

Natural Factors of Isotope Fractionation and the Characterization of Wines

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For characterization of the origin of a wine, the NMR determination of site-specific natural isotope fractionation (SNIF-NMR) provides a new approach. Relative and absolute values of the deuterium/hydrogen ratios of the methyl and methylene sites of the ethanol and of the water extracted from wine, along with computed values of the deuterium content of must water, enable the influence of several wine properties to be analyzed. Effects due to variations in the sugar content of the must are quantified. It is shown that differences due to vine variety can be sorted out in specific conditions of production area and vintage. Factorial discriminant analysis enables geographical origin to be characterized, even down to well-defined areas such as certain French districts. Since the mechanistic effects presiding over the isotopic distribution can be interpreted in physicochemical and biochemical terms, the method constitutes a unique tool for characterizing the origin of wines on a phenomenological basis.

Identification of the geographical origin, of the year of production, and of the vine variety is of great interest for wine consumers and producers since it may provide determinant criteria for guaranteeing quality. Numerous investigations, mainly founded on gas or liquid chromatography (Rapp and G ntert, 1985; Rapp et al., 1977, 1985; Noble, 1980; Liddle and Bossard, 1985; Williams, 1985), have been devoted to the problem of variety characterization. Sensory analysis is also widely used for the same purpose (O'Mahony, 1986), and relations between organoleptic properties of wines and characteristics of the soil have been defined (Asselin et al., 1983). In certain cases the data from such analytical determinations have been the subject of multidimensional analyses in order to classify the wines within specific categories (Kwan et al., 1979; Symonds and Cantagrel, 1982; Scarponi et al., 1982; Medina and Van Zeller, 1984; Williams, 1985; Millery et al., 1986). Although these analytical techniques have been very fruitful for wine characterization, they usually remain of a rather empirical nature due to the difficulty in finding explanatory relationships between the observable and the fundamental concepts of the physical chemistry or biochemistry.

The techniques of *enrichment* by stable or radioactive isotopes have been frequently used for investigating the mechanisms of photosynthesis (Kunesch and Poupat, 1977), and they have been applied to the case of the vine (McWeeny and Bates, 1980; Martin et al., 1981; McCallum et al., 1986). However, a better understanding of the influence of the environmental factors on the biosynthesis of grape in natural conditions can essentially be afforded by the study of *natural* tracers. In this respect mass spectrometry techniques are generally used for determining overall hydrogen, oxygen, and carbon isotope ratios at the natural-abundance level. In fact, in the case of vine products most of these investigations are concerned with the measurement of the D/H and $^{18}\text{O}/^{16}\text{O}$ isotope ratios of *water* contained either in the wine or in the must before

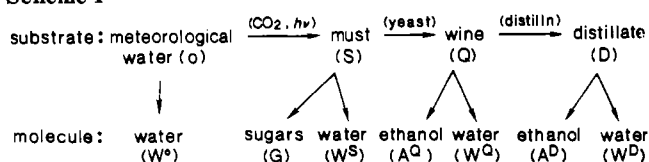
fermentation. The influences of the vine variety, of the harvesting date, of the production technique, and of the year of vintage were discussed by Dunbar (1982a-d) for wines from New Zealand, and, in the same perspective, F rstel (1984, 1985, 1986) considered wines from Germany and from some other countries. Such investigations succeeded in establishing a link between the end product, wine, and the production area. However, since a number of natural factors contribute to isotope fractionation in the course of the history of wine, the isotope parameters of water alone are usually unable to provide unequivocal answers. Additional information was obtained by Bricout (1978) and Bricout et al. (1975) who investigated the total isotope ratios of the main *organic molecules* involved in the production of wine, i.e. sugars and ethanol, and, more recently, Dunbar and Schmidt (1983, 1984) measured the total deuterium content of nitrates of glucose samples extracted from grape musts with a view to detect a sweetening by sucrose.

We have shown that *deuterium* is far from being randomly distributed within organic molecules (Martin and Martin, 1981a,b), and we have developed a new method based on quantitative deuterium NMR for the determination of *site-specific natural isotope fractionation* (SNIF-NMR) (Martin et al., 1981, 1983, 1985, 1986). A procedure for detecting and measuring the enrichment of wines (Martin and Martin, 1983) has been defined on this basis. Moreover, a theoretical analysis of the isotope redistribution factors intervening in the course of a fermentation process was shown to be the source of *mechanistic information* on the transfer of the hydrogen isotopes from glucose and must water to the ethanol and wine water (Martin et al., 1986). In principle the constant character of the isotope redistribution matrix, in the usual conditions of wine production, enables the specific isotope contents of a given wine to become a fingerprint for recognition of its origin and for the elucidation of the role of environmental factors.

In this respect, it can be demonstrated that the site-specific hydrogen isotope parameters may act as probes of climatic significance. This behavior enables plants growing in countries situated at different latitudes in the world and therefore subjected to very different temperatures and precipitations to be distinguished. The purpose of this paper is to examine the influence of genotypic and physiological properties associated to the vine variety. In addition, since natural wines of different alcoholic grade must be compared, we are led to consider possible effects

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Scheme I



of the concentration of sugar in the must. Once these effects are fully appraised, it becomes possible to specify the discriminating power of site-specific isotope parameters in the distinction of wine-producing regions either on a world scale or within a given country such as France which has a variety of climatic features.

MATERIALS AND METHODS

Isotopic Parameters. The isotope filiation leading from the hydrogen atoms of meteorological water to those of ethanol and water of the wine has already been discussed (Martin et al., 1983b, 1986b), and only the different steps and the corresponding notations will be briefly recalled here (Scheme I).

In the SNIF-NMR method only the three mono-deuteriated isotopomers of ethanol have to be considered: CH₂DCH₂OH (I), CH₃CHDOH (II), CH₃CH₂OD (III). The isotope ratio associated with a given molecular site will be specified by a superscript defining the substrate and a subscript defining either the molecule or the specific site. Thus, (D/H)_W^o and (D/H)_I represent the isotope ratios of must water and of the methyl site of wine ethanol, respectively.

The isotope ratio of a given site *i* (D/H)_{*i*} is expressed (ppm) on the international scale V.SMOW (Gonfiantini, 1978). The relationship between (D/H)_{*i*} and the relative parameter δD_{*i*} (‰) is given in eq 1. In fact, due to the

$$\delta D_i (\text{‰}) = 1000[(D/H)_i - (D/H)_{V.SMOW}] / (D/H)_{V.SMOW} \quad (1)$$

chemical exchange between the hydroxylic hydrogens, no independent information is borne by the isotope ratios of wine water and of site III of ethanol since these parameters are related by the thermodynamic constant, *K_e*, of the equilibrium (2).

$$(D/H)_{III}^Q = K_e (D/H)_W^Q \quad (2)$$

In principle, by taking eq 2 into account, the overall deuterium content of ethanol, $(\overline{D/H})^A$, which is accessible independently by mass spectroscopy, can be calculated from the NMR site-specific parameters in eq 3. Rea-

$$(\overline{D/H})^A = \frac{1}{2}(D/H)_I + \frac{1}{3}(D/H)_{II} + \frac{1}{6}K_e(D/H)_W^Q \quad (3)$$

sonable agreement is found between the results of mass spectrometry and the SNIF-NMR method. However, a systematic difference of a few ppm is observed, the reasons for which are probably related to sample preparation (desiccation and combustion) and referencing and are presently under investigation.

Besides the absolute parameters, D/H, a relative parameter, *R*, derived simply from intensity measurements of the methylene (*S_{II}*) and methyl (*S_I*) signals of ethanol has been defined (Martin and Martin 1981) (eq 4). This

$$R = 3S_{II}/S_I = 2(D/H)_{II}/(D/H)_I \quad (4)$$

parameter offers a direct appraisal of the deviation with respect to the statistical value *R* = 2 that would characterize the methylene site, the methyl site being arbitrarily given the probability factor 3.

A natural wine can therefore be characterized by several specific isotope parameters that are more or less closely

Table I. Results of the Variance Analysis of the Data Considered in Figure 1

parameter	(D/H) _I	(D/H) _{II}	<i>R</i>	(D/H) _W ^o
a				
size <i>n</i>	43	43	43	43
moments				
1 (\bar{x})	101.2	131.9	2.60 ₈	160.8
2 (<i>S</i> ²)	0.8	3.8	0.001 ₄	1.8
3	-0.2	-4.5	-0.2 × 10 ⁻⁴	-5.4
4	1.7	47.4	0.6 × 10 ⁻⁵	35.0
asymmetry	-0.2	-0.6	-0.4	-2.2
flatness	2.6	3.3	3.2	10.7
b				
size <i>n</i>	78	78	78	78
moments				
1 (\bar{x})	101.5	131.3	2.58 ₈	160.6
2 (<i>S</i> ²)	0.54	2.78	0.001 ₅	0.535
3	-0.27	0.33	-0.1 × 10 ⁻⁵	0.33
4	1.19	18.9	0.4 × 10 ⁻⁵	1.28
asymmetry	-0.67	0.07	-0.02	0.85
flatness	4.05	2.45	2.15	4.45

connected with the geography and the meteorology of the production area. Since we have only considered natural wines issued from *Vitis vinifera*, a plant with a C3 metabolism, the total ¹³C/¹²C ratios are not expected to exhibit significant variations and they have not been measured.

Samples. Most French wines were obtained in the laboratory by fermenting, in standard conditions and without enrichment (no chaptalization), grapes harvested at a good state of maturation in the main vineyards. Several INRA stations and research centers and several university laboratories have also contributed to collecting precisely known samples. The other wines were prepared in the oenology institutes of countries that are members of the OIV or obtained from laboratories or firms cooperating in the program.

Isotopic Analyses. The site-specific isotope ratios have been measured by the SNIF-NMR method following the procedure already described (Martin et al., 1983). The internal referencing technique used in order to obtain absolute values of the isotope ratios, the technical aspects, and the reproducibility of the NMR data have already been discussed (Martin et al., 1985). The value $(\overline{D/H})$ retained for the working standard, tetramethylurea, is 135.0 ppm. This value will be checked in the course of a circular analysis program so that a systematic adjustment of the present values will eventually be necessary. The isotopic results are collected in a data base administered by an interactive software, ISOLOG (Martin et al., 1987), which can also be used as a diagnosis tool via multidimensional analysis packages.

Statistical Analyses of Data. For all populations of D/H ratios associated with a given factor, either geographical or varietal, the mean values, standard deviation, *S*, and the confidence intervals corresponding to a probability of 95% have been computed. For *n* samples, the confidence interval is calculated for a Student's distribution (*t*) by the formula $tS/n^{1/2}$. The normal character of the distribution of the isotopic variables has been checked for each type of factor by resorting to the histogram representation and to the calculation of the flattening and dissymmetry parameters (Figure 1; Table I).

In order to obtain a meaningful comparison of the mean values associated with different observations of a given parameter (region, variety), a one- or two-factor variance analysis of the data has been performed (Bethea et al., 1975). The *F* values have been calculated by the usual formula (5) in order to appraise the level of significance

$$F = MS_B/MS_E \quad (5)$$

of the differences in the estimation of the mean values,

Table II. Relationships between the Difference in Deuterium Content of Must and Wine Waters and the Alcoholic Grade of the Wine^a (%)

origin		no. of samples	slope ^b	mean of (D/H) _W ^Q ^c	correln coeff ^b
geographical area	year				
Languedoc (Hérault)	1982	12	0.60	155.1	0.81 (0.66)
Pays de la Loire (Maine et Loire)	1983-1984	14	0.53	154.9	0.87 (0.62)
Alsace (Haut-Rhin)	1983	7	0.47	154.8	0.88 (0.80)
all regions	1982-1984	33	0.53	155.0	0.74 (0.44)

^aDifferent vine varieties are considered: Cabernet Sauvignon, Cabernet Franc, Carignan, Ugni Blanc, Syrah, and Cinsault. ^bThe slope of the correlation is given in ppm/degree of ethanol in the wine. The linear correlation is calculated for a null forced intercept, and the critical values of the correlation coefficient are given within parentheses for a level of significance $\alpha = 0.01$. ^cIn ppm.

Table III. Relationships between the Deuterium Content of Wine Water and the Alcoholic Grade for Natural Wines Originating from Different Production Areas and from Several Vine Varieties^d

origin		no. of samples	slope ^a	intercept ^b	correln coeff ^c
geographical area	year				
Provence-Côte d'Azur (Bouches du Rhône)	1982-1983	10	0.63	154.0	0.88 (0.71)
Languedoc (Hérault)	1982-1983	14	0.54	155.5	0.92 (0.62)
Aquitaine (Gironde)	1982-1983	17	0.48	153.5	0.85 (0.58)
Alsace (Haut-Rhin)	1983-1984	12	0.46	154.8	0.82 (0.66)

^aIn ppm/degree. ^bThe intercept of the regression line corresponds in principle to the isotope ratio of the precursor must water. ^cThe critical values of the correlation coefficient are given for a level of significance $\alpha = 0.01$. ^dThe same vine varieties, but not the same samples, as those presented in Table II, were considered here.

Table IV. Isotopic Parameters of the Components of Wines Issued from Different Vine Varieties^a

		<i>n</i> ^b	(D/H) _I ^c	(D/H) _{II} ^c	<i>R</i> ^f	(D/H) ^Q ^e	(D/H) _W ^Q ^d
all countries	Carignan	53	101.9 (0.4)	131.1 (0.5)	2.57 (0.01)	122.1 (0.35)	160.4 (0.35)
	Cabernet Sauvignon	60	101.6 (0.35)	131.5 (0.6)	2.59 (0.01)	122.0 (0.4)	160.4 (0.45)
	Cinsault	18	102.3 (0.6)	130.0 (1.3)	2.54 (0.03)	121.9 (0.5)	160.4 (0.5)
	Grenache Noir	15	101.9 (0.7)	129.9 (1.5)	2.56 (0.03)	121.6 (0.6)	160.2 (1.3)
	Carignan	20	101.7 (0.25)	130.9 (0.7)	2.57 (0.02)	121.8 (0.4)	160.2 (0.3)
France (Hérault)	Cabernet Sauvignon	24	101.0 (0.4)	132.5 (0.8)	2.62 (0.02)	122.2 (0.04)	160.8 (0.4)
	New Zealand	6	102.3 (1.1)	129.7 (1.6)	2.53 (0.03)	121.4 (0.8)	158.4 (0.3)

^aThe confidence intervals computed for a probability of 95% are given within parentheses. ^bNumber of samples. ^c(D/H)_I and (D/H)_{II} are the site-specific isotope ratios of ethanol. ^d(D/H)_W^Q is the isotope ratio of water. ^e(D/H)^Q is the overall isotope ratio of ethanol. ^f*R* is the internal isotope parameter of ethanol (eq 4).

where MS_B and MS_E are the mean squares resulting from the variation between the variables (B) and within the variables (error E). The calculated values of this parameter have been compared to the critical value for a certainty of 99% extracted from the Tables of G. W. Snedecor and W. G. Cochran quoted by O'Mahony (1986).

The confidence intervals for the significant differences between the mean values of the different observations have been computed from the Fischer LSD test (least significant difference) (O'Mahony, 1986).

Factorial discriminant analyses were performed on-line with ISOLOG or off-line with statistical packages developed for Digital E. C. computers (PRO 350 or micro VAX) according to the methods described by Green (1976).

RESULTS AND DISCUSSION

Influence of the Sugar Content of the Must. As we have shown from model experiments (Martin et al., 1986), changes in the nature and in the concentration of the sugars present in the must induce small but significant variations in the isotope values of the fermentation medium. In the case of nonadulterated wines, an influence of the alcoholic grade, t^Q , on the hydrogen isotope ratio of water extracted from the wine, (D/H)_W^Q, is therefore expected. In fact, the deuterium atoms found in wine water may originate either directly from must water and from the exchangeable hydroxylic sites of sugar or indirectly from the nonexchangeable sites of glucose and fructose that end up in the fermentation water. Providing that wines from a given geographical origin are considered, we may therefore check the applicability of a simple linear relationship between the deuterium content of wine water

and both the deuterium content of must water and the alcoholic grade of the wine (eq 6).

$$(D/H)_W^Q = (D/H)_W^S + kt^Q \quad (6)$$

Thus, three series of at least seven musts from several vine varieties originating from three different regions of production have been fermented in standardized conditions. Having at our disposal the isotope ratios of the must wine couples and the values of the corresponding alcoholic grade, we could demonstrate (Table II) that the experimental results satisfactorily fit simple linear regressions with a forced null intercept (eq 7). The slope of the

$$\Delta(D/H)_W^Q = k_0 t^Q \quad (7)$$

correlation has a mean value of 0.5 ppm/% and indicates a slight increase in the deuterium content of wine water with the enrichment in sugar of the must, provided the fermentation is complete. No specific influence of the vine variety has been detected.

Considering now four different series of at least 10 natural wines for which we could only measure the (D/H)_W^Q and t^Q values, we also verify that linear correlations of type 6 are satisfied (Table III). The slopes of the regression lines are still close to 0.5 ppm/%, and the intercepts provide average values of the isotope ratio of must in reasonable agreement with the determinations performed on model systems (Table II).

Influence of Vine Variety. We have considered several hundreds of natural wines issued from different vine varieties from different years and produced in different countries in the world. The results given in Table IV

Table V. Influence of the Vine Variety on the Isotope Parameters of the Methylene Site of Ethanol and of the Water from Wines Produced in Different French Regions

France (region)		variety			
		Cabernet Sauvignon	Carignan	Syrah	Ugni Blanc
Languedoc (Hérault, Gard; Aude, Pyrénées Orientales)	n^c	42	35	5	10
	$(D/H)_{II}$	132.7	131.0	129.4	130.8
	$(D/H)_W^Q$	161.3	160.3	160.5	160.0
Aquitaine (Gironde)	n	6	3		3 ^a
	$(D/H)_{II}$	131.4	129.6		127.0
	$(D/H)_W^Q$	161.1	160.4		160.5
Pays de la Loire (Maine et Loire)	n	3 ^b	3	3	3
	$(D/H)_{II}$	128.8	129.3	127.8	127.3
	$(D/H)_W^Q$	159.4	157.7	158.8	157.7
Alsace (Haut-Rhin)	n	2		2	2
	$(D/H)_{II}$	127.5		127.3	124.2
	$(D/H)_W^Q$	157.2		157.0	156.4

^aIt is interesting to note that 44 wines from the Ugni Blanc variety grown in the same region but in the district Charentes give the same $(D/H)_{II}$ value. ^b20 samples of Cabernet Franc lead to the following values: $(D/H)_{II} = 127.7$ and $(D/H)_W^Q = 158.4$. ^c n is the number of samples.

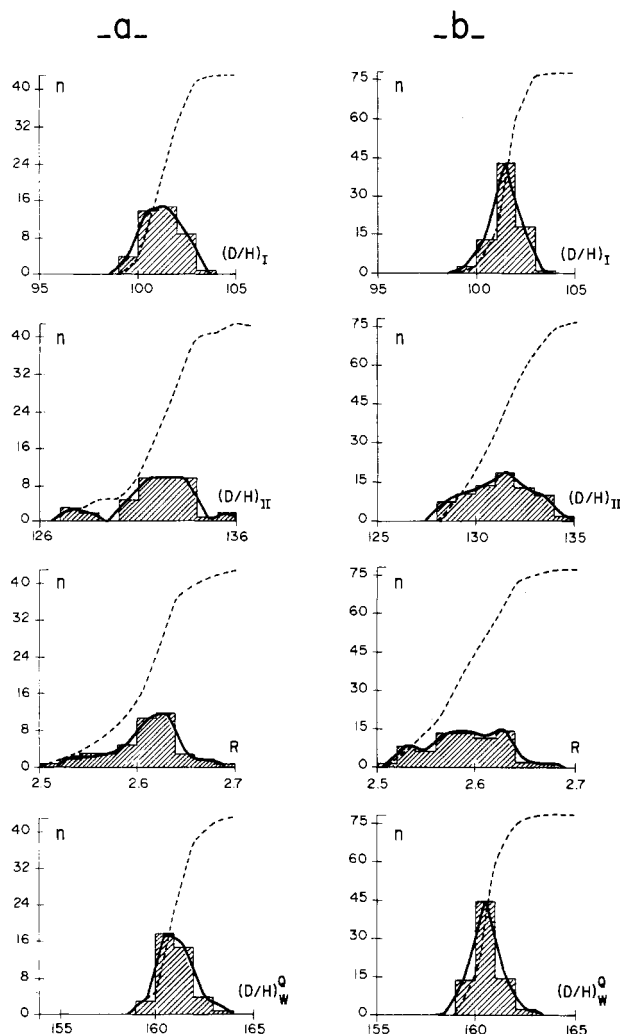


Figure 1. Distribution of isotope ratios associated with the methyl (I) and methylene (II) sites of ethanol and with the water (W^Q) extracted from wine. The ratio, R , defined in eq 4 characterizes the deuterium content in the methylene site, the methyl site being arbitrarily given the statistical value 3. The histograms (a) describe the behavior of Cabernet Sauvignon wines produced in various French districts, and part (b) corresponds to different varieties of vine grown in the district of Herault (in Languedoc). The solid lines represent the envelope of the distribution, and the dashed lines are the cumulative distribution functions of the samples considered.

enable isotopic values representing means of over 15 to more than 50 samples of the four varieties Carignan, Ca-

bernet Sauvignon, Cinsault, and Grenache to be compared. Only small differences are observed, and it can be concluded that averaging over a large number of geographical areas with different climates virtually masks any influence of variety. Even for a country such as France, the two species Cabernet Sauvignon and Carignan for example are barely differentiated since the experimental values of the differences between their isotopic parameters are not higher than the least significant differences (LSD) as shown by the following results:

	$(D/H)_I$, ppm	$(D/H)_{II}$, ppm	$(D/H)_W^Q$, ppm	R
LSD	0.6	1.2	0.8	0.025
exptl diff	0.3	1.2	0.6	0.035

The phenotypic characters of the plant therefore play only a secondary role in the fractionation phenomena as compared to the geographical effects. However, when isotopic values of different varieties grown in a defined geographical area are considered, systematic trends are detected (Table IV). Thus, whatever the producing district, a significant deuterium enrichment of the methylene site of ethanol, a slight depletion of the methyl site of ethanol, and an increase in the R parameter are exhibited by the Cabernet Sauvignon variety as compared to the Carignan variety. More generally, when the isotopic behaviors of the four varieties Cabernet Sauvignon, Carignan, Syrah, and Ugni Blanc, grown in four different regions, are compared, a similar behavior is observed. The former variety Cabernet Sauvignon is characterized by higher values of the $(D/H)_{II}$ and $(D/H)_W^Q$ parameters than the latter one, Ugni Blanc (Table V). Even when the whole population of French wines is considered, the Cabernet Sauvignon and Ugni Blanc are distinguished since the value of the experimental difference between their $(D/H)_{II}$ values, which reaches 4.3 ppm, is noticeably higher than the least significant difference, which is only 1.1 ppm.

Such behavior is probably governed, at least partly, by indirect climatic effects induced by differences in the period of ripening, itself controlled by physiological factors. Thus, it can be considered that, in a given geographical situation subjected to relatively reproducible climatic conditions, Cabernet Sauvignon stores water during a warmer period or for a longer time than Ugni Blanc does. In this respect, it can also be noted that, in identical environmental conditions, nonmatured grapes lead to slightly higher deuterium contents (0.3–0.8 ppm) in the methyl isotopomers of wine ethanol than matured grapes, a situation that probably results from deuterium enrichment in the nonexchangeable sites of early sugars.

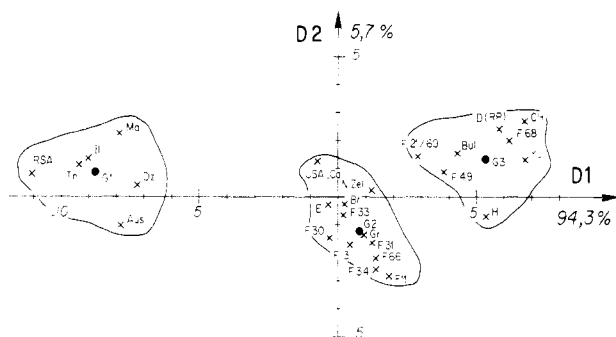


Figure 2. Representation of the discriminant factor analysis classifying three groups of countries whose meteorological data correspond to the following types of climate (long-term monthly average): G1, cold and humid ($T < 11.5$ °C, $P > 60$ mm); G2, cold and dry or hot and humid; G3, hot and dry ($T > 16.5$ °C, $P < 45$ mm). A variance analysis was carried out for each of the different isotopic variables, $(D/H)_I$, $(D/H)_{II}$, $(D/H)_W$, and $(D/H)_W^S$, in order to check the alternate hypothesis of the non-equality of means when different countries are considered. The computed values of the Fischer function ($F = 15-35$) were always larger than the values found in the tables for a 99% confidence level ($F = 2.2-2.4$). Then, the ranges of least significant differences between the means of two different countries were estimated to 0.8/1.5 ppm for $(D/H)_I$ and to 1.2/3.0 ppm for the other isotope ratios. The countries are defined as follows: Aus, Australia; Bu, Bulgaria; CH, Switzerland; Dz, Algeria; F, France; H, Hungary; Ma, Morocco; RSA, Republic of South Africa; Br, Brazil; Ca, California; D, Germany; E, Spain; Gr, Greece; Il, Israel; Nz, New Zealand; Tn, Tunisia; Yu, Yugoslavia. The French wines are referred by using the official number of the district. They originate from the following provinces: F11, Aude (Languedoc); F13, Bouches du Rhône (Provence Côte d'Azur); F21, Côte d'Or (Bourgogne); F30, Gard (Languedoc); F31, Haute-Garonne (Midi-Pyrénées); F33, Gironde (Aquitaine); F34, Hérault (Languedoc); F49, Maine et Loire (Pays de la Loire); F66, Pyrénées Orientales (Midi-Pyrénées); F68, Haut-Rhin (Alsace); F69, Rhône (Rhône-Alpes).

However, from a practical point of view, considering the significant differences exhibited by the isotope parameters of Cabernet Sauvignon wines originating from different countries such as New Zealand and France for example (Table IV), it should be concluded that the geographical factors usually exert a prevailing influence over those of the variety.

Geographical Influence of the Production Area.

The normality of the distribution of the isotopic ratios is illustrated in Figure 1 for samples from a given district but from different vintages and vine varieties on one hand and for samples of a given variety but from different districts and vintages on the other hand. Although the dispersion of the histograms is more pronounced for the $(D/H)_{II}$ and R parameters, the distributions of the isotope ratios obey Gauss-Laplace law and behave quite similarly as shown by the computed values of the different moments (Table I).

In order to check whether the means of the isotopic parameters calculated for the 27 countries or districts under consideration are representative of the production area, an analysis of the variance has been performed (Bethea, 1975). It is shown that the differences between the various means are generally highly significant at the 99% level of confidence since the F -test ranges between 18 and 35 for a theoretical values of 2.2. The multiple comparison of the means, realized by using the Fischer least significant difference test indicates that most of the isotope ratios have a good discriminant power characterized by LSD values of 1-1.5 ppm for $(D/H)_I$ and 1.5-3.5 ppm for $(D/H)_{II}$. Most production areas can therefore be

Table VI. Results from the Analysis of Variance Performed on Wines from 11 Districts of Production in France^a

parameter	$(D/H)_I$	$(D/H)_{II}$	R	$(D/H)_W^S$	
degree of freedom					
total	322	322	322	254 ^c	
between districts	10	10	10	9	
Snedecor test					
F (99%)	2.4	2.4	2.4	2.5	
F (exptl)	4.9	35.1	21.5	26.3	
LSD ranges ^b					
minimum	0.5	0.8	0.02	0.6	
maximum	1.8	3.0	0.07	2.1	
differences, ^d ppm, between means ^e					
F34-F33	EXP	0.7	0.8	0.035	0.9
$n = 85, n = 54$	LSD	0.5	0.8	0.020	0.7
-F68	EXP	0.9	5.4	0.070	3.5
$n = 23$	LSD	0.7	1.1	0.025	0.8
-F16	EXP	0.6	4.0	0.091	<i>f</i>
$n = 56$	LSD	0.5	0.8	0.020	
-F49	EXP	0.2	3.3	0.044	2.6
$n = 36$	LSD	0.6	0.9	0.022	0.7
-F21-69	EXP	0.4	3.1	0.071	4.0
$n = 35$	LSD	1.3	2.2	0.050	1.6

^aSamples from different vintages are considered. ^bThe least significant differences, LSD, are calculated for a 99% significance level. ^cFor technical reasons (the mass spectrometer was not yet available) the determinations of the isotope ratio $(D/H)_W^S$ could not be performed on all samples. ^dExcept for R . ^eThe differences between means are computed for the district Hérault (from the region of Languedoc) with respect to districts of other French regions. The district of Alsace (68), Charentes (16), Anjou (49), and Bourgogne (21-69) are differentiated, but in some cases one of the individual parameters is not discriminating at the selected confidence level. Work is in progress for obtaining analytical parameters from larger numbers of strictly defined samples. ^fThe water extracted from Cognac brandies is not significant for isotopic consideration.

unambiguously distinguished. For example, Swiss and German wines are clearly differentiated from those of Brazil, New Zealand, Greece, and Israel, which in turn also differ from Australian, Tunisian, and South African wines in particular.

This behavior is in fact mainly the result of the climatic significance of the isotope ratios, and the different countries can be tentatively classified in terms of climatic variables. Thus, a discriminant analysis was carried out on three groups of countries associated with the two extreme climates cold-humid, hot-dry, and both cold-dry and hot-humid regions. Figure 2 shows that the three sets of countries are well assigned in their groups of classification. The discriminant power of the two canonical functions computed from four isotopic variables and expressed by the percentages of their eigenvalues is equal to 94% and 6%, respectively. The Bartlett test (70.4) indicates that the first discriminant function (DF1) is highly significant (26.2 for 99%), whereas the second function is much weaker but still significant (Bartlett test 20 for 15.1 at 99%).

In order to investigate the potential of the SNIF-NMR method to characterize a geographical origin, even for a relatively small production area within a given country, we have carried out a variance analysis of wines produced in different French districts. The results given in Table VI show that the value of the Fischer-Snedecor parameter computed on the basis of the experimental values is significantly higher than the test value for a 99% confidence level. Therefore, it may be concluded that there is a definite effect of the production area. Although not all districts are unambiguously distinguished on the basis of these results, which include samples from various varieties and years of production, a clear distinction is possible in

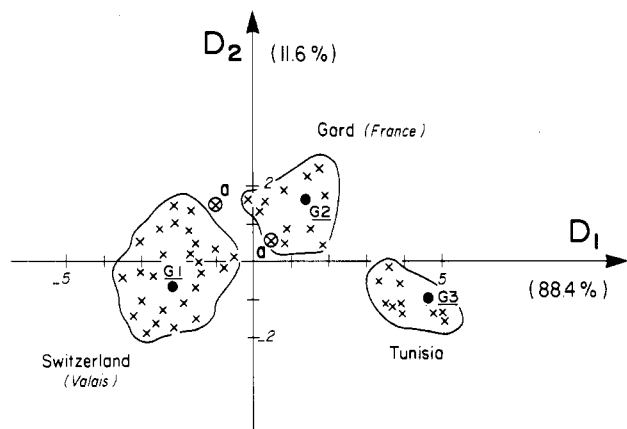


Figure 3. Factorial discriminant analysis of wines produced in the three regions Tunisia, Valais in Switzerland, and Gard in France. The samples correspond to different vine varieties and different years of production. The considered discriminant isotopic variables are $(D/H)_I$, $(D/H)_{II}$, $(D/H)_{III}^Q$, and $(D/H)_{III}^S$. G1, G2, and G3 are the centers of gravity of the three populations. The vine varieties that intervene are Carignan, Grenache, Cinsault (Tunisia), Chasselas, Gamay (Valais) and Ugni Blanc, Cabernet Sauvignon, and Carignan (Gard).

a number of cases. Thus, it is shown in Table VI that the two production districts Herault (Languedoc) and Haut-Rhin (Alsace) are well identified by resorting to the $(D/H)_I$, $(D/H)_{II}$, R , and $(D/H)_{III}^Q$ parameters whereas the distinction between Herault and Gironde (Aquitaine) is based on the three latter parameters.

More generally, it may be concluded that regions subjected to rather different climatic features are clearly differentiated on a statistical basis. In order to further check the applicability of the method to identify a homogeneous wine-producing area, we have performed a discriminant factor analysis on the isotopic data of wines originating from three regions situated within a range of about 8° of latitude and produced during different years. As illustrated in Figure 3, the discrimination is quantitative (100%) between Tunisia and Switzerland (Valais). For the Gard district, whose climatic conditions lie between those of the two other regions, the discrimination is always very significant since it reaches 100% for Tunisia and 93% for Switzerland (2 wines out of 30 are situated closer to the gravity center of Gard than to that of Valais).

For a still more limited geographical area situated within 2° of latitude, a discriminant analysis has been carried out on 50 natural wines produced during the same year, 1984, but corresponding to different vine varieties (Figure 4). The discrimination is also very gratifying since 92% of the wines from Anjou are well assigned to their group and the two other groups, Alsace and Gironde, are 100% well assigned. If we consider, in Anjou, only the wines made from the well-established variety Cabernet Franc, the pattern is much closer to the gravity center of the group and the discrimination is now 100%. The wines corresponding to varieties, which are less well acclimatized in Anjou (Grenache, Pinot, Ugni Blanc) give rise to points that are more scattered on the projection plane.

CONCLUSION

The site-specific isotope fingerprint provides a phenomenological basis, not only for detecting the presence of exogenous sugars (Martin and Martin 1983) but also for investigating the origin of wines. Both geographical and vine variety effects are probably governed by an influence of climatic variables relayed by isotopic responses of the meteorological water and by physiological behavior. Since we have shown that the methylene isotopes of ethanol

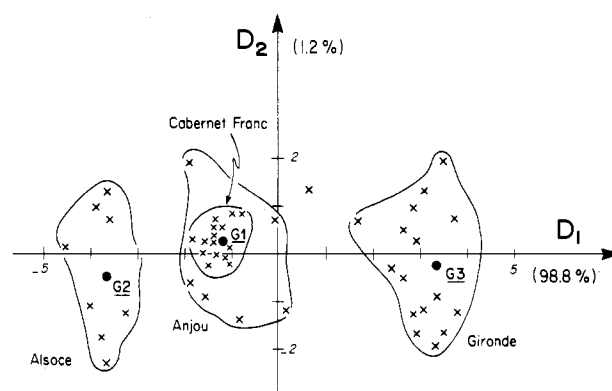


Figure 4. Factorial discriminant analysis of three groups of French wines produced in the regions Alsace, Anjou, and Gironde. The samples correspond to different vine varieties but to the same vintage, 1984. The discriminant variables are $(D/H)_I$, $(D/H)_{II}$, $(D/H)_{III}^Q$, and $(D/H)_{III}^S$. The results corresponding to the species Cabernet Franc cultivated in Anjou are situated within a more restricted area.

originate from the fermentation water and that the methyl site is mainly connected to the nonexchangeable sites of glucose (Martin et al., 1986b), it may be concluded that the isotope ratios constitute complementary witnesses of the physiological and climatological effects intervening all along the photosynthesis of sugars and maturation period of grapes. An effect of the vine variety becomes apparent when results concerning a well-defined production area are considered. From a practical point of view, strategy has been developed for identifying the geographical origin of wine samples. Usually an unambiguous distinction of the main countries is possible through a statistical analysis using the whole populations of samples from different vintages and vine varieties. A "zoom effect" can be realized in order to distinguish regions situated within a limited range of latitudes, even for a country such as France, when the statistical treatment takes into account the vine variety and temporal parameters. The SNIF-NMR method therefore offers to producers, consumers, and official organizations a new tool for the origin labeling of quality wines.

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Gas Chromatographic Analysis of Volatile Components of Ginger Oil (*Zingiber officinale* Roscoe) Extracted with Liquid Carbon Dioxide

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Freeze-dried ginger powder was extracted with liquid carbon dioxide (600-700 psi) for 48 h. Volatile components in the oily extract were fractionated into one hydrocarbon fraction and two oxygenated hydrocarbon fractions by using silica gel column chromatography. Each fraction was analyzed by capillary GC and GC-MS. Out of 168 characterized compounds 90 were identified as far as possible by comparing GC retentions and mass spectral data. There were 58 tentatively identified compounds according to mass spectral data. Effects of liquid carbon dioxide extraction upon the volatile composition of ginger were discussed.

The volatile oil of ginger can be prepared by steam distillation, which is a simple and effective method to obtain essential oils from plants (Lawrence, 1984). However, the high temperature during steam distillation causes considerable problem to the flavor quality. Using supercritical or liquid carbon dioxide as an extractant (Krukonis, 1984; Moyler, 1984) is one of the recent efforts to overcome the thermal effect due to steam distillation.

Volatile ginger oil has been the subject of many research studies (Brooks, 1916; Varma et al., 1962; Nigam et al.,

1964; Connell and Sutherland, 1966; Connell, 1970; Connell and Jordan, 1971; Kami et al., 1972; Bednarczyk and Kramer, 1975; Bednarczyk et al., 1975; Masada, 1976; Sakamura and Hayashi, 1978; Chou et al., 1981; Smith and Robinson, 1981; MacLeod and Pieris, 1984). A recent study by Lawrence (1983) has shown the identification of 114 volatile components; many were reported for the first time in ginger. There were reports showing the profiles of gas chromatographic analyses of ginger oil extracted with supercritical carbon dioxide and solvent (dichloromethane) (Krukonis, 1984) and liquid carbon dioxide (Moyler, 1984). However, there were no specific indications of volatile compounds being identified. Our previous report has shown that, in ginger, not only the pungent principles (primarily gingerol compounds) but also the volatile components could be extracted by liquid carbon dioxide (Chen et al., 1986a).

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