Sesquiterpene Composition of Basil Oil. Assignment of the ¹H and ¹³C NMR Spectra of β-Elemene with Two-Dimensional NMR

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The essential oil of basil from Madagascar was fractionated by distillation and column chromatography, and three sesquiterpenes (γ₂-cadinene, bicyclogermacrene, β-elemene) were isolated in pure form. The sesquiterpene fraction was analyzed by GC and GC–MS, and 31 sesquiterpenes were identified; among them, 11 were found for the fist time in basil oil. The ¹³C NMR chemical shifts of bicyclogermacrene are given, and a complete assignment of the ¹H and ¹³C NMR spectra of β-elemene has been achieved based on data generated from two-dimensional NMR experiments.

Basil oils have quite different aromatic characteristics depending on the growing site (Masada, 1976). Earlier reports in the literature have shown the identification of various constituents in basil oils (Lawrence et al., 1971, 1972, 1980). In 1984, Vernin et al. separated more than 100 constituents in basil oils from various origins, and a review with 175 references has been recently published (Sharma et al., 1987).

The basil oil from Madagascar, obtained by steam distillation of the whole herb of *Ocimum basilicum* L. (family Labiateae), is used in food industries and perfumery. The differences in chemical composition that occur in basil oils play an important role in determining their utilization and value (Masada, 1976). We have investigated (Randriamiharisoa et al. 1986a,b) the chemical composition of 28 basil oils from Madagascar by using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Analyses show that the various essential oils are homogeneous in composition. Among the 36 compounds obtained by GC, methylchavicol was the most abundant with 74–87% of the essential oils, showing therefore that Malagasy samples belong to the tropical group. The sesquiterpene fraction represents less than 2–4% and is of interest from a commercial point of view, but the knowledge of the composition of this compound family is interesting for understanding the biogenesis of some of these compounds in basil oils.

In a study, Lawrence et al. (1971), using fractional distillation and alumina column and silver nitrate column separation, could isolate 23 sesquiterpene hydrocarbons from an exotic oil of *O. basilicum*. Vernin et al. (1984), using GC–MS, identified 10 more new sesquiterpene hydrocarbons.

In the present study, we have developed a fractionation method of basil oil obtained from plants grown in Madagascar for the identification of 11 new sesquiterpenes contained in the hydrocarbon fraction using GC–MS and retention indices. The isolation of pure γ₂-cadinene, bicyclogermacrene, and β-elemene was achieved. Despite

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Table I. Components Identified from the Sesquiterpenic Fraction of Basil Oil and Their Percentage Composition[a]

peak[b]	I_R[c]	component	identificn	rel abund, %
1	1445	α-cubebene	I_R, MS	0.14
2	1472	bicycloelemene	I_R, MS	0.10
3	1480	α-copaene	I_R, MS	0.36
5	1506	α-bourbonene?	I_R	0.17
6	1517	β-bourbonene	I_R, MS	0.05
7	1527	α-gurjunene[d]	I_R, MS	0.52
8	1560	(E)-α-bergamotene	I_R, MS	0.51
9	1577	(Z)-α-bergamotene	I_R, MS	0.35
10	1580	β-elemene	I_R, MS, NMR	18.0
11	1585	β-caryophyllene	I_R, MS	34.6
12	1600	α-guaiene?	I_R	1.10
13	1612	scapanene[d]	I_R, MS	0.23
14	1615	α-elemene[d]	I_R, MS	0.32
15	1623	alloaromadendrene[d]	I_R, MS	0.31
16	1628	eremophyllene	I_R, MS	0.44
17	1634	sesquiterpene		0.32
18	1639	sesquiterpene		0.23
19	1654	α-humulene	I_R, MS	4.67
21	1666	γ-muurolene	I_R, MS	1.89
22	1670	α-amorphene	I_R, MS	2.10
23	1678	β-bisabolene	I_R, MS	0.29
24	1680	(E)-α-bisabolene[d]	I_R, MS	0.75
25	1685	viridiflorene[d]	I_R, MS	0.63
26	1692	germacrene D	I_R, MS	2.77
27	1701	β-selinene	I_R, MS	5.83
28	1706	α-selinene	I_R, MS	1.04
29	1716	bicyclogermacrene[d]	I_R, MS, NMR	3.35
30	1720	(Z)-α-bisabolene[d]	I_R, MS	1.01
31	1733	(E,E)-α-farnesene	I_R, MS	0.15
32	1740	δ-cadinene	I_R, MS	7.70
33	1744	γ₂-cadinene[d]	I_R, MS, NMR	3.02
34	1754	cubenene	I_R, MS	0.17
35	1756	sesquiterpene		1.45
36	1785	α-cadinene[d]	I_R, MS	0.32
37	1806	(Z)-calamenene	I_R, MS	0.46
38	1816	(E)-calamenene?	I_R	0.13
39	1898	α-calacorene[d]	I_R, MS	0.03
40	1928	β-calacorene?	I_R	0.02
41	1960	δ- or γ-calacorene?	I_R	0.04

[a] Percentages were calculated from the peak areas of the hydrocarbon fraction of the essential oil, which represents 3.75% of the oil. FID response factors were not determined. [b] Peak numbers were given in the order of appearance in the programmed temperature GC. Numbers correspond to those in Figure 1. [c] Experimentally determined retention indices on the Carbowax 20M column. [d] Determined for the first time in basil oil.

their common occurrence in essential oils, the complete ¹H and ¹³C nuclear magnetic resonance (NMR) spectra of β-elemene have not yet been reported. The recent introduction of various two-dimensional NMR techniques (Bax, 1982) allowed assignments of ¹H and ¹³C without a large
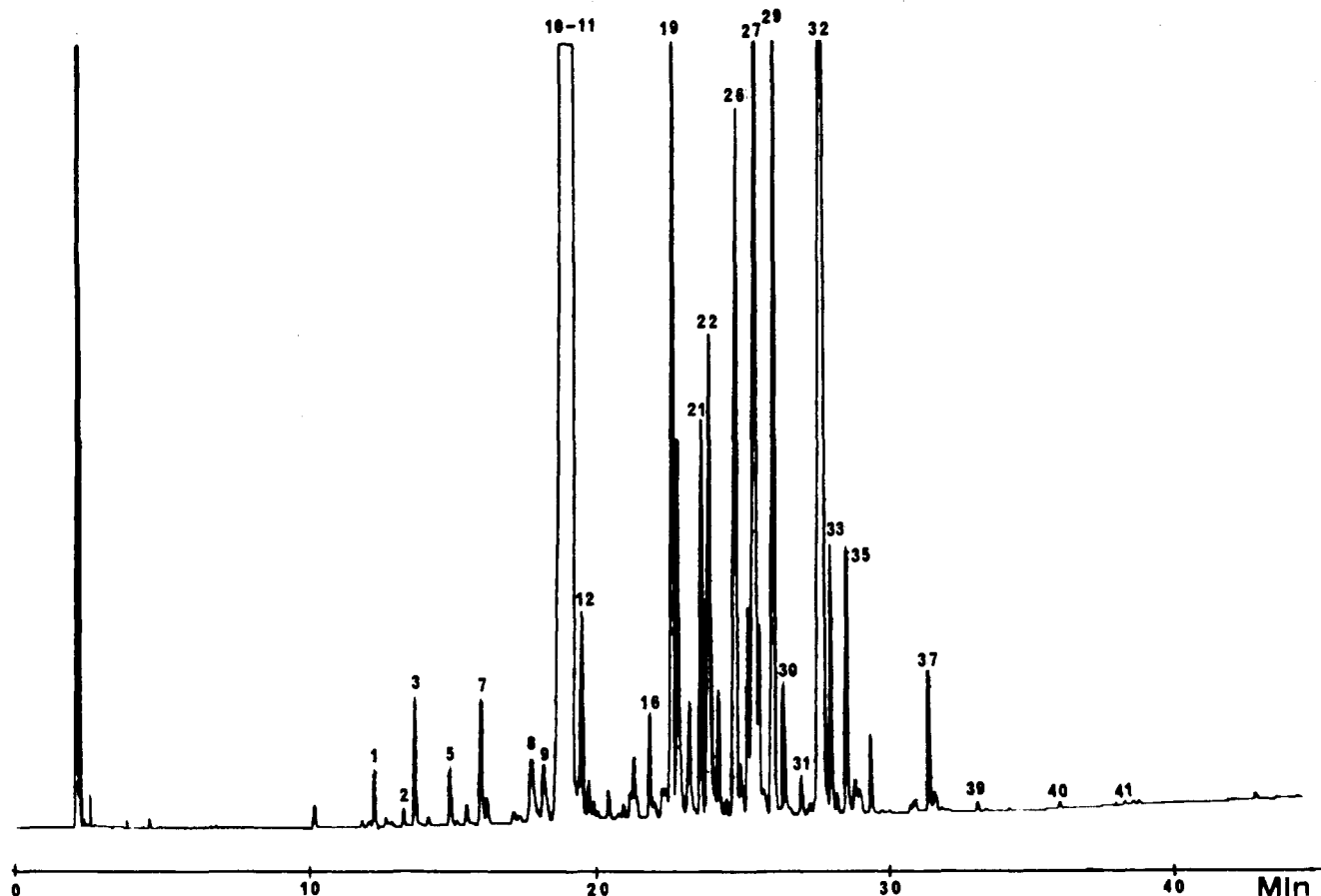
Figure 1. Gas chromatogram of the hydrocarbon fraction of basil essential oil from Madagascar (glass capillary column coated with Carbowax 20M, 50 m, 0.32-mm i.d., 0.15-μm phase thickness; programmed temperature from 70 to 210 °C at 2 °C min^{-1}). See Table I for peak identification.

number of analogues. The application of these new methods to sesquiterpenic compounds has been very limited so far (Faure et al., 1986, 1987).

EXPERIMENTAL SECTION

Materials. The essential oil of basil was obtained from the aerial part of the plant, by industrial steam distillation. This oil was supplied by the Service du Conditionnement et du Contrôle de la Qualité des Produits of Antananarivo (Madagascar), who guarantee its authenticity. This oil was chosen for sesquiterpene investigation among more than 30 samples (Randriamihansoa et al., 1986a,b) for its high content in this hydrocarbon family.

Distillation of the Basil Oil. Fractional distillation of basil oil (116 g) was made on a Nester-Faust NF-200 Perkin-Elmer spinning-band column under reduced pressure (2-3 mmHg). The pot temperature was maintained at 50-60 °C, and the reflux ratio was held around 3-4 drop min^{-1}. The volatiles (mainly monoterpenes, 2.5 g) were obtained from the nitrogen trap. The first four fractions (104.7 g) were composed of oxygenated compounds, mainly methylchavicol (95-98%). The residue (8 g) was distilled at a higher pot temperature (65 °C) on the spinning-band column, and 19 fractions were obtained. Fractions F15-F19 contained more than 95% sesquiterpenic compounds.

Column Chromatography. The distilled fraction F15 (400 mg) was submitted to column chromatography (CC) over silica gel 60 (60 g, 230-400 mesh; E. Merck) impregnated with silver nitrate (10%) according to Gaydou et al. (1986) using a column of 15 cm (18-mm i.d.). Elution of F15 was carried out with hexane (100 mL), 50:50 hexane-benzene (v/v) (100 mL), benzene (100 mL), 80:20 benzene-acetone (v/v) (100 mL), 50:50 benzene-acetone (v/v) (100 mL), and acetone (200 mL) and collected in 35 tubes. Tubes 21-25 (100 mg) contained a hydrocarbon (98% purity by GC) that was conclusively identified as β-elemene by direct comparison with MS retention indices (I_R) and assignment of the ^{1}H and ^{13}C NMR spectra with two-dimensional NMR.

Tubes 16-20 (200 mg) contained a mixture of sesquiterpenes in which bicyclogermacrene was prominent (40%). Purification of bicyclogermacrene was achieved by using CC as described above, and 50 mg of hydrocarbon (96% purity by GC) was obtained, which was submitted for NMR analyses. A mixture of fractions F16-F19 (70 mg) was fractionated by CC using hexane (40 mL) and 80:20 hexane-benzene (v/v) (40 mL) and collected in 16 tubes. Tubes 10-15 contained a sesquiterpene hydrocarbon, 10 mg (88% purity by GC), that was conclusively identified as γ_2-cadinene by direct comparison of I_R with a pure sample, NMR, and MS.

Gas Chromatography and Gas Chromatography-Mass Spectrometry. Analyses by GC of the various fractions of basil oil were done on a FID-type Girdel 30 gas chromatograph. Detector and injector temperatures were set at 220 and 230 °C, respectively. The GC columns used were a Carbowax 20M WCOT glass capillary column (50 m; 0.32-mm i.d.; 0.15-μm phase thickness; programmed temperature, 70-210 °C at 2 °C min^{-1}; inlet pressure of hydrogen used as carrier gas, 1.5 bar) and an OV-101 WCOT glass capillary column (50 m; 0.30 mm i.d.; 0.15-μm phase thickness; programmed temperature, 90-220 °C at 2 °C min^{-1}; inlet pressure of hydrogen used as carrier gas, 1.5 bar). The various constituents were identified by comparison of their I_R and mass spectral data with those of authentic samples purchased if available or taken from our own collection.

Percentages were calculated from the peak area of the hydrocarbon fraction of basil oil by electronic integration (Shimadzu CR 3A). This hydrocarbon fraction was obtained using 0.4 g of oil, which was fractionated by CC over silica gel 60 (30 g, 230-400 mesh; E. Merck). Elution with n-pentane (120 mL) yielded 15 mg (3.75% hydrocarbons).

Combined GC-MS was recorded on a Delsi gas chromatograph linked to a Ribermag R-10-10 C mass spectrometer and coupled with a Sidar data computer. The GC column was a 0.30 mm(i.d.) × 50 m fused silica capillary column coated with Carbowax 20M (0.20-μm phase thickness). The column temperature was programmed from 70 to 210 °C at 2 °C min^{-1} (carrier gas, helium; ion source, 220 °C; ionizing voltage, 70 eV).
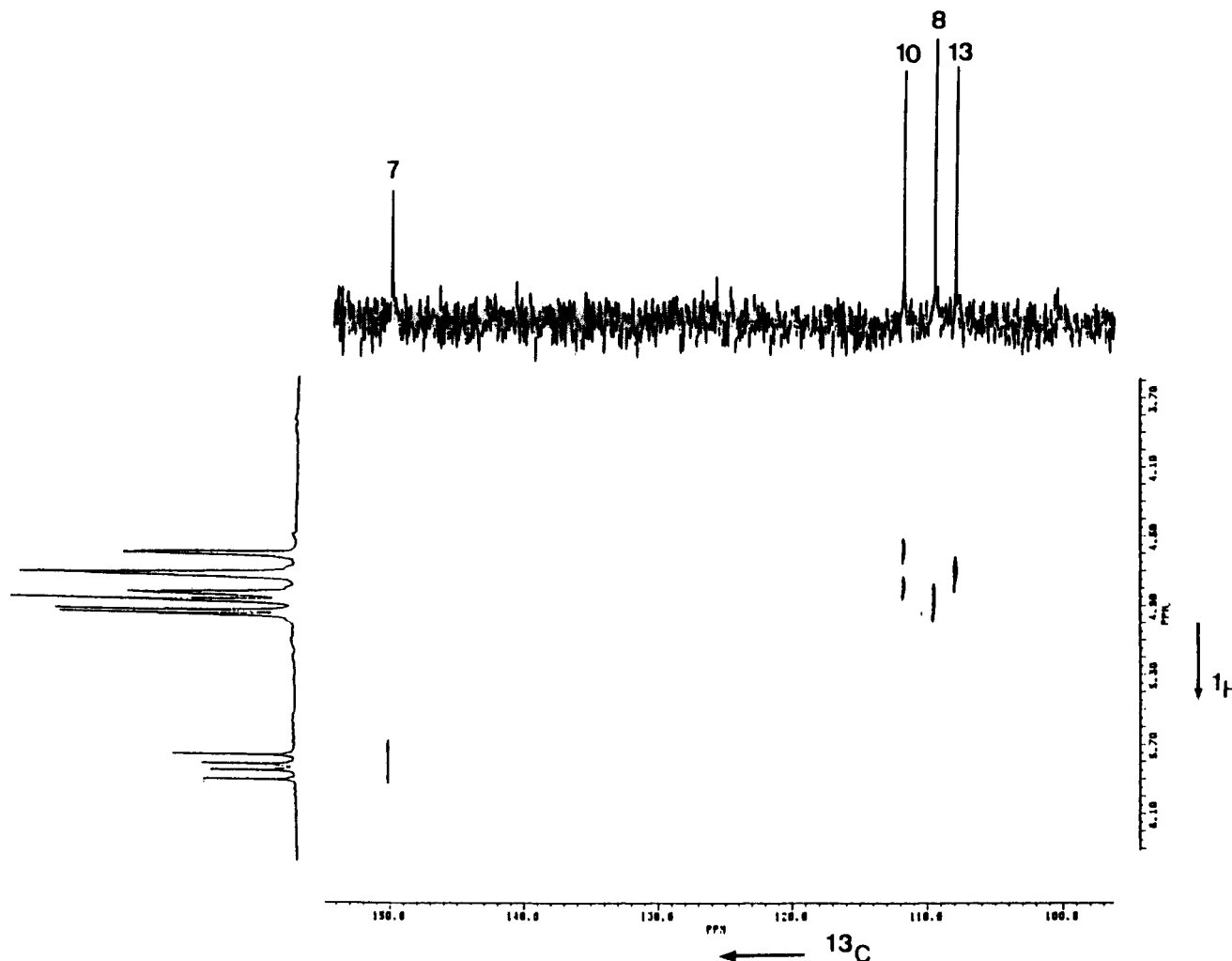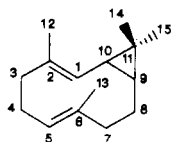
Figure 2. Two-dimensional $^{13}C-^{1}H$ shift correlated diagram of β-elemene (ethylenic part).

Table II. ^{13}C NMR Chemical Shifts of Bicyclogermacrene

δ^a	group^b	assgnt	δ^a	group^b	assgnt
144.51	C	C-2	31.66^c	CH$_2$	C-8
130.95	C	C-6	31.31^d	CH$_2$	C-7
125.41	C	C-5	25.78	CH$_3$	C-14
116.54	CH	C-1	23.88	CH$_2$	C-4
45.52	CH	C-9	23.07	CH$_3$	C-15
41.25	C	C-11	17.62^d	CH$_3$	C-12
39.09	CH	C-10	17.53^d	CH$_3$	C-13
38.73	CH$_2$	C-3			

^{a} With respect to TMS. ^{b} Determined from DEPT subspectra. ^{c,d} These values may be reversed.

Nuclear Magnetic Resonance Spectroscopy. All spectra were recorded with a multinuclear Bruker AM-200 spectrometer (Centre Interuniversitaire de RMN de Marseille). Samples for ^{13}C measurements were prepared in a 10-mm-o.d. tube by mixing 10–30 mg of sesquiterpene with 1.5 mL of CDCl$_3$, while proton measurements were carried out with use of a 5-mm-o.d. tube containing 5 mg of sesquiterpene in 0.4 mL of CDCl$_3$; tetramethylsilane was used as an internal standard in both measurements. Proton coupling constants were extracted from the resolution-enhanced ^1H spectrum by the Gaussian multiplication technique (Ferrige and Lindon, 1978). Resonance multiplicities

for ^{13}C were established via the acquisition of DEPT spectra obtained for proton pulses $P_\theta = 90°$ (CH only) and $P_\theta = 135°$ (CH and CH$_3$ differentiated from CH$_2$). For the DEPT sequence, the width of a ^{13}C 90° pulse was 13 μs, the width of a ^1H 90° pulse was 29 μs, and the $(2J)^{-1}$ delay was set equal to 3.7 ms. In the case of β-elemene, the homonuclear $^1H-^1H$ shift-correlated two-dimensional diagram was obtained by using the COSY-90 pulse sequence (COSY in the Bruker operating software). The spectral widths were $F_2 = 1500$ and $F_1 = \pm750$ Hz, allowing a digital resolution of 1.47 Hz. The spectrum was collected as 2048 × 1024 blocks of data and was processed by sinusoidal multiplication in each dimension followed by symmetrization of the final data matrix. Other parameters were as follows: number of increments in t_1, 512; scans, 32; phase cycling, 16; relaxation delay, 1 s. The heteronuclear two-dimensional $^1H-^{13}C$ chemical shift correlation experiment was obtained by the standard sequence (XH CORR in the Bruker operating software). The spectrum was acquired with 4K × 256 data points $[S(t_1, t_2)]$ and a data acquisition of 16 scans × 128 increments in t_1 and zero-filling in the F_1 dimension. Spectral widths of 8620 and ±720 Hz were employed in the F_2 (^{13}C) and F_1 (^1H) domains, respectively. The data were processed by sine bell functions for weighting in both dimensions. This provided a digital resolution of 4.21 Hz in F_2 and 5.62 Hz in F_1. The refocusing delay was 2 ms, the mixing delay was 4 ms, the relaxation delay was 1 s, and 16 phase cycling steps were employed. The basic pulse sequence (JRES in the Bruker operating software) was used for the two-dimensional homonuclear ^1H J-resolved diagram. The F_2 spectral width was 1538 Hz, and F_1 was ±48 Hz. A 16-phase cycling with 16 scans and 64 increments, followed by zero-filling and weighting with sine bell functions in both dimensions, gave a matrix of 2K × 128 data points, providing a digital resolution of 1.50 Hz in F_2 and 0.75 Hz in F_1; the recycle delay was 1 s.

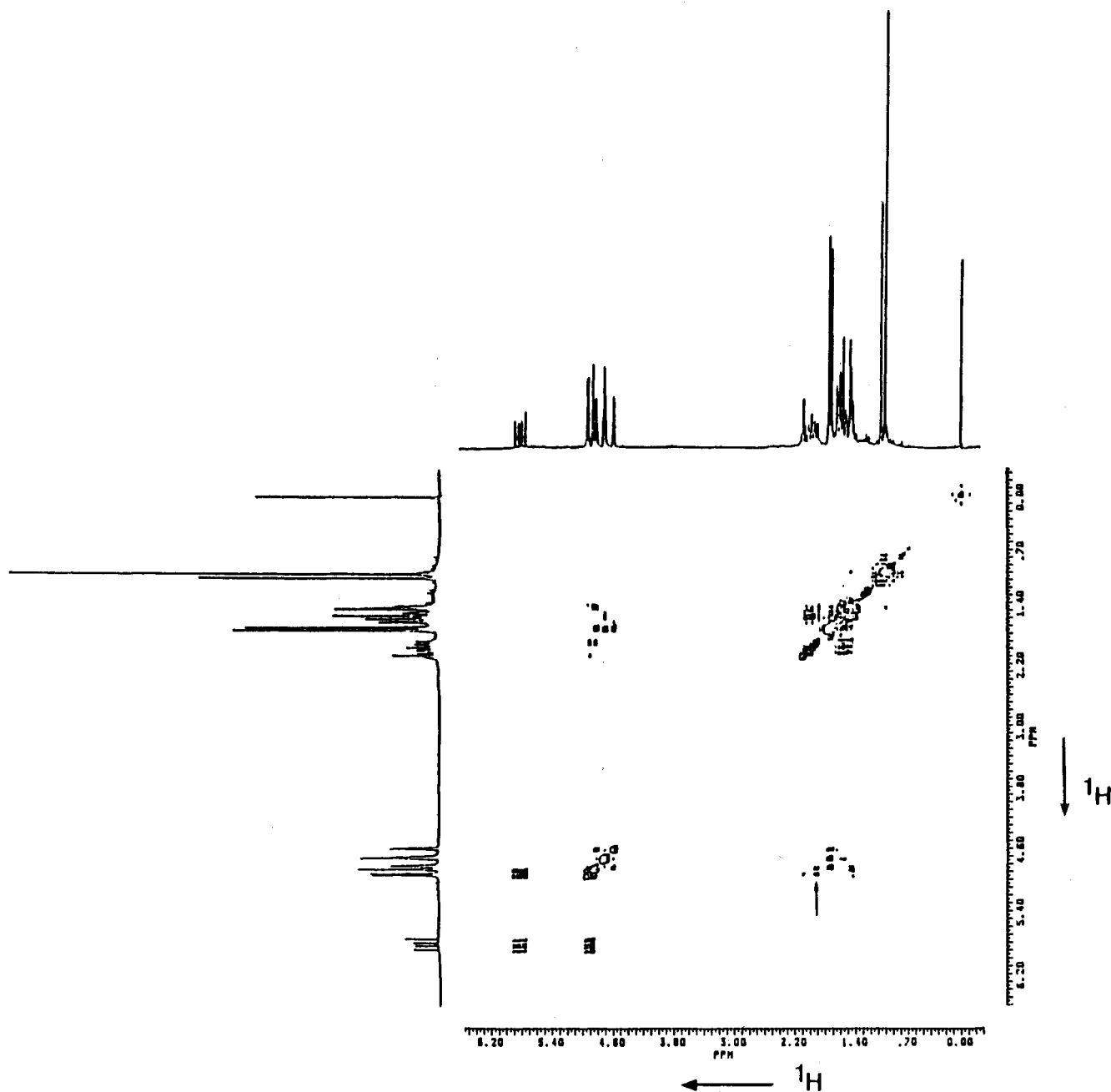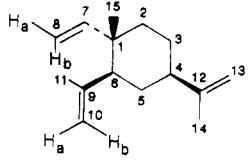**Figure 3.** ^{1}H two-dimensional homonuclear chemical shift correlation spectrum (COSY) of β-elemene. Long-range correlations marked with an arrow are discussed in the text.

RESULTS AND DISCUSSION

Identification of Sesquiterpenes. For the identification of components, the oil was fractionated according to a method previously described (Gaydou et al., 1986) into oxygenated and hydrocarbon fractions. A typical Carbowax 20M capillary gas chromatogram of the hydrocarbon fraction is given in Figure 1. The components were identified in the hydrocarbon fraction by gas chromatography–mass spectrometry and from indices determined on Carbowax 20M and OV 101 columns. Literature data of retention times and mass spectra were taken from Reed (1963), Stenhagen et al. (1974), Andersen et al. (1977), Jennings and Shibamoto (1980), MacLeod and Pieris (1981), Papageorgiou and Argyriadou (1981), Tressl et al. (1983), and Gaydou and Randriamiharisoa (1987). For the isolation of some sesquiterpenes, the complex mixture of basil oil was first submitted to fractional distillation and then to column chromatography over silica gel with silver nitrate. Three sesquiterpenes, γ_2-cadinene, bicyclo-

germacrene, and β-elemene, were obtained in pure form and their structures confirmed by ^{1}H and ^{13}C NMR.

Composition of the Sesquiterpenic Fraction of Basil Oil. Among the 41 peaks obtained by GC, 31 were identified as shown in Table I. α-Gurjunene, scapanene, α-elemene, alloaromadendrene, (E)-α-bisabolene, viridiflorene, bicyclogermacrene, (Z)-α-bisabolene, γ_2-cadinene, α-cadinene, and α-calacorene were identified for the first time in basil oil. Five other sesquiterpenes were identified on the basis of their retention indices. The main components of the sesquiterpene fraction of basil oil were β-caryophyllen (35%), β-elemene (18%), δ-cadinene (8%), β-selinene (6%), and α-humulene (5%). These main components where soon characterized by Lawrence et al. (1971) and Vernin et al. (1984). They represent a small percentage in the Malagasy basil oil type, and only two of them (β-caryophyllene and β-elemene) were used in our study of the variation in the chemical composition (Randriamiharisoa et al., 1986a,b). The occurrence of γ_2-cadinene and bicyclogermacrene, which represents a small

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Table III. ¹H and ¹³C NMR Chemical Shifts of β-Elemene

¹³C			¹H
δ^a	group[b]	assgnt[c]	$\delta^{2,c}$
150.22	CH	C-7	5.82
150.16	C	C-12	
147.66	C	C-9	
112.15	CH₂	C-10	4.82 (a), 4.59 (b)
109.87	CH₂	C-8	4.89 (a), 4.90 (b)
108.33	CH₂	C-13	4.72, 4.70
52.81	CH	C-4	2.01
45.82	CH	C-6	1.91
39.96	CH₂	C-2	1.45, 1.43
39.81	C	C-1	
32.96	CH₂	C-3	1.55
26.89	CH₂	C-5	1.64, 1.56
24.84	CH₃	C-11	1.71
21.05	CH₃	C-14	1.76
16.64	CH₃	C-15	1.00

[a] With respect to TMS. [b] Determined from DEPT subspectra. [c] Information obtained from 2D measurements.

percentage of this oil, is not surprising when the various stereospecific cyclizations that may occur in the biogenesis process as shown in the case of ylang-ylang oils are taken into account (Gaydou et al., 1986).

NMR of Isolated Sesquiterpenes. The ¹H and ¹³C spectral analyses for γ₂-cadinene were in agreement with those given by Burk and Soffer (1971) for ¹H NMR and Biougne et al. (1987) for ¹H and ¹³C NMR. The ¹³C chemical shifts for bicyclogermacrene were deduced by comparison with similar structure, such as α-humulene (Randriamiharisoa et al., 1986b). The results obtained are given in Table II. The ¹H and ¹³C spectral parameters for β-elemene (Table III) were deduced from the concerted use of one-dimensional and two-dimensional nuclear magnetic resonance spectroscopies.

Preliminary information about the number of directly attached protons to each carbon atom and trivial assignment of CH-7 and C-1 was obtained from two DEPT experiments (Doddrell et al., 1982) in which proton pulses of 90° and 135° were employed. Then a convenient mean of extracting ¹H chemical shift information is provided by the 2D J technique (Aue et al., 1976b). The projection of the "tilted" 2D data (Hall and Sukumar, 1980) onto the F_2 axes gives a spectrum that reflects only chemical shifts (Martin et al., 1983) (broad-band proton-decoupled ¹H spectrum) and in which second-order effects due to strong couplings (Patt, 1984) were partially or completely suppressed. Finally, for the complete assignment of ¹H and ¹³C resonances, simultaneous use of the heteronuclear (Bodenhausen and Freeman, 1978) and homonuclear (Aue et al., 1976a; Freeman et al., 1981) chemical shift correlation diagrams proved to be particularly helpful. First, the heteronuclear correlation served to establish the location of each proton attached to a given carbon. Second, the establishment of the proton connectivity is easily available from the homonuclear correlation.

In the conventional 200-MHz ¹H NMR spectrum of β-elemene, H-7 is readily identified as the quadruplet furthest downfield resonating at 5.82 ppm. In the homonuclear ¹H–¹H chemical shift correlated two-dimensional diagram, this signal shows correlated peaks with the protons absorbing at 4.89 and 4.90 ppm, which were therefore assigned, on the basis of cis and trans coupling values, to

H-8a and H-8b, respectively. It is evident from the contour plot of two-dimensional heteronuclear ¹H–¹³C chemical shift correlation diagram (Figure 2) that these later signals correspond to the one at 109.87 ppm in the ¹³C domain. The rest of the ethylenic region of the homonuclear and heteronuclear diagram was analyzed in the same way. Moreover, each methylenic proton exhibits correlation with its corresponding methyl group. Long-range connectivities over four or five bonds were found in the COSY ¹H NMR spectrum (signals maked with an arrow in Figure 3). Of particular interest was the observation of correlation between H-8a and the signal at 1.91 ppm, which in turn is coupled to the CH nucleus responsible for the signal at 45.82 ppm and was therefore assigned to H-6. As a consequence of this zigzag coupling, the vinyl group and H-6 proton are in the trans position. In addition, H-6 is also coupled with a w coupling to the proton at 4.82 ppm, which was ascribed to H-10a. So, location of propenyl groups and assignment of methine signals were found unambiguously. Finally, assignment of the six-membered methylene protons cannot be accomplished on the basis of the COSY spectrum since strongly coupled spin systems are present (Wider et al., 1981). Therefore, utilization of the heteronuclear ¹³C–¹H chemical shift correlation and 2D J-resolved spectroscopy proved particularly helpful for their attribution. The complete results are presented in Table III, and the structure proposed for β-elemene is in agreement with previous results (Ganter and Keller-Wojtkiewicz, 1971).

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