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Authentication of Essential Oils Containing Linalool and Linalyl Acetate by Isotopic Methods

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Site-specific natural isotope fractionation studied by NMR (SNIF NMR) combined with molecular isotope ratio determination by mass spectrometry (IRMS) was used to characterize linalool and linalyl acetate obtained from chemical synthesis or extracted from essential oils of well-defined botanical and geographical origins. In general, the overall carbon-13 or deuterium contents measured by IRMS do not constitute efficient criteria for identifying natural and synthetic samples. In contrast, the nonrandom distribution of deuterium exhibits large variations as a function of the origin of the sample. A discriminant analysis performed in a space defined by the 10 site-specific hydrogen isotope ratios and the overall carbon isotopic parameter enables the natural and synthetic species to be unambiguously distinguished. In spite of the relatively large dispersion of the isotope ratios exhibited by both natural and synthetic families, it is possible to develop an efficient strategy to carry out qualitative and quantitative analyses of the essential oils.

1. INTRODUCTION

Linalool and linalyl acetate are important aroma components. These molecular species exist in a number of essential oils, namely ho-leaf (85%), bois de rose and coriander (70%), and lavender and spike lavender (45%) oils. However, due to the relatively high price of natural linalool and the great demand for this kind of flavor and fragrance, synthetic and hemisynthetic processes have been developed (Clark, 1988) and about 6×10^6 kg of industrial linalool priced at less than $10.0/kg are produced per year. Natural linalool is either extracted (mainly from ho-leaf or bois de rose oils) to constitute a pure natural aroma or directly consumed as a component of high-grade essential oils. For instance, a guaranteed vintage of lavender oil from Haute-Provence was created in 1981 by the French Department of Agriculture.

Given the various possible sources of linalool and linalyl acetate, authentication methods that can specify the natural or synthetic status of the product and even possibly the botanical and geographical origin of the plant from which the essential oil has been prepared are particularly desirable.

Isotopic tracers have been extensively used for investigating the mechanistic pathways leading to terpene compounds. Specific labeling with radioactive (^{3}H, ^{14}C) or stable (^{2}H, ^{13}C) isotopes is a source of information on the metabolic transformations occurring in the course of terpene biosynthesis in plants (Banthorpe et al., 1972; Croteau et al., 1988; Porter and Spurgeon, 1981). Isotopic tracers present at natural abundance are also subject to fractionation effects during biosyntheses in field conditions. As a result, the overall carbon-13 or deuterium contents determined by isotope ratio mass spectrometry (IRMS) for a whole plant organ or for a given molecular species (carbohydrate, vanillin, etc.) are known to differ according to the metabolic cycle of the plant and even to the botanical species (O'Leary, 1988). Only very few results have been published with reference to terpenes. However, significant differences in the overall deuterium content of menthol, limonene, and pulegone samples, for instance, have been detected (Bricout et al., 1973).

Significant deviations with respect to a random distribution of deuterium in natural and synthetic molecules have been directly evidenced by deuterium NMR (Martin and Martin, 1981), and the investigation of site-specific natural isotope fractionation (SNIF NMR) has frequently enabled an unambiguous characterization of the nature of the plant precursor (Martin et al., 1982). In certain cases it has even been possible to identify the geographical area where the plant was grown (Martin and Martin, 1990). Using this method, kinetic isotope effects which intervene in the biosynthesis of pinene and limonene were determined (Pascal et al., 1986; Leopold et al., 1988) and the isotopic distribution of camphor was shown to differ between a natural and a synthetic sample (Grant et al., 1982). The discriminant potential of the site-specific isotope contents was also exploited to characterize the enantiomeric purity of α-pinenes (Martin et al., 1986).

This work presents a systematic study of the role of natural factors on isotope fractionation in linalool and linalyl acetate obtained by chemical synthesis or extracted from essential oils from well-defined botanical and geographical origins. The aim of this investigation was to appraise the influence of mechanistic effects on isotopomeric contents and to estimate the analytical potential of the SNIF NMR method for origin inference of the considered aroma. In a specific case, the environmental influence on isotope ratios will be discussed. The isotopic information provided by the typical linalool example will also form part of a larger study of isotope fractionation occurring in the mevalonate pathway leading to terpenes.

Definitions and Symbolism. The site-specific isotope ratio $(D/H)_i$, defined as the ratio of the number of deuterium atoms to the number of hydrogen atoms associated with a given molecular site, i, characterizes the relative depletion or enrichment in the monodeuterated isotopomer i with respect to the mean isotope content of the whole molecule $(\overline{D/H})$:

$$(D/H)_i = (f_i/F_i)/(\overline{D/H}) \quad (1)$$

The molar fraction f_i of isotopomer i is directly accessible from the signal area, S_i, measured in the deuterium spectrum. F_i is the corresponding statistical molar fraction. If n nonequivalent monodeuterated isotopomers are

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Table I. Main Characteristics of the Investigated Linalool (A) and Linalyl Acetate (B) Samples[a]

no.	raw material: species	product	origin and year	%	opt rot, $[\alpha]_D$	$(\overline{D/H})$, ppm	$\delta^{13}C$, ‰
			(A) Linalool				
1	lavender	EO[b]	Haute-Provence, 1987	31.7	[e]	125.3	−28.0
2		EO[b]		40.9	[e]	125.4	−27.5
3		EO[b]		37.3	[e]	122.4	−27.8
4		EO[b]		29.3	[e]	123.1	−26.9
5		EO[b]	Drôme, 1987	22.1	[e]	122.2	−28.6
6		EO[b]		18.5	−11.5	122.0	−28.1
7		EO[c]	France SE, 1985	37.5	[e]	124.5	−26.4
8		EO[b]	Tasmania	38.0	[e]	123.6	−28.7
9		EO[b]	Bulgaria	29.3	[e]	122.6	−27.0
10		EO[b]	USSR	28.3	[e]	122.6	−26.4
11		EO[b]	USSR	31.7	[e]	121.2	−27.7
12	lavandin	EO[c]	France SE, 1988	39.2	−7.9	130.8	−26.2
13	spike lavender	dist	un	40.2	−17.6	126.6	−24.6
14		dist	Spain	30.3	−17.8	126.6	−24.6
15	bois de rose	dist	Spain, 1987	99.0	−2.2	113.7	−32.0
16		dist	Brazil, 1988	93.2	+1.3	124.7	−28.1
17		dist	Brazil, 1987	[e]	[e]	119.1	−28.6
18	petit grain	dist	Brazil, 1988	25.2	[e]	123.5	−29.3
19		dist	un	(d)	[e]	123.0	−28.3
20	petit grain	dist	un	37.0	[e]	125.8	−28.8
21	bergamot	dist	un	[e]	−4.0	122.4	−26.0
22	bergamot	dist	un	9.1	−11.3	124.2	−25.9
23	bergamot	dist	un	10.9	[e]	130.7	−26.6
24	coriander	dist	un	70.7	+18.2	125.0	−26.2
25	coriander	dist	Drôme, 1989	75.2	+13.0	126.0	−27.3
26	geranium	dist	La Réunion	5.9	[e]	124.9	−27.7
27	geranium	dist	La Réunion	7.8	[e]	130.7	−27.5
28	camphor tree	dist	China, 1988	75.5	−14.0	122.0	−24.6
29	clary sage	dist	un	5.3	−14.2	119.3	−27.6
30	synthetic	chem	Switzerland	98.1	+1.1	130.2	−27.2
31		chem	Switzerland	>98.5	[e]	127.2	−27.6
32	[d]	chem	USA	>98.5	[e]	122.2	−29.2
33		chem	Germany	>98.5	[e]	130.6	−27.0
34		chem	USA	99	[e]	130.6	−27.0
35		chem	USA	99	[e]	130.6	−27.4
			(B) Linalyl Acetate				
36	petit grain	dist[b]	un	48	−6.8	124.1	−28.4
37		dist[b]		45	−5.3	126.2	−28.6
38	bergamot	dist[b]		16	[e]	124.5	−30.6
39		dist[b]		12	−6.3	124.5	−28.0
40	clary sage	dist[b]		86	−7.6	118.2	−27.2
41	synthetic[d]	chem	Germany	98	[e]	127.5	−30.6
42		chem	Switzerland	98	[e]	129.3	−30.1
43		chem	USA	95	1.0	128.3	−30.3

[a] EO, essential oil; dist, distillate; chem, pure chemical; un, unidentified origin. [b] Extracted and purified according to the protocol described in section 2. [c] Steam distillation from concrete. [d] Hemisynthetic origin (see text). [e] Not determined.

distinguished in ^2H NMR

$$f_i = S_i/\sum_n S_i \qquad F_i = P_i/\sum_n P_i \tag{2}$$

P_i being the number of equivalent deuterium positions of type i. Conversely, $(\overline{D/H})$ can be computed from $(D/H)_i$:

$$(\overline{D/H}) = \sum_n F_i(D/H)_i \tag{3}$$

Determination of $(D/H)_i$ by means of eq 1 involves a combination of NMR experiments to obtain f_i (eq 2) and of isotope ratio mass spectrometry (IRMS) measurement of the overall parameter, $(\overline{D/H})$. Alternatively, $(D/H)_i$ can be obtained on the sole basis of NMR experiments by using an internal referencing procedure (Martin et al., 1985).

$$(D/H)_i = \frac{P_{WS}}{P_{Ai}}\frac{M_A}{M_{WS}}\frac{m_{WS}}{m_A}\frac{T_{Ai}}{t_A}(D/H)_{WS} \tag{4}$$

A stands for the product, and WS is a working standard with a known isotope ratio, $(D/H)_{WS}$. M and m are the molecular weight and the weight of A and WS, and t_A is the purity of A (% w/w). T_{Ai} is the ratio of the NMR signal areas associated with site i of A and with the working standard.

The isotope contents are expressed either as isotopic ratios R_i [$(D/H)_i$ for hydrogen and $(^{13}C/^{12}C)_i$ for carbon] or on the relative δ scale defined as

$$\delta_i‰ = [(R_i - R_{ref})/R_{ref}]1000 \tag{5}$$

where ref stands for the international standard.

2. MATERIALS AND METHODS

a. Nature and Origin of the Products. Several samples of essential oils from lavender (*Lavandula angustifolia*) and spike lavender (*Lavandula latifolia*) were provided by industrial firms or purchased from commercial sources. A series of nearly pure samples of linalool or linalyl acetate extracted from bois de rose oil (*Aniba rosaeodora*), bergamot (*Citrus bergamia*), germanium (*Pelargonium graveolens*), clary sage oil (*Salvia sclarea*), petit grain oil (*Citrus aurantium*), coriander (*Coriandrum sativum*), and Formosan camphor oil Shiu oil (*Cinnamomum camphora*) were also obtained from industrial sources. Typical samples of synthetic products were purchased from chemical sources in

Europe and in the United States. To check for the presence of any artifacts which may have occurred during industrial treatment, we also extracted linalool and linalyl acetate from the oils of several well taxonomically identified plants. Altogether 35 samples of linalool and 8 samples of linalyl acetate were investigated, and the main characteristics of these samples are specified in Table I.

b. Purification. For isotopic determinations, checking the purity of the product to be analyzed is of utmost importance. Moreover, where the raw material has undergone physical or chemical treatment, the occurrence of associated isotope effects must be carefully monitored.

Linalool and linalyl acetate were purified by preparative low-pressure liquid chromatography. They were eluted on a polar phase (Silicagel Merck 9385) with increasing amounts of diethyl ether in pentane.

The solvents were then removed carefully to avoid any isotopic fractionation of the residual product.

The possibility of using semipreparative liquid chromatography to obtain quantities of the order of 100–150 mg was also investigated using lavender oils. A Lichrosorb RP18 column fitted with a UV detector (λ_m = 206 and 218 nm for linalool and linalyl acetate) enabled 35 mg of a pure mixture of linalool and acetate to be obtained from 200 μL of essential oil after elution with pure methanol (1.8 mL/min). The two components were isolated by elution with a mixture of methanol and water (80/20). Whatever the preliminary treatment of the material, the purity of the product ready for isotopic analyses was checked by gas chromatography according to the specifications indicated by Bordier (1983) for lavender, lavandin, and spike lavender, Boelens (1986) for spike lavender, Lawrence (1987, 1988) for bergamot and coriander, Swaine (1988) for bitter orange, and Buccellato (1988) for bois de rose. We also checked that the chromatographic purifications did not modify the isotope ratios significantly. The differences observed in the $(D/H)_i$ and $\delta^{13}C$ values of three pure samples either subjected or not subjected to chromatographic treatment were smaller than the range of experimental precision.

In addition, the optical rotation of each of the linalool and linalyl acetate samples from typical origins was determined in an ethanolic solution at 20 °C using either a Perkin-Elmer 241 spectropolarimeter or an Instrulab polarimeter. The results, expressed as specific optical rotations $[\alpha]_D$ are reported in Table I. They can be compared to the values reported in the Merck Index for *d*-linalool (+19.3°) and *l*-linalool (−20.1°).

c. Isotope Ratio Determinations. The determinations of the overall $\overline{D/H}$ and ($^{13}C/^{12}C$) isotope ratios were carried out using VG SIRA 9 and Finnigan Delta E spectrometers, respectively. The deuterium measurements were performed on the hydrogen gas resulting from reduction of water with an on-line Isoprep uranium furnace or with an off-line zinc furnace. The results were referred to the water standard V.SMOW (Gonfiantini, 1978). The ^{13}C determinations were obtained with the help of a Carlo Erba microanalyzer on line with the spectrometer.

Deuterium contents are expressed in terms of $\overline{D/H}$ values. In the case of carbon the results are reported on the δ scale (eq 5) with respect to the carbonate standard PDB (Craig, 1957). The repeatability of the whole experiment is 0.2 ppm in $(\overline{D/H})$ and 0.2 in $\delta^{13}C$. Both scales can be converted by using eq 5 with R (PDB) = 0.011 237 2 and R(V.SMOW) = 155.76 ppm.

The spite-specific isotope ratios $(D/H)_i$ were mostly obtained from ^2H NMR determination of the mole fractions f_i and mass spectrometry measurement of the overall ratio $\overline{D/H}$ according to eqs 1 and 2. An external NMR comparison method (Martin and Naulet, 1988) is not suitable since the signals of some isotopomers of linalool and its acetate partially overlap that of *N,N*-tetramethylurea (TMU) used as a reference. However, to check the consistency of the results, some samples were studied in the presence of TMU and a few drops of $(CF_3CO)_2O$ to shift the hydroxyl isotopomer of linalool. The deuterium spectra were carried out mainly at 61.4 MHz (spectral width 2400 Hz, memory size 32K, acquisition time 6.82 s, number of transients 4400, broadband decoupling). Some spectra were also recorded at 75.7 MHz. Great care must be taken in performing base line adjustment and phase corrections.

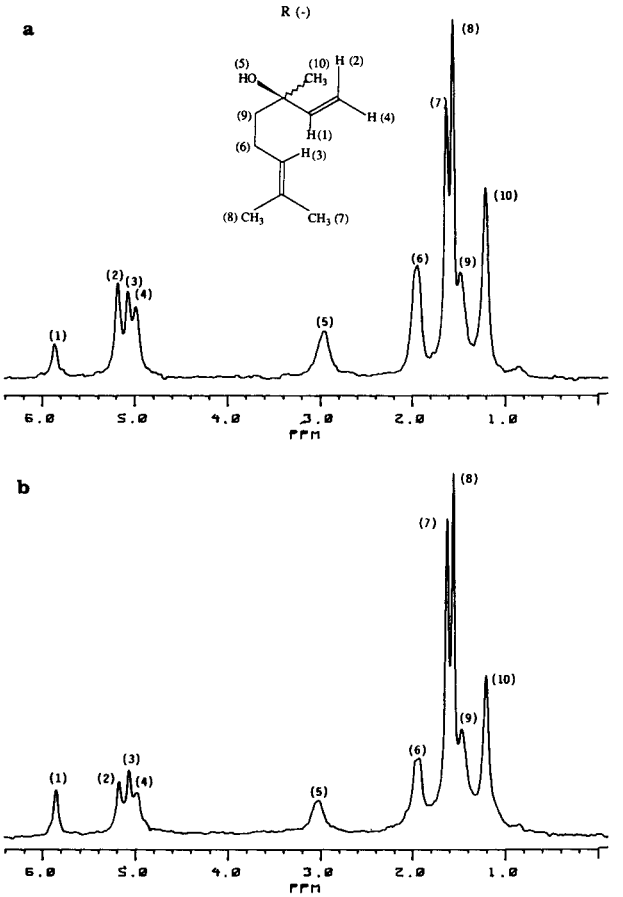


Figure 1. Deuterium NMR spectra recorded at 61.4 MHz of natural (a) and synthetic (b) linalool samples.

Signal areas, S_i, were determined by a curve-fitting procedure which led to the best results in terms of precision and accuracy in case of overlapping signals. The repeatability of the determination of a site-specific isotope ratio depends on the signal-to-noise ratio associated with the corresponding isotopomer and on the degree of overlap. A precision of 2% is obtained for correctly handled spectra in the aliphatic region of chemical shifts. The olefinic part was carefully handled since it carries important information. A dedicated procedure was used to determine the half-height line widths and frequencies of the olefin components corresponding to isotopomers 2–4 (Figure 2). The simulated Lorentzian multiplet was then computed and compared with experimental pattern. An iterative procedure can be started to optimize the fit between the theoretical and experimental multiplet.

3. RESULTS AND DISCUSSION

a. Identification of the Isotopomers of Linalool and Linalyl Acetate. A proper assignment of the ^2H NMR signals (Figure 1) to the monodeuterated isotopomers is a prerequisite to the interpretation of the isotopic fingerprints. The deuterium resonances were assigned on the basis of the proton spectrum analysis. Since a number of discrepancies in the interpretation of the ^1H and ^{13}C NMR spectra of terpenes, and in particular of linalool, exist in the literature, we have performed a complete spectral analysis. The signals numbered 1, 2, 3, 4, 6 and 9 (figure 1) are unambiguously assigned on the basis of the ^1H multiplicities. A 2D (^1H-^{13}C) correlation spectrum enables the corresponding signals to be identified in the ^{13}C spectrum. Assignment of the geminal methyl groups is not straightforward. By taking into account stereochemical substituent effects on the carbon shifts of the olefinic moiety, we confirm (Jautelat et al., 1970; Bohlmann et al., 1975; Wenkert et al., 1975) that the highest carbon

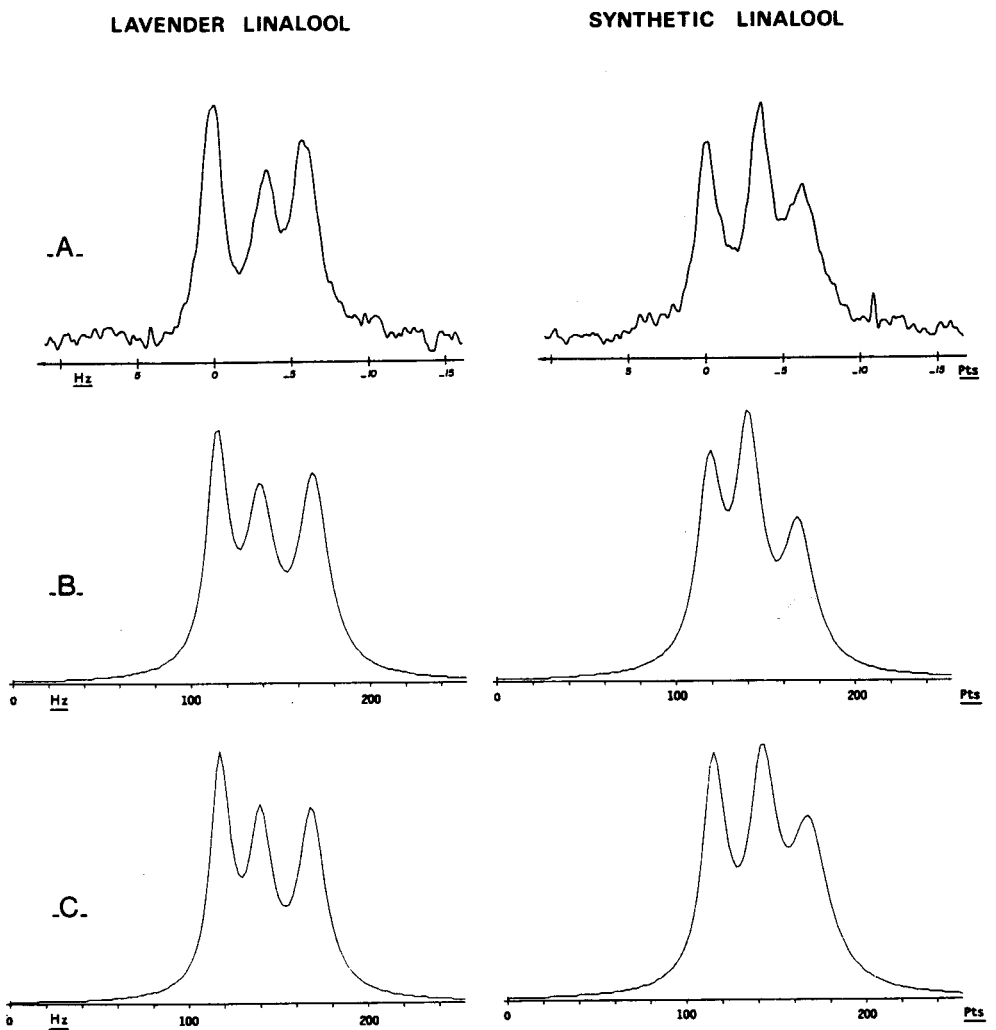


Figure 2. Experimental and simulated fingerprints associated with the ethylenic part of the ^2H NMR spectra of linalool samples from two different origins: lavender and synthesis. (A) Experimental pattern (6.8 points/Hz) recorded at 61.4 MHz on a sample of lavender linalool (no. 3) and a sample of synthetic linalool (no. 35). (B) Simulated pattern (6.8 points/Hz) obtained by using the Lorentzian parameters resulting from curve fitting of (A). (C) Simulated pattern (6.8 points/Hz) suitable for an at-a-glance qualitative identification of lavender and synthetic linalool. The Lorentzian curve fitting was computed using the data corresponding to the whole populations considered.

Table II. Deuterium NMR Parameters of Linalool and Linalyl Acetate[a]

	site i									
	1	2	3	4	5	6	7	8	9	10
					Linalool					
δ^2H (TMS)	5.93	5.25	5.14	5.03	3.47	2.03	1.67	1.60	1.52	1.27
T_1, s	0.1	0.1	0.12	0.07	0.05	0.08	0.38	0.80	0.08	0.1
F_i	0.055	0.055	0.055	0.055	0.055	0.111	0.166	0.166	0.111	0.166
$\Delta\nu_{1/2}$, Hz	3.2	3.9	3.5	5.2	(a)	9	1.7	1.5	7.1	3.6
					Linalyl Acetate					
δ^2H (TMS)	6.08	5.26	5.22	5.18	2.05	2.0	1.84	1.75	1.68	1.61
T_1, s	0.14	0.14	0.14	0.08	0.08	0.84	0.08	0.44	0.75	0.1
F_i	0.050	0.050	0.050	0.050	0.100	0.150	1.100	0.150	0.150	0.150
$\Delta\nu_{1/2}$, Hz	1.7	2.2	2.2	2.8	3.3	0.7	4.2	1.1	0.7	2.2

[a] The numbering of the atoms is given in Figure 1 for linalool. For linalyl acetate the assignment is as follows:

$$CH_2{=}CH{-}C(CH_3)(OCOCH_3){-}CH_2{-}CH_2{-}CH{=}C(CH_3)_2$$
$$2,3 \quad 1 \quad 10 \quad 6 \quad 7 \quad 5 \quad 4 \quad 8,9$$

δ^2H, chemical shift expressed in ppm with respect to tetramethylsilane (TMS); T_1, longitudinal relaxation time in seconds; F_i, statistical population of site i; $\Delta\nu_{1/2}$, half-height line width in hertz. (a) variable (exchanging site).

screening constant belongs to the methyl carbon cis to the methylene fragment. The 2D(^1H–^{13}C) correlation analysis then enabled the corresponding protons or deuterium to be associated with signal 8. The ^2H chemical shifts and the longitudinal relaxation times of linalool and linalyl acetate are given in Table II.

b. Mean ($\overline{D/H}$) and $\delta^{13}C$ Isotope Parameters of Linalool and Linalyl Acetate. The values of the overall carbon and hydrogen isotope ratios determined by mass spectrometry are collected in Table I. No significant differences are observed for the $\delta^{13}C$ values of natural (−27.4% SD 1.7) and synthetic (−27.1% SD 0.6) samples

Table III. Site-Specific Isotope Ratios of Linalool Extracted from Plants or Prepared from Fossil Materials[a]

botanical origin of the samples	$(D/H)_1$	$(D/H)_2$	$(D/H)_3$	$(D/H)_4$	$(D/H)_5$	$(D/H)_6$	$(D/H)_7$	$(D/H)_8$	$(D/H)_9$	$(D/H)_{10}$
lavender, $n = 10$	25.3 (2.5)	123.4 (10)	102.3 (8)	129.8 (12)	169.8 (25)	181.1 (12)	107.8 (5)	116.4 (5)	126.8 (14)	123.1 (15)
spike lavender, $n = 2$	35.3	132.7	109.5	139.9	162.7	162.0	106.7	126.6	120.9	153.5
bois de rose, $n = 3$	23.0 (2)	133.9 (6)	139.3 (21)	138.2 (12)	176.6 (14)	167.9 (9)	93.9 (5)	97.2 (9)	119.3 (35)	135.4 (19)
bitter orange, $n = 3$	41.7 (3)	124.4 (10)	120.7 (14)	134.1 (14)	177.2 (14)	172.3 (18)	111.0 (15)	124.8 (10)	97.0 (25)	130.8 (1.7)
bergamot, $n = 3$	30.4 (5)	135.2 (9)	123.3 (6)	142.6 (5)	168.6 (14)	172.4 (13)	120.9 (21)	117.4 (9)	104.4 (24)	126.3 (4)
coriander, $n = 2$	48	132	150	141	176	~165	92	128	120	130
camphor, $n = 1$	20	132	123	141	173	181	89	140	91	125
natural, $n = 26$	30.2 (9)	128.7 (10)	119.0 (22)	135.3 (11)	171 (17)	174 (14)	105.9 (12)	117.2 (13)	117.2 (20)	130.5 (17)
synthetic, $n = 5$	106.8 (3)	102.6 (6)	126.5 (5)	89.9 (5)	153.7 (12)	128.4 (5)	135.0 (9)	131.9 (14)	140.3 (23)	140.0 (4)
hemisynthetic, $n = 1$	26.4	138.6	167.2	132.0	162.8	172.7	104.9	88.7	122.1	138.6

[a] The numbering corresponds to the isotopomer assignment represented in Figure 1 (decreasing order of chemical shifts). n is the number of investigated samples. The standard deviations are given in parentheses.

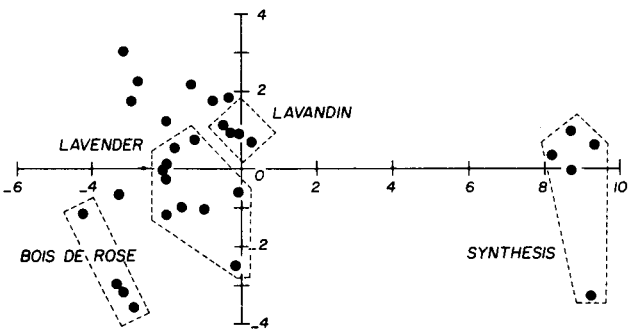


Figure 3. Bidimensional representation of the principal component analysis performed by using 10 site-specific hydrogen isotope ratios and the overall $\delta^{13}C$ parameter. The original data were autoscaled (centered to the mean and reduced by the standard deviation of each variable). The 31 samples are represented in the plane of the two main axes.

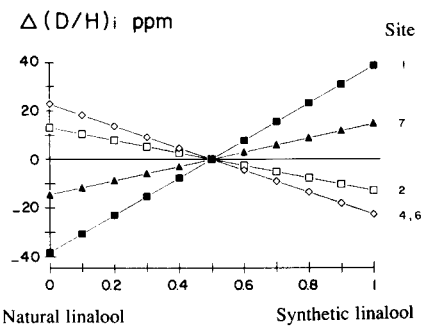


Figure 4. Schematic pattern illustrating the ability of the five most discriminating site-specific isotope ratios $(D/H)_i$ (i = 1, 2, 4, 6, 7) to detect and quantify the addition of synthetic linalool in the natural material. The difference in the values of the isotope ratio of a given isotopomer i, in the natural and synthetic state, $\Delta(D/H)_i$ is represented as a function of the molar ratio of the mixture for the five molecular sites identified in Figure 1. (The $\Delta(D/H)_i$ values have been centered.)

of linalool, although the products extracted from spike lavender and camphor tree seem to be slightly enriched in ^{13}C. It is also noted that linalool has a slightly higher $\delta^{13}C$ value than linalyl acetate. In general, the $\overline{(D/H)}$ isotope ratio seems to be a better probe of the natural status of a linalool or linalyl acetate sample than $\delta^{13}C$ since noticeable differences are observed between compounds of natural and synthetic origins: linalool, natural (123.7 SD 3.4), synthetic (130.1 SD 1.7); linalyl acetate, natural (123.5 SD 3.1), synthetic (128.4 SD 0.9). However, the ranges of $\overline{(D/H)}$ values of the natural samples are rather large, and several exceptions to the rule $\overline{(D/H)}_{syn} > \overline{(D/H)}_{nat}$ are noted. Thus, the natural samples case 12 (spike lavender), case 27 (geranium), and case 23 (bergamot) have values as high as 130.7 ppm. In this respect sample case 32 purchased from a chemical dealer has a very low $\overline{(D/H)}$ ratio (122.2 ppm), suspect of hemisynthetic origin (pinene). It will be confirmed later that the site-specific isotope ratios of this sample are typical of a nonnatural origin. The large range of $\overline{(D/H)}$ values observed for the natural products may be explained in terms of fractionation effects conditioned by the meteorological conditions of plant growth. It is known, for instance, that lavender is found mostly in high, cold, and sunny areas situated between 600 and 1500 m above sea level (Vinot et Bouscary, 1967), whereas spike lavender grows below 800 m in Mediterranean countries with a warmer climate (Boelens, 1986; Peyron, 1983). As a consequence, significantly higher values (128.1 SD 2.3) are exhibited by spike lavender with respect to lavender samples (123 SD 1.4).

c. Site-Specific Isotope Analysis of Linalool and Linalyl Acetate. The results obtained for linalool samples from seven botanical origins are given in Table III, which also summarizes the data concerning the entire population of natural and synthetic samples. In the case of linalyl acetate, the samples from clary sage, bergamot, and petit grain oils exhibit roughly the same $(D/H)_i$ ratios, which strongly differ from those of the synthetic acetates:

natural linalyl acetate

$\overline{(D/H)}$ = 123.2 (3.4); $\delta^{13}C$ = −28.1 (0.6); n = 4

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
40.1	163.7	114.9	114.3	154.5	169.0	94.2	107.2	131.0	104

synthetic linalyl acetate

$\overline{(D/H)}$ = 128.4 (0.9); $\delta^{13}C$ = −30.4 (0.3); n = 3

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
105.3	77.8	140.4	114.7	206.2	126.9	89.9	134.7	131	119.8

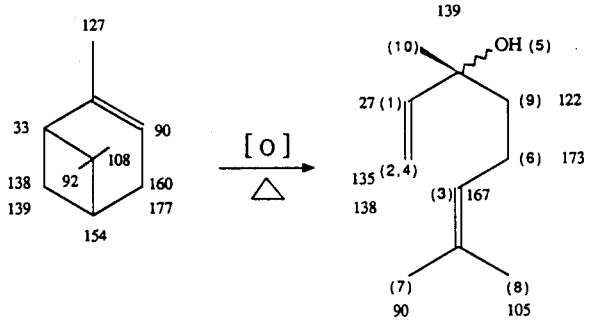
These results demonstrate in particular that the isotopic fingerprint provided by the SNIF NMR method is considerably more powerful than the overall $\overline{D/H}$ and $\delta^{13}C$ parameters obtained by IRMS for characterizing the natural or synthetic status of the sample.

A principal component analysis performed on 31 samples of linalool represented by 11 variables (10 deuterium isotope ratios and $\delta^{13}C$) shows that the whole population space may be reduced to a four dimensional space (75% of the overall variance). The main axis (38% of the

variance) is primarily determined by the isotope ratios of the ethylenic hydrogens, and the second axis (16% of the variance) depends mainly on $\delta^{13}C$ and on the parameters of the two olefinic methyl groups. Some typical origins can be discriminated in the space of the three main axes. Thus, as illustrated in Figure 3, the identification of synthetic linalools is straightforward. A discriminant analysis performed over the natural and synthetic families shows that all of the synthetic samples are assigned to the right group. However, the discrimination between lavender and spike lavender on one hand and bergamot, coriander, bitter orange, and bois de rose on the other hand is only 82% effective.

Natural linalool is characterized by a strong depletion in the heavy isotope in site 1 and by a relatively high enrichment in site 6. Moreover, the two methyl isotopomers 7 and 8 are significantly less abundant than isotopomer 10. In contrast, synthetic linalool is characterized by a rather flat deuterium distribution over sites 3 and 6–10 on one hand (130–140 ppm) and over sites 1, 2, and 4 on the other hand (90–110 ppm). The strong deviations with respect to a random distribution of deuterium are the results of mechanistic fractionation effects which will be discussed in another context. From an analytical point of view the high degree of differentiation between synthetic and natural linalool enables a possible adulteration to be easily quantified.

The case of hemisynthetic linalool deserves some comments. The hemisynthesis of linalool and other terpenes usually starts from pinene, which is a relatively cheap natural material. The pinene is oxidized into 2-pinanol, which leads to linalool by pyrolysis. If we consider the mean values of the site-specific isotope ratios of pinenes (Martin et al., 1986), a good consistency is observed between the corresponding isotopomers in both kinds of compounds. In particular, the isotope contents $(D/H)_i$ of the methyl groups of the pinene bridge resemble closely those of the isopropenyl fragment of linalool.



α-pinene linalool

It should be emphasized that hemisynthetic linalool cannot be confused with the natural compounds since site 3 in particular is greatly enriched in deuterium in the hemisynthetic species as compared to the natural species.

The qualitative distinction between natural, hemisynthetic, and synthetic linalool can therefore be easily carried out by SNIF NMR. In this respect the ethylenic fingerprint provides the basis for a fast at-a-glance identification of an unknown essential oil as exemplified in Figure 2 for lavender and synthetic samples. A more elaborate analysis is required for a quantitative determination of synthetic compounds possibly contained in natural aromas or fragrances.

d. Determination of Synthetic Linalool in Natural Products. To appraise the potential of the NMR method to quantify the adulteration of an essential oil, a variance analysis was carried out on the two groups of natural (n = 25) and synthetic (n = 5) samples. At the 99% level of confidence, five sites (1, 2, 4, 6, and 7) characterized by the following values of the Fischer parameter, F, are highly discriminant ($F1/28$ = 7.64).

Table IV. Site-Specific Isotope Parameters of Linalool Extracted from Lavender Oil either Pure or Adulterated with Synthetic Linalool[a]

	site				
origin	1	2	4	6	7
pure lavender oil	22.9	113.9	135.8	168.7	104.6
synthetic linalool	108.1	101.1	96.4	126.9	134.0
adulterated oil (5%)	34.9	117	136.9	172.2	111.5
adulterated oil (10%)	40.5	113.6	133.6	157.9	113.1

[a] The adulterated mixtures were prepared by adding 5% and 10% of the synthetic component to an essential oil containing 47% linalool.

Table V. Composition Analysis of the Adulterated Linalool Samples Described in Table IV

	% linalool[a]			
	theoretical		experimental	
	% (O)	% (L)	% (L) (mean R)	% (L) (individual R)
mixture 1	5	10	7	12
mixture 2	10	24	16	20

[a] The percentage of added synthetic linalool is expressed with respect to the essential oil (O) and to the total linalool (L). The experimental results of Table III (mean) and of Table IV (individual) were used to compute % (L).

	site				
	1	2	4	6	7
F	361.7	32.4	83.8	45.2	25.3
LSD, ppm	11.1	12.5	13.7	18.8	15.9
ED, ppm	76.6	26.1	45.4	45.6	29.1

The least significant differences (LSD), expressed in parts per million, can be compared to the experimental differences (ED) between the means of the two groups for the five sites.

The expected variations of the different isotope ratios, expressed in the centered data format, are represented in Figure 4 as a function of the percentage of synthetic linalool contained in a natural oil. The discrimination is highly efficient. Moreover, a mixture analysis involves an overdetermined system since five equations are retained for one unknown. Denoting R the matrix constructed from the different isotope ratios of the two pure natural and synthetic species (Table III) and R_m the vector of the isotope ratios of the mixture, the composition A is given by

$$A = (\tilde{R}R)^{-1}\tilde{R}R_m \quad (6)$$

$\tilde{R}$ being the transpose of R.

To check the ability of the method to quantify an adulteration, two mixtures were precisely prepared by adding 5% and 10% synthetic linalool to an authentic lavender oil. The isotopic parameters of the synthetic component and of the linalool obtained after extraction and purification from the natural oil and from the two mixtures are given in Table IV.

The analysis of the mixtures performed according to the formalism described by eq 6 leads to the results in Table V. The theoretical percentage of the synthetic component is expressed both with respect to the essential

oil and with respect to the whole pool of linalool. The latter percentage is also computed either from the matrix of the mean isotope ratios collected in Table III or from the matrix of the isotope parameters measured for the two individual components used in the mixture (Table IV). In spite of the relatively large dispersion of the isotopic parameters of the natural species, the discriminant potential is very satisfactory. It is substantially improved when the origin of the precursors is known.

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LITERATURE CITED

Banthorpe, D. V.; Charlwood, B. V.; Francis, J. O. The biosynthesis of monoterpenes. *Chem. Rev.* **1972**, *72*, 115–149.

Boelens, M. H. The essential oil of spike lavender lavandula latifolia Vill. (L. spica D.C.). *Perfum. Flavor.* **1986**, *11* (5), 43–63.

Bohlmann, F.; Zeisberg; Klein, E. *Org. Magn. Reson.* **1975**, *7*, 426–432.

Bordier, L. Ph.D. Dissertation, Université de Nantes, 1983.

Bricout, J.; Merlivat, L.; Koziet, J. *C. R. Acad. Sci.* **1973**, *277D*, 885–888.

Buccellato, F. Bois de rose oil: a glimpse into the past. *Perfum. Flavor.* **1988**, *13* (4), 35–36.

Clark, G. S. Linalool: an aroma chemical. *Perfum. Flavor.* **1988**, *13* (4), 49–54.

Craig, H. Isotopic standards for carbon and oxygen, and correction factors for mass spectrometric analysis of carbon dioxide. *Geochim. Cosmochim. Acta* **1957**, *12*, 133–149.

Croteau, R.; Satterwhite, D. M.; Wheeler, C. J.; Felton, N. M. Biosynthesis of monoterpenes. Stereochemistry of the enzymatic cyclization of geranyl pyrophosphate to (−)-endo-fenchol. *J. Biol. Chem.* **1988**, *263*, 15449–15453.

Gonfiantini, R. Standards for stable isotope measurements in natural compounds. *Nature* **1978**, *271*, 534–536.

Grant, D. M.; Curtis, J.; Croasmun, W. R.; Dalling, D. K.; Wehrli, F. W.; Wehrli, S. NMR determination of site-specific deuterium isotope effects. *J. Am. Chem. Soc.* **1982**, *104*, 4492–4494.

Jautelat, M.; Grutzner, J. B.; Roberts, J. D. Natural abundance ^{13}C nuclear magnetic resonance spectra of terpenes and carotenes. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *65*, 288–292.

Lawrence, B. M. Progress in essential oils: clary sage oil. *Perfum. Flavor.* **1986**, *11* (5), 111.

Lawrence, B. M. Progress in essential oils. *Perfum. Flavor.* **1987**, *12* (a, bergamot oil) (2), 68; (b, clary sage and coriander oils) (4), 71; (c, spike lavender oil) (5), 62; (d, lavender–lavandin) (6), 62–64.

Lawrence, B. M. Progress in essential oils. *Perfum. Flavor.* **1988**, *13* (a, bergamot oil) (2), 67; (b, coriander oil) (3), 53.

Leopold, M. F.; Epstein, W. W.; Grant, D. M. Natural abundance deuterium NMR as a novel probe of monoterpene biosynthesis: limonene. *J. Am. Chem. Soc.* **1988**, *110*, 616–617.

Martin, G. J.; Martin, M. L. Deuterium labelling at the natural abundance level as studied by high field quantitative ^{2}H-NMR. *Tetrahedron Lett.* **1981**, *22*, 3525–3528.

Martin, G. J.; Naulet, N. Precision, accuracy and referencing of isotope ratios determined by NMR. *Fresenius' Z. Anal. Chem.* **1988**, *332*, 648–651.

Martin, G. J.; Martin, M. L.; Mabon, F.; Bricout, J. A new method for the identification of the origin of natural products. Quantitative ^{2}H NMR at the natural abundance level applied to the characterization of anetholes. *J. Am. Chem. Soc.* **1982**, *104*, 2658–2659.

Martin, G. J.; Sun, X. Y.; Guillou, C.; Martin, M. L. NMR determination of absolute site-specific natural isotope ratios of hydrogen in organic molecules. Analytical and mechanistic applications. *Tetrahedron* **1985**, *41*, 3285–3296.

Martin, G. J.; Janvier, P.; Akoka, S.; Mabon, F.; Jurczak, J. A relation between the site-specific natural deuterium contents in α-pinenes and their optical activity. *Tetrahedron Lett.* **1986**, *27*, 2855–2858.

Martin, M. L.; Martin, G. J. Deuterium NMR in the study of site-specific natural isotope fractionation (SNIF-NMR). *NMR: Basic Prin. Prog.* **1990**, *23*, 1–61.

O'Leary, M. H. Carbon isotopes in photosynthesis. *Bioscience* **1988**, *38*, 328–336.

Pascal, R. A.; Baum, M. W.; Wagner, C. K.; Rodgers, L. R.; Huang, D. S. Measurement of deuterium kinetic isotope effects in organic and biochemical reactions by natural abundance deuterium NMR spectroscopy. *J. Am. Chem. Soc.* **1986**, *108*, 6477–6482.

Peyron, L. *Parfums, Cosmet., Aromes* **1983**, *54*, 55–60.

Porter, J. W.; Spurgeon, S. L. *Biosynthesis of isoprenoids compounds*; Wiley: New York, 1981; Vol. I, pp 165–216.

Swaine, R. L.; Swaine, R. L., Jr. Citrus oils: processing, technology and applications. *Perfum. Flavor.* **1988**, *13* (6), 1–20.

Vinot, M.; Bouscary, A. *Recherches Roure, Paris* **1962–1968.**

Wenkert, E.; Gasic, M. J.; Hagaman, E. W.; Kwart, L. D. Long range substituent effects reflected in the ^{13}C NMR spectra of allyl alcohols and their derivatives. *Org. Magn. Reson.* **1975**, *17*, 51–53.

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