More Clues of the Occurrence and Flavor Impact of Solerone in Wine[†]

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From quantifications of solerone it is evident that its concentration can vary from 0 to 500 ppb in wines. It is more abundant in the French Vin Jaune and is reported for the first time to occur in Rancio wines. Triangular sensory tests were made, and the results demonstrate that this compound can be detected neither at a concentration of 450 ppb in a white wine nor at a concentration of 44 ppm in a winelike synthetic medium. By use of GC–FTIR, GC–MS, and ¹H or ¹³C NMR results to identify synthetic solerone and GC sniffings to provide evidence for its olfactory purity, it can be concluded that solerone is not a key compound of sherry wines as had been claimed.

INTRODUCTION

Wine is a complex medium in which a 10th of all volatiles are thought to contribute to the aroma (Etiévant, 1990). Among these, solerone (5-oxo-4-hexanolide) was studied as an important contributor to flor sherry aroma. Solerone was identified for the first time in a flor sherry (Augustyn et al., 1971). The hypothesis of biosynthesis via glutamic acid was therefore thought to be specific of the metabolism of flor yeasts involved in sherry production. More recent studies on this lactone showed that solerone is also a constituent of red wines (Muller et al., 1973), thus indicating that this metabolism is also involved during alcoholic fermentation (Fagan et al., 1982). Nevertheless, many authors continued to cite solerone as a key compound of wines (Muller et al., 1973) because it was claimed to exhibit an attractive odor similar to that of the "cork of an old premium quality Pinot Noir" (Augustyn et al., 1971). However, more conclusive evidence of the contribution of this compound to the aroma in wines is still missing 19 years later.

The aim of this work, which followed a recent investigation about specific determination of solerone and sotolon in wines (Martin, 1989; Martin and Etiévant, 1990), was to collect more evidence about the olfactory impact of solerone in wines by use of both instrumental and sensory analyses.

MATERIALS AND METHODS

Wine Samples. Samples of Vin Jaune and Savagnin were provided by professional associations, i.e., the Laboratoire départemental d'analyses de Poligny and the Société de Viticulture du Jura. Red Burgundy wines were obtained from the Fédération Interprofessionnelle des Vins de Bourgogne. Other red wines came from the experimental cellar of INRA in Narbonne. Rivesaltes and Banyuls samples were given by the Noilly-Prat Co. Other wines as the white "vin de voile" and the white Burgundy wine were bought directly from producers or purchased in wine specialty shops (Xeres Wines).

Lactones and Reactives. Synthetic solerone was obtained as previously described (Berti et al., 1983). It was then purified by HPLC from successive injections of the product of synthesis on a diol column (Lichrosorb, 250 mm, 10 mm i.d., 5 µm, Interchim, Paris). Elution was made with a 55/45 mixture of dichloromethane and pentane at a flow rate of 4 mL/min. The time of collection of the purified solerone was given by UV detection

at 280 nm. Pure solerone was obtained after evaporation of the pooled collected fractions under vaccuum at 15 °C.

Confirmation of the Structure of Solerone. The structure of the purified synthetic solerone was confirmed by GC–MS, GC–FTIR, and ¹H or ¹³C NMR. The type of instruments and operating parameters are listed in Table I.

Quantification of Solerone. It has been reported that solerone quantities were obtained by two-dimensional gas chromatography (2-D GC) of a liquid–liquid solvent extract of the wine (Martin and Etiévant, 1990). Results were expressed in micrograms of solerone by using an internal standard and a calibration of the 2-D GC (Martin, 1989; Martin and Etiévant, 1990).

Sensory Analysis. *Olfactory and Gas Chromatographic Purities of Synthetic Solerone.* The occurrence of contaminants in synthetic solerone before and after HPLC purification was estimated from GC peak integrations, and olfactory contaminants were identified by GC effluent sniffing (Etiévant et al., 1983).

Determination of the Olfactory Impact in Synthetic Solution and Wine. The odor impact of purified solerone was judged first in a Savagnin white wine (13% ethanol, pH 3.22) at concentrations from 28 to 448 ppb. A second experiment was made with a synthetic solution (12% ethanol, adjusted to pH 3.1 with tartaric acid) containing 44 ppm of the same purified molecule. All solutions (30 mL) were presented in black glass vials closed by inert and odor-free stoppers. Triangular tests were made in which the reference (the pure synthetic solution or the wine) and the samples were given in a random order. All combinations (two references and one sample or two samples and one reference) were presented in the first experiment, and only two references and one sample were used in the second experiment. Judges were asked to spot the odd sample and to give odor comments afterward. Twenty-four people took part in the tests; 12 persons were recruited from the population of the town and trained after selection (Guichard et al., 1991), and the others were staff members.

RESULTS

Results of Instrumental Analyses. The first determination of solerone quantities in different types of wines is given in Table II. The reproducibility of the quantification was estimated from triplicate analyses made on four different samples (Martin, 1989; Martin and Etiévant, 1990). This reproducibility is good (average 6%) and is, as expected, better at high concentrations (4.75% for 123 ppb) than at low concentrations (10.4% for 51 ppb). Samples in which no solerone was detected may contain less than 7 ppb, since this value was determined as the minimum detectable concentration for this method (Martin and Etiévant, 1990). Red wines may also contain solerone as already reported by Sakato et al. (1975). However, this compound is not always found in red wines since only 2 of 15 samples were found to contain some. In these wines solerone occurred at a maximum level of 30 ppb.

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

	GC–MS	GC–FTIR	NMR
instrument	Nermag R10-10C	Bruker IFS85	Bruker WM400
GC	Girdel 31 (Delsi Nermag SA)	G.C. Carlo Erba 5160	
column	DB5 (J&W Scientific)	DB5	
	60 m × 0.32 mm i.d., 1 µm	30 m × 0.32 mm i.d., 1 µm	
injection mode	splitless	on-column	
			solvent, CDCl₃
			¹H, 400.13 MHz
operating parameters and modes	electronic ionization, 70 eV	T° light pipe, 200 °C	¹³C, 100.62 MHz
			5 mm o.d. tube
			ref: CHCl₃ 7.26 ppm/TMS
			¹³C-attached proton test
			J-modulated spin–echo

Table II. Quantification of Solerone in Wines

type of wine	name	amount, µg/L (no. of samples)
red wine	Burgundy Pinot Noir	ND^a–30 (3)
	Carignan	ND (3)
	Grenache	ND (3)
	Cinsault	ND (3)
	Syrah	ND (3)
white wine	Chassagne Montrachet	ND (1)
	Savagnin 1987	ND (3)
	Rivesaltes d'age	18 (1)
	Rivesaltes "Vieux"	70 (1)
	Rancio Picpoul	115–123 (2)
	maderized white wine	97 (1)
sherry	Olorosso	85 (1)
	Amontillado	68 (1)
	Manzanilla	104 (1)
	Fino	44–78 (7)
	experimental flor wine	67–78 (2)
	Vin Jaune	122–492 (6)

^a ND, not detected (lower than 7 ppb).

**Table III. Mass Spectrum and ¹H and ¹³C NMR Spectra of Synthetic Solerone**

MS (eight prominent ions in decreasing abundance)
    85 (100), 29 (88), 43 (70), 57 (14), 128 (M•⁺, 9),
    42 (6) 86 (5), 39 (5)
¹H NMR (CDCl₃, δ relative to TMS)
    4.79 (dd, J = 6.7 and 8 Hz, 1 H, CHO); 2.57–2.44 and
    2.30–2.21 (m, 4 H, CH₂CH₂); 2.28 (s, 3 H, CH₃CO)
¹³C NMR (CDCl₃, δ relative to TMS)
    205.27 (ketone CO); 175.76 (lactone CO); 81.93 (CHO);
    27.32 (CH₂CO); 26.20 (CH₃CO); 24.41 (CH₂)

Solerone was also found in some white wines, thus confirming previous reports or observations (Schreier, 1979; Brock et al., 1984). In these wines, its concentration varied from undetectable concentrations up to 123 ppb. It is also interesting to note that this compound was found for the first time in Rancio wines and in comparatively large amounts (115–123 ppb). Solerone seems to be more abundant in wines aged by flor yeasts as already concluded by Muller et al. (1973). Most of these wines contained more than 50 ppb of solerone, and the French Vin Jaune seems to contain higher quantities which vary from 120 to 500 ppb.

Since the results of the sensory analyses described in this paper were not in agreement with those published earlier, it was first necessary to confirm the structure of the compound we purified from the synthetic mixture.

The mass spectrum (Table III) is characteristic of a 4-substituted γ-lactone, the base peak at m/e 85 arising from the basis γ-lactone ring. It displays the parent peak at m/e 128 and is fully consistent with the published mass spectra of solerone (Augustyn et al., 1971; ten Noever de Braw et al., 1987).

The vapor-phase infrared spectrum (Figure 1) contains
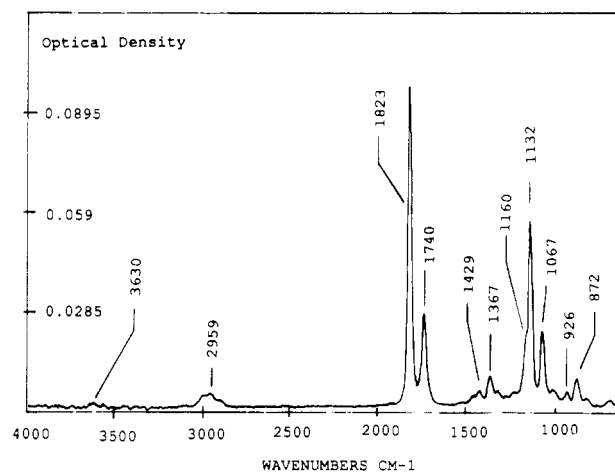
Figure 1. GC–FT/IR spectrum of solerone.

a strong absorption at 1823 cm⁻¹ due to the γ-lactone carbonyl stretching (ν C=O) and medium to strong absorptions at 1132 and 1067 cm⁻¹ which reflect the characteristic stretching of the CCOC groups of the lactone ring (Nyquist, 1984). The absorptions at 1740 and 1160 (shoulder) cm⁻¹ result from the ketone carbonyl stretching (ν C=O and ν CC(O)C, respectively; Nyquist, 1984). The weak absorption at 1367 cm⁻¹ can be assigned to symmetrical deformation of the methyl (CH₃CO) group (δ sym CH₃). This vapor-phase spectrum is therefore consistent with the condensed-phase infrared spectrum published by Augustyn et al. (1971): a weak C—H stretching absorption bond (ν C—H at 2959 cm⁻¹) and a very weak absorption at 3630 cm⁻¹ which can be assigned to the first overtone of the lactone carbonyl stretching frequency (Nyquist, 1984) rather than to a possible intramolecular hydrogen bond as initially described by Augustyn et al. (1971).

Finally, the ¹H and ¹³C NMR spectra (Table III) are fully consistent with the structure of solerone. The highly coupled ¹H spectrum was not completely assigned. However, the deshielded signal at δ 4.79 was attributed to the methine proton of the lactone ring. A selective decoupling experiment showed it was coupled to a proton in the multiplet centered at δ 2.50 and to another proton in the multiplet centered at δ 2.25. These highly coupled multiplets were attributed to the methylene units of the lactone ring. The chemical shift of the singlet at δ 2.28 is consistent with that of a methyl CH₃CO group.

By use of the ¹³C spectrum, the structure of solerone was established. Carbonyl resonances appeared at δ 205.27 and 175.76, revealing a ketone carbonyl and a lactone carbonyl, respectively. The methyl resonance at δ 26.20 was differentiated from the methylene resonances (δ 27.32 and 24.41, CH₂CO and CCH₂C, respectively) by a J-modulated spin–echo experiment. The observed chemical shift is in agreement with the presence of a methyl of an acetyl group. Finally, the single resonance at δ 81.93 was attributed to

Table IV. Olfactive Detection of Solerone in Wine

concn of solerone, ppb	no. of right answers	concn of solerone, ppb	no. of right answers
28	11/24	224	6/24
56	7/24	448	9/24
112	12/24		

a methine carbon adjacent to the lactone oxygen atom. This fixes the 4-position for the substitution of the γ-lactone ring.

Results of Sensory Analyses. The initial chromatographic purity of solerone (96%) was raised to 98.5% by HPLC purification. The olfactory purity was acceptable enough for sensory analysis since no extra odors could be detected from the GC effluent on either DB-5 or DB-1701 columns. A first sensory analysis was made with a wine generally used in the Jura area to produce a sherry (Vin Jaune), in which additions of the purified molecule, leading to final concentrations similar to those observed in wines (Table II), were made. The results of the test, given in Table IV, indicated that our panel was unable to detect solerone in a white wine at naturally found concentrations. The second test was therefore performed in which solerone was added in much higher concentration (44 ppm) in a synthetic medium resembling wine. Only 9 of 24 judges gave the right answer, and it is evident that even at this abnormally high concentration and in a simple medium, judges were not able to identify solerone by its odor.

DISCUSSION

These experiments showed that concentrations of solerone could vary from 0 to 500 ppb in wines, and furthermore that the highest concentrations were found in white and more particularly in flor sherry wines. The concentrations thus determined in wines probably correspond to the concentration of a mixture of the two optical isomers as suggested by Wurz et al. (1988) because we observed that pure enantiomeric solerone could isomerize within a few weeks (E. Guichard and A. J. Mosandl, 1989, Wolfgang Goethe Universität, Frankfurt am Main, unpublished results). Fagan et al. (1981) isolated solerone from a 6-week-old fermented medium by GC and demonstrated that this natural substance was optically active. They concluded from an investigation on the hydrogenated derivatives of solerone that, although (4R)-solerone was obviously produced by enzymatic stereoselective pathways, it could isomerize over time into the 4S isomer (Fagan et al., 1981; Wurz et al., 1988). However, the exact final ratio corresponding to the equilibrium between the two isomers in wine is unknown. We can say that a stereoselective synthesis of pure (4R)-solerone led to a 6:4 mixture of the two isomers after a few weeks. For these reasons, the sensory tests were performed on a purified racemic, the identity of which was fully confirmed by the different spectra (IR, MS, ^{1}H and ^{13}C NMR) reported. The two sensory tests showed that this compound cannot be detected by tasting, neither in a white Savagnin wine at a concentration of 448 ppb nor in a synthetic medium at a much higher concentration (44 ppm).

It is therefore more likely that solerone is not the important flavor compound of wines as previously reported (Augustyn et al., 1971; Muller et al., 1973). As a consequence, the odor reported by Augustyn et al. (1971) had to be due to the presence of some impurities. Since these authors took many precautions when isolating and purifying solerone from wine, it is likely that this odorant impurity arose from thermal degradation of solerone itself in the postcolumn section of their chromatograph. Nev-

ertheless, the concentration of this impurity must have been low because the spectra they reported correspond to those recorded in this study. The decrease of the odor of the purified sample they attributed to degradation of solerone was therefore probably due to the degradation of this impurity. This hypothesis is supported by the stability of the purified sample of solerone and by its very weak odor. Moreover, the purified sample analyzed by GC could not be detected by sniffing the chromatographic effluent.

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