overnight in 95% ethyl alcohol or by refluxing several hours after the addition of a trace amount of HCl to the reaction mixture.

This is another example of how careful one has to be in flavor research to be alert for artifact formation in the various concentration–extraction processes conducted in order to obtain flavor concentrates for analysis.

Klimes and Lamparsky (1976) recently were able to identify the same three ethers found in this study plus the ethyl ether of *p*-hydroxybenzyl alcohol (IV) in the extract of vanilla beans. Since they used methanol as the extracting solvent, one must assume the ethyl ether (IV) to be a natural component and not an artifact. The concentration of *p*-hydroxybenzyl methyl ether (III) in the beans was reported as being about 200 ppm. In studying the ethanolic extract of vanilla beans, Shiota and Itoga (1975) were able to identify vanilla ethyl ether but gave no evidence of finding the corresponding methyl ether or either of the *p*-hydroxybenzyl ethers.

It should be mentioned that the mass spectrum of vanillyl ethyl ether (II) reported by Shiota and Itoga (1975) is not in agreement with ours and is difficult to interpret. We found the base peak for all four ethers to be the aromatic benzyl/tropylium ion formed by the loss of the alkoxide radical. In the case of the vanillyl ethers this peak was m/e 137 in contrast to the base peak m/e 107 found by Shiota et al. The IR and NMR spectra, however, were in complete agreement.

The thresholds of the ethers were determined by tasting successive dilutions of the compounds, individually in water, using experienced panelists. The level at which one-half of the panelists could detect something other than water was selected as the flavor threshold (Table I).

The compounds were found to have sweet, vanilla-like flavor notes with creamy, coconut secondary flavor characteristics. They contribute a character difference to the extract which some people can readily detect whereas others cannot. The overall impact is much less than vanillin, which is present in much higher concentrations.

LITERATURE CITED

Anwar, M. H., *Anal. Chem.* **35**, 1974 (1963).

Bohnsack, H., *Riechst. Aromen, Koerperpflegem.* **17**, 133 (1967).

Bohnsack, H., *Riechst. Aromen, Koerperpflegem.* **21**, 125 (1971a).

Bohnsack, H., *Riechst. Aromen, Koerperpflegem.* **21**, 163 (1971b).

Bohnsack, H., Seibert, W., *Riechst. Aromen, Koerperpflegem.* **15**, 321 (1965).

De Jonge, J., Bibo, B. H., *Recl. Trav. Chim. Pays-Bas* **74**, 1448 (1955).

Gnadinger, C. B., *Ind. Eng. Chem.* **17**, 303 (1925).

Gnadinger, C. B., *Ind. Eng. Chem.* **18**, 588 (1926).

Gobley, M., *J. Pharm. Chim.* **34**, 401 (1858).

Goris, A., *Ind. Parfum.* **2**, 4 (1947).

Klimes, I., Lamparsky, D., *Int. Flavours Food Additives* **7**, 272 (1976).

Mitawa, H., *Bull. Chem. Soc. Jpn.* **27**, 53 (1954).

Ogawa, J., Watanabe, H., Japan Patent 15126 (Sept 26, 1962); *Chem. Abstr.* **59**, 9892f (1963).

Shiota, H., Itoga, K., *Koryo* **No. 113**, 65 (1975).

Steward, F. H., *J. Org. Chem.* **27**, 2662 (1962).

Received for review December 16, 1976. Accepted September 20, 1977.

Comparison of Extracting Solvents for Typical Volatile Components of Eastern Wines in Model Aqueous-Alcoholic Systems

Christine S. Cobb[1] and Maurice M. Bursey*

A model system containing a total of nine (0.028% w/w) previously reported Concord (*Vitis labrusca*) flavor compounds in a 12% (v/v) ethanol–water mixture was extracted with four solvents. The diethyl ether, dichloromethane, 2-methylbutane, and Freon 11 (trichlorofluoromethane) extracts were concentrated by rotary evaporation and analyzed by gas chromatography. Freon 11 extracted and made available for analysis more of the flavor compounds than the other solvents being exceeded by dichloromethane only for 3-methyl-1-butanol. Within the error of the experiment, the presence of 4.4% (w/w) sucrose did not alter extraction and recovery efficiencies.

The most common procedure for studying the volatile components of largely aqueous products such as grape juices and wines involves obtaining a concentrated essence by extraction of the sample with an organic solvent, and removal of most of this solvent, often by rotary evaporation. Although some components are consistently reported by different researchers using this technique with various solvents, certain compounds are unique to each investigation. Table I shows the reporting of four esters, three alcohols, one aldehyde, and one ketone in previous studies of Concord grape juice. Power and Chesnut (1921) and Holley et al. (1955) did not use extraction procedures. Stevens et al. (1965) used diethyl ether solvent and are the sole reporters of 2-methyl-1-propanol, 2-methyl-1-butanol, 1-butanol, no ketones, and no definite aldehydes. Stern et al. (1967) using 2-methylbutane solvent found a unique series of 2-butenoates. Neudoerffer et al. (1965) using ethyl chloride solvent reported 2-butanol and several low molecular weight aldehydes not found by other groups. Although the discrepancy in such reports can perhaps be partially explained by omissions of unidentified peaks, we thought it very likely that the inconsistency follows from significant variation of extraction and recovery efficiencies of the components for different solvents. Each organic solvent may extract components to different extents. Different solvent boiling points may require that extractions be performed at different temperatures. An

---

Venable and Kenan Chemical Laboratories, The University of North Carolina, Chapel Hill, North Carolina 27514.

[1] Present address: Union Carbide Corporation, Bound Brook, N.J. 08805.

0021-8561/78/1426-0197$01.00/0

Table I. Model Compounds Previously Reported in Concord Juice

	Power and Chestnut (1921)	Holley et al. (1955)	Neudoerffer et al. (1965)	Stevens et al. (1965)	Stern et al. (1967)
Ethanol		X	X	X	X
Ethyl butanoate				X	X
2-Methyl-1-propanol				X	
3-Methyl-1-butanol			X	X	
1-Hexanol					X
Benzaldehyde					X
Acetophenone					X
Benzyl formate			X		
2-Phenethyl butanoate			X		
Methyl anthranilate	X	X			X

extraction using a low boiling solvent may need to be performed below room temperature; this, then, would be the normal condition for use of this solvent. In addition, differences in retainment of volatile sample components during rotary evaporation may be observed with different solvents. Each solvent must therefore be presumed to distort the true balance of concentrations of flavor components to some degree. If the extraction and recovery efficiency discriminates strongly against extraction of a component, its concentration may be reduced to a level where it is not easily detected. In spite of the inconsistency of reported components when different solvents have been used, there has been no comparative study of the overall efficiency of extraction and recovery of know mixtures of flavor components to resolve the question of the relative merit of each organic solvent as an extractant for unknown samples under the conditions peculiar to each solvent.

Therefore, an evaluation of several solvents used previously for the extraction of grape juices and wines was undertaken. Two artificial systems were prepared to contain known amounts of flavor components of various functional classes and of widely differing volatility which have previously been reported, a material in which we have further interest. One system contained 4.4% (w/w) sucrose to test the effect of sugar on the extraction. Sugars are present in table wines in very low amounts, bracketed by the two artificial systems. The purpose of using artificial systems was to allow judgment of the losses of each component from the amount originally present.

EXPERIMENTAL SECTION

The model systems were composed of a flavor portion (0.028% (w/w) of total) dissolved in a 12% (v/v) ethanol–water mixture. The percentage was chosen because it was the minimum giving facile preparation of homogeneous solutions. To one system was added 4.4% (w/w) sucrose (170 g) as table sugar. One-fifth gallon of each model system was used with each of four solvents. Each model was composed of 3329 mL of distilled water, 454 mL of absolute undenatured ethanol, 0.25 g of methyl anthranilate (Eastman), and 0.10 g of each of the following: ethyl butanoate (Aldrich), 2-methyl-1-propanol (Eastman), 3-methyl-1-butanol (Mallinckrodt), 1-hexanol (Eastman), benzaldehyde (B & A), acetophenone (Baker), benzyl formate (Eastman), and 2-phenethyl butanoate (K & K). The large amount of methyl anthranilate reflects previous knowledge (Stern et al., 1967) that it is a major component. The four solvents were of the highest purity obtainable: diethyl ether, nanograde (Mallinckrodt); dichloromethane, nanograde (Mallinckrodt); 2-methylbutane, 99+% (Aldrich); and Freon 11 (trichlorofluoromethane), 99.9% (Air Products and Chemicals, Inc.). The diethyl ether (B.P. = 34.5 °C) and dichloromethane (B.P. = 40 °C) extractions were performed at 25 °C while the Freon 11 (B.P. = 23.8 °C) extraction was done in a 5 °C cold room. The system for the 2-methylbutane (B.P. = 27.9 °C) extraction was kept at 5 °C and removed as needed throughout the extraction, which was done at 25 °C because of safety restrictions on the use of the cold room.

To perform the extractions for each solvent, one-fifth of each system at a time (757 mL) was placed in a 1000-mL Teflon stop-cocked separatory funnel and extracted with six 50-mL portions of solvent. Each shaking lasted 2 min, and 4 min were allowed for each separation of the layers. Sodium chloride and gentle stirring were needed to facilitate phase separation in the separatory funnel of all extractions. The combined extracts were rotary evaporated at 80 mm of mercury with minimal heat applied to maintain a 25 °C water bath and then the essences were measured and stored at −25 °C in glass vials with Teflon-lined caps until gas chromatographic analysis. An equal amount of the pure solvent was rotary evaporated to about 13 mL to check for concentrated solvent impurities.

The extracts were not dried over the usual drying agents to prevent possible distortions due to selective adsorption. After rotary evaporation the ether essence separated into two layers, presumably ether and extracted water. In the case of 2-methylbutane, the essence similarly separated upon storage at −25 °C. It also formed an insoluble solid upon prolonged contact with air. The Freon 11 essence was initially stable upon storage, but also began to separate if preserved more than a few days. The dichloromethane essence remained stable upon storage for long periods.

Chromatographic Analysis. Triplicate chromatographic analyses were performed on each of the eight extracts plus the blank. A Hewlett-Packard 402 high-efficiency gas chromatograph equipped with flame ionization detection was used. The analyses were performed on a Carbowax 20M glass open tubular SCOT column, of dimensions 70 m × 0.45 mm i.d., prepared from 2.5% Carbowax 20M, 0.2% Silanox 101, and 0.2% benzyltriphenylphosphonium chloride in nanograde dichloromethane. Percentages are in grams per milliliter of CH_2Cl_2. The injection volume used was 20 nL, because a splitter was not used. The initial column temperature (30 °C) was held for 10 min, then it was programmed at 2 °C/min to 150 °C and maintained. Flow rates were: He, 1 mL/min; H_2, 30 mL/min (for FID); air 240 mL/min. The helium flow rate was 1 mL/min. The detector temperature was 180 °C. The same amount (0.02 g) of *n*-tetradecane was added to each essence before injection, in order to adjust peak areas for variations in injection volumes.

RESULTS AND DISCUSSION

The percent recovery of each compound was determined by dividing the weight recovered by the known initial

$$\mathrm{Wt}_i\ \text{recovered} = \frac{(\mathrm{Area}_i)(\mathrm{Att}_i)/(\mathrm{FID}_i)}{(\mathrm{Area}_s)(\mathrm{Att}_s)/(\mathrm{FID}_s)} \times \mathrm{Wt}_s \qquad (1)$$

Table II. Percentage Recoveries of Model Compounds in Nonsugar System

Compound	Bp, °C	Extracts			
		Freon 11	CH_2Cl_2	Ether	Isopentane
Ethyl butanoate	120	66	43		16
2-Methyl-1-propanol	108	34	55	22	32
3-Methyl-1-butanol	131	63	66	50	48
1-Hexanol	158	85	67	23	38
Benzaldehyde	178	83	54	18	26
Acetophenone	202	53	41	34	20
Benzyl formate	203	75	56	21	25
2-Phenethyl butanoate	254	46	48	25	17
Methyl anthranilate	256	62	59	57	27

Table III. Percentage Recoveries of Model Compounds in Sugar System

Compound	Bp, °C	Extracts			
		Freon 11	CH_2Cl_2	Ether	Isopentane
Ethyl butanoate	120	59	42		12
2-Methyl-1-propanol	108	31	49	17	26
3-Methyl-1-butanol	131	58	63	42	47
1-Hexanol	158	82	61	28	34
Benzaldehyde	178	80	53	24	26
Acetophenone	202	55	35	30	16
Benzyl formate	203	67	47	17	25
2-Phenethyl butanoate	254	44	45	21	11
Methyl anthranilate	256	61	56	55	33

weight of that compound and multiplying by 100. The weight recovered as shown in eq 1 was calculated by multiplying the raw area (determined by triangulation) by the attenuation, dividing by flame ionization detector (FID) response factor for that compound, and then dividing this corrected area by the corrected area for the standard and multiplying by the weight of standard added. Flame ionization response factors were computed from data by Stern et al. (1962). The subscripts i and s refer to the ith component and to the standard, respectively.

Tables II and III list the calculated percentage recoveries of the model compounds in both systems by the four solvents. Average values of two runs are entered. The reproducibility from run to run was within 10%. Small solvent impurities did not cause interferences. A result is not entered for ethyl butanoate with the ether solvent because the peak, if present, was obscured by the solvent peak.

The values in both tables indicate that substantial losses can occur during the isolation process, especially for ether and isopentane. At most, 85% of a component was recovered by the extraction–evaporation method. There is an imperfect trend in recovery toward higher yield of the less volatile components for the Freon 11 and dichloromethane. For ether and 2-methylbutane, the recoveries of the less volatile components are lower, despite their lower volatility. The main sources of loss are insufficiently large extraction efficiencies between solvent and aqueous phase and loss of the more volatile components during rotary evaporation of the solvent. On the basis of the foregoing analysis one may speculate that this latter source is more significant for Freon 11 and dichloromethane and that the former may be more important for the observed recovery differences in ether and isopentane.

Few differences in percentage recoveries exist within the relative error of 10% between the sugar and nonsugar systems. In most cases the sugar system yielded slightly lower recoveries.

Overall, the Freon extracted and made available for analysis more of the model compounds than the other solvents examined. Dichloromethane was next best, with results not very different from those for Freon 11. The ether and isopentane solvents were much poorer. The observed low recovery of 2-methyl-1-propanol by Freon 11 is consistent with observations by Hardy (1969) that Freon 11 has low extraction efficiency for alcohols containing four carbon atoms or less and high efficiencies for extraction of alcohols with five carbon atoms or more. This property is an advantage in working with alcoholic systems.

Although Freon 11 must be handled in a cold room, its high percentage recoveries make it the solvent of choice when the essence will be analyzed soon after extraction. If one must store the essence for more than a few days, it would be better to use dichloromethane because its essences remain stable for long periods, and its percentage recoveries are nearly as good as those of Freon 11. Ether and isopentane are not recommended. Having demonstrated that Freon 11 is the solvent of choice for extractive concentration of flavor volatiles from artificial mixtures approximating the volatiles of Concord wines, we intend to use this solvent in a series of studies of the volatiles of eastern grape juices and wines and recommend its use to other workers in the field.

LITERATURE CITED

Hardy, P. J., *J. Agric. Food Chem.* 17, 656 (1969).

Holley, R. W., Stoyla, B., Holley, A. D., *Food Res.* **20**, 326 (1955).

Neudoerffer, T. S., Sandler, S., Zubeckis, E., Smith, M. D., *J. Agric. Food Chem.* 13(6), 584 (1965).

Power, F. B., Chesnut, V. K., *J. Am. Chem. Soc.* **43**, 1741 (1921).

Stern, D. M., Lee, A., McFadden, W. H., Stevens, K. L., *J. Agric. Food Chem.* 15(6), 1100 (1967).

Sternberg, J. C., Gallaway, W. M., Jones, D. T. L., in "Gas Chromatography", Brenner, N., Callen, J. E., Weiss, M. D., Ed., Academic Press, New York, N.Y., 1962, p 231.

Stevens, K. L., Lee, A., McFadden, W. H. Teranishi, R., *J. Food Sci.* 30(6), 1006 (1965).

Received for review October 25, 1974. Resubmitted April 18, 1977. Accepted September 14, 1977. Presented at the Division of Agricultural and Food Chemistry, 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975.