JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Natural Abundance ^{2}H-ERETIC-NMR Authentication of the Origin of Methyl Salicylate

FLORE LE GRAND,*,† GERARD GEORGE,‡ AND SERGE AKOKA†

LAIEM, UMR 6006 CNRS, Université de Nantes, B.P. 92208, 44322 Nantes Cedex 3, France, and DEGUSSA, Z. I. du Plan, B.P. 82067, 06131 Grasse Cedex, France

Methyl salicylate is a compound currently used in the creation of many flavors. It can be obtained by synthesis or from two natural sources: essential oil of wintergreen and essential oil of sweet birch bark. Deuterium site-specific natural isotope abundance (A_i) determination by NMR spectroscopy with the method of reference ERETIC (^{2}H-ERETIC-NMR) has been applied to this compound. A_i measurements have been performed on 19 samples of methyl salicylate from different origins, natural/synthetic and commercial/extracted. This study demonstrates that appropriate treatment performed on the data allows discrimination between synthetic and natural samples. Moreover, the representation of intramolecular ratios $R_{6/5}$ as a function of $R_{3/2}$ distinguishes between synthetics, wintergreen oils, and sweet birch bark oils.

KEYWORDS: Quantitative ^{2}H NMR; isotope abundance; methyl salicylate; ERETIC

INTRODUCTION

Methyl salicylate is widely used in the flavor industry to create several aromas, such as strawberry, raspberry, banana, tomato, cocoa, mint, cherry, fig, peach, and tea. This molecule can be obtained by synthesis or from two natural sources: essential oil of wintergreen (*Gaultheria procumbens* L.) and essential oil of sweet birch bark (*Betula lenta* L.). The natural extracts are ∼5−7 times more expensive than the synthetic product (also known as "nature identical"). Because consumers are more and more demanding natural origin of foodstuffs and traceability, it is important to differentiate between these origins. To our knowledge, no study, and especially no isotopic study, has been published on the authentication of methyl salicylate.

Quantitative deuterium NMR allows routine determination of site-specific natural isotopic distribution (*1*). This method, also known as site-specific natural isotope fractionation by nuclear magnetic resonance (SNIF-NMR), measures significant variations of deuterium isotopic distribution according to the origin of the molecule (*2*). It has been applied to the authentication of product origin, such as wines (*3*), spirits (*4*), vanilla (*5*), and sugars (*6*). Although using a classical internal reference is not a drawback, the ERETIC reference is a useful alternative to avoid adding an exogeneous compound in a precious sample, available only in a limited quantity. We have shown in previous work (*7*−*9*) that the total experimental time of such analysis could be dramatically reduced by using the ERETIC method (^{2}H-ERETIC-NMR). In the present study, we applied this approach to distinguish natural and synthetic methyl salicylates and between the two essential oils.

* Corresponding author [telephone (33) 493 093 166; fax (33) 493 093 148; e-mail flore.legrand@degussa.com].
† LAIEM.
‡ DEGUSSA.

Table 1. Nature, Origin, and Deuterium Distribution (Averaged A_i in Parts per Million and Its Standard Deviation σ_i in Parts per Million, from Three ^{2}H Spectra) for the 19 Samples Studied

sample	nature/origin[a]	A_2	σ_2	A_3	σ_3	A_4	σ_4	A_5	σ_5	A_6	σ_6
1	W co	130.1	2.3	158.1	2.9	151.4	3.5	137.2	2.6	135.6	1.3
2	SB co	136.3	3.7	138.9	3.7	137.1	2.9	146.2	4.6	146.9	1.2
3	W co	120.2	2.3	150.6	1.9	144.2	0.6	131.1	3.1	131.0	1.3
4	W co	125.8	1.5	159.8	1.9	147.9	3.3	139.0	1.8	136.9	0.7
5	W co	119.6	2.3	148.2	5.6	136.2	2.8	125.5	1.8	126.4	0.3
6	W co	123.4	4.5	155.2	5.9	146.7	1.0	132.1	2.7	134.0	0.9
7	W co	118.7	2.3	153.0	2.0	143.6	4.4	131.8	1.1	129.2	0.4
8	W co	121.3	2.7	152.7	1.3	146.6	5.9	132.4	1.8	131.6	0.3
9	S co	148.4	2.3	147.3	1.3	126.7	2.3	122.2	2.0	138.2	0.6
10	S co	141.2	1.7	138.7	1.3	134.1	4.6	141.8	4.2	149.1	0.3
11	S co	132.3	0.9	131.3	2.0	134.0	6.0	137.4	5.6	128.7	0.6
12	S co	153.9	4.4	155.4	3.2	141.4	2.2	139.8	2.8	133.4	0.7
13	S co	158.4	0.6	159.8	3.1	131.2	1.6	134.8	2.5	144.2	0.7
14	S co	171.3	3.5	171.0	2.5	143.1	1.2	141.5	3.1	154.0	1.6
15	SB lab	129.1	1.0	161.2	1.7	141.1	8.2	119.7	9.0	131.3	1.2
16	SB lab	116.6	2.6	143.3	3.9	123.2	4.6	109.1	4.1	120.5	0.1
17	SB lab	122.1	6.7	151.5	5.5	145.1	6.6	119.9	3.5	144.1	0.4
18	W lab	117.0	3.8	150.2	3.6	135.1	2.3	127.0	3.2	120.8	0.8
19	W lab	120.0	3.6	141.9	7.9	139.6	9.3	113.8	3.8	114.2	0.9

[a] W, wintergreen oil; SB, sweet birch bark oil; S, synthetic; Co, commercial; lab, extracted in the laboratory.

MATERIALS AND METHODS

Methyl Salicylate Samples: Origin and Extraction. Nineteen methyl salicylates from various origins (four sweet birch bark oils, nine wintergreen oils, and six from synthetic origin) were analyzed. Some samples were from commercial origin (purchased from Acros, Aldrich, Alfa Aesar, Berjé, Citrus and Allied Essences, Clariant-Lancaster, Fluka, Rhodia, Roth, and Polarome); others were extracted in the laboratory from raw materials, as detailed in **Table 1**.

10.1021/jf050385a CCC: $30.25
Published on Web 05/24/2005

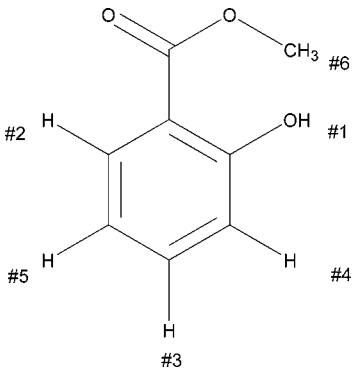

Figure 1. Methyl salicylate molecule and numbering of ^{2}H sites.

When extraction was performed, raw material was dry wintergreen leaves or ground sweet birch bark. One kilogram of raw material was left to soak overnight with 10 L of warm water. Such maceration causes hydrolysis of the primeveroside of methyl salicylate (also known as monotropitin or gaultherin) and frees methyl salicylate, the chief constituent of wintergreen oil and sweet birch bark oil (95−99%). In fact, methyl salicylate does not occur in the plant in the free form, but as a glycoside (*10*). The distillate obtained over 6 h was separated by condensation. Distillation was ended by the addition of 40 mL of cyclohexane. The aqueous phase was extracted two times by 50 mL of cyclohexane. Cyclohexane phases were combined and concentrated under N_2 flow. The purity of samples was checked by ^{1}H NMR.

The molecule of methyl salicylate presents five sites that can be used for A_i measurement by ^{2}H NMR (**Figure 1** and see ref *9* for the ^{2}H spectrum of methyl salicylate), because hydrogen (and deuterium) atoms in site 1 undergo exchange with those of the extraction medium. The tube composition was 2.5 mL of methyl salicylate, 1.5 mL of tetramethylurea (or TMU, a classical internal reference introduced for another study), and 100 μL of lock C_6F_6.

^{2}H NMR Acquisitions. A Bruker DRX 500 spectrometer, with a 500.13 MHz nominal frequency in ^{1}H, was used with a probe dedicated to ^{2}H measurement (10 mm ^{2}H−^{1}H probe, lock ^{19}F). The spectrometer was equipped with three radio frequency channels, permitting the observation of ^{2}H nuclei, the ^{1}H decoupling, and the generation of the ERETIC signal (*11*).

Similar to those previously described for ethanol (*12*−*14*), ^{2}H measurement parameters were as follows: flip angle $\alpha = 90°$, repetition time TR = 6 s (TR $\geq 5 \times T_{1max}$), sampling period AQ = 4 s, number of scans NS = 500, spectral range SW = 1230 Hz, TD = 9842, temperature TE = 308 K. Longitudinal relaxation times were determined by inversion−recovery (eight inversion delays between 5 ms and 8 s); T_{1max} = 1.1 s (*9*). The usual pulse sequence was modified only to introduce the electronic signal ERETIC in the spectra.

^{1}H NMR Acquisitions. Quantitative ^{1}H NMR spectra of samples were performed on the same tubes, with the same probe, from the decoupling coil, following the ^{2}H spectra. Principal acquisition parameters were as follows: pulse width duration ^{1}H PW = 1 μs, TR = 12 s, AQ = 4 s, NS = 12, TD = 60058. The short pulse width is adapted to the high analyte concentrations to avoid FID truncation and receiver saturation, as detailed by Fauhl et al. (*15*). The flip angle is also minimum, and quantitative acquisition conditions are guaranteed for a pulse interval of 12 s. The pulse sequence was modified to introduce the electronic signal ERETIC in the spectra.

Data Processing. After exponential multiplication (line broadening = 1 Hz for ^{2}H spectra, 2 Hz for ^{1}H spectra) and Fourier transform on 32K, the peak areas were determined by fitting the spectrum to Lorentzian shapes with the PERCH NMR software (University of Kuopio, Finland).

For each tube three ^{2}H spectra and four ^{1}H spectra were measured. Data from the four ^{1}H spectra were averaged. Then data from the corresponding three ^{2}H spectra were used in the calculation of A_i and standard deviation σ_i.

Principal componant analysis (PCA) was performed by the Statistica 6.1 software (Statsoft, Inc., Maisons-Alfort, France).

ERETIC. The ERETIC method uses an NMR-like electronically produced signal ("pseudo-FID"), transmitted by a secondary antenna, added to the probe, and received simultaneously with the natural FID of the sample (*11*, *16*). After Fourier transform, the signal gives an additional peak in the spectrum. When variations of sensitivity arise, the global intensities change in the same way for the sample peaks and the ERETIC peak. The ERETIC peak can therefore be used as a reference, like a classical internal reference.

Its amplitude and frequency position can be freely chosen from the spectrometer (*7*, *17*, *18*).

***A*$_i$ Calculation.** ^{2}H and ^{1}H NMR spectra were obtained on samples and on a reference tube, called a "calibration tube", containing the analyzed compound, the isotope abundances A_i^{cal} of which were known (*9*).

In this study, sample 8 was arbitrarily chosen as the reference tube. It contained methyl salicylate from wintergreen oil, the isotopic abundances A_i^{cal} of which were 121.3 ± 2.7, 152.7 ± 1.3, 146.6 ± 5.9, 132.4 ± 1.4, and 131.6 ± 0.3 ppm for sites 2−6, respectively. This was determined by a classical A_i measurement, using TMU as reference (*3*).

RESULTS AND DISCUSSION

The use of a line broadening of 1 Hz for ^{2}H spectra (which have broad lines) and 2 Hz for ^{1}H spectra (which have narrow lines) can be surprising. However, the line broadening in ^{2}H spectra was restricted to 1 Hz to avoid peak overlap, especially for sites 4 and 5, which were only 8 Hz distant. On the other hand, the line brodening of 2 Hz for ^{1}H spectra was not optimal in terms of signal-to-noise ratio; however, it was not a restrictive parameter, and the influence of magnetic field inhomogeneity on peak shape was minimized.

The results of the NMR determination of A_i, calculated according to eq 1, are given in **Table 1**.

$$A_i^{c} = A_{cal} \times \frac{S_i^{c}}{S_{eretic}} \times \frac{S'_{eretic}}{S^{cal}} \times \frac{\sum_{eretic}}{\sum^{c}} \times \frac{\sum^{cal}}{\sum'_{eretic}} \qquad (1)$$

At each site, the standard deviation σ_i depends essentially on the corresponding signal-to-noise ratio. The range of A_i variation (difference between $A_{i,max}$ and $A_{i,min}$) observed for the natural samples is 30.6 ppm, whereas for synthetic samples a slightly narrower range of 21.7 ppm is found. Sample 2 (commercial sweet birch bark oil) has a lower range of A_i variation than the other natural samples. For synthetic samples, A_2 and A_3 are always very similar. A_4 and A_5 seem also more comparable for synthetic samples than natural ones.

Recently, Breena et al. (*19*) reported a detailed investigation of the ^{2}H isotope pattern of salicin and salicyl alcohol of different origins, using suitable derivatives. These molecules are very close to our molecule of interest (*o*-hydroxybenzyl alcohols) and are synthesized in nature according to the same metabolic pathway. It has been proved that the ^{2}H distribution along the aromatic ring in phenylpropanoids is strongly influenced by the ^{2}H labeling of the sugar fragments providing shikimic acid (*20*−*23*). Their data showed that for natural samples the deuterium enrichment on the aromatic ring follows the trend $A_{para} > A_{meta3} \sim A_{meta5} > A_{ortho}$ (positions are referred to the alkyl chain). It is different from the trend observed on natural phenylpropanoids: $A_{para} > A_{ortho} > A_{meta}$. This inversion cannot be explained by a NIH shift during hydroxylation, as for para-hydroxylated propanoids, but is explained by an equilibrium with water at some time of the compound history. If these positions are converted to our numeration by decreasing chemical shift, it brings for natural ortho-hydroxylated propanoids $A_3 > A_4 \sim A_5 > A_2$. In contrast, the same compounds

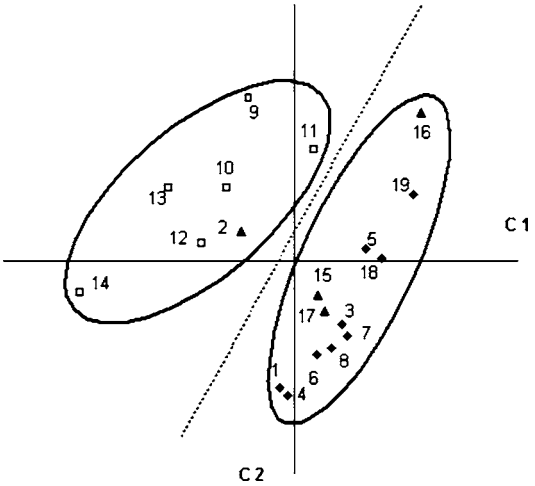

Figure 2. Representation of the PC plane of PCA carried out on 19 methyl salicylate samples and with the five isotope abundances A_i measured: ◆, wintergreen oil; ▲, sweet birch bark oil; □, synthetic.

of synthetic origin displayed very similar deuterium contents in all positions.

Among our samples, these from wintergreen oils show the trend $A_3 > A_4 > A_5 > A_2$ except for sample 19, which had a quite similar profile: $A_3 > A_4 > A_5 \sim A_2$. The samples from sweet birch bark oil follow the trend $A_3 > A_4 > A_5 \sim A_2$ except for sample 2: $A_3 \sim A_4 \sim A_5 \sim A_2$. For synthetic methyl salicylates, the deuterium enrichment is equally distributed on all positions: $A_3 \sim A_4 \sim A_5 \sim A_2$ or $A_3 \sim A_4 > A_5 \sim A_2$. A clear distinction is also observed between the compounds of different origins, and sample 2 is ambiguous, very close to synthetic ones. Furthermore, our results are consistent with data previously published by Breena et al. (*19*).

A PCA was carried out on the 19 samples (13 naturals and 6 synthetics) in the space of the five variables A_i. The plan of the two principal components is presented **Figure 2**. The first component, C1 (which expresses 61.2% of the variance), is mainly derived from A_2 and A_6, and the second component, C2 (17.8% of the variance), depends mainly on A_3 and A_4. In this graphical representation of the data, samples are divided into two groups: the first one contains all of the synthetic samples 9−14 and sample 2 (commercial sweet birch bark); the second contains all of the natural samples 1, 3−8, and 15−19, except sample 2.

The clear-cut way of determining the naturalness of methyl salicylate has been indicated by a separation line. Synthetic and natural samples are clearly differentiable except for the ambiguous sample 2, which was our only commercial sweet birch bark oil. It should be noted that the botanical origin of samples obtained by extraction in the laboratory is sure, whereas it could be uncertain for commercial samples. Furthermore, for commercial samples, only the raw material is declared, but not details about the conditions of extraction or geographical origin. Therefore, results on commercial samples must be carefully interpreted.

Unfortunately, the two natural origins of essential oils are not distinguished here. Ten intramolecular ^{2}H distributions were therefore calculated, $R_{3/2}$, $R_{4/2}$, $R_{5/2}$, $R_{6/2}$, $R_{4/3}$, $R_{5/3}$, $R_{6/3}$, $R_{5/4}$, $R_{6/4}$, $R_{6/5}$, and for the 19 samples studied:

$$R_{i/j} = n_j^c \times \frac{A_i^c}{A_j^c} \qquad (2)$$

One of these parameters shows a clear rule to determine the origin of methyl salicylate: $R_{3/2}$ (**Figure 3**). Considering $R_{3/2}$, samples are again divided into the same two groups: synthetics, samples 9−14 (plus sample 2) with $0.95 < R_{3/2} < 1.05$, and naturals (samples 1, 3−8, and 15−19) with $1.15 < R_{3/2} < 1.30$. Actually, for synthetic samples, A_2 and A_3 are always very similar, thus meaning $R_{3/2}$ is close to 1 (mean = 0.997; SD = 0.011). In the case of natural samples (except sample 2), $R_{3/2}$ average is 1.247 with SD = 0.029. The $R_{3/2}$ parameter seems to give, in the simplest way, the same information as a PCA performed on the A_i data: separation of natural samples from synthetic ones. Unfortunately, the two botanical origins of the natural molecule were never distinguished.

Couples of $R_{i/j}$ were graphically reported. The representation of $R_{6/5} = f(R_{3/2})$ is the most interesting (**Figure 4**). Data are now divided into three groups. As previously observed, natural samples ($R_{3/2} > 1.15$) are distinguished from synthetics ($R_{3/2} < 1.05$), but sweet birch bark oils ($R_{6/5} > 1.07$) are separated from wintergreen oils ($R_{6/5} < 1.07$). A direct way of distinguishing

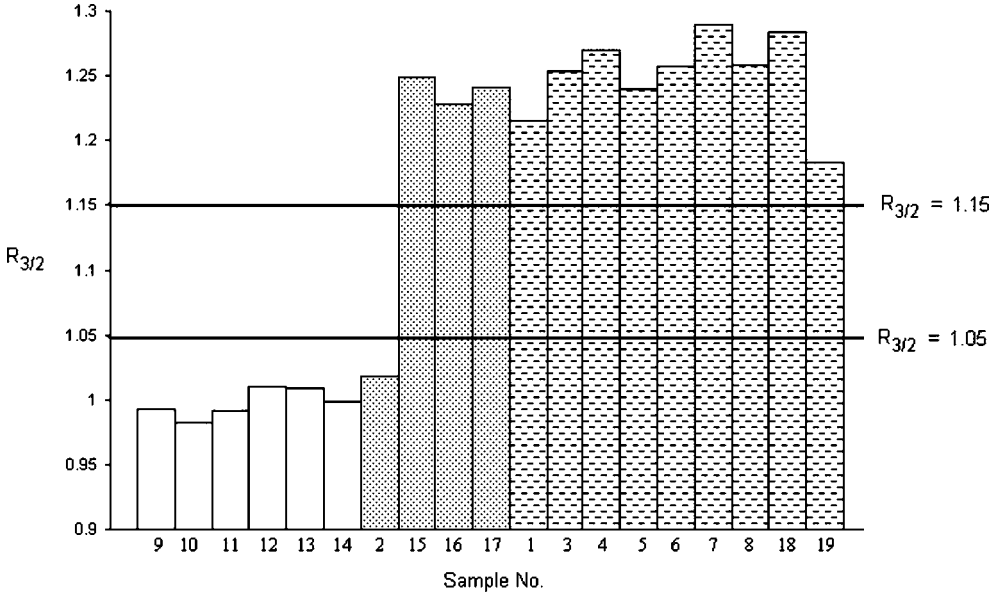

Figure 3. Parameter $R_{3/2}$ for the 19 samples studied: white bar, synthetic; dotted bar, sweet birch bark oil; dashed bar, wintergreen oil.

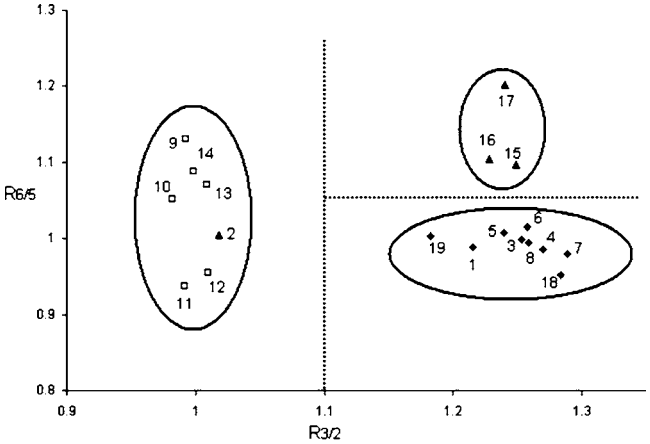

Figure 4. $R_{6/5}$ as a function of $R_{3/2}$ on 19 methyl salicylate samples: ◆, wintergreen oil; ▲, sweet birch bark oil; □, synthetic.

synthetic samples, wintergreen oils, and sweet birch bark oils has therefore been found.

Within the circle of synthetics, one can observe two groups: samples 11, 12, and 2, on the one hand, and samples 9, 10, 13, and 14, on the other. Unfortunately, the information at our disposal on commercial samples was not sufficient to interpret this observation.

PCA was then performed on the 19 samples with the 10 $R_{i/j}$ as variables. The first two dimensions of the graphical representation explain 85.9% of the overall variance. The first component, C1 (which expresses 62.4% of the variance), depends mainly on $R_{3/2}$ and $R_{4/2}$, and the second component, C2 (23.5% of the variance), depends mainly on $R_{5/3}$ and $R_{5/2}$. PCA was therefore performed with the 10 $R_{i/j}$ as variables, but only for 12 natural samples: 1, 3−8, and 15−19. The first two dimensions of the graphical representation explain 74.2% of the overall variance (first component, C1, 43.9% of the variance and second component, C2, 30.3% of the variance). Finally, no more information in terms of authentication was obtained with two successive PCA than with the simple representation of $R_{6/5} = f(R_{3/2})$.

This study shows the application of the ^{2}H NMR method in determining the naturalness of an important flavor, methyl salicylate. PCA performed from isotopic abundance A_i allows the discrimination between natural samples and synthetic ones. A simpler method is to observe the $R_{3/2}$ parameter: a value lower than 1.05 indicates a synthetic origin, whereas when it is higher than 1.15, a natural origin is indicated. The three origins of methyl salicylate (synthetic, wintergreen oil, and sweet birch bark oil) are distinguished by the graphical representation of $R_{6/5} = f(R_{3/2})$. A combination of compositional and isotopic analytical variables (^{2}H, ^{13}C, ^{14}C, etc.), computed in a larger databank, could improve the performance of methyl salicylate authentication. Further work is now underway to quantify potential adulterations.

ACKNOWLEDGMENT

M. Aubanel and J. Rivollier (DEGUSSA, Organic Research, Grasse) are acknowledged for helpful advice and technical assistance for raw material extractions.

LITERATURE CITED

(1) 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.

(2) Martin, G. J.; Martin, M. L. Deuterium NMR in the study of natural isotope fractionation (SNIF-NMR). In *NMR Basic Principles and Progress*; Diehl, P., Fluck, E., Günther, H., Kosfeld, R., Seelig, J., Eds.; Springer-Verlag: Berlin, Germany, 1990; pp 1−61.

(3) Martin, G. J.; Guillou, C.; Martin, M. L.; Cabanis, M.-T.; Tep, Y.; Aerny, J. Natural factors of isotope fractionation and the characterization of wines. *J. Agric. Food Chem.* **1988**, *36*, 316−322.

(4) Martin, G. J.; Martin, M. L.; Mabon, F.; Michon, M.-J. A new method for the identification of the origin of ethanols in grain and fruit spirits: high-field quantitative deuterium nuclear magnetic resonance at the natural abundance level. *J. Agric. Food Chem.* **1983**, *31*, 311−315.

(5) Remaud, G.; Martin, Y.-L.; Martin, G. G.; Martin, G. J. Detection of sophisticated adulterations of natural vanilla flavors and extracts: application of the SNIF-NMR method to vanillin and *p*-hydroxybenzaldehyde. *J. Agric. Food Chem.* **1997**, *45*, 859−866.

(6) Martin, G. J.; Danho, D.; Vallet, C. Natural isotope fractionation in the discrimination of sugars origins. *J. Sci. Food Agric.* **1991**, *56*, 419−434.

(7) Billault, I.; Akoka, S. Kinetic constant measurement by NMR using a calibrated internal signal (ERETIC). *Instrum. Sci. Technol.* **2000**, *28*, 233−240.

(8) Le Grand, F.; Akoka, S. Détermination des abondances isotopiques spécifiques par RMN-^{2}H et la méthode ERETIC. *C. R. Acad. Sci. II C* **2004**, *7*, 233−239.

(9) Le Grand, F.; Georges, G.; Akoka, S. How to reduce the experimental time in isotopic ^{2}H NMR using the ERETIC method. *J. Magn. Reson.* **2005**, *174*, 171−176.

(10) Guenther, E. Oil of wintergreen, oil of sweet birch. In *The Essential Oils*; Van Nostrand: New York, 1952; pp 3−15.

(11) Akoka, S.; Trierweiler, M. Improvement of the ERETIC method by digital synthesis and addition of a broadband antenna inside the NMR probe. *Instrum. Sci. Technol.* **2002**, *30*, 21−29.

(12) Martin, G. J.; Martin, M. L.; Mabon, F.; Michon, M.-J. Identification of the origin of natural alcohols by natural abundance hydrogen-2 nuclear magnetic resonance. *Anal. Chem.* **1982**, *54*, 2380−2382.

(13) Martin, G. J.; Zhang, B.-L.; Naulet, N. Deuterium transfer in the bioconversion of glucose to ethanol studied by isotope labeling at the natural abundance level. *J. Am. Chem. Soc.* **1986**, *108*, 5116−5122.

(14) Martin, G. J.; Trierweiler, M.; Ristow, R.; Hermann, A.; Belliardo, J. J. The certification of the three reference ethanols for SNIF-NMR: BCR certified reference materials. *BCR Inf.−Ref. Mater.* **1994**, *EUR 15347 EN*, 1−30.

(15) Fauhl, C.; Wittkowski, R. On-line 1H NMR to facilitate tube preparation in the SNIF-NMR analysis. *Z. Lebensm. Unters. Forsch.* **1996**, *203*, 541−545.

(16) Akoka, S.; Barantin, L.; Trierweiler, M. Concentration measurement by proton NMR using the ERETIC method. *Anal. Chem.* **1999**, *71*, 2554−2557.

(17) Barantin, L.; Akoka, S.; Le Pape, A. A new method for absolute quantification of MRS metabolites. *Magn. Reson. Med.* **1997**, *38*, 179−182.

(18) Silvestre, V.; Goupry, S.; Trierweiler, M.; Robins, R.; Akoka, S. Determination of substrate and product concentrations in lactic acid bacterial fermentations by proton NMR using the ERETIC method. *Anal. Chem.* **2001**, *73*, 1862−1868.

(19) Brenna, E.; Fronza, G.; Fuganti, C.; Gatti, F.; Pinciroli, M.; Serra, S. Differentiation of extractive and synthetic salicin. The ^{2}H aromatic pattern of natural 2-hydroxybenzyl alcohol. *J. Agric. Food Chem.* **2004**, *52*, 7747−7751.

(20) Schmidt, H.-L.; Werner, R. A.; Eisenreich, W. Systematics of ^{2}H patterns in natural compounds and its importance for the elucidation of biosynthetic pathways. *Phytochem. Rev.* **2003**, *2*, 61−85.

(21) Aleu, J.; Fronza, G.; Fuganti, C.; Serra, S.; Fauhl, C.; Guillou, C.; Reniero, F. Differentiation of natural and synthetic phenylacetic acids by ^{2}H NMR of the derived benzoic acids. *Eur. Food Res. Technol.* **2002**, *214*, 63−66.

(22) Hagedorn, M. L. Differentiation of natural and synthetic benzaldehydes by ^{2}H nuclear magnetic resonance. *J. Agric. Food Chem.* **1992**, *40*, 634−637.

(23) Brenna, E.; Fronza, G.; Fuganti, C.; Pinciroli, M. Differentiation of natural and synthetic phenylalanine and tyrosine through natural abundance ^{2}H nuclear magnetic resonance. *J. Agric. Food Chem.* **2003**, *51*, 4866−4872.

Received for review February 18, 2005. Revised manuscript received May 3, 2005. Accepted May 3, 2005. The contribution of the Scientific Council of the Region of the Pays-de-la-Loire to the purchase of a 500 MHz spectrometer is gratefully acknowledged.

JF050385A