231. Food-Related Applications of High-Resolution NMR

Part II[1])

Differentiation between Natural and Synthetic Vanillin Samples Using ^{2}H-NMR

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Summary

Differences in the distribution of deuterium at individual atomic sites in vanillin samples are revealed by ^{2}H-NMR measurements, and can be used to determine whether the sample is of natural or synthetic origin.

Distinguishing between compounds of natural origin and their so-called 'nature-identical' equivalents prepared by chemical synthesis is difficult. In fact, by traditional chemical and physical methods, it is impossible. In recent years, isotope mass spectrometry has given hope of a solution through its capacity to measure the total ^{2}H- or ^{13}C-content of a sample, and this has been demonstrated in certain cases, including vanillin [2]. However, using specific ^{2}H- or ^{13}C-labelled reagents, it is now easy to synthesise compounds which emulate the natural isotopic content, and alternative methods are required to detect fraudulent practice and misrepresentation.

The proportion of ^{2}H in plants varies according to the latitude at which the plant is cultivated, diminishing as a function of the distance from the equator [3]; it also varies according to the species of plant [4]. The ^{2}H content of natural products isolated from these plants also varies accordingly, not only in total, but more important, as a function of the different hydrogen-substituted atomic sites within the molecule.

Martin et al. [5] have recently proposed the use of natural-abundance ^{2}H-NMR to measure the ^{2}H content at individual atomic sites. They reported that in the case of anethole, samples of natural and synthetic origins could be clearly distinguished one from the other.

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We present here the results of a study undertaken on vanillin samples, three synthetic products and three isolated from vanilla beans, which supports the contention that ^{2}H-NMR can be used as a diagnostic tool to investigate origins.

Results and Discussion. – *Fig. 1* shows the ^{2}H-NMR spectrum of a natural vanilla sample, namely sample 4 of the *Table* where a description of the characteristics of the six vanillins analysed is presented, together with their ^{13}C-contents as measured by isotope mass spectrometry. The probability of finding a single ^{2}H-atom in a given vanillin molecule is approximately 1:700, and as such, the probability of finding a di-deuterated molecule is essentially negligible. The five peaks in the spectrum of *Fig. 1* thus correspond to six separate monodeuterated structures, **I–VI**, with **II** and **III** giving a single unresolved peak. Only the peaks corresponding to structures **I–V** are used to compare samples, because the signal from the deuteroxy group of **VI** shows wide variations from sample to sample. As with hydroxy signals in ^{1}H-NMR spectra, this variability probably results from intermolecular solute-solute and solute-solvent interactions and from exchange phenomena which result in line broadening and chemical shift displacements. This signal may still be of diagnostic value, but many more samples would need to be analysed to test this.

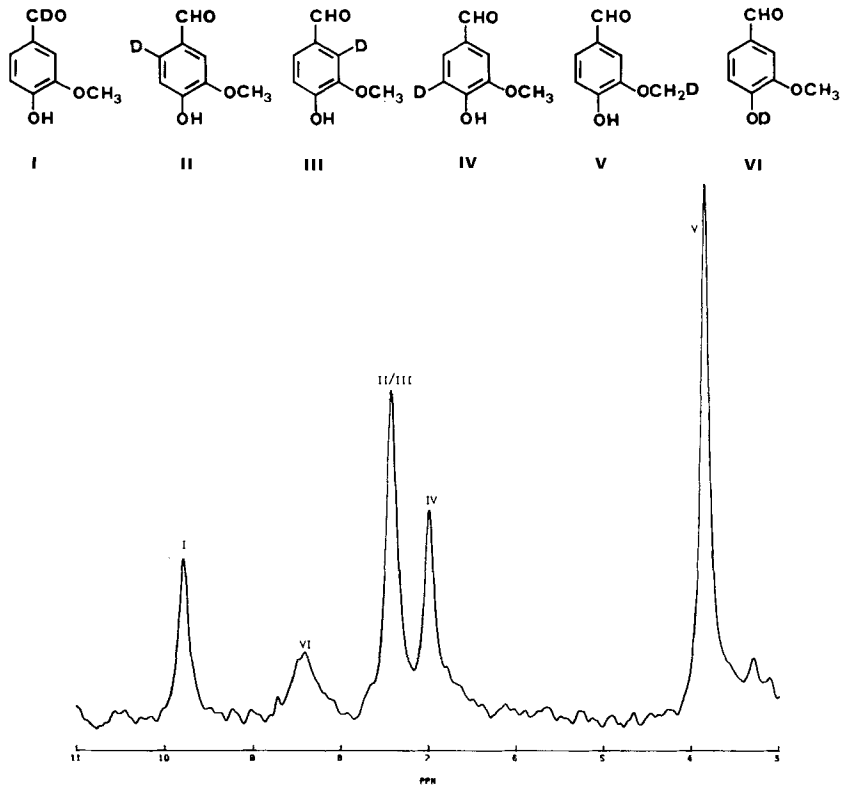

Fig. 1. *The natural-abundance ^{2}H-NMR spectrum of an authentic natural vanillin* (sample 4) (35% w/v solution in acetone, recorded at 38.4 MHz)

Table. *Vanillin Samples Analysed, Indicating Origins*

Sample	Origin	Prepared from:	$\delta^{13}C$ [‰ PDB][a])
1	synthetic	lignin[b])	−27.1
2	synthetic	guaiacol[b])	−31.0
3	synthetic	[c])	−27.2
4	natural	Madagascar vanilla beans – 1980 crop	−20.6
5	natural	Madagascar vanilla beans – 1981 crop	−21.0
6	natural	commercial vanilla beans, unspecified origin or year	?

[a]) ^{13}C-deficiency relative to PDB standard (*Belemite*, S. Carolina) as measured by isotope mass spectrometry.
[b]) Starting materials commonly used in the synthesis of vanillin.
[c]) Purchased from *Fluka*, Buchs (CH).

If all the different ^2H-positions in **I–V** were uniformally substituted, the four corresponding peaks of *Fig. 1* would have relative areas of 1:2:1:3. The differences from these statistical proportions observed in reality reflect $^1H/^2H$ isotope discrimination which differs from site to site in the different samples according to the mode of formation. The relative amounts of **I–V** present in each sample can be measured from spectra such as *Fig. 1* using either peak areas or peak intensities, and although these alternative forms of measurement do not indicate the same distribution of **I–V** in a given sample, the differences are consistent from sample to sample, and as such, both methods appear to have the same diagnostic value in defining sample origins. Because they are easier to measure directly from the spectra recorded, we have chosen here to express relative quantities of **I–V** in terms of peak intensities.

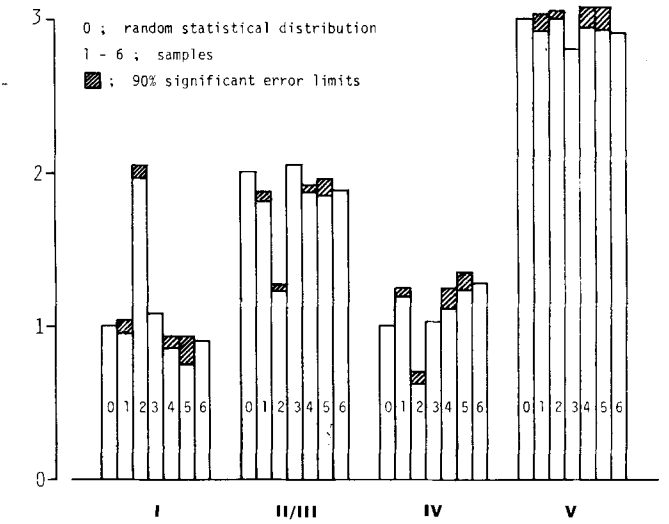

Fig. 2. *The distributions of the different monodeuterated vanillins* **I–V** *in the samples analysed, compared with the distribution expected if all H-sites in vanillin were uniformally deuterated* (90% significant limits were calculated by multiplying the standard deviation in peak heights by the appropriate *Student*'s t coefficient for three degrees of freedom, namely 2.35)

The distributions of **I–V** thus measured in the samples of the *Table* are shown in histogram form in *Fig. 2,* compared to the statistical distribution of 1:2:1:3. In the natural samples, 4–6, the ^{2}H-content of **I** is depleted by 10–15%, and of **IV** is enriched by 20–25% relative to a uniform distribution, whereas **II/III** shows a slight depletion. Only **V** appears to exhibit the statistical proportion of 3 ^{2}H-atoms for the same number of equivalent atomic sites. Samples 4 and 5 on which standard deviations in the relative proportions of **I–V** were measured, show consistent distributions with differences lying within the 90% significant error limits shown in *Fig. 2.* This is to be expected since both samples come from the same latitude and the same plants, differing only in the year in which they were harvested. Looking at the synthetic samples 1–3, it is immediately obvious that sample 2, prepared by introducing an aldehyde group into guaiacol, clearly distinguishes itself from all the other samples whether synthetic or natural. Sample 1, synthesised from lignin, shows a significantly higher amount of **I** and a lower amount of **II/III** than the natural samples, suggesting that it also can be recognised through its ^{2}H-distribution, even if the differences are much less apparent than for sample 2. Samples 3 and 6 were of non-certified origins, but were claimed to be synthetic and natural respectively. Their deuterium distribution maps in *Fig. 2* evidently confirm this, with sample 3 also showing a distribution much different to that of the authentic natural samples.

We conclude that ^{2}H-NMR can be used as a probe to diagnose the origins of vanillin, in particular through the amount of ^{2}H found in the aldehyde group which is augmented in synthetic vanillin relative to its authentic natural counterpart. To generalise the method, several other samples must be studied to establish the limits of variation of ^{2}H in natural samples, and thus to define norms of authenticity. We are presently undertaking such a study involving about 100 samples of diverse origins, both natural and synthetic, where we will also use a higher-resolution NMR spectrometer at 360 or 400 MHz, in addition to the 250-MHz instrument on which the present results were obtained. We will thus achieve complete separation of the two peaks corresponding to **II/III** and **IV** in the aromatic region of the spectrum, which will allow improved definition of the relative populations of **I–V**. This, coupled with total ^{2}H-measurements, is expected to permit the unambiguous classification of samples.

Experimental. – Approximately 1 g of each sample was dissolved in 2 ml acetone, this solvent being chosen because it gives a single ^{2}H-absorption signal at δ 2.08 which does not interfere with the vanillin spectrum. ^{1}H-decoupled spectra were recorded on a *Bruker AM 250* superconducting NMR spectrometer at 38.417 MHz using aquisition parameters already described [5], namely, as the sum of 2500 transients, with a 90° pulse (22.5 μs), and an aquisition time of 6.8 s. For samples 1, 2, 4 and 5, four separate spectra were recorded to permit estimation of statistical error limits in peak intensities. For samples 3 and 6, peak intensities were determined from a single spectrum representing the sum of 11,000 scans.

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