JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Linalool from *Lippia alba*: Study of the Reproducibility of the Essential Oil Profile and the Enantiomeric Purity

ANTONIO C. SIANI,*,† MARCELO R. R. TAPPIN,† MÔNICA F. S. RAMOS,†,‡ JOSÉ L. MAZZEI,§ MARIA CONCEIÇÃO K. V. RAMOS,|| FRANCISCO R. DE AQUINO NETO,|| AND NÉLSON FRIGHETTO⊥

Laboratório de Química de Produtos Naturais, Instituto de Tecnologia em Fármacos, Fundação Oswaldo Cruz, Rua Sizenando Nabuco 100, Manguinhos, Rio de Janeiro, RJ, Brazil, 21041-250; Departamento de Medicamentos, Faculdade de Farmácia, Escola de Química, and Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Centro de Pesquisas Químicas, Biológicas e Agrícolas da UNICAMP, Campinas, Brazil

A new chemotype of the aromatic Verbenaceae species *Lippia alba* Mill. N. E. Br. from southeastern Brazil has recently been shown to have a high content of linalool in the leaf essential oil. Vegetative propagation of this chemotype was conducted at six different locations in Brazil, and the variation of the content and the optical purity of linalool in the oils were verified. Yields (0.6−0.9%, hydrodistillation), chemical composition, linalool content, and optical purity of the oils from all the plants were compared, using GC−FID, GC−MS, chiral chromatography, and retention index calculation. No plant exceeded the matrix in linalool content (46.5 to 90.7%), and the chemical profile of the oils was the same for all the samples. Purification of linalool to a content close to 100% was effected by vacuum distillation of the crude oil. Chiral analysis showed exclusively the presence of *S*-linalool in all the crude oils and in the distilled samples.

KEYWORDS: ***Lippia alba*; Verbenaceae; (+)-linalool; essential oil; chiral gas chromatography**

INTRODUCTION

Linalool (3,7-dimethyl-1,6-octadien-3-ol) is one of the most important compounds in the industrial flavor field. It is used as a substitute for bergamot or French lavender oil because the levorotatory form has an odor similar to these oils. Linalool occurs naturally as two isomeric optical forms, 3*R*-(−)-linalool or 3*S*-(+)-linalool (**Figure 1**), each with quite distinctive odors. The levorotatory isomer (licareol) has a flowery-fresh, lavender aroma, reminiscent of lily of the valley, whereas the dextrorotatory isomer (coriandrol) has a herbaceous, musty green smell, often described as having a citric note (*1*).

The most important plant source of linalool is still the trunk of the Brazilian rosewood species of the genus *Aniba*. This tree is facing imminent extinction (*2*) and therefore alternative natural sources are urgently needed in order to establish a rational production of this substance or of similar oils. Besides the cheaper synthetic material, the oils from some other plant species are currently marketed as sources of linalool. This is the case of the Chinese Lauraceae "Ho" (*Cinnamomum camphora*),

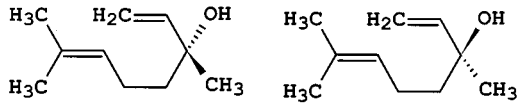

Figure 1. Structural representation of the linalool isomeric optical forms.

which produces a lower-priced linalool compared to that from *Aniba* species (*3*). Part of the reason for this is that the Ho oil also contains camphor which is frequently not acceptable to perfumists. Oils from Ho and rosewood are different in optical properties and odor. Linalool produced by Ho is specifically levorotatory, and the oil from rosewood contains both the levo- and dextrorotatory forms in variable proportions which determines the optical activity. The mixture makes a decisive contribution to its pleasant odor (*3*).

The search for an alternative source of linalool to supply the market demand, and at the same time preserve the native flora, triggered the investigation of *Lippia alba* as a potential industrial source of this monoterpene alcohol. *Lippia alba* Mill N. E. Br., an aromatic plant widely distributed in temperate Central and South America, commonly known as "erva-cidreira", "melissa", or "salvia morada", has sedative properties and status as a medicinal plant for digestive problems (*4*). The species contains from a poor to good essential oil content (yields ranging from 0.1 to 1.2 wt %) but exhibits wide variation of composition.

* To whom correspondence should be addressed. E-mail: siani@far.fiocruz.br. Tel: +55-21-2270-0764. Fax: +55-21-2270-3912.
† Laboratório de Química de Produtos Naturais, Instituto de Tecnologia em Fármacos, Fundação Oswaldo Cruz.
‡ Departamento de Medicamentos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro.
§ Escola de Química, Universidade Federal do Rio de Janeiro.
|| Instituto de Química, Universidade Federal do Rio de Janeiro.
⊥ Centro de Pesquisas Químicas, Biológicas e Agrícolas da UNICAMP.

10.1021/jf011592n CCC: $22.00 © 2002 American Chemical Society
Published on Web 05/03/2002

Table 1. Geographic Locations of the Chemotype Planting Sites, Linalool Content, and Refractive Index of the Oils of *Lippia alba* from Different Sites of Cultivation

code of site (municipality)	latitude/longitude	altitude (m)	linalool content (%)[a]	η_D^{25}
LA-VA (Valinhos)[b]	22°58′ S/46°59′ W	900	59 ± 4	1.4730
LA-C (Campinas, matrix)	22°48′ S/47°07′ W	669	89 ± 9	1.4735
LA-CO (Conchal)	22°20′ S/47°10′ W	513	64 ± 4	1.4719
LA-AC (Assis Chateaubriand)	24°25′ S/53°29′ W	440	80 ± 8	1.4700
LA-CU (Cuiabá)	15°35′ S/56°06′ W	165	79 ± 12[c]	1.4738
LA-RJ (Rio de Janeiro)	22°52′ S/43°14′ W	25	67 ± 7	1.4701
LA-CS (Caxias do Sul)	29°09′ S/51°10′ W	773	56 ± 8	n.d.
LA-MA (Manaus)	03°08′ S/60°01′ W	72	54 ± 4	1.4710
distilled oil[d]			98 ± 3	1.4615

[a] $n = 6$, $p < 0.05$. [b] Re-collected species in the original chemotype site (Ref. *18*). [c] Calculated on dried plant basis. [d] Optimized conditions for the sample LA-AC.

This has been reported since the 1960s for plants cultivated in Argentina (*5*−*8*), Cuba (*9*), Uruguay (*10*), and Brazil (*8*, *11*): in the dry northeast (*8*, *12*−*15*), the hot humid north (*16*), and the temperate or sub-tropical southern regions (*8*, *17*). From these reports, and based on their own work, Zoghbi et al. (*16*) identified at least three chemotypes of this plant, in terms of the main monoterpene constituents in the essential oil which each have distinct odors. The first is characterized by a high content of 1,8-cineole, limonene, and carvone; the second is characterized by limonene, carvone, and myrcene; and the third is represented mainly by geranial and neral.

Morphological differences are insufficient to affirm the existence of more than one species (*5*). The chemical composition of the essential oil of *Lippia alba* may also vary with the stage of development of the plant, the parts of the plant used (the composition varied between aerial parts or leaves with or without flowers, dried or fresh), on the geographic location of collection site, the general physicochemical characteristics of the soil, climate (humidity, oxygen, and sunlight), and other ecological conditioning factors (*16*). The influence of those factors on the accumulation of distinct terpene metabolites in a *Lippia* species defines its chemotype (*18*).

A recently reported chemotype of *L. alba* from Valinhos, São Paulo State, in the southeastern region of Brazil, afforded a good yield of essential oil (1.0% w/w) from fresh leaves with a high content (67−83 wt %) of linalool. The production of linalool by this plant was apparently not affected by seasonal factors (*18*). Because of its good oil yield, its content of linalool, and the rapid growth cycle of this Verbenaceae species, this chemotype may be considered as a promising commercial source of essential oil to supply the perfume and flavor industries, both of which require a standard flavor/aroma behavior, which means a stable terpene composition in the volatile oils.

The present study was conducted to test the variation of the linalool content in this *L. alba* chemotype (designated LA-VA) by random cultivation of the plant at six different sites of Brazil. This study also aimed to establish the optical constancy of the oil and to verify its geographical variation. Enantiomeric analysis of the oils by chiral chromatography was used for this purpose.

MATERIALS AND METHODS

Cultivars. The original Valinhos chemotype described by Frighetto et al. (*19*) was reproduced by vegetative propagation in the city of Campinas, about 60 km from the original wild chemotype location, to establish the cultivar matrix LA-C. Using the same procedure, LA-C was propagated to six other widely distributed sites in Brazil, listed in **Table 1**. In all cases the planting was made between October 1997 and March 1998 (spring and summer months) and the plants were harvested after three to four months growth. The original chemotype LA-VA was also re-collected and analyzed.

Reagents. Commercial standards (±)-linalool (97% purity) and R-(−)-linalool were obtained from Aldrich Chemical Co. (Milwaukee, WI) and Fluka Chemie GmbH (Buchs, Switzerland), respectively.

Essential Oils. The volatile compounds of the leaves were extracted by hydrodistillation during 2 h by using a modified Clevenger-type apparatus. Fresh leaves were used except for plants cultivated in Cuiabá (LA-CU), which were previously oven-dried at 45 °C. After removal of water by centrifugation, the oils were stored in sealed tubes in a freezer. The mean contents of linalool in each oil was calculated from chromatographic results using flame ionization detection (FID).

Distillation. Distillation experiments were carried out with the sample LA-AC (160 mg), in a ball-tube Kugelrohr apparatus (Aldrich) coupled to a VacoBox B-177 with vacuum controller B-720 (Büchi Labortechnik AG; Flawil, Switzerland). Twenty assays were carried out varying the pressure, temperature, and time of the distillation, and the optimal condition for the highest purity of linalool (80 °C, 50 ± 10 mbar, during 1 h) was reproduced in quadruplicate.

Refractive Index. Three aliquots of each oil were measured without dilution, in an Abbé apparatus (Carl Zeiss; Jena, Germany) thermostated at 25 °C.

Optical Rotation. Optical rotation of six aliquots of each oil was measured, without dilution, in a digital polarimeter model JASCO DIP-370, at 589.3 nm (sodium D-line) (Hachioji; Tokyo, Japan) and room temperature in a 10-mm cell. The values of optical rotation were determined by assuming a standard deviation less than 3%.

Gas Chromatography. Constituents of the oils were characterized in a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA) coupled to a mass spectrometer (GC−MS), with a HP-5 MS capillary column, 30 m × 0.32 mm i.d., 0.25 μm film thickness, in the following conditions: helium as carrier gas at 0.5 mL/min; injector split at 250 °C (split ratio 1/20); ion source at 250 °C and electron impact ionization at 70 eV; oven temperature programmed from 70 °C (held for 5min) to 250 °C at 3 °C/min. Individual components were characterized by comparison of mass spectra with the Wiley Library Software 59943B, and calculation of the retention index, with reference to a linear C_8−C_{36} *n*-alkane series (*20*). The content of linalool in the oil was determined using FID at 290 °C in a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 3396 integrator ; oven temperature programmed from 60 °C to 240 °C at a rate of 2 °C/min with a HP-5 capillary column 60 m × 0.25 mm i.d., 0.25 μm film thickness. Other chromatographic conditions as above. Chloroform solutions (2.0 mg in 500 μL) of five weighed aliquots of each oil and of a standard solution of commercial racemic linalool were injected, using the latter as an external standard for quantification.

Enantiomeric analysis of linalool was carried out in a Hewlett-Packard 5890 gas chromatograph by using a "home-made" glass manufactured (Duran 50; Bayeruth, Germany) capillary column, 16 m × 0.3 mm i.d., prepared according to the literature (*21*). This column was coated with a chiral stationary phase (0.3 μm film thickness) composed of 10% 6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin (TBCD) diluted in SE-54 phase (Chrompack, 5% phenyl, 1% vinylmethyl-polysiloxane; Bergen op Zoom, The Netherlands). The oven temperature was programmed from 70 °C (held for 15 min) to 150 °C at 5 °C/min. Samples of 1.0 μL from a solution of 1 mg/mL of *L. alba* oil in CH_2Cl_2 were injected at 260 °C in a split ratio of 1:100. Hydrogen was used as a carrier gas at a linear velocity of 50 cm/s. The detection was by FID at 280 °C.

Statistical Analysis. The statistical significance differences of the cultivation on the optical rotation and linalool content of the cultivars was evaluated using ordinary analysis of variance (ANOVA, $p < 0.05$) (*22*).

RESULTS AND DISCUSSION

General. Oil yields ranged from 0.6 to 0.9 wt %, independent of the growing site. The same behavior was observed for their refractive indices which maintained a narrow range of 1.4700−1.4738 (**Table 1**). No significant variation among the aliquots of each oil was observed. Mono- and sesquiterpenes were characterized by matching the results from the GC−MS and

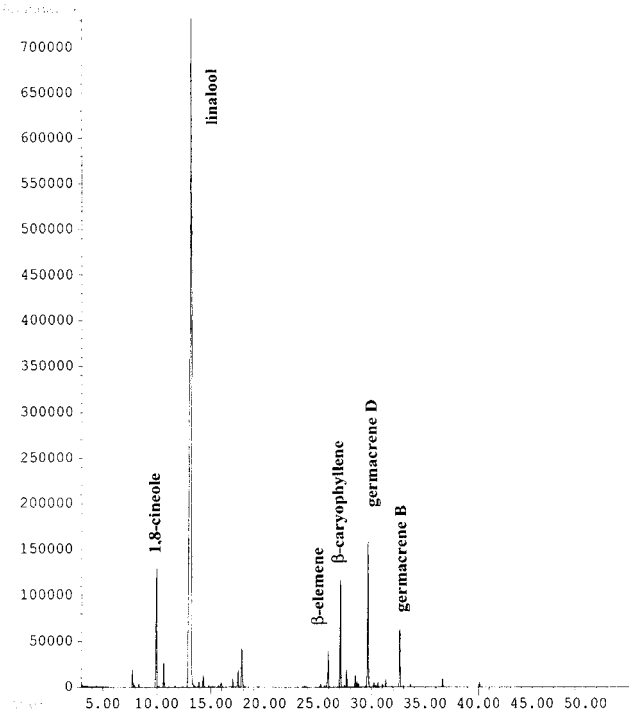

Figure 2. GC–MS of *L. alba* oil and other relevant substances. Analytical conditions are described in the text.

the retention index determination. A typical chromatogram and the more relevant substances are shown in **Figure 2**. Apart from linalool, the most relevant compounds in the oils are 1,8-cineole and β-caryophyllene, followed by germacrene D, germacrene B, and β-elemene. Traces of neral and geranial were found in LA-AC, and trans-dihydrocarvone (RI 1203) and trans-carveol (RI 1213) were minimally present in LA-AC and LA-RJ. The qualitative profiles of all the oils were quite similar to that described for the original chemotype (*18*), which is good evidence for the biosynthetic stability of this *Lippia alba* linalool chemotype under several different environmental conditions, when propagated vegetatively.

The Kugelrohr experiment afforded 75% yield of the oil in the more volatile fraction, almost exclusively linalool with traces of cineole. This result also suggests that distillation could represent a very convenient procedure for obtaining a high purity linalool from this source, although a large-scale distillation would have to be assayed to confirm this assertion.

Linalool Content and Enantiomeric Analyses. In terms of the statistical significance, five groups can be established with regard to linalool content. These are: LA-CS and LA-MA (≅ 55%); LA-VA and LA-CO (55−65%); LA-RJ (65−75%); LA-CU and LA-AC (75−80%); and LA-C (matrix, > 80%). A preliminary drying experiment involving LA-RJ (oven-dried, 45 °C, 4 days) showed that the leaves were 77% water. No clear correlation between the linalool content and any climatic parameter or geographic location of the planting was found.

On the other hand, significant differences in the optical rotations of the oils from the different sites cannot be rationalized

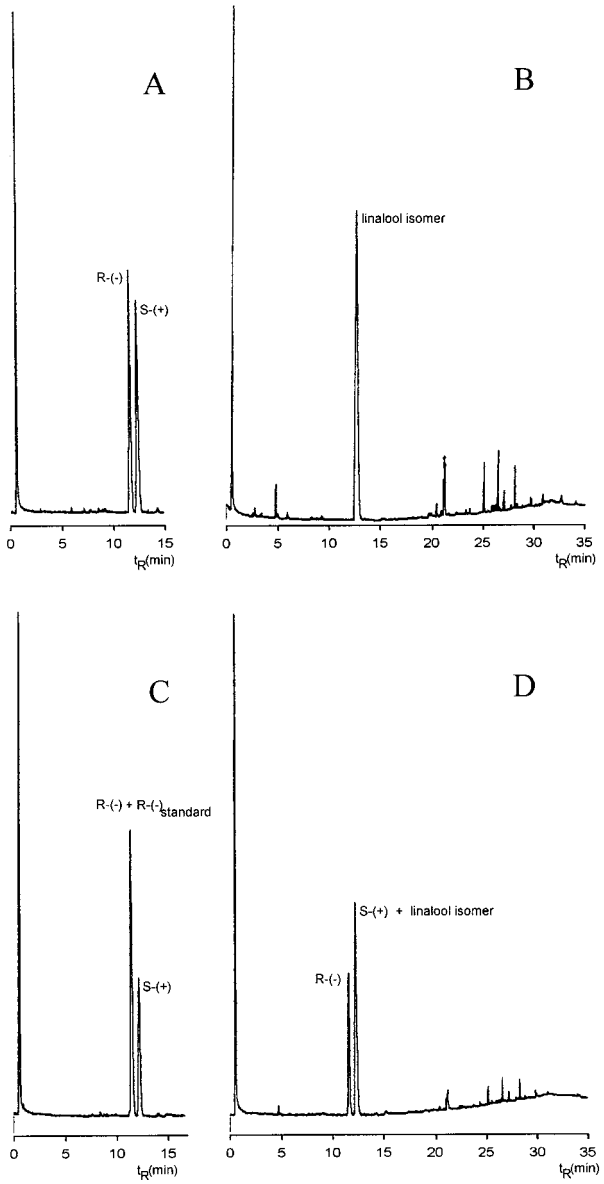

Figure 3. Chiral high-resolution gas chromatography: A (±)-linalool standard; B typical *Lippia alba* oil; C co-injection result of (±)-linalool and 3R(–)-linalool; D co-injection result of (±)-linalool and *Lippia alba* oil. For experimental conditions see Materials and Methods.

Table 2. Content (%) of the Main Sesquiterpenes Present in the Essential Oil of *Lippia alba* and Optical Rotation of the Oils[a]

experiment	β-elemene	β-caryophyllene	germacrene D	germacrene B	sesqui total	α_D
LA-MA	1.7	4.4	2.1	2.4	10.6	0.126 ± 0.008
LA-VA	2.2	4.3	2.7	3.2	12.4	0.134 ± 0.005
LA-CO	2.1	4.0	2.8	3.0	11.9	0.296 ± 0.006
LA-RJ	2.0	3.7	2.5		8.2	0.369 ± 0.007
LA-CS	1.6	1.9	2.0		5.5	0.465 ± 0.029[b]
LA-CU	3.3	5.0	3.2		10.5	0.510 ± 0.015
LA-AC	2.6	5.0	2.2		9.8	0.697 ± 0.014
LA-C	2.8	6.0	2.0		10.8	0.710 ± 0.013
distilled LA-CS						1.269 ± 0.005

[a] The same statistic approach for the calculation of linalool content was applied to individual sesquiterpenes (*21*). Retention indices for β-elemene, β-caryophyllene, germacrene D, and germacrene B were 1395, 1423, 1485, and 1561, respectively. [b] Obtained in solution (100 μL of the oil in 2.5 mL of $CHCl_3$).

straightforwardly in terms of the differences in the *S*-linalool content. Large variations in the optical rotation values of the oils are rather related to the relative amount of sesquiterpenes present in the samples (**Table 2**), with special emphasis on the germacrene D, β-caryophyllene, and β-elemene present in all of the samples that show larger absolute optical rotation values (*23*). This fact corroborated indirectly the higher optical rotation measured for the distilled oil (**Table 2**), which contains around 98% of linalool content, and is the nearest to the specific absolute value described for *S*-linalool. This evidence invalidates optical rotation determination as a practical tool for measuring linalool content in the oils. The same reason may also explain the unusually low value of the refractive index of the distilled sample (**Table 1**).

Chiral gas chromatography (**Figure 3A**) resulted in good resolution of the enantiomers from the racemic standard solution, the first peak eluting at 11.7 min and the second eluting at 12.4 min. The 3*R*-(−)-linalool standard had the same retention time as the first peak of the racemic solution. Co-injection of racemic linalool and standard 3*R*-(−)-linalool resulted in selective increase of the peak corresponding to the *R* isomer, and confirmed the elution order as *R*, *S*. The *L. alba* oils were injected under the same chromatographic conditions and all the samples showed only one peak. Further co-injection of each oil and the racemic linalool showed exclusively an increase of the peak corresponding to the *S* isomer. These results indicated that all the oils showed only the presence of the 3S-(+)-linalool isomer, justifying the dextrorotatory property of the oils analyzed. Thus, in contrast to Ho and rosewood, this chemotype of *L. alba* produces specifically dextrorotatory linalool, independent of the different growing sites.

The linalool chemotype of *L. alba* is thus shown to be an excellent potential industrial source of dextrorotatory 3*S*-(+)-linalool, and also may be easily cultivated by vegetative propagation without any serious detriment to the oil quality. Furthermore, the distillation experiment points to a quick, economic, and reliable way to enrich linalool in the oil to near 100%, as a measure to improve its odor properties as well as to establish it as a commercial high-purity source. Moreover, the oil content might be improved by standardizing cultivation parameters and also possibly by genetic intervention.

ACKNOWLEDGMENT

We thank the following persons for valuable help in the planting: Dr. J. A. Cabral (LA-MA), Dr. L. A. Serafini (LA-CS), J. R. Machado (LA-RJ), I. P. Camargo (LA-CU), and M. D. Scarranaro (LA-AC).

LITERATURE CITED

(1) Koppenhoefer, B.; Behnisch, R.; Epperlein, U.; Holzschuh, H.; Bernreuther, A. Enantiomeric odor differences and gas chromatographic properties of flavors and fragrances. *Perfum. Flavor.* **1994**, *19*, 1−14.

(2) Dias, B. F. S. *Primeiro Relatório Nacional para a Convenção sobre Diversidade Biológica do Brasil*. COBIO, Ministério do Meio Ambiente, dos Recursos Hídricos e da Amazônia Legal: Brasília, 1998, p 283.

(3) Ohashi, S. T.; Rosa, L. dos S.; Santana, J. A.; Green, C. L. Brazilian rosewood oil: sustainable production and oil quality management. *Perfum. Flavor.* **1997**, *22*, 1−5.

(4) Gupta, M. P., Ed. *270 Plantas Medicinales Iberoamericanas* (270 Ibero-American Medicinal Plants); CYTED-SECAB, Editorial Presencia: Santafé de Bogotá, 1995; p 617.

(5) Fester, G.; Retamar, J.; Ricciardi, A.; Cassano, A. Algunas esencias volatiles (Some volatile oils). *Rev. Fac. Ing. Quim.* **1961**, *14a* Comunicacion, 5−10.

(6) Fester, G.; Fonseca, L. R.; Ricciardi, A.; Cassano, A.; Burgos, J. Algunas esencias volatiles (Some volatile oils). *Rev. Fac. Ing. Quim.* **1961**, *15a* Comunicacion, 11−14.

(7) Catalán, C. A. N.; Merep, D. J.; Retamar, J. A. El aceite de *Lippia alba* (Miller) N. E. Brown de la provincia de Tucumán (The *Lippia alba* (Miller) N. E. Brown oil from Tucumán province). *Rev. Ital. EPPOS* **1977**, *59*, 513−518.

(8) Gomes, E. C.; Ming, L. C.; Moreira, E. A.; Miguel, O. G.; Miguel, M. D.; Kerber, V. A.; Conti, A.; Weiss-Filho, A. Constituintes do óleo essencial de *Lippia alba* (Mill.) N. E. Br. (Verbenaceae) (Constituents of the essential oil from *Lippia alba* (Mill.) N. E. Br. (Verbenaceae)). *Rev. Bras. Farm.* **1993**, *74*, 29−32.

(9) Pino, J. A.; Ortega, A.; Rosado, A. Chemical composition of the essential oil of *Lippia alba* (Mill.) N. E. Brown from Cuba. *J. Essent. Oil Res.* **1996**, *8*, 445−446.

(10) Dellacassa, E.; Soler, E.; Menéndez, P.; Moyna, P. Essential oils from *Lippia alba* (Mill.) N. E. Brown and *Aloysia chamaedrifolia* Cham. (Verbenaceae) from Uruguay. *Flavour Fragrance J.* **1990**, *5*, 107−108.

(11) Fun, C. E.; Svendsen, A. B. The essential oil of *Lippia alba* (Mill.) N. E. Br. *J. Essent. Oil Res.* **1990**, *2*, 265−217.

(12) Craveiro, A. A.; Alencar, W.; Matos, F. J.; Andrade, H. S.; Machado, M. I. L. Essential oils from Brazilian Verbenaceae. Genus *Lippia*. *J. Nat. Prod.* **1981**, *44*, 598−601.

(13) Craveiro, A. A.; Fernandes, A. G.; Andrade, C. H. S.; Matos, F. J. A.; Alencar, J. W.; Machado, M. I. L. *Óleos Essenciais de Plantas do Nordeste*; UFC: Fortaleza, Ceará, 1981; pp 106−209.

(14) Matos, F. J. A. As ervas cidreiras do Nordeste do Brasil. Estudo de três quimiotipos de *Lippia alba* (Mill.) N. E. Brown (Verbenaceae) (The "ervas cidreiras" at Northeast of Brazil. Study of three chemotypes of *Lippia alba* (Mill.) N. E. Brown (Verbenaceae)). *Rev. Bras. Farm.* **1996**, *77*, 137−141.

(15) Matos, F. J. A.; Machado, M. I. L.; Craveiro, A. A.; Alencar, J. W. Essential Oil composition of two chemotypes of *Lippia alba* grown in Northeast Brazil. *J. Essent. Oil Res.* **1996**, *8*, 695−698.

(16) Zoghbi, M. D. G. B.; Andrade, E. H. A.; Santos, A. S.; Silva, M. H. L.; Maia, J. G. S. Essential oils of *Lippia alba* (Mill) N. E. Br. growing in the Brazilian Amazon. *Flavour Fragrance J.* **1998**, *13*, 47−48.

(17) Ming, L. C. Influência da adubação orgânica na produção de biomassa e teor de óleos essenciais de *Lippia alba* (Organic fertilization influence on the biomass production and on the content of the essential oils in *Lippia alba*). *Hortic. Bras.* **1994**, *12*, 49−52.

(18) Retamar, J. A. Variaciones fitoquimicas de la especie *Lippia alba* (salvia morada) y sus aplicaciones en la quimica fina. *Essenze Deriv. Agrum.* **1994**, *12*, 55−60.

(19) Frighetto, N.; Oliveira, J. G.; Siani, A. C.; Chagas, K. C. *Lippia alba* Mill N. E. Br. (Verbenaceae) as a source of linalool. *J. Essent. Oil Res.* **1998**, *10*, 578−580.

(20) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*; Allured Publishing: Carol Stream, IL, 1995; p 470.

(21) Grob, K. *Making and Manipulating Capillary Columns for Gas Chromatography*; Huethig: Heidelberg, Germany, 1986; p 232.

(22) Box, G. E. P.; Hunter, W. G.; Hunter, J. S. *Statistics for Experiments*. John Wiley & Sons: New York, 1978.

(23) Buckingham, J., Ed. *Dictionary of Natural Products*, 1st ed.; Chapman & Hall: New York, 1994; vols. 1−6,, entries C-482, E-156, and G-273.

Received for review December 4, 2001. Revised manuscript received March 11, 2002. Accepted March 12, 2002. We thank the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/PIBIC), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Universitária José Bonifácio (FUJB) for their financial support.

JF011592N