More Clues about Sensory Impact of Sotolon in Some Flor Sherry Wines

Bruno Martin, Patrick X. Etiévant,* Jean Luc Le Quéré, and Pascal Schlich

Laboratoire de Recherches sur les Arômes, Institut National de la Recherche Agronomique (INRA), 17 Rue Sully, 21034 Dijon Cedex, France

The olfactory impact of sotolon [4,5-dimethyl-3-hydroxy-2(5*H*)-furanone] in wines was first demonstrated in botrytized wines. In sherry wines it was recently found to occur at concentrations varying from 0 to 500 ppb. The olfactory detection threshold of a sample of purified racemic sotolon was determined to be lower (15 ppb) in white wine than its concentration in most sherries. Its flavor impact in sherries was calculated to be between 1 and 25 OUV. Using MCA of the data obtained from 22 wines (white wines, rancios, Spanish and French sherries) during two tasting sessions by wine professionals, significant positive correlations were found between the concentration of sotolon and the typicality as well as between the concentration and the persistence in the mouth of a nutty flavor.

INTRODUCTION

4,5-Dimethyl-3-hydroxy-2(5*H*)-furanone is a powerful aromatic compound, commercially known as sotolon. This compound was found as a natural compound in cane molasses, fenugreek seeds, soya sauce, and other protein hydrolysates and also in sake and in certain particular wines (Koyabashi, 1989). In all of these products, sotolon was supposed or proven to be responsible for the burnt, spicy, sweet, walnut, or curry olfactory characteristics. Masuda et al. (1984) demonstrated its contribution to the particular sweet aroma of botrytized wines at concentrations greater than 2.5 ppb. This compound was also found in flor sherry wines (Dubois et al., 1976), and a more recent study showed that its concentration in such wines varied from a few to 500 ppb (Martin, 1989; Martin et al., 1990; Martin and Etiévant, 1991). Since it could not be detected in red wines, white wines (except when made from botrytized grapes), or oxidized wines as rancios, it is considered as characteristic of flor sherry wines (Etiévant, 1991).

Sotolon was found significantly more abundant in the French vin jaune (average 114.8 ppb) than in the Spanish fino sherries (average 41.5 ppb). As already suggested by Dubois et al. (1976), this compound is most probably responsible for some of the characteristic odors of flor sherry wines, but to date, no sensory analyses have been performed to confirm this contribution.

The aim of this study was to obtain sensory information (odor unit values, relation between sotolon presence and sensory tasting of wines) about sotolon's contribution to the flavor of flor sherry wines and more particularly to the flavor of the French vin jaune. This is now possible since a previous quantification of this compound by two-dimensional gas chromatography was published (Martin et al., 1990).

EXPERIMENTAL PROCEDURES

Wine Samples. Foreign wines were purchased in wine shops, and others were provided by wine producers.

Sotolon Synthesis. A stable sample of sotolon was obtained according to the method of Sulser et al. (1972), with the exception that the acidic decarboxylation was changed for a thermal degradation (Martin et al., 1990).

Purification of Sotolon. Synthetic sotolon was purified by HPLC on a Lichrosorb diol column (25 cm × 10 mm i.d., 5 μm, Interchim) using a 85/15 mixture of dichloromethane and pentane (3 mL min^{-1}) as eluent. Collection of purified sotolon in the effluent was made possible by UV detection at 235 nm. The fractions collected from repetitive injections were pooled, and the solvent was evaporated at a temperature of 15 °C using a micro vacuum evaporator (Rotavapor, Buchi). As previously described for solerone (Martin et al., 1991), chromatographic and olfactory purities were estimated by gas chromatography (GC and GC sniffing) on two different bound phases.

Instrumental Analysis. The identity of the purified synthetic compound was confirmed by mass spectrometry, FT-IR/HRGC, ^1H NMR, and ^{13}C NMR. Types of instruments and conditions involved during analyses are given in Table I.

Quantification of Sotolon. The quantity of sotolon was estimated in wines by capillary two-dimensional gas chromatography of solvent wine extracts as reported previously (Martin and Etiévant, 1991).

Sensory Analyses. The determination of the olfactory detection threshold of sotolon was made using the ascending method in a savagnin white wine as previously described (Etiévant et al., 1983), except that each triangle was made of one sample and two references. Purified sotolon was added in the five wine solutions. Concentrations increased from 13 ppb to 333 ppm geometrically (step 4).

The estimation of the importance of sotolon in the typicality and the persistence of the aroma of flor sherry wines was made from the results of two tasting sessions in which, respectively, 10 and 17 assessors (producers or professionals of vin jaune) took part. Only four participants came to both sessions. The samples, described in Table II, were presented randomly in coded black glasses covered with a glass top. Assessors were asked to swallow the wines and to score the typicality and the aldehydic and the maderized characteristics on a scale of 1–5 (1, absence; 2, weak; 3 medium; 4 high; 5, very high intensity). The persistence of the nutty flavor was scored seconds after the sample was swallowed.

Statistical Analyses. Detection threshold was calculated as described by Frijters and Bemelmans (1977). For the persistence of the nutty flavor, the answers of each taster were first converted into a 1–4 score. This was done for each taster separately by considering the maximum score as the upper limit of a continuous scale and by dividing this scale into four equal segments.

The results of the two sessions were processed by multiple correspondence analysis (MCA) using the SPAD statistical package (Lebart and Morineau, 1985).

RESULTS

The mass spectrum (Table III) of synthetic sotolon, identical with that of the natural product, has a base peak at 83 *m/e* arising from the basic unsaturated γ-lactone ring. It displays the parent peak at 128 *m/e* and a fragment ion at 113 *m/e* (6%) corresponding to a loss of a methyl group from the parent. Fragment ions at 31 (10%) and 45 (8%) *m/e* arise from the hydroxyl group. Finally, the mass spectrum is identical to the mass spectrum initially published by Dubois et al. (1976).

0021-8561/92/1440-0475$03.00/0

Table I. Instruments and Operating Conditions Used in the Structural Determination of Sotolon

	GC–MS	GC–FTIR	NMR
instrument	Nermag R10-10C	Bruker IFS85	Bruker WM400
GC	Girdel 31 (Delsi Nermag SA)	Carlo Erba 5160 GC	
column	DB5 (J&W Scientific)	DB5 column	
	60 m × 0.32 mm i.d., 1 μm	30 m × 0.32 mm i.d., 1 μm	
injection mode	splitless	on column	
			^{1}H: 400.13 MHz, $CDCl_3$
operating parameters and modes	electronic ionization, 70 eV	light pipe, 200 °C	^{13}C: 100.62 MHz, D_2O
			5-mm o.d. tube
			ref: $CHCl_3$ 7.26 ppm/TMS for ^{1}H
			ref: TMSP-d_4 for ^{13}C

Table II. Characteristics of Wine Samples Evaluated by Sensory Analysis

sample code	description of the wine	sotolon concn, ppb	group affectation
X1	xeres fino	22	1
X2	xeres manzanilla	22	1
X3	xeres amontillado	0	1
W1, W2	French white savagnin	0	1
W3	oxidized savagnin	15	1
W4	W1 after air bubbling	0	1
W5	W1 plus 150 ppb of sotolon	150	2
W6	W2 after biological ageing	72	2
W7	W1 plus 530 ppm of acetaldehyde	0	2
W8	W7 plus 150 ppb of sotolon	150	2
W9	rancio wine	0	1
J1	French vin jaune	75	2
J2	French vin jaune	ND[a]	
J3	French vin jaune	138	3
J4, J5	French vin jaune	ND	
J6	French vin jaune	123	3
J7	French vin jaune	143	3
J8	French vin jaune	142	3
J9–11	French vin jaune	ND	
J12	French vin jaune	50	2
J13	J12 plus 100 ppb of sotolon	150	3
J14	maderized French vin jaune	25	1
J15	J14 plus 125 ppb of sotolon	150	3

[a] ND, not determined.

Table III. Mass Spectrum, Vapor-Phase Infrared Spectrum, and ^{1}H and ^{13}C NMR Spectra of Synthetic Sotolon

MS (8 prominent ions in dec abundance)	83 (100), 43 (99), 55 (89), 128 (49), 57 (47), 39 (25), 72 (12), 85 (12)
GC-FTIR (main absorptions), cm^{-1}	1792 (conjugated lactone νC=O), 1150 and 1067 (lactone ν(C—C—O—C) and hydroxyl νC—O), 3555 (hydroxyl νOH)
^{1}H NMR ($CDCl_3$, δ relative to TMS)	5.28 (1 H, O*H*), 4.82 (qq, J = 6.64 and 1.25 Hz, 1 H, CH_3CH), 1.89 (d, J = 1.25 Hz, 3 H, CH_3C=), 1.41 (d, J = 6.64 Hz, 3 H, CH_3CH)
^{13}C NMR (D_2O, δ relative to TMSP-d_4)	174.9 (lactone *C*O), 141.1 (HO*C*=C), 138.5 (C=*C*CH_3), 81.8 (*C*HO), 20.1 (CH_3C=C), 11.5 (CH_3)

The vapor-phase infrared spectrum (Table III) shows a strong absorption at 1792 cm^{-1} due to the conjugated γ-lactone carbonyl stretching (νC=O) and medium to strong absorptions at 1150 and 1067 cm^{-1} consistent, respectively, with the stretchings of the CCOC group of the lactone ring and of the hydroxyl C—O bond (Nyquist, 1984).

A medium absorption at 3555 cm^{-1} (νOH) confirmed that, at least in the gas phase, sotolon is an enol. This spectrum is also identical to the vapor-phase spectrum of 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone already published by Pouchert (1989).

The ^{1}H and ^{13}C NMR spectra (Table III) are fully consistent with the structure 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone. In the proton NMR spectrum, the deshielded quadruplet of quadruplet at δ 4.82 was attributed unambiguously to the methine proton of the lactone ring. It is coupled (^3J = 6.64 Hz) to the methyl doublet at δ 1.41 and to another methyl doublet at δ 1.89 (^4J = 1.25 Hz). The last chemical shift is characteristic of a methyl group bonded to a carbon–carbon double bond.

The ^{13}C spectrum in D_2O was fully consistent with the proposed enol structure. Only one carbonyl resonance appeared at δ 174.9 characteristic of a lactone carbonyl. Two olefinic resonances were found at δ 141.1 and 138.5, and the methyl resonances appeared at δ 20.1 and 11.5. Finally, the single resonance at δ 81.8 was characteristic of a methine carbon adjacent to the lacton oxygen atom. Varying the pH of the solution between 3 (pH of wines) and 7 failed to detect any diketone form. Therefore, sotolon is always present as an enol, either in the gas phase or in organic or aqueous media.

Additionally, ^{1}H NMR spectroscopy with the chiral shift reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol was applied to the synthetic sotolon. When the amount of the chiral reagent was increased, the asymmetric methine quadruplet signal was split into a poorly resolved quintuplet. Meanwhile, the methyl doublet signal at δ 1.41 was separated into two signals of equal intensity corresponding to the two enantiomers.

This result was later confirmed by enantiomeric capillary GC on a modified β-cyclodextrine phase (FS-cyclodex). Moreover, preliminary results obtained with natural sotolon in various vin jaune wines (Guichard, 1991) indicate that sotolon occurs mainly as a racemic mixture in these wines, thus validating the present sensory study on synthetic (racemic) sotolon.

The sample of purified sotolon we used in the sensory tests was estimated to be free of contaminants from HRGC analyses and HRGC sniffings.

The determination of the olfactory detection threshold of sotolon in wine was made from the following regression equation calculated from the logarithm of the concentration: $u = 0.712 \log_{10} C - 0.827$; $r = 0.9836$. Its value was determined to be 15 ppb.

Two MCAs were first run separately using the results of the two different testing sessions. In the first session, three tasters were eliminated from further analyses because they systematically provided very different results as compared to other tasters. The results obtained in the second session were more homogeneous, and all tasters were selected for further analyses. Since the plots obtained from MCA for the two sessions were similar (not shown), this analysis was applied simultaneously to the results of both tasting sessions. The result is given in Figure 1, in which the different points are the baricenters of the points obtained for each sample or each assessor. It can be first observed that modalities for typicality and persistence of the nutty flavor are roughly distributed along a parabola (Gutman effect), thus showing that the scores given for these two descriptors vary similarly. The maderized modalities roughly follow the same pattern as the typicality but in opposite directions. The first level of the maderized descriptor is very close to the origin of the plot since most of the wines do not have this characteristic. Low

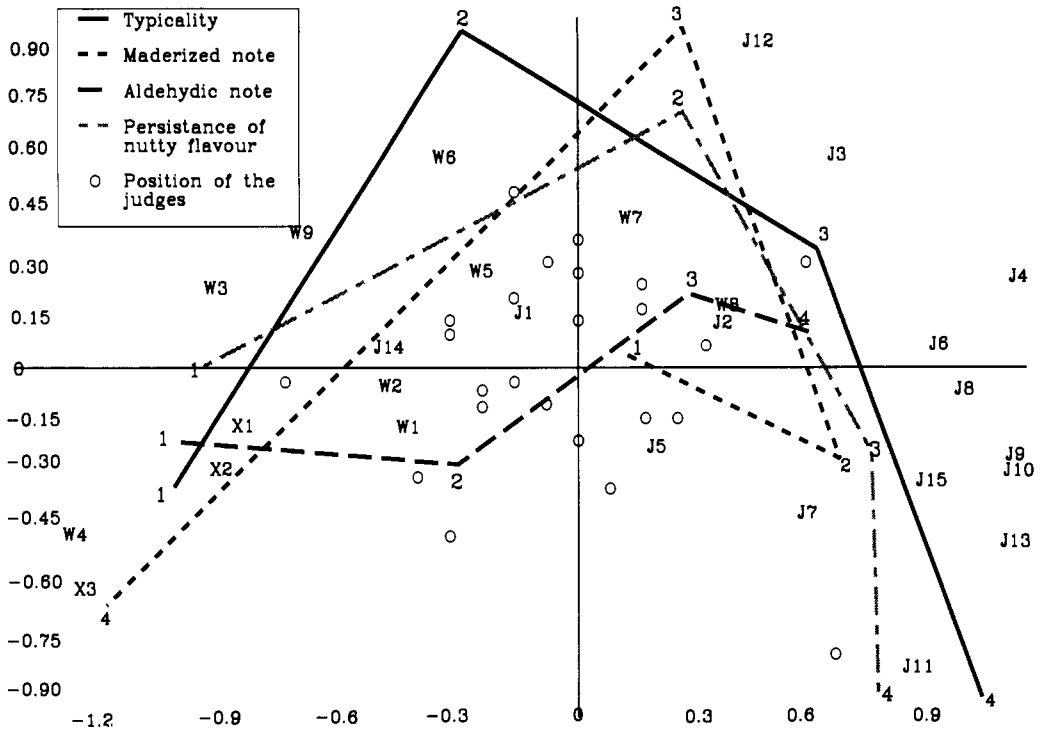

Figure 1. MCA of the results of the two tasting sessions. Sample code significations are given in Table II. Numbers on continuous or dotted lines correspond to sensory scores.

intensities of the maderized descriptor are therefore judged as favorable for the typicality of the wines, but higher scores are judged unfavorable. Except for one sample (J11), all assessors are visualized in the center of the figure. This demonstrates that, after selection, no assessor showed a particular sensitivity toward a particular sensory descriptor. The characterization of the samples by the descriptors is consequently not due to one individual giving odd high scores but corresponds to a consensus for most panelists.

It is interesting to note that all wines resulting from an oxidative ageing process, such as the amontillado (X3), the manzanilla (X2), the oxidized white wines (W3, W4), and the rancio (W9), are all situated on the left part of the graph. This means that they had a highly maderized characteristic. The vin jaune coded J14 was located in the same region. This is explained by an accidental oxidation of this sample by direct contact with air caused by a yeast film degeneration at the surface of the wine. It can be noted that the fino sherry wine X1 is also located in the left part of the graph. This shows a low persistence of the nutty aroma and a low typicality as referred to by the ideal vin jaune.

The white wines and some of the vin jaune wines are located in the center of the graph, showing the average scores for the different descriptors.

A third group of samples is found on the right part of the graph, which contains mainly samples of vin jaune and samples in which sotolon was adjusted to 150 ppb.

Twenty-two of these samples were analyzed for their sotolon content, and the results of this quantification are given in Table II. A simple analysis of variance, performed on the three groups determined from an MCA plot (group 1, X1–3, W1–4, W9, J14; group 2, J1, J5, J12, W5–8; group 3, J2, J6–8, J13, J15), demonstrated a significant difference in the amounts of sotolon (group 1, mean 9.33 ppb; group 2, mean 81.7 ppb; group 3, 141 ppb; $p < 0.025$). Samples showing high typicality and persistence of nutty flavor therefore contain higher concentrations of sotolon and the inverse is true.

Table IV. Spearman's Rank Correlation Test

variables involved in the correlations		coeff of correlation	critical value
typicality and sotolon concn	first session	0.8694	0.8471[a]
	second session	0.8520	0.8471[a]
persistence of nutty aroma and sotolon concn	first session	0.8315	0.7348[b]
	second session	0.5127	NS[c]
typicality and ethanal concn		0.7630	0.7348[b]
persistence of nutty aroma and ethanal concn		0.5750	NS

[a] Significant at the 99.9% level. [b] Significant at the 99.0% level. [c] NS, not significant.

To test the significance of this apparent correlation, a Spearman rank correlation test was applied. The results, given in Table IV, demonstrate a significant correlation between the amount of sotolon in wines and their typicality for both sessions ($p < 0.001$) and between the same amount and the persistence of the nutty aroma in the first session ($p < 0.01$).

DISCUSSION AND CONCLUSION

The results of the quantification of sotolon show that this compound is only found in detectable amounts (> 7 ppb) in wines which underwent a specific ageing with a development of flor yeasts, either as a film (in jaune) or as a submerged culture (fino sherries). The production of this particular compound seems related to the metabolism of certain strains of *Saccharomyces cerevisiae* species in a medium containing few residual sugars but high amounts of ethanol (12–15%). This is confirmed by sample W6, which is similar to W2 but which underwent a biological ageing for 2 weeks after inoculation with selected yeast species (Laboratoire d'analyses de Poligny, France). Wines that underwent such a process for a shorter time, such as amontillado wines, do not contain detectable amounts of sotolon.

The first important clue about sotolon flavor impact is that the amount in wines is often higher than the detection threshold. It is therefore probable that it could influence some of the olfactory characteristics of the French vin jaune with odor unit values varying from 1 to 25. It must be noted that this threshold (15 ppb) is perfectly consistent with the thresholds determined earlier by Sulser et al. (1972) and Kobayashi (1989), who found 1 ppb in water, and by Masuda et al. (1984), who found 2.5 ppb in an alcoholic synthetic solution. This result is similar to the conclusion given by Masuda et al. (1984) that sotolon is likely to give a sweet aroma to botrytized wines at concentrations exceeding 15 ppb.

A second clue about the importance of sotolon is provided by the MCA of the results of two tasting sessions in which mainly French sherries (vin jaune) were tasted. MCA was chosen since each of the sensory attributes had been noted on an ordinal scale. A good correlation between the persistence of the nutty flavor and typicality of the French vin jaune is demonstrated by this analysis. It shows the importance of the aldehydic characteristic on the typicality of these wines. Acetaldehyde has been considered as a factor of quality in the vin jaune for some decades, and its concentration, which is typically higher than 100 ppm and lower than 700 ppm, is already used as an indicator of efficient activity of the flor yeasts during the biological ageing. It is interesting to note from Figure 1 that an addition of 150 ppb of sotolon to a white wine or a vin jaune (J12, J14, W1, W7) shifts the sample location in the graph to the right (J13, J15, W5, W8), thus indicating that the wines are then judged more typical and less maderized, with a more persistent nutty aroma. This shift, which is much greater when sotolon is added to a vin jaune than to a white wine, is another clue showing that sotolon does have an impact on the sensory characteristics of wines at natural concentrations (150 ppb). The difference in the shift caused by sotolon addition in wines which underwent a biological ageing with flor yeasts (J12, J13; J14, J15) or which did not (W1–5, W7, W8) could be due to different initial amounts of acetaldehyde, which may enhance the perception of the odor of sotolon. Spanish sherries (X1–3) do not seem to have the same unique flavor and do not leave a nutty aroma in the mouth after swallowing, although two samples contained about 20 ppb of sotolon. This amount, which is close to the threshold we determined in wine, is probably too low for the pure compound to be recognized.

A third indication of the olfactory impact of sotolon is given by the correlations calculated for wines between the scores given for the three descriptors chosen and the amount of sotolon in 22 wines. They indicate that sotolon with ethanal is a variable which may explain the typicality defined as a nutty flavor of the wines. Sotolon could be responsible for the persistence of this flavor in the mouth, although this was not confirmed by the second tasting session.

Irrefutable arguments are difficult to provide for the sensory impact of volatile compounds in the aroma of a complex medium such as wine. Nevertheless, the different experiments we performed indicate that sotolon is an important contributor to the aroma of the French vin jaune. This contribution is not so evident for Spanish sherries, although it is still present in the fino samples we analyzed.

ACKNOWLEDGMENT

This work was supported by grants from the Conseil Régional and the Conseil Général from Franche Comté, France.

LITERATURE CITED

Dubois, P.; Rigaud, J.; Dekimpe, J. *Lebensm. Wiss. Technol.* **1976**, *9*, 366–368.

Etiévant, P. X. Wine. In *Volatile Compounds in foods and beverages*; Maarse, H., Ed.; Dekker: New York, 1991.

Etiévant, P.; Issanchou, S.; Bayonove, C. The flavour of Muscat wines: the sensory contribution of some volatile compounds. *J. Sci. Food Agric.* **1983**, *34*, 497–504.

Frijters, J. E. R.; Bemelmans, J. M. H. Flavor sensitivity for chloroanisoles in coagulated egg yolk. *J. Food Sci.* **1977**, *42*, 1122–1123.

Guichard, E. A. Enantiomeric separation of chiral lactones from wine and microbiological media by multidimensional gas-chromatography. *Proceedings of the 13th International Symposium on Capillary Chromatography*; Sandra, P., Ed.; Huëthig: Heidelberg, 1991; pp 253–259.

Kobayashi, A. Sotolon, identification, formation and effect on flavor. In *Flavor chemistry, trends and development*; ACS Symposium Series 388; American Chemical Society: Washington, DC, 1989.

Lebart, L.; Morineau, A. *SPAD: système portable pour l'analyse des données*; CESIA: Paris, 1985.

Martin, B. Thesis, University of Paris-sud, Orsay, 1989; 227 pp.

Martin, B.; Etiévant, P. Quantitative determination of solerone and sotolon in flor sherry wines by two-dimensional-gas-chromatography. *J. High Resolut. Chromatogr.* **1991**, *14*, 133–135.

Martin, B.; Etiévant, P.; Henry, R. The chemistry of sotolon: a key parameter for the study of a key component of flor sherry wines. In *Flavour science and technology*; Bessière, Y., Thomas, A., Eds.; Wiley: Chichester, U.K., 1990.

Martin, B.; Etiévant, P. X.; Le Quéré, J. L. More clues of the occurrence and flavor impact of solerone in wine. *J. Agric. Food Chem.* **1991**, *39*, 1501–1503.

Masuda, M.; Okawa, E.; Nishimura, K. Identification of 4,5-dimethyl-3-hydroxy-2(5H)-furanone (Sotolon) and 9-hydroxynonanone in botrytised wine and evaluation of the roles of compounds characteristic of it. *Agric. Biol. Chem.* **1984**, *48*, 2707–2710.

Nyquist, R. A. *The interpretation of vapor-phase infrared spectra. Vol. 1. Group Frequency Data*; Sadtler-Heyden: Philadelphia, 1984.

Pouchert, C. J. *The Aldrich Library of FT-IR Spectra, Vol. 3. Vapor Phase*; Aldrich Chemical Co.: Milwaukee, WI, 1989; p 753.

Sulser, H.; Habegger, M.; Buchi, W. *Z. Lebensm. Unters. Forsch.* **1972**, *148*, 215–220.

Received for review September 9, 1991. Accepted December 13, 1991.

Registry No. Sotolon, 28664-35-9.