Compositional analysis of the leaf oils of Piper callosum Ruiz & Pav. from Peru and Michelia montana Blume from India

Spectroscopy 14 (1999) 51–59
IOS Press

Compositional analysis of the leaf oils of *Piper callosum* Ruiz & Pav. from Peru and *Michelia montana* Blume from India

Marcel H.P. van Genderen [a], Piet A. Leclercq [b], Hermann Silva Delgado [c], Purnendu B. Kanjilal [d] and Ramesh S. Singh [d]

[a] *Laboratory of Macromolecular and Organic Chemistry, Department of Chemical Engineering, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands*
[b] *Laboratory of Instrumental Analysis, Department of Chemical Engineering, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands*
[c] *Traditional Medicinal Institute, Peruvian Social Security Institute, Pasaje San Lorenzo No. 205, Guayabamba, Iquitos, Perú*
[d] *Division of Plant Sciences and Ecology, Regional Research Laboratory, Jorhat 785 006, Assam, India*

Received August 1998
Accepted October 1998

Abstract. The leaf oils of *Piper callosum* from Peruvian Amazon and *Michelia montana* from Assam, India, were prepared by hydrodistillation and analyzed by a combination of GC and GC/MS. Twenty five and thirty components have been identified, representing 96.3 and 100.0% of the respective oils. The major constituents were found to be asaricin (syn. sarisan) (35.9 and 81.8%, respectively) and safrole (20.2 and 13.0%). The oil of *P. callosum* contained in addition eugenyl methyl ether (9.7%) and (E)-asarone (7.8%), compounds not detected in the *M. montana* oil. The identity of the principal compound, an isomer of myristicin, was unequivocally established by ^{13}C-NMR spectrometric techniques, especially long-range ^{1}H–^{13}C correlation.

Keywords: *Piper callosum*, *Michelia montana*, essential oil composition, asaricin, sarisan, safrole

1. Introduction

Piper callosum Ruiz & Pav., Piperaceae, is a shrub plant with a height of 0.5–1 m, with internodes 3–15 cm long. Alternate oval elliptical leaves 5–16 cm long, 3–6 cm wide. Top acuminate and sharply pointed; glabrous and bright base. Inflorescence in short spikes 1–2.5 cm long and 3–4 mm in diameter. Tiny yellowish flowers; bracts subpeltate, androecium with 4 stamen, pistel with 3 stigmata on short and thick stylets. Drupe fruit glabrous and subglabrous. The plant is native to the Peruvian Amazon and the Andes mountains.

A decoction of *P. callosum* leaves has diuretic and depurative properties. The crushed leaves are applied as hemostatic [1]. Chemical investigation of the roots of *P. callosum* revealed the presence of the amides piperovatine, pipercallosine and pipercallosidine [2]. This plant has also been reported as a new source of safrole [3].

Michelia montana Blume, Magnoliaceae, is a fairly large evergreen tree up to 9 m tall. Bark grey with horizontal winkles. Leaves 15–19 cm long, coriaceous, ovate, elliptical or obovate, glabrous and shining

both surfaces. Flowers white, axillary, solitary, buds cylindrical. Sepals and petals about 8, oblanceolate or lanceolate, acute. Seeds 3–5, reddish brown, faceted, suspended in an elastic cord. Flowers during summer and fruits during winter [4]. This species is found scattered in tropical evergreen forests in the sub-Himalayan tracts and lower hills of Northeast India, particularly in Assam and Arunachal Pradesh, up to 1000 m above sea level.

The bark of this species is a bitter tonic useful against fevers [5]. The essential oil of fresh leaves of *Michelia montana* consisted mainly of safrole and the trunk bark oil yielded asaricin [6]. No other reports on the uses and chemical composition of these plants were found in the literature.

2. Experimental

2.1. Plant material

Piper callosum: Cultivated plants from the botanic garden of the Traditional Medicinal Institute (TMI), Peruvian Social Security Institute, Iquitos, Perú, were used. A voucher specimen of this plant was deposited in the herbarium of TMI for further reference purposes.

Michelia montana: Fresh leaves were collected from Joypur Reserve Forest, Dibrugarh District, Assam. A voucher specimen was deposited in the herbarium of the Regional Research Institute, Jorhat, Assam.

Fresh leaves of *P. callosum* and *M. montana* were washed and hydrodistilled to produce oils in 0.35 and 0.9% yield (w/w), respectively.

2.2. Compositional analysis

The oils were analyzed by a combination of capillary GC and GC/MS, using Shimadzu GC-17A and GCMS-QP5000 instruments. The GC columns (25 m $\times$ 0.25 mm, 0.25 m CP-Sil 5 CB) were used with the following temperature program: 2.5 min at 35 °C, 5 °C/min to 280 °C). Split injector, FID detector and GC/MS interface temperatures were maintained at 280 °C. Helium carrier gas was pressure controlled to give linear gas velocities of 30 cm/s (GC/FID) and 44 cm/s (GC/MS), respectively. The percentage composition of the oil was calculated from electronic integration measurements using FID detection without response factor correction. Temperature programmed, linear retention indices of the compounds were determined relative to n-alkanes. 70 eV electron ionization mass spectra were acquired over the mass range of 10–400 Da at a rate of 4 spectra/s. The constituents of the oils were identified by matching their mass spectra and retention indices with reference libraries [7–16].

NMR measurements were performed at 400.13 MHz (^{1}H) or 100.62 MHz (^{13}C) on a Bruker AM 400 spectrometer, equipped with an Aspect-3000 computer. All chemical shifts are given in ppm downfield of TMS. Reference for ^{1}H spectra: TMS = 0 ppm, for ^{13}C spectra: residual $CHCl_3$ = 77.0 ppm. ^{1}H-NMR spectra were recorded with 32 K points, a digital resolution of 0.27 Hz/point, a relaxation delay of 1 s and 8 scans. ^{13}C-NMR spectra were recorded with 32 K points, a digital resolution of 1.53 Hz/point, a relaxation delay of 60 s, 860 scans, and inverse-gated decoupling. APT spectra were recorded with 32 K points, a digital resolution of 1.53 Hz/point, a 5 s relaxation delay, 64 scans, and a delay corresponding to an average 1-bond J_{XH} of 125 Hz (8 ms). The optimal long-range coupling constant was determined with a refocussed, decoupled INEPT experiment with 32 K points, a digital resolution of 1.53 Hz/point, a 3 s relaxation delay and 32 scans. Optimal peak intensities for all quaternary carbons were found for delay values of 30 and 15 ms ($1/(4J_{XH})$ and $1/(8J_{XH})$), respectively, indicating an average long-range

J_{XH} of 8.3 Hz. This value was then used in a long-range HETCOR experiment. For the ^{13}C dimension (F2), the parameters were: 2 K points, 9 kHz spectral width, digital resolution 8.8 Hz/point, relaxation delay 3 s, and 32 scans. For the Fourier transform in this dimension, a line broadening window (LB = 2) was used. For the ^{1}H dimension (F1), the parameters were: 512 experiments, zero-filled to 1 K points, 1.6 kHz spectral width, digital resolution 1.6 Hz/point.

3. Results and discussion

The compounds identified in the essential oils are listed in Table 1. Using GC and GC/MS data, the major constituent of the oils could only be characterized as an isomer of myristicin. Further structural elucidation by various NMR techniques was necessary to reveal the correct isomer.

The structure of myristicin (4-methoxy-6-(2-propenyl)-1,3-benzodioxole) is given in Fig. 1(B). We will designate the main component in both oils (36 and 82%, respectively) as X. Safrole (5-(2-propenyl)-1,3-benzodioxole), present in amounts of 20 and 13%, respectively, is designated here as S. Other components are present at a level of less than 10%.

Both ^{1}H- and ^{13}C-NMR studies were performed to determine which isomer of myristicin was the major constituent of the oil of *Piper callosum*. Six possible isomers can be thought of (designated A–F, see Fig. 1). Based on the respective positions of the methoxy and propenyl substituents, the following short-hand identification holds: A = 4,5 (croweacin); B = 4,6 (myristicin); C = 4,7; D = 5,4; E = 5,6 (asaricin); F = 6,4. The numbering scheme used for discussing the NMR results is as follows: positions 2, 4–7 are as normal for numbering in the compound. C_a and C_b refer to the quaternary atoms between the benzene and dioxole rings. Positions c, d and e are used to designate atoms in the substituent $–CH_2–CH{=}CH_2$, respectively. Finally, f refers to the methoxy group (not present in S).

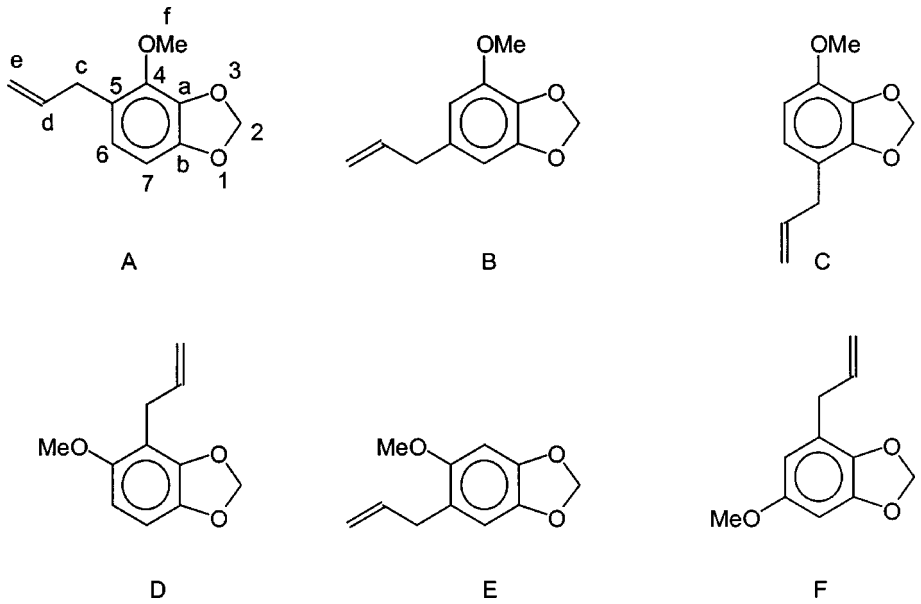

Fig. 1. Structures of six possible isomers of myristicin (B).

Table 1

Constituents of the leaf oils of *Piper callosum*, Piperaceae, from Peru and *Michelia montana*, Magnoliaceae, from India

Component	RI[a]	*Piper callosum*[b]	*Michelia montana*[b]
α-pinene	928	0.2	0.5
camphene	940	–	tr
sabinene	962	1.5	–
β-pinene	967	–	2.3
myrcene	982	–	tr
1.8-cineole	1016	–	0.1
limonene	1019	0.2	0.3
(Z)-β-ocimene	1027	0.5	tr
(E)-β-ocimene	1038	0.6	1.1
γ-terpinene	1047	–	tr
terpinolene	1077	–	tr
linalool	1084	1.1	–
terpinen-4-ol	1158	0.7	0.1
α-terpineol	1169	–	0.1
piperitone	1223	0.8	–
safrole	1259	20.2	13.0
p-eugenol	1327	0.4	–
α-copaene	1370	–	tr
eugenyl methyl ether	1371	9.7	–
β-elemene	1384	0.2	tr
β-caryophyllene	1411	–	0.2
α-santalene	1414	–	tr
(Z)-isoeugenyl methyl ether	1415	0.4	–
trans-α-bergamotene	1429	–	0.1
α-humulene	1444	–	tr
(E)-β-farnesene	1445	–	tr
asaricin (sarisan)	1457	35.9	81.8
α-selinene	1486	2.1	–
β-bisabolene	1499	0.7	0.1
n-pentadecane	1500	0.3	–
γ-cadinene	1501	0.2	–
δ-cadinene	1510	–	tr
elemicin	1518	1.1	–
elemol	1530	–	tr
(Z)-isoelemicin	1537	3.3	–
(E)-nerolidol	1547	1.5	–
spathulenol	1557	–	tr
caryophyllene oxide	1562	–	tr
(Z)-asarone	1584	3.2	–
hinesol	1607	–	0.1
β-eudesmol	1620	–	0.1
selin-11-en-4α-ol	1629	0.4	0.1
α-cadinol	1632	0.2	–
α-eudesmol	1635	–	tr
(E)-asarone	1644	7.8	–
6-isopropenyl-4,8A-dimethyl-4A,5,6,7,8,8A-hexahydro-2(1H)-naphtalenone[c]	1739	4.2	–
other compounds		3.7	0.0

[a] Measured linear retention indices on the nonpolar CP-Sil 5 column, relative to n-alkanes.
[b] Area (%); tr = trace (<0.1%).
[c] Correct isomeric form not identified.

3.1. ^{1}H-NMR spectroscopy

The main components (X and S) are clearly visible in a proton spectrum. The highest peaks are due to X: 3.3 (2H, d, H_c), 3.72 (3H, s, OCH_3), 5.0 (2H, m, H_e), 5.85 (2H, s, H_2), 5.9 (1H, m, H_d), 6.5 (1H, s, aromatic H), 6.6 (1H, s, aromatic H). The next highest peaks are due to S: 3.35 (2H, d, H_c), 5.1 (2H, m, H_e), 5.9 (3H, s, H_2), ca. 6.0 (1H, m, H_d), 6.6–6.7 (3H, m, aromatic H). Various other small signals are due to the minor components in the mixture.

The first clue to the identity of X are the aromatic signals, which are both singlets. Therefore, no ortho position for the aromatic protons is possible, due to the then expected large coupling. Only three isomers would then remain: B, E and F. However, the proton spectrum is too crowded for further analysis.

3.2. ^{13}C-NMR spectroscopy

In order to obtain a quantitative ^{13}C-NMR spectrum, we performed an inverse-gated measurement with a relaxation delay of 60 s. This allows all peaks (including quaternary carbons) to be visible at their true intensity, so peaks due to X can be distinguished from those of S (similar but weaker). An APT (attached proton test) measurement [17] allowed the assignment of CH_3, CH_2, CH and quaternary C for both X and S.

The peaks of S can be identified from a known spectrum in $CDCl_3$ [18]: 39.8 (C_c, lit: 39.9), 100.7 (C_2, lit: 100.74), 108.0 (aromatic CH, lit: 108.11), 109.0 (aromatic CH, lit: 109.5), 115.5 (C_e, lit: 115.59), 121.2 (aromatic CH, lit: 121.25), 133.7 (aromatic C, lit: 133.81), 137.5 (C_d, lit:137.55), 145.7 (aromatic C, lit: 145,80), 147.5 (aromatic C, lit: 147.62).

The remaining highest peaks must then be due to the unknown major constituent X. The number of resonances (11) and their approximate location is as expected for a myristicin isomer: 35.8 (C_c), 56.3 (C_f), 94.7 (aromatic CH), 100.8 (C_2), 109.5 (aromatic CH), 115.1 (C_e), 120.5 (aromatic C), 137.1 (C_d), 140.8 (aromatic C), 146.2 (aromatic C), 151.9 (aromatic C). However, these data are not in good agreement with the known ^{13}C-NMR spectrum of myristicin (isomer B) [19]: 40.2 (C_c), 56.4 (C_f), 101.2 (C_2), 102.8 (C_5), 108.9 (C_7), 115.6 (C_e), 134.2 (C_d), 134.7 (C_a), 138.0 (C_6), 144.1 (C_b), 149.6 (C_4). Especially the low chemical shift of 94.6 ppm for an aromatic carbon is not found for myristicin, indicating a different substitution pattern of the aromatic ring. However, the literature spectrum was recorded in C_6D_6, so discrepancies may occur.

The results up to now would exclude isomer B, and together with the proton NMR data would only leave isomers E and F, but arguments based solely on chemical shifts should be treated with caution, especially since we do not have spectra of the other isomers.

3.3. ^{1}H–^{13}C correlation spectroscopy

The best proof of the structure can be obtained by determining the substitution pattern directly from experimental evidence. Assignment of aromatic carbons in X could be done by INADEQUATE (^{13}C–^{13}C correlation) measurements, but the sample size was insufficient. Therefore, heterocorrelation (^{1}H–^{13}C) was chosen [20].

The use of long-range coupling constants was necessary, in order to obtain correlations for the quaternary aromatic carbons. Refocussed, decoupled INEPT ^{13}C spectra were measured with varying delays to determine the optimum long-range coupling constant for the quaternary carbons. This gives $J = 8.3$ Hz:

a typical value for 3-bond aromatic couplings (C–C–C–H) or 3-bond couplings over a hetero-atom (C–O–C–H) [21]. The heterocorrelation experiment allowed to identify various long-range and also residual one-bond couplings.

The residual one-bond couplings are the following. The aromatic CH at 94.7 ppm with the proton at 6.5 ppm. The aromatic CH at 109.5 ppm with the proton at 6.6 ppm. The methylene carbon at 100.6 ppm with the protons at 5.8 ppm, as expected for C_2 with H_2. The methylene carbon at 115 ppm with the protons at 5.0 ppm, as expected for C_e.

Several long-range correlations were also visible. The expected correlations in the propylene fragment were seen: a 2-bond coupling of C_d (137.1 ppm) with H_c (3.3 ppm), and a 3-bond coupling of C_e (115.1 ppm) with H_c (3.3 ppm). The proton signal of H_d is too small in intensity to give correlations, due to the strong multiplet splitting of the proton peak.

The aromatic C at 151.9 ppm is the only one with a correlation to the CH_3O protons, so this must be the carbon atom that carries the methoxy substituent. The aromatic carbons at 140.8 ppm and 146.2 ppm are the only ones with correlations to the protons on C_2, so these must be C_a and C_b, although we do not yet know which is which. These carbons also *both* have a correlation with *both* aromatic protons (6.5 and 6.6 ppm), indicating that the latter nuclei can not be further away than C_4 and C_7 (4-bond couplings are very small). This would already strongly suggest isomer E.

In agreement with this isomer, where the methoxy and propenyl substituents would be ortho, is the correlation between the aromatic C at 151.9 ppm (carrying the methoxy) and the protons H_c (3.3 ppm) in the propenyl moiety. The only quaternary C left (120.5 ppm) must be carrying the propenyl substituent, and indeed has a 2-bond correlation with H_c (3.3 ppm). The proton signal of H_d is too small to give correlations, due to the strong multiplet splitting.

Isomer E is further corroborated by 3-bond couplings between the aromatic carbon at 120.5 ppm and the proton at 6.5 ppm, the carbon at 109.5 ppm and the protons at 3.3 ppm (H_c), and the carbon at 151.9 ppm and the proton at 6.6 ppm. In conclusion, the pattern of long-range ^1H–^{13}C cross peaks is only compatible with the 5-methoxy-6-(2-propenyl) isomer (E).

The same conclusion can be drawn from a systematic analysis of the number of possible coupling constants over 2 and 3 bonds in the six isomers: the only isomers where a carbon atom can have four coupling constants (as the 151.9 ppm peak has) are D, E and F. The 151.9 ppm resonance must also have a coupling with the methoxy group, which only occurs in isomers D and E. In isomer D, a strong coupling should be seen between the resonances at 140.8 and 146.2 ppm with the H_c protons, since the propenyl moiety is located near the dioxole ring. However, this cross peak is not present. Also, the coupling of both C_a and C_b (140.8 and 146.2 ppm) with both aromatic protons (6.5 and 6.6 ppm) is not possible in isomer D, since it involves a 4-bond coupling. And finally, isomer D is ruled out by the ^1H-NMR spectrum (see above). This approach also yields isomer E as the only consistent structure for X.

The final assignment of the peaks in the ^{13}C-NMR spectrum of X can be made with the observation that the carbon at 151.9 ppm has a weak (2-bond) coupling with the proton at 6.5 ppm, and that the cross peak of the carbon at 140.8 ppm with the proton at 6.5 ppm is much stronger (so, 3-bond coupling) than the cross peak with the proton at 6.6 ppm (so, 2-bond coupling): 35.8 (C_c), 56.3 (C_f), 94.7 (C_4), 100.8 (C_2), 109.5 (C_7), 115.1 (C_e), 120.5 (C_6), 137.1 (C_d), 140.8 (C_b), 146.2 (C_a), 151.9 (C_5). These shifts are consistent with the known effects of alkoxy substituents on aromatic rings: deshielding on the ipso position (high shifts for C_5, C_a and C_b), and shielding on the ortho and para positions (low shifts for C_4 and C_7).

A subsequent literature survey revealed the empirical name asaricin for the major constituent (X = E) of the oils, and a report on the structure elucidation with ^1H-NMR and IR spectroscopy of this compound,

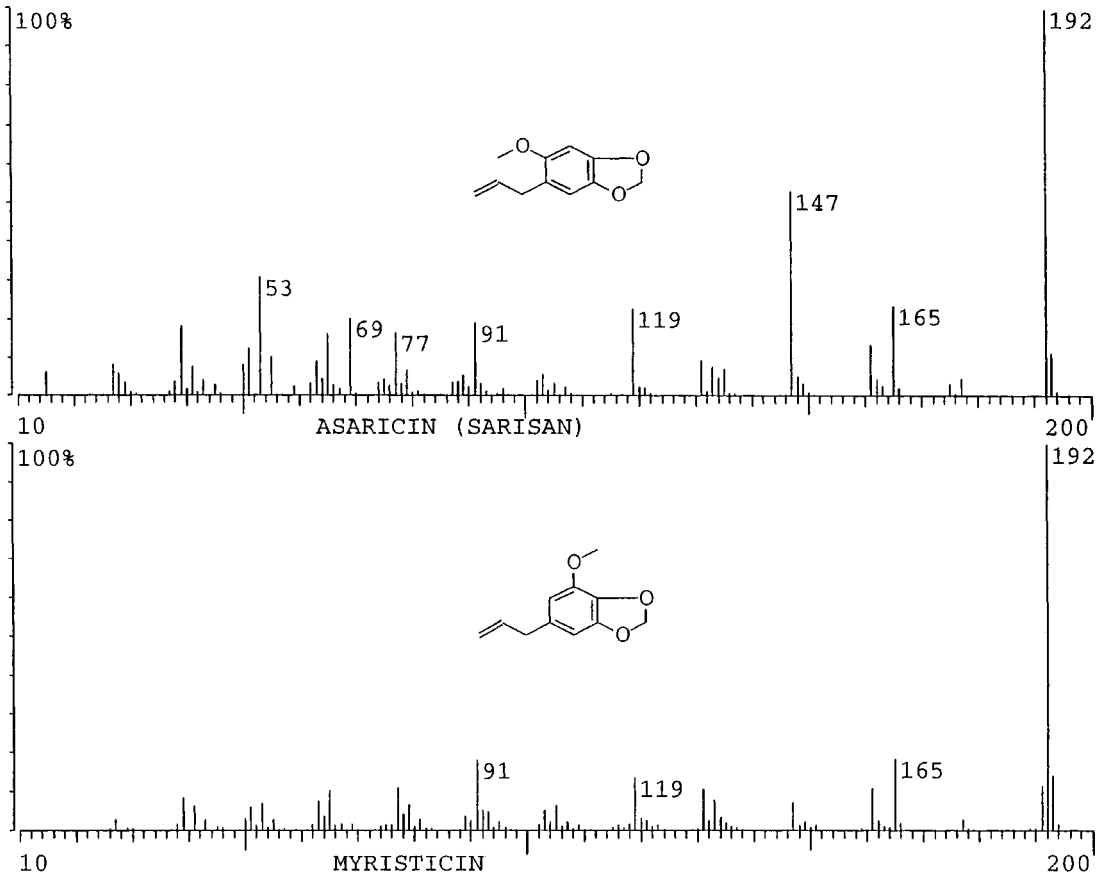

Fig. 2. 70 eV EI mass spectra of asaricin and myristicin.

which was the principal component of the leaf oil of *Beilschmiedia miersii* [22]. Our ^{1}H-NMR data were found to match those given in [22].

The phenylpropanoid asaricin (syn. sarisan) has also been found earlier in the oils of four Piperaceaea species: Columbian *Piper lenticellosum* leaf oil [23], Peruvian *P. aduncum* oil [24], Nigerian *P. guineese* fruit oil [25] and Japanese *P. sarmentosum* leaf oil [26]. Asaricin has most commonly been found, however, in the oils of plants of the *A(sia)sarum sp.* (syn. *Heterotropa sp.*), Aristolochiaceae [27–44], but also in *Illicium sp.* [45–47], *Cornus officinales* [48], *Elsholtzia sp*. [49], *Crowea exalata* [50] and *Ligusticum pteridophyllum* [51].

The 70 eV EI (quadrupole) mass spectra of asaricin and myristicin are presented in Fig. 2.

References

[1] M. Pinedo, E. Rengifo and T. Cerruti, *Plantas Medicinales de la Amazonia Peruana*, Estudio de su uso y cultivo, Iquitos, 1997.
[2] B.G. Pring, *J. Chem. Soc. Perkin Trans*. **1** (1982), 1493.
[3] J.G. Maia, M.P.E. Goeldi, B.C.L. Green and M.J. Milchard, *Perfum. Flavor*. **18**(2) (1993), 19.
[4] U.N. Kanjilal, P.C. Kanjilal and A. Das, *Flora of Assam*, Vol. I, Periodical Expert Book Agency, Delhi, 1992, p. 25.
[5] K.R. Kirtikar and B.D. Basu, *Indian Medicinal Plants*, Vol. I, Periodical Book Agency, Delhi, 1976, p. 59.

[6] S.C. Dutta, R.K. Mathur, A.K.S. Baruah and J.N. Baruah, *Planta Med*. **53** (1987), 505.
[7] Sadtler Research Laboratories, *The Sadtler Standard Gas Chromatography Retention Index Library*, Bio-Rad Laboratories, Philadelphia, PA, 1986.
[8] N.W. Davies, *J. Chromatogr*. **503** (1990), 1.
[9] P. Sandra and C. Bicchi, *Capillary Gas Chromatography in Essential Oil Analysis*, Hüthig, Heidelberg, 1987.
[10] Y. Masada, *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*, Wiley, New York, 1967.
[11] R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, Allured Publ., Carol Stream, IL, 1995.
[12] L.M. Libbey, *J. Essent. Oil Res.* **3** (1991), 193.
[13] S.K. Ramaswani, P. Briscese, R.J. Gargiullo and T. von Geldern, in: *Flavors and Fragrances: A World Perspective*, B.M. Lawrence, B.D. Mookherjee and B.J. Willis, eds, Elsevier, Amsterdam, 1988, p. 951.
[14] D. Henneberg, B. Weimann and W. Joppek, *Mass Spectrometry Library Search System MassLib*, Version 7.4 (for Ultrix), Max-Planck-Institut für Kohlenforschung, Mülheim/Ruhr, 1994. Using MassLib the following data bases were searched:
(a) F.W. McLafferty and D.B. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data*, 4th edn., Wiley–Interscience, New York, 1988;
(b) D. Henneberg, B. Weimann and W. Joppek, *MPI Library of Mass Spectral Data*, Max-Planck-Institut für Kohlenforschung, Mülheim/Ruhr, 1994;
(c) M.C. ten Noever de Brauw, J. Bouwman, A.C. Tas and G.F. La Vos, *Compilation of Mass Spectra of Volatile Compounds in Food*, TNO-HVV-CSIA, Zeist, 1988;
(d) M.A. Posthumus and C.J. Teunis, *WAU Library of Mass Spectra of Natural Products*, Wageningen Agricultural University, Wageningen, 1997;
(e) P.A. Leclercq and H.M.J. Snijders, *EUT Library of Mass Spectra*, Eindhoven University of Technology, Eindhoven, 1997.
[15] National Institute of Standards and Technology, *PC Version of the NIST/EPA/NIH Mass Spectral Data Base*, Version 4.5, U.S. Department of Commerce, Gaithersburg, MD, 1994.
[16] F.W. McLafferty and D.B. Stauffer, *Mass Spectrometry Library Search System BenchTop/PBM*, Version 3.0, Palisade Co., Newfield, NY, 1993. Using BenchTop/PBM the following datbase was searched:
F.W. McLafferty and D.B. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data*, 5th edn., Wiley, New York, NY, 1991.
[17] S.L. Patt and J.N. Shoolery, *J. Magn. Res.* **46** (1982), 535.
[18] C.J. Pouchet and J. Behnke, *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*, Vol. 2, 1st edn., Aldrich Chemical Company, Milwaukee, WI, 1993, p. 232.
[19] V. Formacek, Ph.D. Thesis, University of Würzburg, 1979; found in the SpecInfo database, version 3.1.5.1, 1996, by Chemical Concepts, Germany.
[20] A. Bax and G. Morris, *J. Magn. Res.* **42** (1981), 501.
[21] M. Hesse, H. Meier and B. Zeeh, *Spektroskopische Methoden in der organischen Chemie*, 4th edn., Georg Thieme Verlag, Stuttgart, 1991, p. 147.
[22] J. Kumamoto and R.W. Scora, *J. Agr. Food Chem.* **18** (1970), 544.
[23] P.P.D. Diaz and J.V. Dorado, *Rev. Latinoam. Quim.* **17** (1986), 58.
[24] J.C. Burgos Macedo and S. Gibaja Oviedo, *Bol. Soc. Quim. Peru* **53** (1987), 228.
[25] O. Ekundayo, I. Laakso, R.M. Adegbola, B. Oguntimein, A. Sofowora and R. Hiltunen, *J. Agr. Food Chem.* **36** (1988), 880.
[26] T. Masuda, A. Inazumi, Y. Yamada, W.G. Padolina, H. Kikuzaki and N. Nakatani, *Phytochemistry* **30** (1991), 3227.
[27] Y. Saiki, T. Saito, H. Sasaki and S. Fukushima, *Yakugaku Zasshi* **87** (1967), 1524.
[28] Y. Saiki, Y. Akahori, K. Morinaga, T. Taira, T. Noro, S. Fukushima and T. Harada, *Yakugaku Zasshi* **87** (1967), 1535, 1539, 1544.
[29] Y. Saiki, A. Ueno, H. Sasaki, T. Morita, K. Suzuki, T. Saito and S. Fukushima, *Yakugaku Zasshi* **88** (1967), 185.
[30] C. Tien, S.N. Tung, P.Y. Wang and C.C. Lo, *Yao Hsueh Tung Pao* **16** (1981), 53.
[31] Z. Tian, S.N. Dong, B.R. Wang and Z.C. Lou, *Pei-ching I Hsueh Yuan Hsueh Pao* **13** (1981), 179.
[32] Z. Tian and Z. Lou, *Yaoxue Tongbao* **16**(8) (1981), 59.
[33] Z. Tian, S. Dong, B. Wang and Z. Lou, *Beijing Yixueyuan Xuebao* **13** (1981), 282.
[34] Z. Shen, *Yaowu Fenxi Zazhi* **2** (1982), 335.
[35] Z. Xu, J. Pan, G. Wang, C. Yang and Z. Chunshu, *Zhongyao Tongbao* **9** (1984), 27.
[36] N. Hayashi, K. Maeshima, T. Murakami and H. Komae, *Z. Naturforsch. C* **39** (1984), 705.
[37] J. Pan, Z. Xu, G. Wang, C. Yang and J. Zhang, *Zhongyao Tongbao* **9** (1984), 175.
[38] Z. Wang, X. Heng, Q. Zhou and G. Liu, *Zhongcaoyao* **16** (1985), 297.
[39] Z. Xu, J. Pan, Q. Zhu, G. Wang, C. Yang and J. Qhang, *Zhongyao Tongbao* **11** (1986), 46.
[40] C. Yang, J. Zhang, Q. Pan, Z. Xu, Q. Zhu and G. Wang, *Zhongyao Tongbao* **11** (1986), 423.
[41] Y. Saika, E. Fukuyama and T. Tsuneya, *Yakugaku Zasshi* **107** (1987), 219.

[42] H. Komae, N. Hayashi and T. Sakao, *Dev. Food Sci.* **18** (1988), 259.
[43] N. Hayashi, J. Ding, Z. Ding, Z. Chen, Y. Yi and H. Komae, *Z. Naturforsch. C* **45** (1990), 32.
[44] Y. Oka, K. Otsuki, M. Katagi and H. Tsuchihashi, *Yakugaku Zasshi* **111** (1991), 234.
[45] M. Shibuya, K. Abe, Y. Nakahashi and S. Kubota, *Chem. Pharm. Bull.* **26** (1978), 2671.
[46] W. Schultze, A. Zaenglein, G. Lange and K.H. Kubeczka, *Deutsch. Apoth. Ztg.* **130** (1990), 1194.
[47] C. Yang, C. Liu and F. Wang, *Zhongguo Yaoxue Zazhi* **25** (1990), 583.
[48] M. Miyazawa and H. Kameoka, *Agr. Biol. Chem.* **53** (1989), 3337.
[49] G. Zhu, *Zhongguo Zhongyao Zazhi* **15** (1990), 677.
[50] J.J. Brophy, R.J. Goldsack, A. Punruckvong, P.I. Forster and C.J.R. Fookes, *J. Essent. Oil Res.* **9** (1997), 401.
[51] G. Rao, Y. Dai, L. Wang, F. Cai, Z. Lin and H. Sun, *Yunnan Zhiwu Yanjiu* **13** (1991), 233.