

## Some Benzyl Ethers Present in the Extract of Vanilla (*Vanilla planifolia*)

William G. Galetto\* and Patrick G. Hoffman

A flavorful concentrate was prepared by the continuous pentane extraction of a commercially prepared Bourbon vanilla extract. Combined GLC-MS analysis led to the identification of four benzyl ethers: vanillyl ethyl ether, vanillyl methyl ether, *p*-hydroxybenzyl ethyl ether, and *p*-hydroxybenzyl methyl ether. The methyl ethers were present in trace quantities with the ethyl being major components. However, a direct GLC examination of several commercial vanilla extracts that had not been extracted with pentane showed that *p*-hydroxybenzyl methyl ether is normally the major ether with lesser quantities of vanillyl methyl ether and vanillyl ethyl ether present. No *p*-hydroxybenzyl ethyl ether was detected in the commercial extracts that had not been subjected to liquid-liquid extraction with pentane. The ethers contribute to the overall character of vanilla but do not have the flavor strength and impact of vanillin and are present in much lesser amounts.

Vanilla is the fruit of an orchid plant. Each long slender pod or bean is picked while still green. It then undergoes a drying and curing process during which time its characteristic aroma and flavor are developed. The aqueous-alcohol extraction of the cured beans results in a product with a delicate, complex, and eminently desirable flavor that has been in wide use for centuries. In 1975, 2.12 million pounds of vanilla beans were imported into the U.S., the major portion from Madagascar.

The major flavor constituent of vanilla extract is generally recognized to be vanillin (4-hydroxy-3-methoxybenzaldehyde) which was first isolated from vanilla by Goble in 1858. This material, while very important, alone does not account for the flavor strength and extremely complex nature of this delicate flavoring material.

Surprisingly little has been published on the less abundant, volatile flavor constituents of vanilla. Gnadinger (1925, 1926) noted the presence of 4-methylbenzaldehyde, 4-methoxybenzyl alcohol, piperonal, cinnamic aldehyde, and cinnamic alcohol; Goris (1947) found vanillyl alcohol and 3,4-dihydroxybenzaldehyde in vanilla extracts. In addition to 4-hydroxybenzaldehyde, Anwar (1963) identified 4-hydroxy-3-methoxycinnamic alcohol using paper chromatographic techniques. Bohnsack and Seibert (1965) found 4-hydroxybenzyl alcohol and 4-hydroxybenzyl methyl ether, and several years later (Bohnsack, 1967) reported the presence of isobutyric acid, acetophenone, benzaldehyde, 2,3-butanedione, furfural, and 5-methylfurfural. The same author (Bohnsack, 1971a,b) reported the identification of octanic acid, decanoic acid, *o*-cresol, *p*-cresol, ethyl cinnamate, ethyl octanoate, ethyl decanoate, furfuryl acetate, furfuryl butyrate, and 4-methoxybenzyl acetate. Numerous phenolic acids have also been identified in vanilla extracts.

Subsequent to our completing this work and submitting it for publication, the results of an intensive study on vanilla by two Swiss workers (Klimes and Lamparsky, 1976) were reported. In addition, a study on vanilla bean components (Shiota and Itoga, 1975) became available from the Japanese literature. The latter workers identified, among other components, vanillyl ethyl ether in an extract of Madagascar beans. Klimes and Lamparsky (1976), also working with Bourbon vanilla beans, identified 169 components, most of them being present in a concentration range below 1 ppm in the beans. Included in their findings were the methyl and ethyl ethers of both vanillyl and *p*-hydroxybenzyl alcohols.

Despite the highly popular and desirable flavor characteristics of vanilla, the key constituents responsible for its characteristic and easily recognized flavor remain undefined. We report here the results of our chemical investigations conducted in order to help better understand this complex flavoring material.

### EXPERIMENTAL SECTION

**Preparation of Vanilla Concentrate.** Four liters of 3.33-fold natural vanilla extract prepared from Bourbon beans (Madagascar) was continuously extracted with pentane for 4 weeks. The extracting solvent was changed twice weekly. The combined extracts were concentrated using a rotary vacuum evaporator. Several crops of vanillin crystals were removed prior to reaching a final volume of about 5 mL.

**Preparative Gas Chromatography.** The vanilla concentrate was chromatographed on a Hewlett Packard 5750 gas chromatograph equipped with a thermal conductivity (TC) detector. The injection port temperature was 250 °C and the detector temperature 260 °C. The column was an 8 ft × 0.25 in. glass column packed with 5% Carbowax 20M on Chromosorb W.A.W. The flow rate was approximately 60 mL/min, and the column oven temperature was programmed from 80–230 °C at 4 °C/min.

Peaks of interest were collected from the original pentane concentrate in glass capillary tubes and rechromatographed on a Varian 90P gas chromatograph equipped with a TC detector and a 9 ft × 0.25 in. glass column packed with 5% SF-96 on Chromosorb W.A.W. The injection port temperature was 250 °C, and the flow rate was 40 mL/min, with the column oven temperature being programmed from 90 to 220 °C using a programmer power setting of 40%. Samples purified in this manner were again collected in glass capillary tubes prior to further spectroscopic analysis.

**Spectroscopic Analysis.** Infrared (IR) analyses were run on a Perkin-Elmer Model 257 spectrometer equipped with a 4X beam condenser and etched NaCl and KBr plates or KBr pellets.

Mass spectra (GC-MS) were obtained on a du Pont Model 490 mass spectrometer equipped with a solid probe inlet system and coupled to a Varian 1400 FID gas chromatograph. Spectra were scanned at a rate of 4 s/decade and at an ionizing voltage of 70 eV and recorded on a Bell & Howell Model 50134 datagraph oscilloscopic recorder.

The NMR spectra were obtained using either a Varian A60 or EM360 60 MHz spectrometer using CDCl<sub>3</sub> as solvent and Me<sub>4</sub>Si as the internal standard.

Research and Development Laboratories, McCormick & Co., Inc., Hunt Valley, Maryland 21031.

**Reference Compounds. General Synthetic Method.**

The etherification method employed was that of Steward (1962) using a water- and alcohol-washed, acidic ion-exchange resin as a catalyst. It was found to be more advantageous to run several small reactions rather than a large-scale preparation due to the tendency of the crude product to polymerize during extended workup. Each reaction utilized 30–40 mmol of *p*-hydroxybenzyl alcohol or vanillyl alcohol (Aldrich Chemical Co.), 20–25 mL of the etherifying alcohol, either anhydrous methanol or ethanol, and 6–8 g of Dowex 50W-X8 ion-exchange resin. The reactions were run in a stoppered flask at room temperature with occasional swirling. Two to four days were required for complete etherification which was monitored by thin-layer chromatography (Silica Gel, Eastman, 3:1 petroleum ether–ether). The reaction mixture was filtered, the resin was washed with the appropriate etherifying alcohol, and the combined solvent was removed under reduced pressure. The product was immediately purified by column chromatography.

**Vanillyl Methyl Ether (I)** (yield, 39%). IR (liquid) 3400, 3070, 1608, 1515, 1465, 1455, 1430, 1368,  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  3.36 (3 H, s), 3.82 (3 H, s), 4.36 (2 H, s), 6.07 (1 H, s), 6.80 (3 H, m); UV  $\lambda$  max (MeOH) 280 ( $\epsilon$  2700); 239 nm ( $\epsilon$  6600); MS [ $m/e$  (%)] 168 (69), 167 (18), 137 (100), 122 (16), 45 (34), 43 (15), 32 (23), 31 (70), 28 (57).

**Vanillyl Ethyl Ether (II)** (yield, 53%). IR (liquid) 3415, 3070, 1608, 1515, 1465, 1455, 1433, 1372, 1352  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  1.21 (3 H, t), 3.51 (2 H, q), 3.83 (3 H, s), 4.42 (2 H, s), 6.09 (1 H, s), 6.84 (3 H, m); UV  $\lambda$  max (EtOH) 282 ( $\epsilon$  2800), 233 nm ( $\epsilon$  6800); MS [ $m/e$  (%)] 182 (60), 138 (29), 137 (100), 122 (16), 93 (20), 65 (18), 28 (35).

***p*-Hydroxybenzyl Methyl Ether (III)** (yield, 64%). Mp 83.5–86.0 °C (lit. 82.5–83.5 °C De Jonge and Bibo, 1955; 85–86 °C, Ogawa and Watanabe, 1962); IR (KBr pellet) 3290, 3015, 1613, 1595, 1512, 1445, 1439, 1385, 1350, 1340  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  3.38 (3 H, s), 4.39 (2 H, s), 6.10 (1 H, s), 6.74 (2 H, d), 7.20 (2 H, d); UV  $\lambda$  max (MeOH) 280 nm ( $\epsilon$  1500), 229 nm ( $\epsilon$  9000); MS [ $m/e$  (%)] 138 (71), 137 (37), 107 (100), 106 (24), 77 (15).

***p*-Hydroxybenzyl Ethyl Ether (IV)** (yield 42%). Mp 50.5–53.5 °C (lit. 50–51 °C, Steward, 1962, Mitawa, 1954; 56.5–57.5 °C, De Jonge and Bibo, 1955); IR (KBr pellet) 3240, 3013, 1610, 1596, 1512, 1444, 1343  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  1.20 (3 H, t), 3.54 (2 H, q), 4.42 (2 H, s), 6.08 (1 H, s), 6.72 (2 H, d), 7.18 (2 H, d); UV  $\lambda$  max (EtOH) 280 nm ( $\epsilon$  1500), 230 nm ( $\epsilon$  9000); MS [ $m/e$  (%)] 152 (52), 123 (18), 108 (20), 107 (100), 106 (23), 95 (15), 77 (18), 28 (21).

**Purification by Column Chromatography.** A glass column (20 mm  $\times$  1000 mm) was packed with 150 g of dry 60–200 mesh Silica Gel (W.R. Grace) and washed with petroleum ether. The combined crude product from two separate reactions (7–10 g) was dissolved in 20 mL of diethyl ether and placed on the column which was then eluted with petroleum ether containing increasing larger amounts of diethyl ether. The fractions, as checked by thin-layer chromatography, containing a single compound were combined, filtered, and concentrated at vacuum pump pressures to yield the pure products. In each case, the IR, UV, MS and NMR spectra were in full accord with the proposed structures.

**RESULTS AND DISCUSSION**

The initial isolation and identification work was conducted on the pentane concentrates which had a powerful, characteristic vanilla aroma. GC-MS and NMR analyses of one of the more abundant purified components indicated the material to be vanillyl ethyl ether (II). A second component was subsequently identified, based primarily

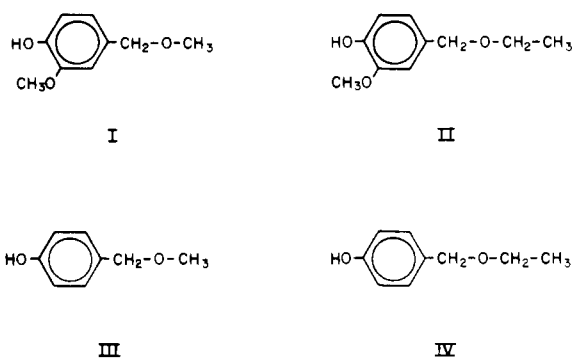
**Table I. Approximate Concentration and Flavor Threshold of Vanilla Benzyl Ethers**

Compound	Rel. ret. time <sup>a</sup>	Approx. concn in vanilla, ppm <sup>b</sup>	Threshold tasted in water, ppm
I. Vanillyl methyl ether	0.85	13	2.0
II. Vanillyl ethyl ether	0.87	3	1.0
III. <i>p</i> -Hydroxybenzyl methyl ether	0.96	143	0.1
IV. <i>p</i> -Hydroxybenzyl ethyl ether	0.98		0.1

<sup>a</sup> Carbowax 20M, vanillin = 1.00. <sup>b</sup> Reported on a onefold basis.

on MS data, as *p*-hydroxybenzyl ethyl ether (IV). The two corresponding methyl ethers (I and III) were also identified; they were present only in trace concentrations. All identifications were confirmed by comparisons with authentic samples (see Experimental Section) using retention time and spectroscopic information.

Bohnsack and Seibert (1965) had previously found *p*-hydroxybenzyl methyl ether (III) to be present in Bourbon vanilla beans. They made no mention of the other aromatic ethers (I, II, and IV). The interpretation



of our results was perplexing in that Bohnsack and Seibert, while giving no quantitative data, were able to confirm their identification of *p*-hydroxybenzyl methyl ether by melting point and mixed melting point, indicating at least a moderate amount being present in the extract under investigation. Our pentane concentrate contained only trace amounts of methyl ether (III) and much larger amounts of the ethyl ethers.

In order to get more accurate qualitative and quantitative data on an unaltered extract, samples of two tenfold and a 12.5-fold commercially prepared vanilla extracts were analyzed by GLC using direct injection thus avoiding any concentration-extraction steps. The results (Table I) clearly show that *p*-hydroxybenzyl methyl ether (III) is indeed the major naturally occurring ether in vanilla. Its average concentration (on a onefold basis) was 143 ppm with a range of 115 to 187 ppm. The vanillyl methyl and ethyl ethers (I and II) were present in much lesser quantities. No *p*-hydroxybenzyl ethyl ether (IV) was detected in the vanilla samples analyzed by direct injection.

The abundance of the ethyl ethers in the pentane concentrate can possibly be explained by either etherification of the free vanillyl and *p*-hydroxybenzyl alcohol present in the extract and/or by transesterification of the methyl ethers present in vanilla. Both processes are likely to happen because of the large quantities of ethyl alcohol present, but the latter best explains why only trace amounts of the methyl ethers could be detected in the pentane concentrate.

We, in fact, were able to partially convert *p*-hydroxybenzyl methyl ether to the ethyl ether by simply refluxing

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, Koerperpflagem.* **17**, 133 (1967).  
Bohnsack, H., *Riechst. Aromen, Koerperpflagem.* **21**, 125 (1971a).  
Bohnsack, H., *Riechst. Aromen, Koerperpflagem.* **21**, 163 (1971b).  
Bohnsack, H., Seibert, W., *Riechst. Aromen, Koerperpflagem.* **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 15 126 (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<sup>1</sup> and Maurice M. Burse<sup>\*</sup>

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-butenolates. 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.

<sup>1</sup>Present address: Union Carbide Corporation, Bound Brook, N.J. 08805.