Wine Aroma Composition: Identification of Additional Volatile Constituents of Red Wine

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Eleven additional components not previously identified in wine volatiles were characterized from an oxygen-rich fraction of the aroma extracts obtained by liquid–liquid extraction of Burgundy Pinot Noir red wines. The compounds identified by coupled gas chromatography–mass spectrometry were ethoxy and acetyloxy derivatives of hydroxy ketones and hydroxy esters: 3-ethoxy-2-butanone, 3-ethoxy-2-pentanone, 3-acetyloxy-2-butanone, ethyl 2-acetyloxyacetate, ethyl 2-acetyloxypropanoate, ethyl 3-acetyloxybutanoate, ethyl 4-acetyloxybutanoate, 3-methylbutyl 4-acetyloxybutanoate, ethyl 2-acetyloxy-3-methylbutanoate, ethyl 2-acetyloxy-4-methylpentanoate, and diethyl 2-acetyloxysuccinate.

The analysis of wine aroma by using modern and efficient techniques such as gas chromatography and mass spectrometry has resulted in the identification of hundreds of volatile constituents in white and red wines as well as in sherries (Webb and Muller, 1972a; Schreier, 1979). In regard to red wines produced from *Vitis vinifera* varieties, the interest has been especially focused on Cabernet wines (Kepner et al., 1969, 1972; Webb et al., 1969; Muller et al., 1971, 1972; Bayonove et al., 1975), characteristic red wine constituents such as phenolics (Dubois and Brulé, 1970; Dubois et al., 1971) or particular techniques, e.g., headspace analysis (Bertuccioli and Viani, 1976). Only little information is available on the aroma composition of Pinot Noir red wines (Meunier and Bott, 1979). This paper deals with the identification of some volatile constituents in an aroma fraction separated from Burgundy Pinot Noir containing compounds rich in oxygen. These components have not yet been identified as wine (Webb and Muller, 1972a; Schreier, 1979) or partly as food aroma constituents (Van Straten, 1977).

EXPERIMENTAL SECTION

Samples. Five Burgundy Pinot Noir red wines (Côte de Beaune, 1963, 1964, 1972, 1973, 1976) were investigated.

Isolation of Volatile Compounds. Five hundred milliliters of each wine was used, and the volatiles were isolated by continuous liquid–liquid extraction for 8 h, using 100 mL of freshly distilled pentane–dichloromethane mixture (2:1) (Drawert and Rapp, 1968). The aroma extracts were dried over sodium sulfate, and the solvent was removed by distillation (45 °C) using low hold-up Vigreux columns as described by Drawert et al. (1969). The concentrates (about 3 mL) were put into a column (1.8 i.d. × 40 cm) of silica gel 60 (Merck), activity grade II, and the extracts were separated in three fractions by using a pentane–diethyl ether solvent system (elution rate, 60 mL/h) as described by Schreier et al. (1979). Fraction II, containing compounds rich in oxygen, eluted with 20% diethyl ether in pentane, was concentrated in a Vigreux column (45 °C) to 0.5 mL, and the concentrated eluate was then subjected to analysis by GLC and combined GLC–MS.

Gas Chromatography and GLC–MS Analysis. Analytical GLC separations were affected by the use of a 5 m × 2 mm i.d. glass column, packed with 80–100-mesh Varaport 30 coated with 5% FFAP, on a Varian Aerograph, Model 2700, equipped with flame ionization detectors. The column was temperature programmed from 70 to 250 °C at 2 °C/min. The operation conditions were as follows: injection port temperature, 200 °C; detector temperature, 280 °C; carrier gas (N_2) flow, 20 mL/min; hydrogen flow, 30 mL/min; air flow, 300 mL/min.

GLC–MS data were obtained by using a Varian MAT Model CH 7 mass spectrometer (Varian MAT, Bremen) coupled via a Watson Bieman separator with a Varian Aerograph, Model 1201. The gas chromatograph was fitted with a 5 m × 1 mm i.d. glass "mikropak" column, packed with 5% FFAP on Varaport 30 (100–120 mesh). The injector was held at 200 °C, and the column oven was programmed from 70 to 100 °C at 1 °C/min and from 100 to 240 °C at 2 °C/min. A helium flow of 4.1 mL/min was used. The ion source temperature was 250 °C. Mass spectra, recorded on a Siemens oscillograph, were obtained at 70 eV.

Reference Substances. Ethoxy ketones were prepared according to Mayer (1977) by etherification of α-hydroxy ketones with ethanol, adding the ion exchanger Amberlyst 15-H as catalyst. After separation of the ion exchanger by filtration, extraction of the ethoxy compounds, using a pentane–dichloromethane mixture (2:1), drying over sodium sulfate, and removal of the solvent, the ethoxy ketones were purified by distillation.

3-Ethoxy-2-butanone ($C_6H_{12}O_2$), bp 30–32 °C (12 mm); lit. bp 60–80 °C (80 mm) (Nikiforov and Schmidt, 1974).

3-Ethoxy-2-pentanone ($C_7H_{14}O_2$) was synthesized after reduction of 2,3-pentandione with Zn/H_2SO_4 (Diehls and Stephan, 1907) by reaction of the formed isomer hydroxy compounds with ethanol as mentioned above. A mixture of 3-ethoxy-2-pentanone and 2-ethoxy-3-pentanone was obtained by distillation; bp 45–48 °C (15 mm). GLC analysis showed two peaks.

Acetyloxy compounds were prepared according to Prochazka and Palecek (1970) by reaction of acetic anhydride with the corresponding hydroxy compound, adding small amounts of concentrated sulfuric acid.

3-Acetyloxy-2-butanone ($C_6H_{10}O_3$), bp 58–60 °C (15 mm); lit. bp 86–95 °C (50 mm) (Burton and Wiese, 1968).

Ethyl 2-acetyloxyacetate ($C_6H_{10}O_4$), bp 44–48 °C (10 mm).

Ethyl 2-acetyloxypropanoate ($C_7H_{12}O_4$), bp 76–79 °C (12 mm); lit. bp 81–82 °C (25 mm) (Cohen et al., 1963).

Ethyl 3-acetyloxybutanoate ($C_8H_{14}O_4$), bp 95–98 °C (12 mm); lit. bp 92–94 °C (10 mm) (Prochazka and Palecek, 1970) and bp 92–94 °C (8 mm) (Cohen et al., 1963), respectively.

Ethyl 4-acetyloxybutanoate ($C_8H_{14}O_4$), bp 98–102 °C (12 mm); lit. bp 103–105 °C (14 mm) (Mattioda, 1969).

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Table I. Additional Compounds Identified in an Oxygen-Rich Fraction of Aroma Extracts of Pinot Noir Red Wines

compound[a]	mass spectral data[b]
3-ethoxy-2-butanone, MS, RT	27 (6), 29 (10), 31 (2), 43 (24), 45 (100), 46 (2), 72 (2), 73 (18)
3-ethoxy-2-pentanone, MS, RT	27 (11), 29 (22), 31 (29), 39 (6), 41 (23), 43 (49), 59 (100), 87 (28)
3-acetyloxy-2-butanone, MS, RT	27 (4), 42 (5), 43 (100), 45 (15), 86 (3), 87 (33), 88 (2), 130 (16)
ethyl 2-acetyloxyacetate, MS, RT	27 (5), 29 (17), 31 (4), 42 (6), 43 (100), 73 (8), 74 (7), 101 (6)
ethyl 2-acetyloxypropanoate, MS, RT	27 (4), 29 (9), 42 (1), 43 (100), 87 (18), 88 (2), 115 (3), 116 (2)
ethyl 3-acetyloxybutanoate, MS, RT	29 (15), 42 (7), 43 (100), 45 (6), 61 (6), 69 (39), 114 (6), 131 (7)
ethyl 4-acetyloxybutanoate, MS, RT	29 (33), 42 (15), 43 (100), 45 (10), 61 (10), 85 (13), 87 (45), 88 (11)
3-methylbutyl 4-acetyloxybutanoate, MS, RT	29 (12), 41 (24), 42 (14), 43 (81), 55 (17), 70 (50), 71 (100), 87 (60)
ethyl 2-acetyloxy-3-methylbutanoate, MS, RT	27 (3), 29 (7), 41 (2), 43 (100), 55 (2), 73 (2), 104 (1), 115 (10)
ethyl 2-acetyloxy-4-methylpentanoate, MS, RT	27 (3), 29 (6), 41 (3), 43 (100), 55 (2), 69 (9), 129 (6), 142 (6)
diethyl 2-acetyloxysuccinate, MS, RT	27 (5), 29 (12), 43 (100), 55 (3), 71 (7), 89 (3), 127 (3), 159 (4)

[a] MS, RT = mass spectral and GLC retention evidence, respectively. Data consistent with that of an authentic sample measured on the same instrument. [b] The eight most intense ions, percent intensities in parentheses.

3-Methylbutyl 4-acetyloxybutanoate ($C_{11}H_{20}O_4$), bp 172–174 °C (10 mm).

Ethyl 2-acetyloxy-3-methylbutanoate ($C_9H_{16}O_4$), bp 90–93 °C (12 mm).

Ethyl 2-acetyloxy-4-methylpentanoate ($C_{10}H_{18}O_4$), bp 96–99 °C (10 mm).

Diethyl 2-acetyloxysuccinate ($C_{10}H_{16}O_6$), bp 148–150 °C 10 mm); lit. bp 107–108 °C (2 mm) (Liu et al., 1975) and bp 129–130 °C (4 mm) Cohen et al., 1966), respectively.

RESULTS AND DISCUSSION

The volatile constituents in Burgundy Pinot Noir red wines were obtained by liquid–liquid extraction. An oxygen-rich fraction was then separated by selective adsorption on silica gel, and the components of this fraction were characterized by using the information from GLC–MS analysis. Packed column GLC separation was also used to confirm and aid the mass spectral identification. Table I lists the components identified for the first time in wine aroma along with the mass spectral data.

The ethoxy and acetyloxy derivatives of hydroxy ketones and hydroxy esters summarized in Table I were detected in all the wines investigated, in which they have been determined in varying amounts, in particular in the ppb range. Details of the quantitative aroma composition of Burgundy Pinot Noir wines, including the individual amounts of the compounds mentioned in this paper as well as the contents of more than 60 aroma substances, are given elsewhere (Schreier et al., 1980).

The ethoxy and acetyloxy compounds may result from fermentation byproducts through secondary reactions, e.g., the hydroxy ketones acetoin and 3-hydroxy-2-pentanone occurring in relatively high concentrations in red wines along with the corresponding vicinal diketones (Kavadze et al., 1977; Leppänen et al., 1979) may be considered as precursors for the ethoxy ketones identified in this study. α-Hydroxy ketones are well known to be very susceptible to etherification in the presence of an acidic catalyst (Mayer, 1977).

It may be presumed that acetoin is also the precursor for 3-acetyloxy-2-butanone, an aroma substance very common in food volatiles (Oser and Ford, 1978), and known to be formed generally in Maillard-type reactions (Piloty and Baltes, 1979).

Among the acetyloxy esters which may be considered as derivatives of the corresponding hydroxy esters arising through fermentation processes (Maw and Coyne, 1966), ethyl 2-acetyloxypropanoate is quantitatively the main component (Schreier et al., 1980). The occurrence of 4-acetyloxybutanoate is of particular interest due to its analgesic effect, which has been found to be stronger than that of acetylsalicylic acid (Nordmann and Mattioda, 1970). The anesthetic effect of the precursor of this ethyl ester, 4-hydroxybutanoic acid, has been described previously (Makoto and Hoshino, 1976). 4-Substituted-γ-butyrolactones have also been shown to be physiologically active (Webb et al., 1972b, 1976).

As mentioned above, it is fairly easy to assume that the ethoxy compounds identified in this study were formed through a nonenzymatic reaction mechanism. On the other hand, the acetyloxy derivatives listed in Table I may possibly result from hydroxy compounds through enzymatic side reactions in the course of fermentation steps which include the activities of yeasts as well as bacteria, due to the traditional methods of winemaking in Burgundy (Meunier and Bott, 1979). Although the coenzyme A dependent enzymatic acetylation of primary OH or NH_2 groups are metabolic steps widely distributed in nature (Decker, 1959), the occurrence of the corresponding acetylation of secondary hydroxyl groups is rather limited. The formation of acetylcarnitine from carnitine is considered to be a classic example (Friedman and Fraenkel, 1955).

Taste qualities and odor thresholds of the components identified in this study have not yet been investigated. The silica gel fraction II, in which the ethoxy and acetyloxy components were eluated, showed a phenolic and smoke-like odor, caused by relatively high amounts of 4-ethylphenol and 4-ethylguaiacol, both also detected in this fraction (Schreier et al., 1980). Recently, Schreier et al. (1980) demonstrated that the bouquet of Burgundy Pinot Noir wines is characterized by a specific quantitative distribution of volatile substances which influence each other in their sensory effect in a way that is still unknown. The components identified in this work should be included in future studies dealing with analyses of correlations of instrumental and taste panel results.

LITERATURE CITED

Bayonove, C., Cordonnier, R., Dubois, P., *C. R. Hebd. Seances Acad. Sci., Ser. D* **281**, 75 (1975).
Bertuccioli, M., Viani, R., *J. Sci. Food Agric.* **27**, 1035 (1976).
Burton, P. E., Wiese, H. K., U.S. Patent 3404176, 1968.
Cohen, S. G., Crossley, J., Khedouri, E., Zand, R., Klee, L. H., *J. Am. Chem. Soc.* **85**, 1685 (1963).
Cohen, S. G., Neuwirth, Z., Weinstein, S. Y., *J. Am. Chem. Soc.* **88**, 5306 (1966).
Decker, K., "Die Aktivierte Essigsäure", Enke, Stuttgart, 1959.
Diehls, O., Stephan, E., *Ber. Dtsch. Chem. Ges.* **40**, 4338 (1907).
Drawert, F., Rapp, A., *Chromatographia* **1**, 446 (1968).
Drawert, F., Heimann, W., Tressl, R., Emberger, R., *Chromatographia* **2**, 57 (1969).
Dubois, P., Brulé, G., *C. R. Hebd. Seances Acad. Sci. Ser. D* **271**, 1597 (1970).
Dubois, P., Brulé, G., Ilic, M., *Ann. Technol. Agric.* **20**, 131 (1971).
Friedman, S., Fraenkel, G., *Arch. Biochem. Biophys.* **59**, 491 (1955).
Kavadze, A. V., Rodopulo, A. K., Egorov, I. A., *Prikl. Biokhim. Mikrobiol.* **13**, 199 (1977).

Kepner, R. E., Webb, A. D., Maggiora, L., *Am. J. Enol. Viticult.* **20**, 25 (1969).
Kepner, R. E., Webb, A. D., Muller, C. J., *Am. J. Enol. Viticult.* **23**, 103 (1972).
Leppänen, O., Ronkainen, P., Koivisto, T., Denslow, J., *J. Inst. Brew.* **85**, 278 (1979).
Liu, M. T. H., Banjoko, O., Yamamoto, Y., Moritani, I., *Tetrahedron* **31**, 1645 (1975).
Makoto, M., Hoshino, M., *Jikeikai Med. J.* **23**, 189 (1976).
Mattioda, G. D., Ger. Offen. Patent 1903076 (1969).
Maw, G. A., Coyne, C. M., *Arch. Biochem. Biophys.* **117**, 499 (1966).
Mayer, D., in "Houben/Weyl, Methoden der Organischen Chemie", Vol. VII/2c, 4th ed., Müller, E., Ed., Thieme, Stuttgart, 1977, p 2232.
Meunier, J. M., Bott, E. W., *Chem. Mikrobiol. Technol. Lebensm.* **6**, 92 (1979).
Muller, C. J., Kepner, R. E., Webb, A. D., *Am. J. Enol. Viticult.* **22**, 156 (1971).
Muller, C. J., Kepner, R. E., Webb, A. D., J. Agric. Food Chem. **20**, 193 (1972).
Nikiforov, A., Schmidt, U., *Monatsh. Chem.* **105**, 1044 (1974).
Nordmann, J., Mattioda, G. D., Fr. M. Patent 7593 (1970).
Oser, B. L., Ford, R. A., *Food Technol.* **32**(2), 60 (1978).
Piloty, M., Baltes, W., *Z. Lebensm.-Unters.-Forsch.* **168**, 374 (1979).
Prochazka, M., Palecek, M., *Collect. Czech. Chem. Commun.* **35**, 1399 (1970).
Schreier, P., *CRC Crit. Rev. Food Sci. Nutr.* **12**, 59 (1979).
Schreier, P., Drawert, F., Winkler, F., *J. Agric. Food Chem.* **27**, 365 (1979).
Schreier, P., Drawert, F., Abraham, K. O., *Lebensm.-Wiss. Technol.* in press (1980).
Van Straten, S., Ed., "Volatile Compounds in Food", 4th ed, CIVO, TNO, Zeist, 1977.
Webb, A. D., Kepner, R. E., Maggiora, L., *Am. J. Enol. Viticult.* **20**, 16 (1969).
Webb, A. D., Muller, C. J., *Adv. Appl. Microbiol.* **15**, 75 (1972a).
Webb, A. D., Muller, C. J., Kepner, R. E., Eriksson, K., Nährli, M., *Am. J. Enol. Viticult.* **23**, 121 (1972b).
Webb, A. D., Eriksson, K., Muller, C. J., Kepner, R. E., *Am. J. Enol. Viticult.* **27**, 27 (1976).

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Volatile Ester Hydrolysis or Formation during Storage of Model Solutions and Wines

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The effects of temperature, ethanol concentration, and pH on the rate of hydrolysis of common volatile esters of wine (ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isobutyl acetate, isoamyl acetate, hexyl acetate, and 2-phenylethyl acetate) were investigated in model situations. The pseudo-first-order rate constants for temperature and pH effects were calculated as well as the second-order rate constants for $[H^+]$ effect on model solution ester hydrolysis. The effect of acid catalysis of species other than H^+ was calculated and found to be minor. Ethanol concentration differences in amounts of 10–14% v/v had little effect on the rates. Activation energies and thermodynamic activation constants were calculated for the esters. Several wines were also analyzed for changes in ester concentration with time at several different temperatures.

Volatile esters are introduced into wine primarily by yeast during fermentation. Although small quantities of esters are present in grapes prior to fermentation, the amounts are negligible compared to those introduced enzymatically by the yeast (Schreier et al., 1976; Stevens et al., 1969; Usseglio-Tomasset and Bosia, 1978; Van Wyk et al., 1967; Webb, 1973). Ethyl esters of straight-chain, saturated fatty acids and acetate esters of higher alcohols predominate, since these compounds are present in high concentrations in the fermenting medium and within the yeast cell (Majaama, 1978; Nelson and Wheeler, 1939; Nordstrom, 1963, 1964a). These esters have pleasant, fruitlike aromas which are particularly pronounced in new wines.

While the enzymatic mechanism of formation of esters and the factors affecting the quantities synthesized have been studied extensively in wine, beer, and synthetic media, little work has been done on their fates following fermentation or on the rates at which they are hydrolyzed or formed. Studies have been made of the rates of hydrolysis of various esters in strongly acidic media (>85% H_2SO_4), but this is of limited applicability to a buffered, weakly acidic solution such as wine, since the reaction mechanism differs.

The probable mechanism of hydrolysis of esters in wine or solutions modeled after wine is that of Bamford and Tipper (1972) and Isaacs (1974) and is given below. The reaction is entirely reversible, one direction resulting in ester hydrolysis and the other in esterification of the component acid and alcohol, so that factors affecting the reaction rate in one direction will affect the reverse reaction similarily. The catalyst may be either a free hydrogen ion or an undissociated proton of an organic acid.

The first studies of esterification rates in model solutions approximating wine were completed by Ribéreau-Gayon and Peynaud (1936). Polyprotic acids such as tartaric, malic, and succinic esterified more rapidly than the monoprotic acids acetic, propanoic, and butanoic. Generally, the more complicated the acid within each category, that is, the higher its molecular weight, the more slowly it was esterified. Using the equilibrium constant of 4 calculated for esterification by Berthelot (Berthelot and Saint-Gilles, 1962–1963) $[K_e = [\text{ester}][H_2O]/([\text{acid}][\text{alcohol}])]$, they calculated the theoretical limit of esterification and found that none of the acids studied were esterified to that limit.

Nordstrom (1963, 1964a,b) provided a kinetic analysis of ester formation by yeast in synthetic media but employed enzyme kinetics.

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