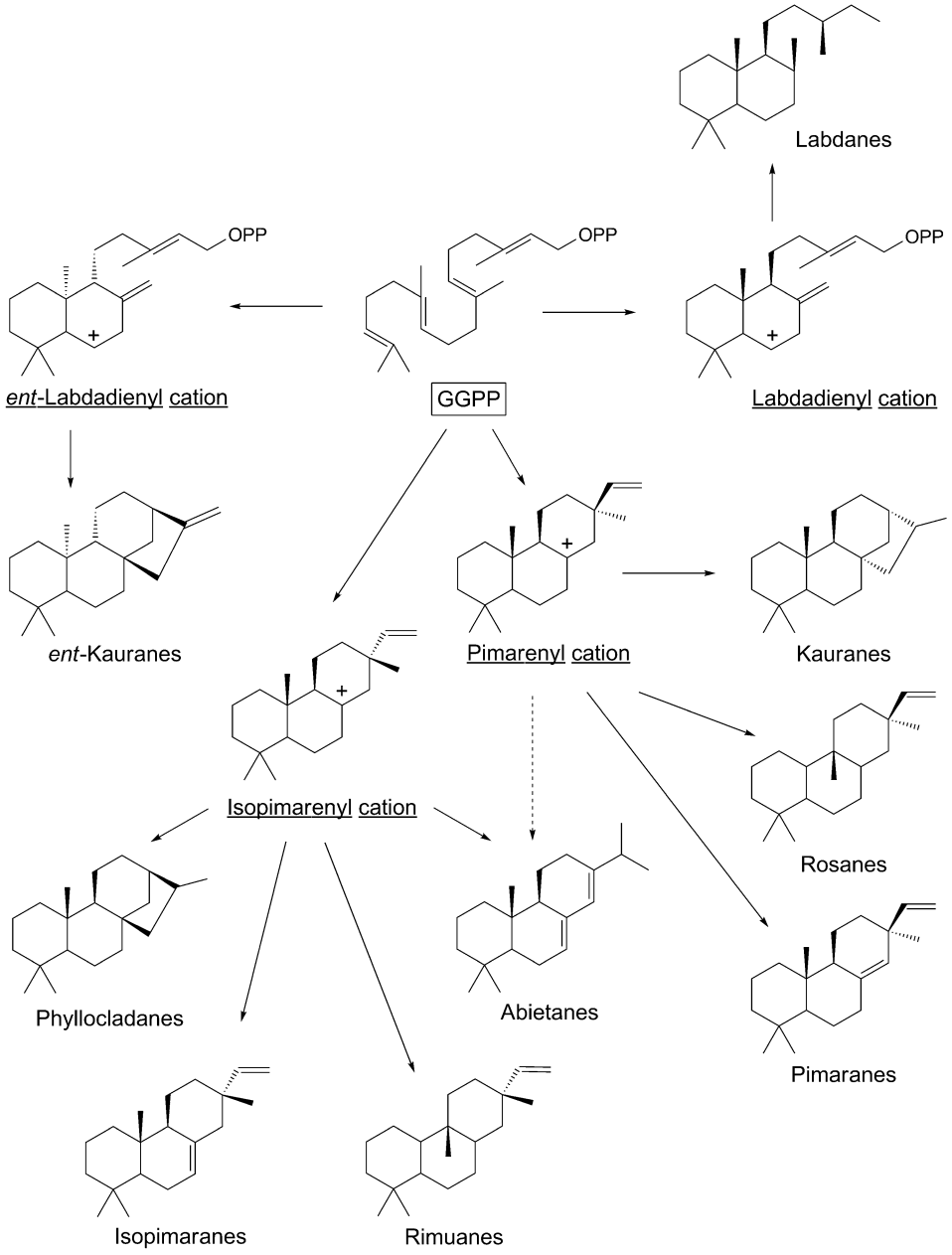


these oils according to their terpene composition. The essential oils obtained from *A. pancheri* and *F. taxoides* leafs were poor in sesquiterpenes. In contrast, the *Dacrydium* species contained a majority of sesquiterpene compounds. The cyclic sesquiterpenes may be divided into five classes, *viz*., himachalane-, bisabolane-, cadalane-, humulane-, and germacrane-related sesquiterpenes (*Scheme 1*). In the oils of the investigated *Dacrydium* species, germacrane-type compounds were the most represented, followed by cadalane- and humulane-type ones (*Table 3*). Humulane derivatives characterized the oils of the two species *D. balansae* and *D. araucarioides*.

The oils of all the species studied showed some similar characteristics which are typical of leaf oils of the Podocarpaceae family, *i.e.*, a predominance of cadalane- and germacrane-type sesquiterpenoids and the absence of bisabolane- and farnesane-type sesquiterpenoids. Sesquiterpenes of the himachalane class have only been found in *D. cupressinum* oil [14].

Concerning the diterpene classes, the *A. pancheri* and *F. taxoides* oils showed a majority of compounds with abietane and phyllocladane skeletons, derived from the isopimarenyl cation. The oils of *D. araucarioides* and *D. balansae* contained no diterpenoids. Conversely, the *D. guillauminii* oil was characterized by a significant amount of compounds with a rosane-type skeleton, while the *D. lycopodioides* oil contained high amounts (41.6%) of compounds with a rimuane-type skeleton, issued from the isopimarenyl cation.

Scheme 2. *Biosynthetic Pathways of Diterpenoids* [25]



Multivariate Statistics. To clarify the relationship between the essential oils of the six different species of Podocarpaceae studied, a cluster analysis was employed on the basis of the biosynthetic origin of their sesquiterpenes and diterpenes. The dendrogram of

Table 3. *Content of the Terpene Groups in the Leaf Essential Oils of Endemic* Dacrydium, Falcatifolium, *and* Acmopyle *Species from New Caledonia, Classified on the Basis of the Biosynthetic Pathways of the Terpenes*[a])

	Content [%]					
	Ap[b])	*Ft*	*Da*	*Db*	*Dg*	*Dl*
Monoterpenes	3.0	1.6	2.8	0.8	19.1	0.5
Sesquiterpenes						
(2*Z*,6*E*)-Farnesyl pyrophosphate						
Bisabolyl cation → Bisabolanes	3.5	0.7	0.3	–	–	–
(*Z*,*E*)-Humulyl cation → Himalachanes	0.5	–	–	–	–	–
(*Z*,*E*)-Germacradienyl cation → Cadalanes	4.7	4.9	14.5	17.2	0.2	5.1
(2*E*,6*E*)-Farnesyl pyrophosphate						
(*E*,*E*)-Humulyl cation → Humulanes	1.3	0.6	39.2	50.7	–	–
(*E*,*E*)-Germacradienyl cation → Germacranes	11.3	4.7	38.7	28.0	66.8	38.2
Diterpenes						
Geranylgeranyl pyrophosphate						
Labdadienyl cation → Labdanes	–	–	–	–	2.1	–
Pimarenyl cation → Pimaranes	4.0	5.3	–	–	–	0.4
→ Rosanes	0.5	–	–	–	7.0	–
→ Kauranes	8.3	0.2	–	–	–	–
Isopimarenyl cation → Abietanes	16.5	4.8	–	–	0.2	0.2
→ Phyllocladanes	33.2	65.8	–	–	0.2	8.0
→ Rimuanes	–	–	–	–	–	41.6
→ Isopimaranes	1.4	3.5	–	–	–	0.6

[a]) All identified compounds were classified according to their chemical classes (monoterpenes, sesquiterpenes, and diterpenes) and, furthermore, the most abundant components (sesquiterpenes and diterpenes) were distinguished on the basis of their biosynthetic pathways, *cf. Schemes 1* and *2*. [b]) Species abbreviation: *Ap* = *Acmopyle pancheri*, *Ft* = *Falcatifolium taxoides*, *Da* = *Dacrydium araucarioides*, *Db* = *Dacrydium balansae*, *Dg* = *Dacrydium guillauminii*, *Dl* = *Dacrydium lycopodioides*.

the ascendant hierarchical clustering analysis representing the chemical composition dissimilarities of the New Caledonian *Dacrydium*, *Falcatifolium*, and *Acmopyle* species is given in *Fig. 2*. and shows that the cluster analysis allowed the classification of the essential oils into three groups. The first one was composed of the oils of the two closely related species *D. araucarioides* and *D. balansae*, which were characterized by high contents of sesquiterpenes belonging to the cadalane, germacrane, and humulane structural classes (especially β-caryophyllene) and by the absence of volatile diterpenes. These results are consistent with previously published data on the phylogeny of *Dacrydium* species, which showed a well-supported clade with *D. araucarioides* and *D. balansae* [4].

The second group was formed by the oils of *D. guillauminii* and *D. lycopodioides*, which were characterized by the lack of humulane, bisabolane, himachalane, and farnesane derivatives and a predominance of germacrane-type sesquiterpenoids, mainly germacrene D for the *D. lycopodioides* oil and bicyclogermacrene for the *D. guillauminii* oil. The differences within this group were more pronounced regarding their diterpene composition. The diterpene fraction of the *D. guillauminii* oil was

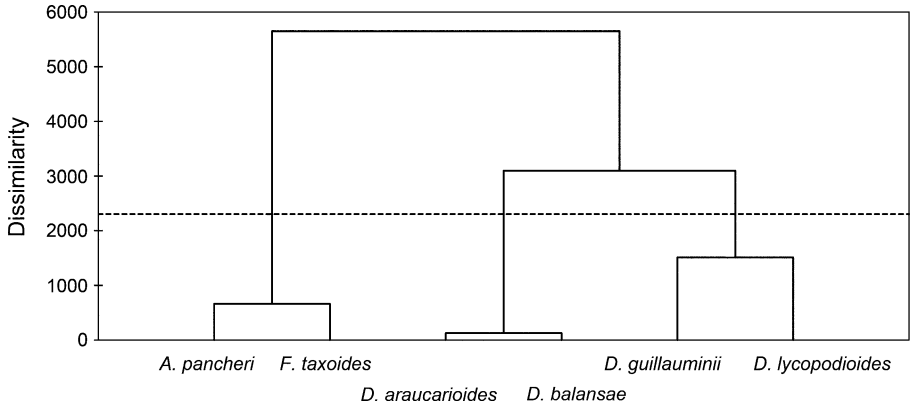


Fig. 2. *Dendrogram obtained by ascendant hierarchical clustering, representing the chemical composition dissimilarity of the studied leaf essential oils of* Dacrydium, Falcatifolium, *and* Acmopyle *species endemic to New Caledonia*

principally composed of diterpenes belonging to the rarely observed (but characteristic of the Podocarpaceae) rosane structural class (rosa-5,15-diene, 7%), while the *D. lycopodioides* oil contained mainly compounds of the rimuane (rimuene, 41.6%) and phyllocladane (phyllocladene, 8%) classes.

Finally, the last group comprised the oils of *A. pancheri* and *F. taxoides*, which had a similar sesquiterpene compositions with small amounts of cadalane, humulane, bisabolene, and germacrane-type compounds. Both species shared also a similar diterpene composition represented by compounds with phyllocladane, kaurane, abietane, and pimarane skeletons. The oil compositions of *A. pancheri* and *F. taxoides* were characteristic of the Podocarpaceae family, but diverged from those of the two groups of *Dacrydium* species from New Caledonia. Surprisingly, in terms of the essential-oil composition, *F. taxoides* was closer to *A. pancheri* than to the studied *Dacrydium* species, even though *Falcatifolium* and *Dacrydium* are two genera belonging to the Dacrydioid clade, while the genus *Acmopyle* is a sister of this clade [5].

The terpene compositions of the oils of the four New Caledonian *Dacrydium* species presented a few similarities with those of previously studied *Dacrydium* species from Fiji [15] and New Zealand [13][14].

D. guillauminii, one of the world's rarest conifers, is found only in a few localities on the banks of rivers and lakes in the south of New Caledonia. Its essential oil is mainly composed of bicyclogermacrene (38.7%) and does not share the same biosynthetic pathways with the other New Caledonian *Dacrydium* species, considering its major compounds. The oil of this species appears to be closer to those of *Dacrydium* species from New Zealand and Fiji (*D. cupressinum* and *D. nidulum*), because it is the only one to share with them compounds biosynthetized from the bicyclogermacrane skeleton precursor. The *D. guillauminii* oil had only β-elemene in a small amount jointly with the oil of New Zealand species. Regarding the diterpene composition of the essential oils, *D. guillauminii* shared compounds as sclarene, phyllocladene, and abietatriene with *D. cupressinum*, but its main diterpene, rosa-5,15-diene (a diastereoisomer of rimuene) was never found before in the *Dacrydium* genus. Moreover, concerning the presence of

monoterpenes, only the *D. guillauminii* oil contained significant amounts of α-pinene, which was the main monoterpene occurring in the leaf oils of *D. nidulum* and *D. nausoriense* from Fiji. Camphene, another major monoterpene of *D. nausoriense* oil, was totally absent from the New Caledonian *Dacrydium* oils.

The *D. lycopodioides* oil had a closer chemical composition to that of the oils of *Dacrydium* species from Fiji, due to the significant presence of diterpenes, including rimuene (41.6%). *D. lycopodioides* oil, as well as those of *D. balansae* and *D. araucarioides*, had common sesquiterpenes with the New Zealand rimu tree, *D. cupressinum*. Some sesquiterpenes found in *D. cupressinum* oil, as α-longipinene, longifolene, or longibornyl acetate, were not present in the oils of the New Caledonian species. Surprisingly, none of the sesquiterpenes of the *D. lycopodioides* oil were shared with the Fijian *Dacrydium* oils, while these sesquiterpenes (mainly germacrene D, β-elemene, β-selinene, and α-selinene) occurred in the other New Caledonian species. The main diterpene of *D. lycopodioides* was rimuene, which was also present in large amounts in the oils of New Zealand and Fijian *Dacrydium* species. Laurenene, the unusual diterpene of the fenestrane structural class from *D. cupressinum*, was not present in the New Caledonian *Dacrydium* oils.

Concerning the volatile terpene composition at the genera level, particularly that of diterpenes, only weak relationships between *Dacrydium* species on the one hand and *Acmopyle* and *Falcatifolium* species on the other hand were observed. Indeed, in the case of the studied species, *D. araucarioides* and *D. balansae* oils did not contain any diterpenes, while the *D. lycopodioides* as well as the *D. guillauminii* oils were characterized by different major diterpene structural classes, which were different from those identified in the *A. pancheri* and *F. taxoides* oils. This also applies for the oils of the other species of *Dacrydium*, as *D. cupressinum* from New Zealand and the two Fijian species, which have a diterpene composition different from those of the *Acmopyle* and *Falcatifolium* species.

Finally, the oils of *A. pancheri* and *F. taxoides* shared the same major component, phyllocladene (29.4 and 65.5%, resp.), which, from a biosynthetic point of view, is related to the isopimarane skeleton. Hence, these species seem close from a chemotaxonomic point of view, with the common marker phyllocladene, possibly indicating a genetic proximity.

Conclusions. – The chemical composition of the leaf essential oils of six Podocarpaceae species endemic to New Caledonia was reported. The cluster analysis based on the biosynthetic origin of the volatile terpenes led to the description of three distinct groups. The two groups that characterized the *Dacrydium* species were in agreement with the phylogeny of this genus [5]. Concerning the essential-oil composition, *D. araucarioides* and *D. balansae* revealed to be the most similar species, while *D. guillauminii* and *D. lycopodioides* formed a less homogenous group. The differentiation between the species of *Dacrydium* from New Caledonia concerned mainly the diterpenes and the sesquiterpenes of the humulane structural class. The same variability was observed, when taking into account the chemical composition of the other species of *Dacrydium* in the Pacific islands. The New Caledonian species presented a terpene-class distribution different from that of the New Zealand and Fijian species, even though they may share some compounds, as rimuene, which is the

major diterpene of the oils of *D. lycopodioides*, *D. cupressinum*, and the two Fijian species (*D. nidulum* and *D. nausoriense)*. Finally, the last and well consistent group was composed of *F. taxoides* and *A. pancheri*, even if the *Falcatifolium* genus is genetically closer to the *Dacrydium* than to the *Acmopyle* genus. This study showed that the chemotaxonomy gives supplementary information for the classification of species that are phylogenetically close. To deepen our understanding of the variability of terpenes in the Podocarpaceae family, it would be necessary to study other species belonging to the *Falcatifolium* and *Acmopyle* genera occurring outside New Caledonia. This research strategy will be applied for future studies that will consist in the evaluation of other species of Podocarpaceae in New Caledonia, especially species belonging to the *Podocarpus* and *Retrophyllum* genera of the Podocarpoid clade.

We wish to thank the team of the *Herbarium NOU* of the *IRD center of Noumea* and *Jean-Pierre Butin* of the *Department of Water and Forests of the North Province* for the confirmation of the voucher-specimen identifications. This work was supported by funding of the *Ministère des Outre-mers.*

Experimental Part

Plant Material. Leaves of *Dacrydium araucarioides* Brongn. & Gris, *Dacrydium balansae* Brongn. & Gris, *Dacrydium guillauminii* J. Buchholz, *Dacrydium lycopodioides* Brongn. & Gris, *Falcatifolium taxoides* (Brongn. & Gris) de Laub., and *Acmopyle pancheri* (Brongn. & Gris) Pilg. were collected in the main island of New Caledonia under scientific authorization of the South Province (N°10919-2009 and N°2188-2010) and North Province (N°60912-1777-2010). All leaves used for extraction were collected from bulk collections of five individual adult trees. Within any one collection, there was a gradation in the size of leaf, which was assumed to relate to the age of the leaf and/or the leaf position within the seasonal growth flush. They were not treated differently. Voucher samples have been deposited with the center of Noumea of the Research Institute for Development, New Caledonia.

Essential-Oil Extraction. The essential oils were obtained by hydrodistillation of lots of 200–300 g of fresh leaves using a *Clevenger*-type apparatus for 7 h. After decantation, the oils were dried (anh. Na_2SO_4) and stored in glass vials in a fridge at 4°. The oil yield was expressed as g/100 g of fresh plant material (% (*w*/*w*)).

GC-FID Analysis. The GC/FID analyses were performed with a *Varian* gas chromatograph, model *CP-3380* equipped with a flame-ionization detector (FID) and a *HP-5* (*J&W Agilent*) silica cap. column (5% phenyl, 95% methyl polysiloxane; 30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temp. was programmed rising from 60 to 200° at 3°/min and then kept isothermal at 200° for 20 min; injector temp., 220°; detector temp., 250°; carrier gas, N_2 (0.8 ml/min); manual injection of 1 μl of a 10% soln. of essential oils in CH_2Cl_2; split mode, 1:100.

GC/MS Analysis. The GC/MS analyses were performed with a *Hewlett Packard 5890 II* gas chromatograph interfaced with a quadrupole detector (*Model 5972*) and equipped with the same *HP-5* cap. column as described above. Also the oven temp. program was the same as that used for the GC/FID analyses (*cf. GC-FID Analysis*); injector temp., 220°; MS transfer-line temp., 250°; carrier gas, He (0.6 ml/min). Diluted samples (10:100 (*v*/*v*) in pentane) of 1 μl were injected manually using a split mode of 1:100. The same samples were analyzed under the same experimental conditions on a *DB-Wax* fused-silica cap. column (30 m × 0.25 mm i.d., film thickness 0.25 μm) for the calculation of the linear retention index on a polar phase (LRI_p). The MS was operated in the EI mode at 70 eV, over the *m*/*z* range of 35–300; electron multiplier, 1460 eV; scan rate, 2.96 scan/s.

Qualitative and Quantitative Analysis. The identification of the constituents was assigned on the basis of the comparison of their linear retention indices (*LRI*s), calculated with reference to a series of *n*-alkanes (C_9–C_{20}), and their mass spectra with those found in the literature [20–22] and listed in the *NBS75K* database and the *Wiley 7th NIST 98 EPA/NIH* mass-spectral library upgrade (provided by *Hewlett Packard* with the GC/MS control and data processing software). Their relative contents in the

essential oils expressed as percentages were determined by assuming that all response factors were equal to 1.

Cluster Analysis. Ascendant hierarchical clustering (AHC) was performed using the Excel program plug-in XLSTAT. The relative contents of the identified compound classes that exceeded 0.1% of the total oil composition were used as variables. The AHC was performed using *Euclidian* distance dissimilarity and *Ward*'s method as aggregation criteria.

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Received December 15, 2014