# Occurrence of Various Chemotypes in Niaouli (Melaleuca quinquenervia) Essential Oils from Madagascar Using Multivariate Statistical Analysis

**ARTICLE** *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · APRIL 1994

Impact Factor: 2.91 · DOI: 10.1021/jf00041a024



**4 AUTHORS**, INCLUDING:

Emile M Gaydou
Aix-Marseille Université
**352** PUBLICATIONS **3,914** CITATIONS

SEE PROFILE

# Occurrence of Various Chemotypes in Niaouli (*Melaleuca quinquenervia*) Essential Oils from Madagascar Using Multivariate Statistical Analysis†

Panja A. R. Ramanoelina,‡ Josette Viano,§ Jean-P. Bianchini,‡,‖ and Emile M. Gaydou*,§

Department IAA, ESSA, Université d'Antananarivo, BP 175, 101 Antananarivo, Madagascar, and Laboratoire de Phytochimie de Marseille, Faculté des Sciences et Techniques de Saint Jérôme, Avenue Escadrille Normandie Niémen, 13397 Marseille Cedex 20, France

The chemical composition of niaouli (*Melaleuca quinquenervia*) essential oils have been characterized by GC/MS and relative retention times. The leaf oil samples (144 samples obtained from leaves, collected during three seasons, on 48 different trees growing in Madagascar) were investigated for compound content, and 24 compounds were submitted to multivariate statistical analyses. Factorial discriminant analysis revealed the presence of four chemotypes: a chemotype having a high content of 1,8-cineole (37%); a chemotype relatively rich in 1,8-cineole (23%), viridiflorol (20%), and terpinolene (5%); a viridiflorol (48%) chemotype; and an (*E*)-nerolidol (87%) chemotype. The seasonal influence is negligible versus the chemical compound content of the chemotypes. The 1,8-cineole and (*E*)-nerolidol chemotypes represent 70% of the tree population.

## INTRODUCTION

Native to the Moluque islands, the niaouli tree grows in tropical areas and particularly in Australia, Madagascar, Indonesia, Malaysia, and New Caledonia. From a botanical point of view, the literature of this plant seems a little confused, although some reviews have been done (Cherrier, 1983), especially on the genus *Melaleuca* from Australia (Jones and Haenke, 1938; Blake, 1968; Byrnes, 1984) and more recently from New Caledonia (Dawson, 1992). Therefore, different names are used for the botanical identification of niaouli trees. According to the Blake revision, it appears that the niaouli from Madagascar must be called *Melaleuca quinquenervia*, since the name *M. viridiflora* Gaertn. given by Humbert (1953) and Cabanis *et al.* (1970) is incorrect. Our recent investigation on this subject has shown that niaouli from Madagascar (local names: oli, ahambo, kinindrano, kinimbohaka) is *Melaleuca quinquenervia* (Cav.) S. T. Blake, from a comparison of various herbarium samples from Madagascar and New Caledonia deposited at the Natural History Museum of Paris.

Like most plants belonging to the Myrtaceae family, an essential oil can be obtained by hydrodistillation of the leaves.

Among the various niaouli essential oils recently described, investigations reveal the existence of several chemotypes: a chemotype rich in 1,8-cineole (40–50%) (Ekundayo *et al.*, 1987; Todorova *et al.*, 1988; Motl *et al.* 1990; Ramanoelina *et al.*, 1992); a chemotype rich in (*E*)-nerolidol (40–80%) (Beylier, 1979); two chemotypes rich in (*E*)-methylisoeugenol (up to 88%) or in methyleugenol (up to 99%) (Brophy and Lassak, 1988); and a viridiflorol type (48%) from Madagascar (Ramanoelina *et al.*, 1992). A linalool type was earlier described by Guenther (1950).

† Financial support and a grant (P.A.R.) from UNESCO/BAD Project 702-MAG-10 and the Fonds d'Aide et de Coopération (France) is gratefully acknowledged.

‡ Université d'Antananarivo.

§ Laboratoire de Phytochimie de Marseille.

‖ Present address: Université Française du Pacifique, Tahiti, Polynesia.

Since niaouli essential oils are characterized by pharmaceutical and antimicrobial properties (Beylier, 1979; Valnet, 1984), the chemical composition of these oils may affect microbial growth.

In this paper, we have followed the chemical composition change of niaouli leaf oils from Madagascar of known trees at various times of the year to ascertain the occurrence of several chemotypes and the seasonal influence on the main component contents. Multivariate statistical analyses were applied to 144 leaf essential oil samples, for chemotype identification, using chemical compound content. Such methods have been successfully applied in essential oil research such as ylang-ylang grade classification (Gaydou *et al.*, 1988).

## MATERIALS AND METHODS

**Plant Material.** The leaves of *M. quinquenervia* were collected in 1988–1989 on trees growing near Toamasina (eastern part of Madagascar). Among the various trees, 48 were randomly chosen for essential oil determinations. The leaf harvest was done in July (winter), December (spring/summer), and March (autumn), giving 144 samples. Fresh leaves (200 g) were steam distilled during 6 h to give crude oils with an average yield of 1.0%.

**Compound Identification.** For compound identification, a mixture of an aliquot (1 mL) of each niaouli essential oil sample was made. This mixture was then fractionated using column chromatography according to known procedures (Gaydou *et al.*, 1986). Compounds were characterized by GC/MS and retention indices on two GC columns and NMR, when purity of the compounds was higher than 90% by GC. The method of calculation for retention indices was described in NF T 75-401 norm (Afnor, 1986). Complete assignment of $^{1}H$ and $^{13}C$ NMR spectra of viridiflorol has been described (Faure *et al.*, 1991).

**Gas Chromatography.** A Girdel 300 gas chromatograph equipped with a flame ionization detector (FID) was used for compound separations with a glass capillary column (0.26 mm i.d., 50 m) coated with Superox 4 (phase thickness, 0.10 μm; column temperature, 70–220 °C at 3 °C $min^{-1}$) or a glass capillary column (0.27 mm i.d., 50 m) coated with OV-1 (phase thickness, 0.20 μm; column temperature, 80–240 °C at 3 °C $min^{-1}$). Detector and inlet temperatures were 250 °C. Nitrogen was used as carrier gas at an inlet pressure of 0.45 bar. The injections averaged 0.1–0.2 μL of crude essential oil. Combined GC/MS spectra were recorded on a Delsi TI-700 gas chromatography linked to a

0021-8561/94/1442-1177$04.50/0 

**Table 1. Compound Identification and Content Range of Niaouli Essential Oil from Madagascar**

| compound[a] | RI[b] SPX-4 | RI[b] OV1 | content[c] (%) min | max | mean | SD |
|---|---|---|---|---|---|---|
| hydrocarbons | | | | | | |
| α-pinene | 1020 | 934 | 0.0 | 21.5 | 3.36 | 3.70 |
| α-thujene | 1020 | 923 | | | | |
| unknown | 1025 | | 0.0 | 7.57 | 0.14 | 0.65 |
| camphene | 1060 | 948 | 0.0 | 0.50 | 0.0 | |
| β-pinene | 1105 | 975 | 0.0 | 1.65 | 0.87 | |
| sabinene | 1114 | 977 | 0.0 | 4.11 | 0.06 | 0.37 |
| δ3-carene | 1135 | 1109 | 0.0 | 0.19 | 0.02 | 0.04 |
| myrcene | 1144 | 981 | 0.0 | 2.96 | 0.33 | 0.47 |
| α-phellandrene | 1151 | 1000 | | | | |
| α-terpinene | 1164 | 1012 | 0.0 | 2.10 | 0.32 | |
| limonene | 1192 | 1027 | 0.85 | 6.54 | 3.75 | |
| γ-terpinene | 1228 | 1051 | 0.0 | 13.47 | 1.65 | |
| p-cymene | 1249 | 1015 | 0.0 | 3.32 | 0.42 | |
| terpinolene | 1263 | 1081 | 0.0 | 8.33 | 0.75 | 1.73 |
| α-cubebene[d,e] | 1445 | | 0.0 | 0.18 | 0.01 | |
| α-ylangene | 1460 | | 0.0 | 0.18 | 0.0 | |
| α-copaene | 1467 | | 0.0 | 0.27 | 0.0 | |
| α-gurjunene | 1512 | 1406 | 0.0 | 1.23 | 0.29 | 0.28 |
| β-caryophyllene | 1569 | 1415 | 0.0 | 24.6 | 4.08[g] | 3.93 |
| sesquiterpene | 1574 | | 0.0 | 0.25 | 0.0 | |
| aromadendrene | 1578 | 1435 | 0.0 | 0.53 | 0.02 | |
| sesquiterpene | 1582 | | 0.0 | 0.32 | 0.0 | |
| alloaromadendrene | 1612 | 1460 | 0.0 | 0.51 | 0.27 | |
| α-humulene | 1637 | 1453 | 0.0 | 0.51 | 0.09 | 0.11 |
| α-amorphene[d] | 1654 | 1435 | | | | |
| viridiflorene | 1663 | 1494 | 0.0 | 17.1 | 5.48[f] | 4.46 |
| germacrene D | 1673 | 1485 | 0.0 | 0.48 | 0.0 | |
| β-selinene | 1682 | 1485 | 0.0 | 0.35 | 0.05 | 0.06 |
| (δ+γ)-cadinenes | 1721 | 1515 | 0.0 | 2.16 | 0.09 | 0.21 |
| cadina-1,4-diene | 1742 | 1527 | 0.0 | 0.62 | 0.0 | |
| α-cadinene | 1752 | 1546 | 0.0 | 0.27 | 0.0 | |
| (E)-calamene | 1788 | 1509 | 0.0 | 0.27 | 0.0 | |
| oxygenated compounds | | | | | | |
| 1,8-cineole | 1200 | 1025 | 0.03 | 51.5 | 19.7 | 17.4 |
| fenchone[d] | 1375 | 1072 | 0.0 | 0.05 | 0.0 | |
| benzaldehyde | 1486 | 932 | 0.0 | 1.32 | 0.38 | |
| linalool | 1512 | 1085 | 0.0 | 2.28 | 0.16 | 0.21 |
| fenchol | 1537 | 100 | 0.0 | 0.32 | 0.0 | |
| thujilic alcohol[d,e] | 1558 | | 0.0 | 0.21 | 0.0 | |
| 4-terpineol | 1567 | 1068 | 0.0 | 24.60 | 5.08[g] | 3.93 |
| methyl benzoate | 1637 | 1070 | 0.0 | 0.56 | 0.08 | |
| neral[d] | 1654 | 1212 | 0.0 | 1.02 | 0.05 | |
| α-terpineol | 1668 | 1177 | 0.0 | 17.10 | 5.48[f] | 4.46 |
| unknown | 1788 | | 0.0 | 0.56 | 0.07 | 0.09 |
| sesquiterpene oxide | 1862 | | 0.0 | 0.64 | 0.08 | |
| sesquiterpenol | 1882 | | 0.0 | 1.01 | 0.05 | |
| caryophyllene oxide | 1931 | | 0.06 | 6.13 | 0.82 | 0.77 |
| ledol | 1978 | 1596 | 0.0 | 9.44 | 2.39 | 2.12 |
| (E)-nerolidol | 2000 | 1550 | 0.0 | 92.4 | 26.1 | 39.0 |
| globulol | 2025 | 1569 | 0.0 | 1.14 | 0.0 | |
| viridiflorol | 2036 | 1589 | 0.07 | 66.4 | 19.9 | 17.4 |
| δ-cadinol | 2127 | 1529 | 0.0 | 2.40 | 0.17 | 1.03 |
| sesquiterpenol | 2165 | | 0.0 | 0.68 | 0.06 | 0.08 |
| sesquiterpenol | 2171 | | 0.0 | 0.76 | 0.06 | 0.09 |
| sesquiterpenol | 2183 | | 0.0 | 2.29 | 0.54 | 0.49 |
| selin-11-en-4-ol[d,e] | 2195 | | 0.0 | 0.57 | 0.09 | |
| sesquiterpenol | 2249 | | 0.0 | 0.84 | 0.16 | 0.17 |

[a] Compound identification realized on a composite sample of all 144 oil samples, after fractionation into hydrocarbon and oxygenated components followed by GC and GC–MS determinations. [b] Experimentally determined retention indices on Superox 4 (SPX-4) and OV1 glass capillary columns, calculated according to NF T 75-401 norm (Afnor, 1986). [c] Area percentage normalizations obtained from the injection of individual oil samples (144 crude essential oil) using SPX-4 column. [d] Newly identified in niaouli. [e] Tentative. [f] Sum of α-terpineol + viridiflorene contents. [g] Sum of β-caryophyllene + 4-terpineol contents.

Ribermag R-10-10C mass spectrometer and coupled with a Sidar data computer. The GC column was a 0.25 mm (i.d.) × 50 m fused silica capillary column coated with Carbowax 20M (phase thickness, 0.20 μm). The column temperature was 70–210 °C at 3 °C $min^{-1}$; carrier gas, helium; ion source, 220 °C; and ionizing voltage, 70 eV.

**Statistical Analyses.** Principal component analysis (PCA) has been performed by using the data set transformed into centered and reduced variables (standardized PCA). The data sets were first composed by all essential oil samples (144) and 56 variables corresponding to internal normalization of peak chromatograms obtained on the Superox 4 column. Some variables having a high correlation coefficient were deleted, and 24 of them were used for final statistical analyses. In a second attempt, factorial discriminant analysis (FDA) (Fischer, 1936) was performed to classify them into six, five, and then four populations (chemotypes). The analysis was realized on centered and reduced variables with Mahalanobis's (1936) metric. The attraction of samples to the populations was done with a distance criterion of samples to the center of gravity. In a third experiment, samples

**Table 2. Chemical Composition of Niaouli Oils from Madagascar**

| GC peak no.[a] | component | chemotype I[b] min | chemotype I[b] max | chemotype I[b] mean | chemotype II[c] min | chemotype II[c] max | chemotype II[c] mean | chemotype III[d] min | chemotype III[d] max | chemotype III[d] mean | chemotype IV[e] min | chemotype IV[e] max | chemotype IV[e] mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | α-pinene + α-thujene | 0.81 | 21.5 | 5.84 | 1.31 | 7.91 | 4.87 | 0.21 | 6.29 | 2.62 | 0.0 | 4.48 | 0.51 |
| 2 | unknown | 0.00 | 7.57 | 0.13 | 0.00 | 0.82 | 0.09 | 0.00 | 1.30 | 0.16 | 0.00 | 0.94 | 0.15 |
| 4 | sabinene | 0.00 | 4.11 | 0.13 | | | | 0.00 | 0.39 | 0.07 | | | |
| 5 | δ3-carene | | | | 0.06 | 0.19 | 0.13 | | | | | | |
| 6 | myrcene + α-phellandrene | 0.00 | 2.96 | 0.56 | 0.05 | 2.80 | 0.68 | 0.00 | 0.80 | 0.12 | 0.00 | 0.14 | 0.01 |
| 10 | 1,8-cineole | 19.1 | 51.5 | 37.1 | 0.39 | 31.1 | 22.8 | 0.17 | 34.5 | 8.17 | 0.03 | 6.56 | 1.13 |
| 13 | terpinolene | 0.00 | 0.86 | 0.17 | 1.35 | 8.33 | 5.19 | 0.00 | 3.19 | 0.32 | 0.00 | 0.18 | 0.03 |
| 16 | α-gurjunene | 0.00 | 1.23 | 0.34 | 0.00 | 0.97 | 0.47 | 0.00 | 1.09 | 0.47 | 0.00 | 0.39 | 0.02 |
| 17 | linalool | 0.00 | 2.28 | 0.18 | 0.06 | 0.24 | 0.19 | 0.00 | 0.55 | 0.16 | 0.00 | 0.67 | 0.12 |
| 18 | β-caryophyllene | 0.18 | 14.0 | 5.04 | 2.21 | 8.18 | 3.67 | 1.31 | 24.6 | 8.53 | 0.46 | 8.68 | 3.57 |
| 21 | α-humulene | 0.00 | 0.51 | 0.13 | 0.00 | 0.35 | 0.08 | 0.00 | 0.29 | 0.09 | 0.00 | 0.46 | 0.04 |
| 23 | α-terpineol + viridiflorene | 0.00 | 17.1 | 9.31 | 2.59 | 7.55 | 6.32 | 1.54 | 13.6 | 4.71 | 0.00 | 1.48 | 0.23 |
| 27 | β-selinene | 0.00 | 0.35 | 0.05 | 0.00 | 0.13 | 0.02 | 0.00 | 0.35 | 0.08 | 0.00 | 0.21 | 0.03 |
| 29 | (δ+γ)-cadinene | 0.00 | 0.36 | 0.48 | 0.00 | 0.52 | 0.33 | 0.00 | 2.16 | 0.17 | 0.00 | 0.08 | 0.01 |
| 31 | sesquiterpenol | 0.00 | 0.32 | 0.07 | 0.00 | 0.17 | 0.02 | 0.08 | 0.39 | 0.09 | 0.00 | 0.53 | 0.07 |
| 32 | caryophyllene oxide | 0.09 | 1.90 | 0.69 | 0.2 | 1.95 | 0.67 | 0.08 | 4.20 | 1.31 | 0.06 | 6.13 | 0.76 |
| 35 | ledol | 0.18 | 9.44 | 2.69 | 1.45 | 5.12 | 3.66 | 1.71 | 8.28 | 4.44 | 0.00 | 0.77 | 0.17 |
| 36 | (*E*)-nerolidol | 0.05 | 38.4 | 1.21 | 0.08 | 2.93 | 1.01 | 0.14 | 3.32 | 1.15 | 75.7 | 92.5 | 86.7 |
| 37 | viridiflorol | 21.2 | 47.9 | 24.2 | 8.38 | 34.4 | 19.9 | 26.1 | 66.5 | 47.8 | 0.07 | 1.74 | 0.51 |
| 38 | δ-cadinol | 0.00 | 0.45 | 0.11 | 0.00 | 0.25 | 0.09 | 0.00 | 0.32 | 0.15 | 0.00 | 2.50 | 0.31 |
| 39 | sesquiterpenol | 0.00 | 0.30 | 0.05 | 0.00 | 0.10 | 0.05 | 0.00 | 0.68 | 0.13 | 0.00 | 0.30 | 0.05 |
| 40 | sesquiterpenol | 0.00 | 0.25 | 0.05 | 0.00 | 0.16 | 0.05 | 0.00 | 0.36 | 0.11 | 0.00 | 0.76 | 0.04 |
| 41 | sesquiterpenol | 0.00 | 2.22 | 0.49 | 0.14 | 1.37 | 0.55 | 0.30 | 2.29 | 1.22 | 0.00 | 0.76 | 0.19 |
| 42 | sesquiterpenol | 0.00 | 0.61 | 0.17 | 0.00 | 0.84 | 0.21 | 0.00 | 0.67 | 0.34 | 0.00 | 0.29 | 0.02 |

[a] Peak number on the chromatogram obtained with a SPX-4 column. Area percentage normalizations obtained from the injection of individual oil samples. [b] Mean of 59 samples. [c] Mean of 17 samples. [d] Mean of 26 samples. [e] Mean of 42 samples.

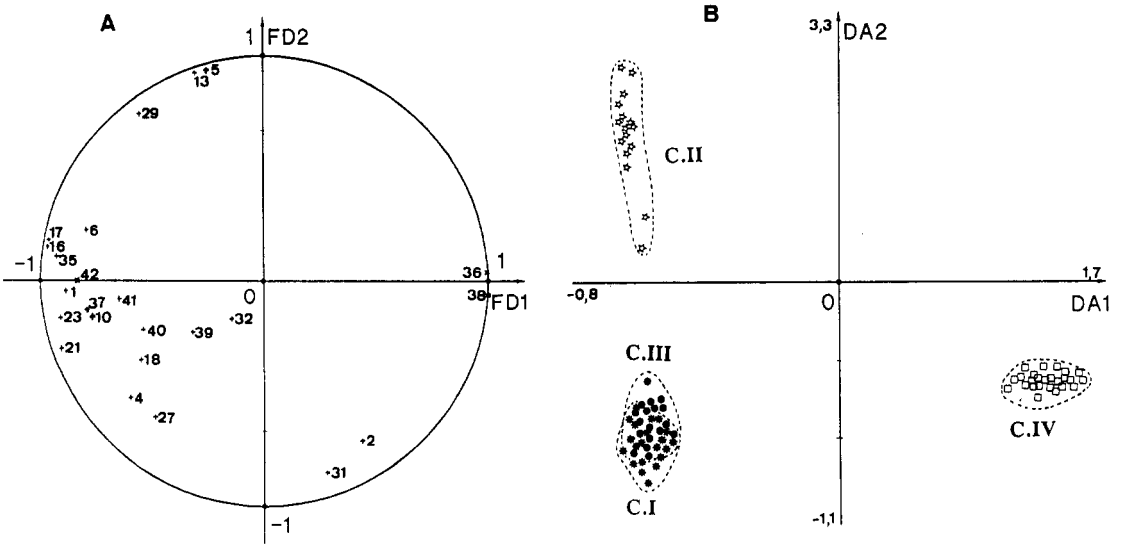


**Figure 1.** Niaouli essential oil chemotype differentiations using FDA of chemical composition obtained by gas chromatography. (A) Factor loading of variables on the two first discriminant axes. For compound identification see Table 2. (B) Two-dimensional plot of chemotypes I (*), II (☆), III (●), and IV (□) on FDA axes 1 and 2.

were classified using FDA into three groups corresponding to the seasons of collection. The whole process was done using the ANDON program (Llinas and Ruiz, 1986; Gaydou *et al.*, 1988) on an AT 486 microcomputer.

## RESULTS AND DISCUSSION

**Compound Identification.** Niaouli trees (*M. quinquenervia*) were randomly chosen to follow their chemical composition during a year. In the eastern part of Madagascar, two seasons are generally considered, but we retained three periods of time, for best control of the chemical content changes. Fresh leaf samples were hydrodistilled and gave 144 green-yellow essential oil samples. Compound identification was done starting from a mixture of these samples, which was first submitted to a known column chromatography procedure (Gaydou *et al.*, 1986) to obtain a hydrocarbon fraction and an oxygenated faction. Retention indices were determined on two glass capillary columns (Table 1), and the two fractions were analyzed using gas chromatography/mass spectrometry (GC/MS). Peaks were mostly identified by their accepted trivial names. Among the 56 peaks characterized (Table 1), 46 were identified as far as possible or tentatively identified on the basis of their retention indices and mass spectral data. With regard to the literature on niaouli essential oil composition (Guenther, 1950; Beylier, 1979; Ekundayo *et al.*, 1987; Todorova *et al.*, 1988, Brophy and Lassak, 1988; Motl *et al.*, 1990; Ramanoelina *et al.*, 1992), three compounds were newly identified: α-amorphene, fenchone, and neral. Three compounds were tentatively identified: α-cubebene, thujilic alcohol, and selin-11-en-4-ol.

**Chemical Range Determination.** Each niaouli oil sample was analyzed on the Superox 4 column without fractionation. Table 1 lists area percentage normalizations

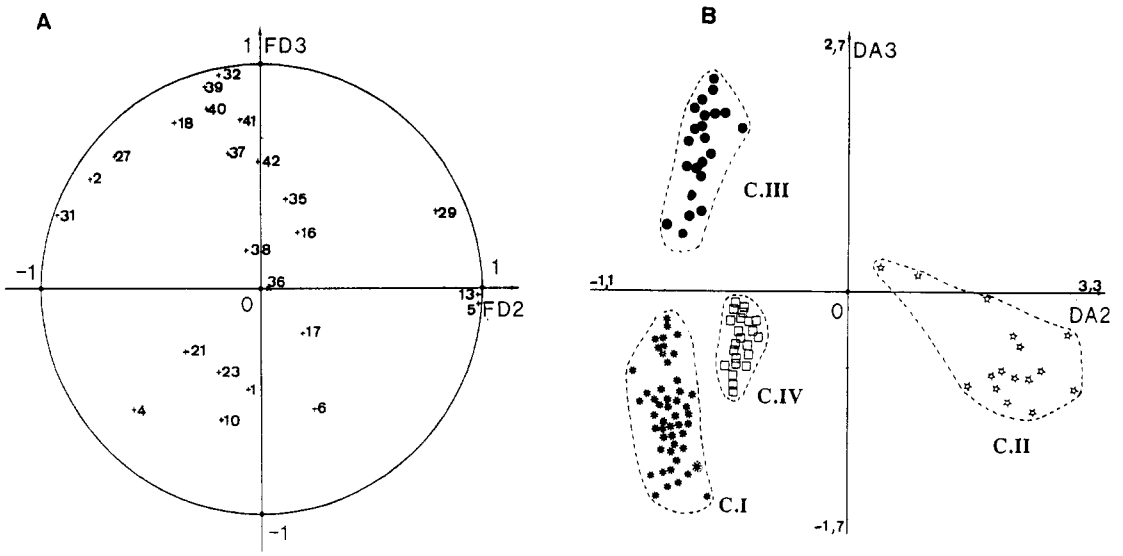


**Figure 2.** Differentiation of chemotypes I and II using FDA of chemical composition obtained by gas chromatography. (A) Factor loading of variables on discriminant axes 1 and 3. For compound identification see Table 2. (B) Two-dimensional plot of chemotypes I (*), II (☆), III (●), and IV (□) on FDA axes 1 and 3.

**Table 3. Seasonal Influence on the Chemical Composition of Niaouli Essential Oil Chemotypes from Madagascar**

| | chemotype I[b] | | | chemotype II[c] | | | chemotype III[d] | | | chemotype IV[e] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GC peak no.[a] | W[f] | S[g] | A[h] | W[f] | S[g] | A[h] | W[f] | S[g] | A[h] | W[f] | S[g] | A[h] |
| 1 | 5.94 | 5.95 | 5.87 | 5.02 | 3.99 | 5.74 | 2.74 | 1.90 | 3.29 | 0.73 | 0.52 | 0.26 |
| 2 | 0.09 | 0.13 | 0.12 | 0.10 | 0.09 | 0.08 | 0.15 | 0.16 | 0.17 | 0.16 | 0.15 | 0.14 |
| 4 | 0.12 | 0.10 | 0.13 | | | | 0.02 | 0.03 | 0.01 | | | |
| 5 | | | | 0.45 | 0.91 | 0.69 | | | | | | |
| 6 | 0.41 | 0.52 | 0.61 | 0.15 | 0.10 | 0.15 | 0.09 | 0.18 | 0.16 | 0.01 | 0.01 | 0.01 |
| 10 | 38.0 | 37.4 | 36.4 | 22.6 | 20.4 | 25.8 | 8.17 | 7.89 | 8.45 | 0.94 | 1.74 | 0.72 |
| 13 | 0.08 | 0.22 | 0.20 | 4.97 | 4.73 | 6.03 | 0.25 | 0.46 | 0.23 | 0.01 | 0.03 | 0.05 |
| 16 | 0.34 | 0.29 | 0.41 | 0.54 | 0.41 | 0.46 | 0.52 | 0.43 | 0.46 | 0.01 | 0.06 | 0.01 |
| 17 | 0.12 | 0.23 | 0.19 | 0.19 | 0.18 | 0.21 | 0.16 | 0.14 | 0.20 | 0.14 | 0.17 | 0.07 |
| 18 | 4.99 | 4.90 | 5.24 | 3.26 | 4.90 | 2.68 | 8.73 | 9.49 | 7.24 | 3.18 | 2.99 | 4.54 |
| 21 | 0.13 | 0.14 | 0.10 | 0.13 | 0.10 | 0.01 | 0.11 | 0.04 | 0.13 | 0.04 | 0.00 | 0.08 |
| 23 | 8.64 | 10.3 | 9.02 | 6.28 | 5.95 | 6.83 | 4.56 | 4.80 | 4.77 | 0.19 | 0.27 | 0.24 |
| 27 | 0.05 | 0.05 | 0.05 | 0.02 | 0.02 | 0.01 | 0.12 | 0.05 | 0.06 | 0.03 | 0.01 | 0.05 |
| 29 | 0.06 | 0.03 | 0.04 | 0.34 | 0.32 | 0.34 | 0.35 | 0.07 | 0.07 | 0.00 | 0.01 | 0.01 |
| 31 | 0.08 | 0.01 | 0.12 | 0.00 | 0.03 | 0.05 | 0.12 | 0.04 | 0.09 | 0.10 | 0.01 | 0.12 |
| 32 | 0.95 | 0.34 | 0.74 | 0.77 | 0.72 | 0.51 | 1.70 | 0.92 | 1.31 | 1.14 | 0.40 | 0.75 |
| 35 | 2.92 | 2.85 | 2.21 | 3.58 | 3.96 | 3.40 | 4.31 | 4.37 | 4.64 | 0.16 | 0.18 | 0.17 |
| 36 | 2.01 | 1.17 | 0.33 | 0.78 | 1.38 | 0.84 | 0.40 | 1.42 | 1.70 | 86.8 | 85.9 | 87.3 |
| 37 | 28.4 | 22.4 | 22.1 | 20.5 | 22.2 | 16.6 | 46.3 | 48.9 | 48.0 | 0.42 | 0.61 | 0.49 |
| 38 | 0.10 | 0.11 | 0.11 | 0.08 | 0.10 | 0.07 | 0.10 | 0.17 | 0.17 | 0.03 | 0.89 | 0.01 |
| 39 | 0.06 | 0.05 | 0.04 | 0.05 | 0.05 | 0.04 | 0.14 | 0.09 | 0.16 | 0.03 | 0.07 | 0.05 |
| 40 | 0.07 | 0.05 | 0.03 | 0.05 | 0.05 | 0.04 | 0.14 | 0.08 | 0.12 | 0.01 | 0.07 | 0.03 |
| 41 | 0.39 | 0.60 | 0.46 | 0.52 | 0.50 | 0.52 | 0.88 | 1.48 | 1.29 | 0.15 | 0.30 | 0.13 |
| 42 | 0.12 | 0.20 | 0.18 | 0.25 | 0.19 | 0.17 | 0.28 | 0.44 | 0.31 | 0.00 | 0.01 | 0.05 |

[a] For peak identification see Table 2. Area percentage normalizations obtained from the injection of individual oil samples. [b] 59 samples. [c] 17 samples. [d] 26 samples. [e] 42 samples. [f] Mean of collection in July (winter). [g] Mean of collection in December (spring/summer). [h] Mean of collection in March (autumn).

(mean and range) of the major compounds. The percentages were determined by the method of area normalization and without the application of response factor corrections. Results obtained were submitted to statistical analyses. As shown in Table 1, the range content of some compounds is very large. This is the case for four hydrocarbons, α-pinene (0.01–21.5%), limonene (0.85–6.54%), γ-terpinene (0.00–13.47%), and β-caryophyllene (0.00–24.6%), and four oxygenated compounds, 1,8-cineole (0.03–51.5%), α-terpineol + viridiflorene (0.00–17.1%), (*E*)-nerolidol (0.00–92.4%), and viridiflorol (0.04–66.4%). Other compounds were found in lower amounts and only ledol has a mean content higher than 1%. The structure of viridiflorol was confirmed by $^{1}H$ and $^{13}C$ NMR (Faure *et al.*, 1991).

**Multivariate Statistical Analyses.** The data set composed of 144 samples and 56 chemical compounds was submitted to multivariate statistical analyses for chemotype determination. Using principal component analysis (PCA), the correlation matrix reveals some intercorrelations between variables. Therefore, compounds showing strong correlation coefficients were deleted for subsequent analyses. The 24 variables retained are given in Table 2. The peak numbers in this table show the elution order on the Superox 4 column. The graphical representation of the projection of samples onto the two first principal components shows a population rich in compound **36** [(*E*)-nerolidol]. Some other populations seems to appear on the second and third principal components. Therefore, factorial discriminant analysis (FDA) was applied for the characterization of these populations.

In a first experiment six groups were considered on the basis of the chemical range content: α-pinene + α-thujene (1); 1,8-cineole (2); β-caryophyllene (3); α-terpineol +

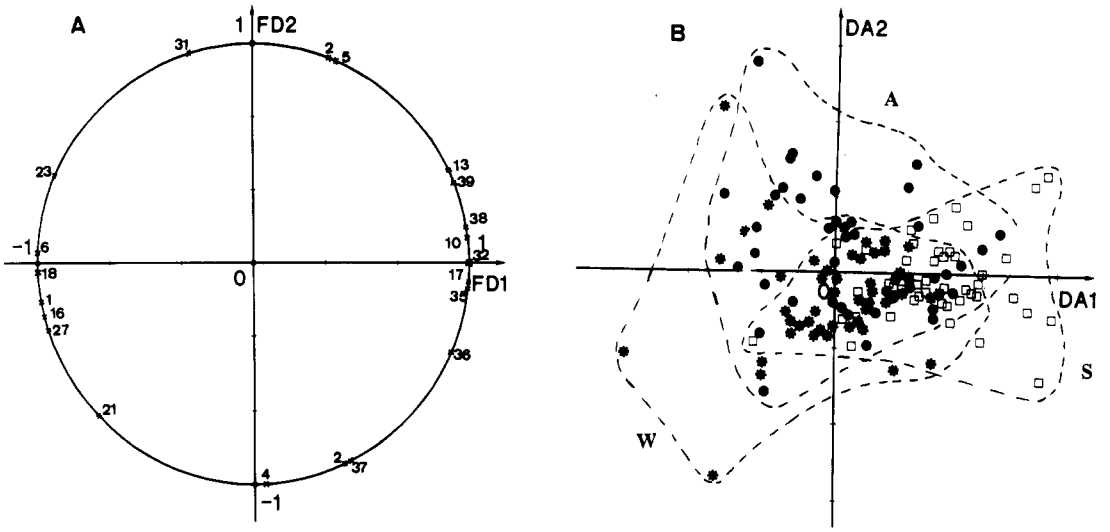


**Figure 3.** Attempt of niaouli essential oil seasonal differentiations using FDA of chemical composition obtained by gas chromatography. (A) Factor loading of variables on the two first discriminant axes. For compound identification see Table 2. (B) Two-dimensional plot of samples collected in winter (*), summer (□), and autumn (●).

viridiflorene (4); (*E*)-nerolidol (5); and viridiflorol (6). FDA showed that the two hydrocarbon groups 1 and 3 could not be considered as chemotypes on the basis of their repartition content in the other groups. Finally, four chemotypes were considered, and Table 2 summarizes the data for each of the chemotypes. Chemotype I is a cineole type (37.1%) with a relatively high content in viridiflorol (24.2%). α-Terpineol + viridiflorene (9.3%), α-pinene + α-thujene (5.8%), and β-caryophyllene (5.0%) are the other main components. Chemotype II is characterized by δ3-carene (0.7%), found only in this chemotype, and a high content in terpinolene compared to the other groups (5.2%). The 1,8-cineole and viridiflorol contents are about 20% each. Chemotype III is a viridiflorol type (47.7%) recently described (Ramanoelina *et al.*, 1992). Chemotype IV is an (*E*)-nerolidol type (86.7%) with very low content in 1,8-cineole (1.1%) and viridiflorol (0.5%). Such an (*E*)-nerolidol type was earlier described by Beylier (1979).

Using FDA and considering four chemotypes, only three discriminant axes are built up. Plots of variables and samples for the two first discriminant axes are given in Figure 1. Axis 1, strongly positively loaded with (*E*)-nerolidol, differentiates chemotype IV from the others. Chemotype II was differentiated from chemotypes I and III, since this axis is highly positively loaded with δ3-carene and terpinolene. The differentiation of chemotypes I and III occurred on axis 3 (Figure 2) since this axis is positively loaded with viridiflorol and negatively loaded with 1,8-cineole (variables 37 and 10, respectively, Figure 2A).

**Seasonal Influence on the Chemical Composition of Chemotypes.** Since chemical composition may be modified by agroclimatic conditions, leaf collect was done every 4 months. The means of the chemical compositions for these time periods and for each chemotype are given in Table 3. As can be ssen, the range change is poor, showing that chemical composition is not significatively modified by the data of collection. The (*E*)-nerolidol content ranges from 85.9% to 87.3% for chemotype IV, and that of 1,8-cineole, in chemotype I, ranges from 36.4% to 38.0%. The three-season differentiation using FDA was attempted. In this case only two discriminant axes are needed. As shown in Figure 3, seasonal differentiation does not occur, showing therefore that the niaouli essential oil compositions are fairly stable all year long.

**Conclusion.** If we consider that trees were randomly chosen, the 1,8-cineole chemotype is well represented in Madagascar (41% of the trees). This chemotype seems very common since it has been described by Jones and Haencke (1937), Guenther (1950), Ekundayo *et al.* (1987), Todorova *et al.* (1988), and Motl *et al.* (1990). The (*E*)-nerolidol chemotype represents about 30% of the tree population with a low oil content. The linalool type and the linalool + (*E*)-nerolidol type described by Guenther (1950) was not observed in this area of Madagascar. Finally, the viridiflorol chemotype (19% of the trees) was confirmed (Ramanoelina *et al.*, 1992) and seems to be "native" to Madagascar since, to our knowledge, it was never described. This chemotype should be considered from a pharmaceutical point of view.

ACKNOWLEDGMENT

We gratefully acknowledge E. Andrianary, SINPA, Toamasina, B. Jaosolo, FOFIFA, Andasibe, and A. Ranaivoson, ESSA, Antananarivo, for collecting samples and providing facilities.

LITERATURE CITED

Afnor. *Recueil de Normes Françaises des Huiles Essentielles*, 2nd ed.; Association Française de Normalisation: Paris, 1986.

Beylier, M. F. Bacteriostatic activity of some Australian essential oils. *Perfum. Flavor.* **1979**, *4*, 23–26.

Blake, S. T. Contributions from the Queensland herbarium. 1. *A revision of Melaleuca leucadendron and its allies (Myrtaceae)*; Queensland herbarium, Department of primaries industries: Brisbane, Australia, 1968.

Brophy, J. J.; Lassak, E. V. *Melaleuca leucadendra* L. leaf oil: two phenylpropanoid chemotypes. *Flavour Fragrance J.* **1988**, *3*, 43–46.

Byrnes, N. B. A revision of *Melaleuca* L. (Myrtaceae) in Northern and Eastern Australia. *Austrobaileya* **1984**, *1*, 65–76.

Cabanis, Y.; Cabanis, L.; Chabouis, F. *Végétaux et Groupements Végétaux de Madagascar et des Mascareignes*; BDPA: Tananarive, 1970; Tome 1.

Cherrier, J. F. *Les essences forestières exploitables en Nouvelle Calédonie: Botanique, Technologie, Usages*; Service des Eaux et Forêts: Paris, 1983.

Dawson, J. W. Flore de Nouvelle-Calédonie, *Myrtaceae. Bull. Mus. Hist. Nat. Paris* **1992**, *14*, 216–229.

Ekundayo, O.; Laakso, I.; Hiltunen, R. Volatile components of *Melaleuca leucadendron* (cajuput) oils. *Acta Pharm. Fenn.* **1987**, *96*, 79–84.

Faure, R.; Ramanoelina, A. R. P.; Rakotonirainy, O.; Bianchini, J. P.; Gaydou, E. Two-Dimensional Nuclear Magnetic Resonance of Sesquiterpenes. 4. Application to complete as-

signment of $^{1}H$ and $^{13}C$ NMR Spectra of some Aromadendrene Derivatives. *Magn. Reson. Chem.* **1991**, *29*, 969–971.
Fischer, R. A. The Use of Multiple Measurements in Taxonomics Problems. *Ann. Eugen. (London)* **1936**, *7*, 179–188.
Gaydou, E. M.; Randriamiharisoa, R.; Bianchini, J. P. Composition of the Essential Oil of Ylang-Ylang (*Cananga odorata* Hook Fil. et Thomson, *forma genuina*) from Madagascar. *J. Agric. Food Chem.* **1986**, *34*, 481–487.
Gaydou, E. M.; Randriamiharisoa, R. P.; Bianchini, J. P.; Llinas, J. R. Multidimensional Data Analysis of Essential Oils. Application to Ylang-Ylang (*Cananga odorata* Hook Fil. et Thomson, *Forma Genuina*) Grades Classification. *J. Agric. Food Chem.* **1988**, *36*, 574–579.
Guenther, E. *The essential oils*; Van Nostrand: New York, 1950; Vol. IV, pp 537–540.
Humbert, H. Flore de Madagascar et des Comores. Perrier de la Bâthie, H., Ed. *Mus. Hist. Nat., Paris* **1953**, 74–75.
Jones, T. G. H.; Haenke, W. L. Essential oils from the Queensland Flora: IX. *Melaleuca viridiflora. Proc. R. Soc. Queensl.* **1937**, *48*, 41–44.
Jones, T. G. H.; Haenke, W. L. Essential oils from the Queensland flora: X. *Melaleuca viridiflora. Proc. R. Soc. Queensl.* **1938**, *49*, 95–98.
Llinas, J. R.; Ruiz, J. M. Multivariate Analysis of Chemical Data Set. In *Computer Aids to Chemistry*; Vernin, G., Chanon, M., Eds.; Ellis Horwood: Chichester, U.K., 1986; pp 200–256.
Mahalanobis, P. C. On the Generalized Distance in Statistics. *Proc. Natl. Inst. Sci. Ind.* **1936**, *12*, 49–55.
Motl, O.; Hodakova, J.; Ubik, K. Composition of vietnamese cajuput essential oil. *Flavour Fragrance J.* **1990**, *5*, 39–42.
Ramanoelina, A. R. P.; Viano, J.; Bianchini, J. P.; Gaydou, E. M.; Andriantsiferana, M. Chemical Composition of Niaouli Essential Oils from Madagascar. *J. Essent. Oil Res.* **1992**, *4*, 657–658.
Todorova, M.; Ognyanov, I.; Pham Trong, T. T. Composition of Vietnamese Essential oil from *Melaleuca leucadendron* L. *Perfum. Flavor.* **1988**, *13* (1), 17–18.
Valnet, J. *Aromathérapie: Traitement des maladies par les essences des plantes*; Maloine, S. A.: Paris, 1984.

Received for review November 16, 1993. Accepted February 18, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1994.