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Consistency of NMR and Mass Spectrometry Determinations of Natural-Abundance Site-Specific Carbon Isotope Ratios. The Case of Glycerol

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Quantitative determinations of natural-abundance carbon isotope ratios by nuclear magnetic resonance (SNIF-NMR) have been optimized by appropriate selection of the experimental conditions and by signal analysis based on a dedicated algorithm. To check the consistency of the isotopic values obtained by NMR and mass spectrometry (IRMS) the same glycerol samples have been investigated by both techniques. To have access to site-specific isotope ratios by IRMS, the products have been degraded and transformed into two derivatives, one of which contains carbons 1 and 3 and the other carbon 2 of glycerol. The sensitivity of the isotopic parameters determined by IRMS to fractionation effects possibly occurring in the course of the chemical transformations has been investigated, and the repeatability and reproducibility of both analytical chains have been estimated. The good agreement observed between the two series of isotopic results supports the reliability of the two different approaches. SNIF-NMR is therefore a very attractive tool for routine determination, in a single nondestructive experiment, of the carbon isotope distribution in glycerol, and the method can be applied to other compounds. Using this method, the isotopic distributions have been compared for glycerol samples, obtained from plant or animal oils, extracted from fermented media, or prepared by chemical synthesis. Typical behaviors are characterized.

The investigation of site-specific natural-isotope fractionation by nuclear magnetic resonance (SNIF-NMR)[1] has now been widely exploited in different areas such as the determination of kinetic or thermodynamic isotope effects,[2] the study of biochemical pathways,[3] and the origin inference of various materials.[2] In this respect, deuterium NMR was shown to be well suited to the quantification of monodeuterated isotopomers at natural abundance, and the isotope ratios, $(D/H)_i$, of molecular sites or clusters of sites *i* are often accessible with levels of precision compatible with the natural dispersion. Although carbon-13 is about 70 times more abundant than deuterium, the NMR determination of carbon isotope ratios is less favorable due, in particular, to relatively large relaxation times and to perturbations introduced by imperfect broad-band decoupling, possibly associated with incomplete suppression of nuclear Overhauser effects. Taking into account the relatively small range of variation of the carbon isotope ratios at natural abundance (currently less than 40‰ as compared to more than 500‰ for ^2H), very high levels of experimental accuracy are required. It was shown in 1991 that reliable values could be obtained in favorable cases,[4] but significant progress is expected from improvements in the technical performance of the spectrometers and from the development of dedicated algorithms for signal analysis.[5]

Isotope ratio mass spectrometry (IRMS) is recognized as a sensitive and precise method of determination of natural-abundance carbon isotope ratios. However, since the product is combusted prior to the determination, only an overall molecular value is measured on the resulting CO_2. In practice, site-specific natural-isotope ratios, $(^{13}C/^{12}C)_i$, are accessible by IRMS but suitable degradation reactions of the product are required in order to isolate the different molecular positions.[6−8] These transformations must be carried out under conditions devoid of spurious isotope effects or under conditions where appropriate corrections can be properly estimated. This IRMS strategy has been used in the case of glycerol, and large deviations with respect to a statistical distribution of carbon-13 among the three carbon sites have been measured.[9] A recent SNIF-NMR investigation of $(^{13}C/^{12}C)_i$ ratios of glycerol samples from various origins has determined trends in the isotopic distribution that are in good agreement with the independent IRMS observations.[10] Consequently, it may be expected that the NMR technique has reached

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a level of quantitative performance which makes it a convenient tool for determining the carbon isotopic fingerprint at a level of precision sufficient to distinguish samples from different origins.

To check the reliability of the SNIF-NMR and IRMS results corresponding to a given product, we have developed analytical chains that are suitable for the accurate determination, by both methods, of site-specific carbon isotope ratios. The example of glycerol has been chosen since it is easily degraded, in view of the IRMS measurements, into derivatives representing carbon positions 1 and 3, on one hand, and 2, on the other hand. Moreover, this compound is a major constituent of food and beverages and a great variety of different metabolic origins can be examined. We have therefore compared the isotopic distributions measured by the two methods in a series of samples from different origins and characterized by different relative ^{13}C contents. From a practical point of view, the SNIF-NMR approach is obviously attractive for routine authentication of a product since the site-specific parameters are obtained in a single nondestructive experiment.

EXPERIMENTAL SECTION

Analytical Methods. Carbon isotopic distribution can be characterized by several types of parameters. The isotopic abundance at a specific position i of a molecule G, A_i^G, is defined as the ratio of the number of ^{13}C atoms to the total number of ^{12}C and ^{13}C atoms at site i

$$A_i^G = \frac{[^{13}C]_i^G}{[^{12}C]_i^G + [^{13}C]_i^G} \quad (1)$$

At natural abundance, the isotopic abundance slightly differs from the isotope ratio, R_i^G, which is equal to the ratio of the numbers of ^{13}C and ^{12}C atoms at position i

$$R_i^G = \frac{[^{13}C]_i^G}{[^{12}C]_i^G} = \left(\frac{^{13}C}{^{12}C}\right)_i^G = \frac{A_i^G}{1 - A_i^G} \quad (2)$$

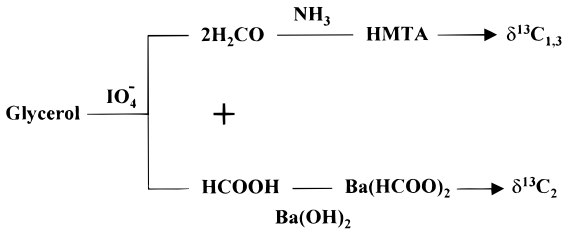
In the relative δ scale commonly used in IRMS, the isotope ratios are referred to that of the international reference, V.PDB (Vienna Pee Dee Belemnite):[11]

$$\delta^{13}C_i^G\ (‰) = \frac{R_i^G - R_{PDB}}{R_{PDB}} \times 1000 = \left[\frac{A_i^G(1 - A_{PDB})}{A_{PDB}(1 - A_i^G)} - 1\right] \times 1000 \quad (3)$$

The absolute isotopic abundance of V.PDB is $A_{PDB} = 1.111\,233 \times 10^{-2}$. This value is practically equal to that of the standard pioneered by Craig.[12] In practice, secondary working standards calibrated with respect to V.PDB have been used.

Isotope Ratio Mass Spectrometry (*IRMS*). The overall carbon-13 content of glycerol, $\delta^{13}C^G$, was measured on carbon dioxide resulting from previous combustion of the sample. To determine site-specific parameters, chemical degradation of the starting material must be carried out. Glycerol was first transformed by periodic oxidation into formaldehyde and formic acid (Scheme 1). These molecules contain respectively carbons 1 and 3 and carbon 2 of the starting glycerol. Formaldehyde is then transformed into hexamethylenetetramine on which $\delta^{13}C_{1,3}$ is measured. The isotope ratio of site 2 of glycerol, $\delta^{13}C_2$, is determined on barium formate derived from the formic acid (Scheme 1). In principle, the measured parameters faithfully represent the initial isotopic contents of sites 1, 3 and 2 of glycerol only under conditions of complete transformation or in the absence of isotopic fractionation introduced by the chemical reactions.

Scheme 1



Nuclear Magnetic Resonance (*SNIF-NMR*). The site-specific isotopic abundance can be measured by referring the area, S_i^G, of signal i of compound G to the signal area of a working standard, the isotopic abundance of which has been carefully calibrated.

Since our aim was mainly to compare the isotope contents in sites 1, 3 and 2, we have calculated the specific abundances, A_i^G, from the molar fractions, f_i^G, of the ^{13}C isotopomers monolabeled at positions 1, 3 and 2 and from the overall isotopic abundance of glycerol, A^G:

$$A_i^G = \frac{f_i^G}{F_i^G} A^G \quad (4)$$

F_i^G are the statistical molar fractions which would characterize a random distribution of ^{13}C ($F_{1,3}^G = {}^2/_3$ and $F_2^G = {}^1/_3$). This equation assumes, as further discussed below, that the relative isotopic abundances of the bilabeled species are the same as those of the monolabeled ones. The actual molar fractions f_i^G were determined by ^{13}C NMR from the area of the glycerol signals

$$f_i^G = \frac{S_i^G}{\sum_i S_i^G} \quad (5)$$

The overall isotopic abundance A^G was measured by conventional IRMS.

Materials. The glycerol samples derived from lipids of plant or animal origin have been prepared as described in ref 10. Fermentation glycerol has been extracted from wine. Commercial samples were purchased from different companies. Synthetic glycerol industrially produced from chloropropylene was kindly provided by L'Oreal-France.

Chemical Degradation of Glycerol. Glycerol (0.3 g) was added to a mixture composed of 7 mL of water, 3.2 mL of an H_3PO_4

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solution (10%), 1.7 g of periodic acid, and 1 mL of methanol. After 30 min of reaction at room temperature under stirring, $Ba(OH)_2$ was added and the pH was adjusted to 7. The mixture was then filtered, and about 10 mL of an NH_3 solution (35%) was added to the residual filtrate. After 30 min under stirring, the solvent was removed by rotary evaporation under vacuum. The residue was extracted with 50 mL of chloroform, and the liquid phase was recovered by filtration. Chloroform was removed from the filtrate by rotary evaporation under vacuum. The hexamethylenetetramine residue was obtained with a yield of about 50%. The solid part contained both barium formate and barium hydroxide.

To check the influence of the yield, a modified procedure leading to a yield of 70% was also carried out. No methanol was added at the beginning of the preparation, and before extraction with chloroform, a second portion of NH_3 solution was added and evaporated to dryness by rotatory evaporation under vacuum.

IRMS Measurements. The carbon isotope parameters of glycerol, HMTA, and barium formate were measured on a Finnigan Delta E spectrometer coupled with a Carlo Erba NA 1500 elemental analyzer. The precision of the determinations is usually better than 0.3%.[13]

Carbon SNIF-NMR Measurements. The NMR spectra were recorded on a Bruker DRX-500 spectrometer equipped with a 5 mm $^1H/^{13}C$ dual probe. The experimental conditions were usually the following: recording frequency, 125.76 MHz; frequency window, 8000 Hz; memory size, 32K; pulse sequence, inverse gated decoupling; 90° pulse width, 4.5 μs; acquisition time, 2 s; delay time, 20 s; scan number, 128−600; decoupling sequence, Waltz-16; pulse composite pulse decoupling, 135 μs; temperature, 308 K; exponential multiplication corresponding to a line broadening of 1 Hz.

As a result, in particular, of differences in the purity of the samples, some minor adjustments of the experimental conditions were required for every sample. The quantitative performance is especially sensitive to the decoupling parameters (power and offset) and to temperature stability. Instabilities resulting from filtering and field drifts are eliminated with modern spectrometers. For most samples, several determinations were made at different times. In each experiment, 5−20 spectra were recorded. The signal-to-noise ratio ranged from 2600 to more than 4500. The signal was analyzed by means of a dedicated program based on a complex least-squares treatment which rigorously integrates all phases and baseline parameters.[5] Being fully automated, this curve-fitting program avoids perturbations due to the operator (Eurospec program from Eurofins Scientific, Nantes, France).

The glycerol sample mixed with D_2O (1/2 v/v) was contained in a 5 mm o.d. tube, and one or two drops of HCl (2 N) were added in order to accelerate exchange of the hydroxyl groups. No paramagnetic reagent was added. The relaxation times measured under these conditions were $T_1 = 0.9$ s for carbons 1, 3 and $T_1 = 1.9$ s for carbon 2.

RESULTS AND DISCUSSION

In a first step, mass spectrometry and NMR analytical chains were defined. Appropriate protocols for preparation of the samples were selected, and spectrometric experimental conditions were optimized (Experimental Section). A comparison of the isotopic distributions determined by the two different approaches could then be carried out.

Table 1. Repeatability of IRMS Determinations of Site-Specific Isotope Parameters, $\delta^{13}C_i$ (‰), of Glycerol[a]

glycerol sample	expt[b]	$\delta^{13}C^G_{exp}$ [c]	yield of HMTA (%)	$\delta^{13}C_{1,3}$-(HMTA)	$\delta^{13}C_2$[Ba-(OOCH)$_2$]	$\delta^{13}C^G_{calc}$ [c]
S_1, synthetic	1	−30.1	53	−31.1	−26.5	−29.6
	2		63	−31.5	−25.8	−29.6
	3		23	−32.6	−27.2	−30.8
	4		48	−31.7	−26.7	−30.0
	5		70	−32.7	−26.7[d]	−30.7
	mean			−31.9	−26.6	−30.1
	(SD)			(0.7)	(0.5)	(0.6)
S_2, corn oil	1	−15.7	61	−23.7	−5	−17.4
	2		46	−22.5	−4.3	−16.4
	3		65	−23.3	*e*	
	mean			−23.2	−4.7	−16.9
	(SD)			(0.6)	(0.5)	(0.7)

[a] Two glycerol samples were investigated; sample S_1 was prepared by chemical synthesis, and sample S_2 was obtained from corn oil. [b] Treatment of the whole analytical chain, including chemical degradation, preparation of the sample, and IRMS measurements, was repeated five times for S_1 and three times for S_2 at different periods of time. The mean and standard deviations (SD) were estimated. [c] $\delta^{13}C^G_{exp}$ and $\delta^{13}C^G_{calc}$ are the overall isotopic deviations of the glycerol sample measured by IRMS and calculated from the site-specific parameters (eqs 3 and 6), respectively. [d] Measured on ammonium formate. [e] Not determined.

Repeatability of the Isotopic Determinations. The performance of the isotopic determinations was investigated on two samples of glycerol from different origins in terms of repeatability defined by the International Standard Organization, ISO 5725.[14] One sample was produced by chemical synthesis, and the second was obtained from corn oil. The experimental conditions finally retained are described in the Experimental Section. Several series of experiments were performed on these samples, which were subsequently used as references for controlling the stability of the instrumental conditions.

Mass Spectrometry Determination of Site-Specific Isotope Ratios (*IRMS*). Chemical degradation of glycerol by periodic acid enables carbon sites 1, 3 to be found in formaldehyde, whereas carbon site 2 is found in formic acid (Scheme 1). The isotopic deviation, $\delta^{13}C_{1,3}$, is then measured on hexamethylenetetramine derived from formaldehyde, and $\delta^{13}C_2$ is determined on barium formate obtained from formic acid. The internal repeatability of the IRMS measurement on a given sample is very good.[13] However, due to kinetic isotope effects possibly intervening in the course of the reaction pathway, the repeatability of the whole analytical chain is likely to be degraded. Five different treatments of the synthetic sample, conducted according to the protocol described in the Experimental Section but characterized by different yields in HMTA, were performed. Similarly, the sample obtained from corn oil was subjected to three different degradations at different periods of time (Table 1). The purity of HMTA was checked by ^1H NMR. It should be noted that the yield of barium formate is not easy to determine, since this product is mixed with excess

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(14) ISO Norm No. 5725, International Standard Organization, Geneva, 1985.

barium hydroxide. Whereas the combustion of HMTA in the elemental analyzer is fast and complete, that of barium formate is often imperfect in the presence of too high a concentration of $Ba(OH)_2$. This difficulty may be the source of some inaccuracy in the $\delta^{13}C_2$ value. In a test experiment (experiment 5 in Table 1), barium formate was transformed through reaction with ammonium sulfate into ammonium formate, which is more efficiently combusted.

No regular variation of $\delta^{13}C_{1,3}$ and $\delta^{13}C_2$ as a function of the yield is detected, and the standard deviation remains below 0.7‰. It is therefore concluded that the analytical chain leads to satisfactory performance in terms of repeatability r ($S(r) = 0.6$‰ and $r = 1.7$‰). This r value, calculated for means which range between −4.7‰ and −31.9‰, does not depend on the level of the test. The reliability of the site-specific determination is checked by a comparison of the overall ^{13}C content, $\delta^{13}C^{G}_{exp}$, measured on the starting glycerol with the mean value, $\delta^{13}C^{G}_{calc}$, calculated from the individual parameters (eqs 2, 3, and 6).

$$A^{G}_{calc} = {}^{2}/_{3}A_{1,3} + {}^{1}/_{3}A_{2} \qquad (6)$$

The agreement is reasonable (Table 1), but some differences may be higher than 1.5‰. Since $\delta^{13}C_2$ is expected to be more affected than $\delta^{13}C_{1,3}$ by spurious isotope effects, the NMR results will also be correlated in the following with parameters, $\delta^{13}C_{2(calc)}$, calculated from the experimental values $\delta^{13}C^{G}_{exp}$ and $\delta^{13}C_{1,3}$ (HMTA) by a balance equation of type 6.

SNIF-NMR Determination of the Isotopic Distribution. Due to symmetry degeneracy, only two signals are observed in the ^{13}C NMR spectrum of glycerol. These signals are accompanied by several satellite lines which are assigned to modulations associated with the rotation of the NMR tube and to the one-bond coupling constant, $^{1}J_{^{13}C-^{13}C}$, intervening in the bilabeled isotopomers present at a relative concentration of about 10^{-4} at natural abundance. Only the isotopomeric sidebands are observed in the absence of rotation of the sample tube (Figure 1A). The spectrum of the molecules labeled at positions 1−2 and 2−3 has been isolated in the 1D INADEQUATE spectrum of glycerol (Figure 1B). The measured value of $^{1}J_{^{13}C-^{13}C}$ is 41.1 Hz, and the carbon isotope effects on the methylene and methine carbon chemical shifts are respectively equal to about 0.01 and 0.005 ppm toward higher shielding. Because of their low signal-to-noise ratios, these satellite signals would not be quantified with an accuracy sufficient to detect possible differences in the natural abundances of the bilabeled isotopomers. Moreover, on a relative basis, taking these differences into account would introduce correcting contributions negligible with respect to the precision of the measurements. In a first step, all satellite lines were therefore excluded from the integrals by the curve-fitting treatment. However, it was then necessary to correct the measured signal area, $S_{1,3}$, of the methylenic carbons for the presence of a single line pertaining to the magnetically equivalent carbons of the isotopomers bilabeled at positions 1 and 3. The intensity of this signal has been given the statistical percentage (≃1.1%) with respect to the main $C_{1,3}$ signal. After correction of the experimental $S_{1,3}$ value for this contribution the "true" area, $S^{corr}_{1,3}$, corresponding to the monolabeled isotopomers was obtained.

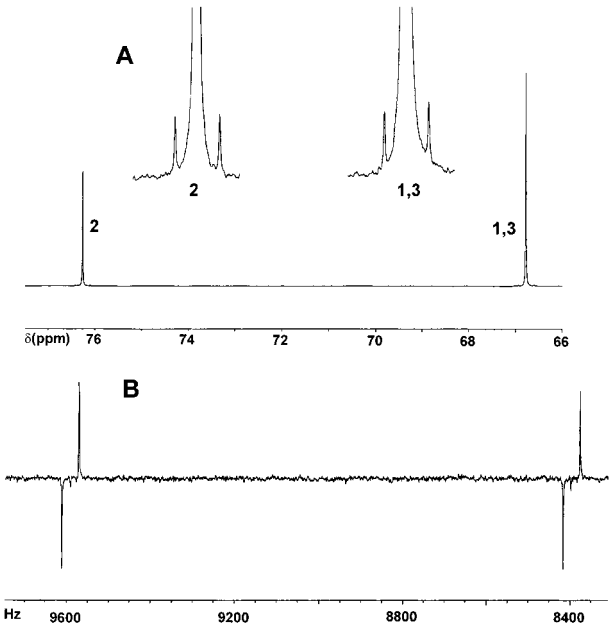

Figure 1. ^{13}C NMR spectra of a sample of synthetic glycerol. Part A shows the conventional ^{13}C spectrum run at 125.8 MHz in the absence of rotation of the sample tube. The expanded spectra have been amplified 500 times. Part B shows the 1D INADEQUATE spectrum of the same sample.

Table 2. Repeatability of NMR Determinations of Site-Specific Isotope Parameters of Glycerol[a]

glycerol sample	expt[b]	no. of spectra[c]	$R_{1,3}$[d] mean value	$R_{1,3}$[d] SD[c]	molar fractions $f_{1,3}$	molar fractions f_2
S_1, synthetic	1	5	1.9871	0.0020	0.6652	0.3348
	2	8	1.9904	0.0014	0.6656	0.3344
	3	8	1.9910	0.0009	0.6657	0.3343
	4	6	1.9908	0.0022	0.6656	0.3344
	5	6	1.9878	0.0007	0.6653	0.3347
	6	6	1.9873	0.0010	0.6652	0.3348
	7	5	1.9876	0.0006	0.6653	0.3347
	8	10	1.9840	0.002	0.6649	0.3351
	mean		1.9881		0.6653	0.3347
S_2, corn oil	1	20	1.9547	0.0020	0.6616	0.3384
	2	6	1.9568	0.0007	0.6618	0.3382
	3	15	1.9596	0.0010	0.6621	0.3379
	4	6	1.9554	0.0022	0.6616	0.3384
	mean		1.9566		0.6618	0.3382

[a] The same glycerol samples, S_1 and S_2, as in the IRMS experiments (Table 1) were investigated. [b] Seven (S_1) and four (S_2) experiments were performed on the same sample at different periods of time. [c] In each experiment, 5−20 spectra were registered successively; SD is the standard deviation on these repetitions. [d] The relative parameter $R_{1,3}$ is equal to the ratio of the signal areas $S_{1,3}/S_2$ corrected for the presence of the bilabeled isotopomers.

The two glycerol samples S_1 and S_2 studied by IRMS (Table 1) were considered as reference compounds for defining the acquisition parameters (Experimental Section). These parameters have been adjusted in order to optimize the repeatability of the determinations. Seven and four series of experiments were carried out on the synthetic and corn oil samples, respectively. For each experiment, the data were averaged over $n = 5-20$ series of accumulated spectra (Table 2). The molar fractions $f_{1,3}$ and f_2 of the isotopomers naturally labeled at position 1 or 3 and position 2, respectively, have been directly computed from the signal areas

Table 3. Carbon Isotope Site-Specific Parameters Determined on Samples of Glycerol from Different Origins by Mass Spectrometry (IRMS) and Nuclear Magentic Resonance (SNIF-NMR)

		NMR results[c]							IRMS results[d]			
			$R_{1,3}$		$\delta^{13}C_{1,3}$		$\delta^{13}C_2$			$\delta^{13}C_{1,3}$	$\delta^{13}C_2$	
glycerol sample[a]	expt[b]	n	mean	SD	mean	SD	mean	SD	$\delta^{13}C^{G}_{exp}$	HMTA	formate	calc
synthetic[e]	8	54	1.9881	0.0024	−32.0	0.4	−26.2	0.8	−30.1	−31.9	−26.6	−26.5
corn oil A[f]	4	47	1.9566	0.0022	−23.0	0.4	−1.1	0.7	−15.7	−23.2	−4.7	−0.7
corn oil B	1	20	1.9484	0.0032	−24.0	0.5	2.1	1.1	−15.3	−23.4	−4.6	0.9
	2	6	1.9504	0.0027	−23.7	0.5	1.4	0.9	−15.3	−23.4	−4.6	0.9
red wine	1	15	1.9762	0.0020	−35.0	0.3	−23.3	0.7	−31.1	−34.2	−24.0	−24.9
olive oil	1	6	1.9521	0.0039	−38.2	0.7	−14.3	1.3	−30.2	−36.1	−19.1	−18.4
	2	24	1.9577	0.0031	−37.2	0.5	−16.2	1.0	−30.2	−36.1	−19.1	−18.4
	3	18	1.9603	0.0036	−36.8	0.6	−17.0	1.2	−30.2	−36.1	−19.1	−18.4
fish oil	1	5	1.9637	0.0019	−30.0	0.3	−11.9	0.6	−24.0	−29.2	−15.2	−13.6
	2	20	1.9623	0.0061	−30.3	1.0	−11.5	2.1	−24.0	−29.2	−15.2	−13.6
chicken A[g]	1	5	1.9651	0.0029	−18.1	0.5	−0.5	1.0	−12.2	−18.8	−2.6	1.0
	2	20	1.9641	0.0038	−18.2	0.6	−0.1	1.3	−12.2	−18.8	−2.6	1.0
chicken B[h]	1	10	1.9578	0.0020	−31.8	0.3	−10.7	0.7	−24.8	−31.1	−14.9	−12.2
	2	10	1.9602	0.0044	−31.4	0.7	−11.5	1.5	−24.8	−31.1	−14.9	−12.2
	3	6	1.9558	0.0034	−32.2	0.6	−10.0	1.2	−24.8	−31.1	−14.9	−12.2
sunflower oil A	1	5	1.9549	0.0022	−38.1	0.4	−15.6	0.7	−30.6	−36.9	−18.7	−18.0
	2	19	1.9579	0.0033	−37.6	0.6	−16.6	1.1	−30.6	−36.9	−18.7	−18.0
sunflower oil B	1	20	1.9602	0.0040	−35.8	0.7	−16.0	1.3	−29.2	−35.7	−17.1	−16.2
	2	6	1.9564	0.0037	−36.4	0.6	−14.7	1.2	−29.2	−35.7	−17.1	−16.2
commercial A	1	20	1.9834	0.0020	−29.3	0.3	−21.1	0.7	−26.6	−29.2	−23.2	−21.4
commercial B	1	20	1.9533	0.0001	−34.6	0.0	−11.2	0.0	−26.8	−34.8	−19.0	−10.8
commercial C	1	20	1.9664	0.0020	−31.4	0.3	−14.7	0.7	−25.8	−31.2	−19.0	−15.0
mixture A[i]	1	16	1.9654	0.0016	−26.2	0.3	−8.9	0.5	−20.4	−26.1	−8.2	−9.0
mixture B[j]	1	16	1.9802	0.0016	−29.2	0.3	−19.4	0.5	−25.9	−29.6	−19.3	−18.5

[a] A, B, C denote samples of a given species obtained from different pools of raw materials. [b] Several experiments (1, 2, or 3) were carried out on the same sample at different periods of time. [c] n is the number of spectra repeated for each sample and used to calculate the mean values and standard deviations (SD) of the $R_{1,3}$ ratio and $\delta^{13}C$(‰) parameters. [d] $\delta^{13}C^{G}_{exp}$ (‰) is the overall deviation of the glycerol sample. The site-specific deviations $\delta^{13}C_i$ (‰) were measured on HMTA and on formate derived from the starting glycerol; $\delta^{13}C_2$ (‰) was also calculated from $\delta^{13}C^{G}_{exp}$ and $\delta^{13}C_{1,3}$(HMTA) (eq 8). [e] Sample S_1 of Tables 1 and 2. [f] Sample S_2 of Tables 1 and 2. [g] Chicken fed with wheat. [h] Chicken fed with maize. [i] 0.996 g of corn oil glycerol + 0.446 g of synthetic glycerol. [j] 0.603 g of corn oil glycerol + 1.425 g of synthetic glycerol.

$S^{corr}_{1,3}$ and S_2 (eq 5). The relative parameter $R_{1,3} = S^{corr}_{1,3}/S_2$ represents the number of ^{13}C atoms at positions 1, 3 in a situation where position 2 is arbitrarily given the probability factor 1. In both cases, this ratio is different from the value 2 which would correspond to a statistical distribution of ^{13}C. The standard deviation on $R_{1,3}$ associated with a given experiment involving a consecutive series of n spectra is usually lower than 0.003. The standard deviation of repeatability is $S(r) = 0.0015$ and the repeatability is $r = 0.0043$. This performance, which is on the order of 0.2% in relative value, is comparable to that of the IRMS determinations.

The extension of the concept of reproducibility[14] to the data sets of Table 2 by considering that the experiments have been carried out by different operators on different spectrometers during a large period of time permits a standard deviation of reproducibility $S(R) = 0.0030$ and a reproducibility $R = 0.0085$ to be computed.

When transformed into δ (‰) units values by applying eqs 2−4 using the overall $\delta^{13}C$ values −30.1‰ and −15.7‰, respectively (Table 1), the NMR parameters lead to mean values $\delta^{13}C_{1,3} = -32.0$‰, $\delta^{13}C_2 = -26.2$‰ for the synthetic glycerol and $\delta^{13}C_{1,3} = -23.0$‰, $\delta^{13}C_2 = -1.1$‰ for the corn oil sample. The very good agreement with the IRMS values given in Table 1 is a strong argument in favor of the reliability of the determinations carried out by both methods.

Correlation between SNIF-NMR and IRMS Determinations. Fifteen glycerol samples from different origins have been investigated in parallel by SNIF-NMR and IRMS. To check the stability of the NMR performance, the two reference samples characterized above were integrated in every series of experiments conducted at a given period of time. Any change in the data obtained for the reference samples led to the invalidation of the whole series of experiments and to appropriate adjustment of the experimental conditions (Experimental Section).

The overall ^{13}C contents and the values of the site-specific parameters $\delta^{13}C_{1,3}$ and $\delta^{13}C_2$ measured by IRMS on hexamethylenetetramine and on formate are given in Table 3. The corresponding isotopic values directly determined on glycerol by SNIF-NMR are also given. To further check the reproducibility of the NMR determinations, two or three series of experiments were carried out on certain samples. These results, detailed in Table 3, confirm the reproducibility performance estimated above.

As illustrated in Figure 2, a good agreement is observed between the isotopic distributions determined by SNIF-NMR and IRMS. The isotopic deviations are linearly correlated by an equation of type 7.

$$\delta^{13}C_i(\text{NMR}) = a + b\delta^{13}C_i(\text{IRMS}) \qquad (7)$$

for $i = 1, 3$: $a = 1.7$ (0.6); $b = 1.07$ (0.02);

$$S(\delta^{13}C_{NMR}) = 0.5,\ F = 2978$$

for $i = 2$: $a = 4.3$ (1.0); $b = 1.08$ (0.06);

$$S(\delta^{13}C_{NMR}) = 2.0,\ F = 304$$

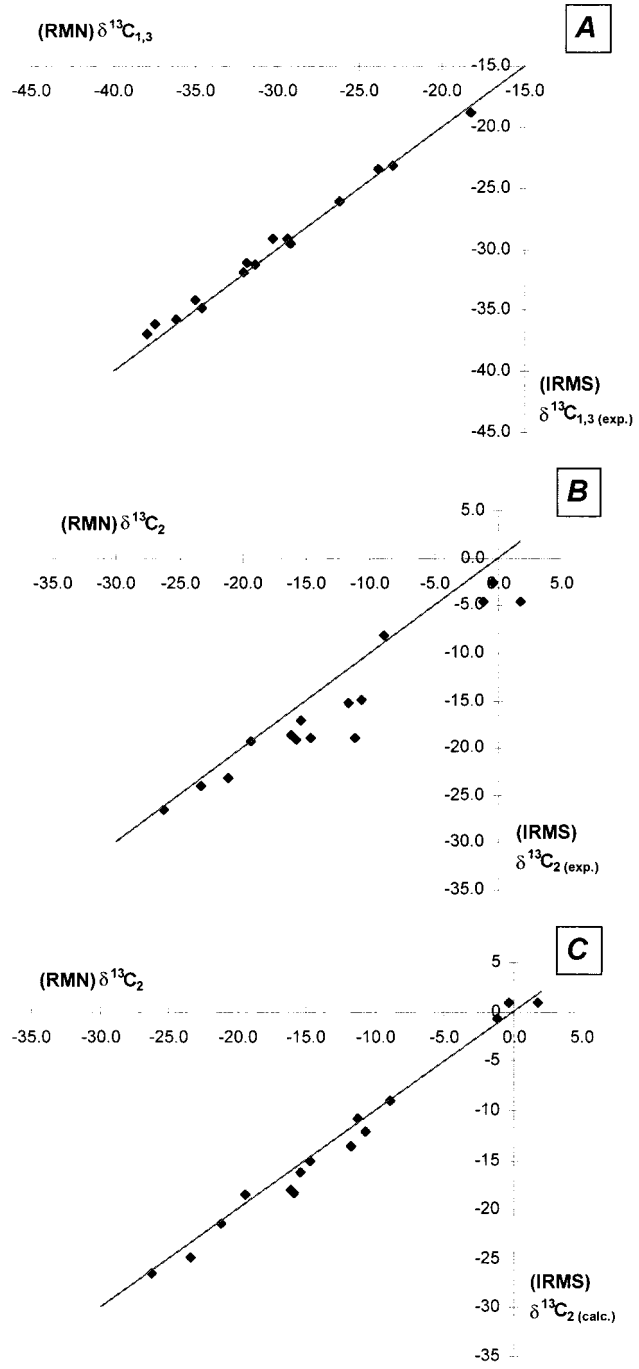

Figure 2. Correlation between the site-specific deviations of glycerol samples from different origins determined by IRMS and SNIF-NMR for carbons 1, 3 (A) and carbon 2 (B, C).

The lowest quality of the correlation for site 2 may be partly due to spurious fractionation effects intervening in the IRMS experiments carried out on barium formate. To check this hypothesis, the $\delta^{13}C_2$ deviation was also estimated by means of eqs 2, 3, and 8.

$$A_{2(\mathrm{calc})} = 3A^{G}_{\mathrm{exp}} - 2A_{1,3}(\mathrm{HMTA}) \qquad (8)$$

An improvement in the correlation (eq 7) is achieved by using these calculated $\delta^{13}C_{2(\mathrm{calc})}$ values instead of the experimental values measured on formate:

a = not significant; b = 0.931 (0.015);

$$S(\delta^{13}C_{\mathrm{NMR}}) = 1.2,\ F = 987$$

These results further illustrate the discriminating potential of the carbon isotopic contents of glycerol.[9,10,15,16] Whereas the ^{13}C distribution is nearly random for the synthetic sample, significant ^{13}C impoverishments of carbons 1, 3 with respect to carbon 2 are observed in all other samples. The isotopic values measured for the two mixtures composed of corn oil glycerol and synthetic glycerol (Table 3) verify the expected weighted averaging of the isotopic abundances.

CONCLUSION

It has been demonstrated in the case of glycerol that analytical chains adapted to the determination of site-specific parameters by IRMS and by SNIF-NMR give very consistent results. IRMS is recognized as a sensitive and precise method, but in order to provide site-specific information, it requires degradation of the product into suitable derivatives. If incomplete, these transformations introduce the risk of inaccuracies resulting from fractionation effects. It was shown that such perturbations are small in the case of the degradation of glycerol and that the reproducibility is satisfactory. However, dedicated chemical and analytical chains must be established and checked for every kind of molecule. In contrast, carbon NMR gives direct and simultaneous access to all site-specific parameters of the product but suffers from the reputation of being poorly sensitive and poorly accurate. We have shown, in the case of glycerol, that reproducible results can be obtained by resorting to modern NMR spectrometers, careful selection of the experimental conditions, and appropriate signal analysis. By addition to the sample of a known quantity of a previously calibrated standard, the site-specific isotope ratios can be determined solely by the NMR technique. However, this method requires the selection of a standard appropriate to the considered product in terms of chemical shift, relaxation time, volatility, etc. and both monolabeled and bilabeled isotopomers must be taken into account. Consequently, it may be more convenient in many cases to estimate the site-specific deviations from the molar fractions obtained by NMR spectroscopy and the overall ^{13}C contents measured by IRMS. The proposed analytical strategy provides a fast and simple tool which can be very helpful for authentication purposes.

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