Identification of 4,5-Dimethyl-3-hydroxy-2(5H)-furanone (Sotolon) and Ethyl 9-Hydroxynonanoate in Botrytised Wine and Evaluation of the Roles of Compounds Characteristic of It

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Two new components of botrytised wine were identified: 4,5-dimethyl-3-hydroxy-2(5H)-furanone (Sotolon) and ethyl 9-hydroxynonanoate. Sotolon, the key substance of cane sugar aroma, was identified as the sugary flavor substance of botrytised wine by means of gas chromatography-mass spectrometry after column chromatographic separation on DEAE-Sephadex and silica gel. Ethyl 9-hydroxynonanoate was identified by chemical ionization and electron impact mass spectrometry. To evaluate the role of 17 volatile and 5 nonvolatile compounds characteristic of botrytised wine, these compounds were added to a normal wine. This produced a sweet, honey-like flavor similar to that of botrytised wine. The importance of Sotolon and the role of each group of flavor substances in producing this flavor was clarified by omission tests.

Botrytised wine, or noble wine, is produced when Botrytis cinerea fungus attacks ripe grapes under special climatic conditions leading to loss of moisture from the ripe berries and increased sugar concentrations in the juice. This wine is highly prized because of its noble flavor and availability in only small quantities.

To characterize the noble flavor of botrytised wine, we analyzed sugars, alcohols, organic acids and volatile compounds of botrytised and other wines from various countries. We reported1,2 that botrytised wine has significantly larger amounts of 4 organic acids (gluconic acid, glucuronic acid, etc.), 2 sugars and 18 volatile compounds (benzaldehyde, 4-nonanolide etc.), but smaller amounts of 20 volatile compounds (isoamyl alcohol, ethyl octanoate, etc.) than normal wines.

In this study, one of the unknown compounds found in our previous study which was characteristic of botrytised wine was identified as ethyl 9-hydroxynonanoate. 4,5-Dimethyl-3-hydroxy-2(5H)-furanone (Sotolon) was identified as the sugary flavor substance of botrytised wine. Sensory evaluations clarified the roles of the components characteristic of botrytised wine including these two compounds in producing the flavor of botrytised wine.

MATERIALS AND METHODS

1) Wines. Botrytised wine from Vitis vinifera var. Riesling and Sémillon harvested at our company’s vineyards in 1978 was used for the isolation of compounds. The following botrytised and normal wines from Japan, France and Germany were analyzed for the quantitative determination of Sotolon. Japan: 4 Botrytised wines; Riesling, Sémillon. 4 Normal wines; Riesling, Sémillon. France: 2 Botrytised wines; Premier Grand Cru (Sauterne). 2 Normal wines; Sec (Graves). Germany: 2 Botrytised wines; Riesling, Trockenbeerenauslese (Rhein). 2 Normal wines; Riesling, Kabinet (Rhein).

2) Reference compounds. The chemicals used were the purest available on the market. Volatile compounds were examined by gas chromatography and thin-layer chromatography in order to ensure that they contained less than 1~2% impurities. Sotolon was a gift from Professor A. Kobayashi of Ochanomizu University and some was also synthesized according to Sulser et al.3

3) Isolation of ethyl 9-hydroxynonanoate. One liter of
Botrytised wine was extracted by liquid-liquid extraction with 400 ml of distilled n-pentane at 45°C for 20 hr. The extract was washed with 100 ml of sat. NaHCO₃ and 100 ml of distilled water and then dried over Na₂SO₄ overnight. The solvent was removed under reduced pressure, and the residue was chromatographed on 5 g of silica gel, eluting successively with 50 ml of 5% ether in n-pentane, 20% ether in n-pentane, and ether. The fraction eluted with 20% ether in n-pentane was analyzed by gas chromatography-mass spectrometry (GC-MS) by electron impact (EI) and chemical ionization (CI) methods.

4) Preparation of ethyl 9-hydroxynonanoate. Ethyl oleate (15.5 g) was oxidized by ozone following the method of Noller and gave 8 g of ethyl 9-oxononanoate. To 40 ml of dry ethanol solution of ethyl 9-oxononanoate (6.1 g), sodium borohydride (1.37 g) was added at 0°C and the mixture was stirred at room temperature for 18 hr. Ethanol was removed in vacuo, and the resulting mixture was mixed with 1 N HCl (40 ml) and extracted with ether. The extract was washed with sat. NaHCO₃ and distilled water, and then dried and concentrated in vacuo, yielding 2.2 g of ethyl 9-hydroxynonanoate.

5) Isolation of Sotolon. Botrytised wine (1.2 liters) was extracted by liquid-liquid extraction with 600 ml of ether-n-pentane-dichloromethane (1:2:2) at 45°C for 20 hr. The extract was washed with 360 ml of 1 N NaOH, and the aqueous layer was acidified with 210 ml of 2 N HCl and then extracted with two 600 ml of the same solvent. The extract was dried and concentrated in vacuo. The acidic part was chromatographed on a 60 ml DEAE-Sephadex A-25 (OH⁻ form) column which had been conditioned with methanol and ether-methanol-water (89:10:1). The column was eluted successively with 200 ml of ether-methanol-water (89:10:1), four 200 ml portions of CO₂-saturated ether-methanol (9:1) and four 200 ml portions of 4% formic acid in ether-methanol (9:1). The third fraction eluted with CO₂-saturated ether-methanol solution was chromatographed on a 13 g neutral silica gel (Mallinckrodt SILICAR CC-7) column eluted with benzene-CHCl₃-acetone (60:40:5).

6) Quantitative analysis of Sotolon. Sotolon content in botrytised and normal wines was analyzed by the same method described in section 5 using 200 ml of wine. The eluate from the DEAE-Sephadex column was concentrated to 50 μl in vacuo and 5 μl was subjected to gas chromatography-mass fragmentography. Ions at m/z 128 were used.

7) GC-MS. The Finnigan 4000 GC-MS system was used at an ionization temperature of 250°C and electron voltage of 70 eV. For the identification of ethyl 9-hydroxynonanoate, a CP-Wax 51 (Chrompac, Holland) glass capillary column (50 m x 0.25 mm i.d.) was used under the conditions of: column temperature, 100~220°C (2°C/min); injection temperature, 250°C; 1 ml of He/min. Isobutane (0.1 Torr) was used as the reaction gas for CI.

RESULTS AND DISCUSSION

7) Identification of ethyl 9-hydroxynonanoate

One of the unknown compounds characteristic of botrytised wine found in our previous study appeared on GC with a tₚ of 43.0 min and was identified from its EI and CI mass spectra. The EI mass spectrum (Fig. 1) had ion

![Fig. 1. EI Mass Spectrum of Ethyl 9-Hydroxynonanoate.](image1)

8) Sensory evaluation. The tasters taking part were researchers for wine making and quality evaluation from our company. Descriptive tests were used to evaluate the role of compounds characteristic of botrytised wine. Triangular tests were used to determine the flavor threshold of Sotolon, and the threshold was taken as that concentration producing six correct identifications with seven testers.
peaks at \( m/z \) 88 and 101 indicating that it was an ethyl ester. In the CI mass spectrum (Fig. 2), the protonated molecular ion appeared at \( m/z \) 203 and some fragments like \( M-17, M-45 \) and \( M-63 \) indicated the presence of hydroxyl and ethoxyl groups. These data and the presence of the ion at \( m/z \) 172 (\( M-30 \)) in the EI mass spectrum led to this compound's being identified as ethyl 9-hydroxynonanoate. The identification was confirmed by synthesis.

This is the first time that ethyl 9-hydroxynonanoate has been found in botrytised wine. This compound has also recently been found in Alban wine from Italy.\(^5\)

2) Identification and determination of Sotolon

Analysis of the aroma compounds of botrytised and other wines demonstrated that botrytised wine contained an acidic substance that gave it a very sweet and sugar-like aroma. As this sweet aroma disappeared under alkali conditions and was slowly regenerated upon the addition of acids, this substance was assumed to be an enolic or lactonic compound. It was thus subjected to the following procedure. The acidic part of botrytised wine was chromatographed over DEAE-Sephadex, resulting in separation of the phenolic and enolic part which had a very sweet aroma. This part was then fractionated by neutral silica gel column chromatography. The fraction which had a sweet and burnt aroma was analyzed by GC-MS. Analysis of the mass spectrum (Fig. 3) indicated that 3-hydroxy-4,5-dimethyl-2(5H)-furanone was present.

This is the first time that 3-hydroxy-4,5-dimethyl-2(5H)-furanone has been found in botrytised wine. This compound has been identified in sugar molasses\(^6\) as a key substance on the sugary flavor and is called Sotolon. It has also been found in aged rice wine (sake)\(^7\) and flor sherry\(^8\) and was reported to contribute to the flavor of the beverages containing it.

The amount of Sotolon in botrytised and other wines was determined by mass fragmentography \( (t_R = 9.5 \text{ min}) \). Sotolon content was 5 ~ 20 ppb in botrytised wines but lower than 1 ppb in normal wines. The same levels were found in the botrytised wines examined from Japan, France and Germany.

3) Organoleptic evaluation of the compounds characteristic of botrytised wine

Ethyl 9-hydroxynonanoate has little aroma. Sotolon has a sugar-like and caramel-like aroma and its threshold value was determined
to be 2.5 ppb in 12% alcohol solution. Its threshold value was one of the lowest among the sugar-like aroma substances.

The roles of the characteristic substances of botrytised wine, including Sotolon, in producing the noble flavor were examined. First, normal wines were supplemented with the 22 compounds which were present in higher amounts in botrytised wines, as shown in Table I. Next, omission tests were conducted in which only certain groups of compounds were omitted in order to evaluate the role played by each group. The results showed that when all characteristic compounds were added, the honey-like aroma and sweetness of the botrytised wines could be reproduced to a certain extent. This confirmed that these substances play important roles in producing the characteristic aroma and flavor of botrytised wine. When sugars were omitted, there was a lack of sweetness and of strength in the aroma. This indicated that sugars contribute not only to the taste but also to the volatility of the aroma compounds. When acids were omitted, there was a slight lack of sourness. When aroma compounds other than Sotolon were omitted, a sweet-associated aroma was present, but it was similar to that of normal wines. When Sotolon was omitted, that special sweet-associated fragrance of botrytised wines was missing. This showed that Sotolon plays an important role in the aroma of botrytised wine.

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REFERENCES