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Sweetness and Bitterness of Some Aliphatic α,ω -Glycol D-Glucopyranosides

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Mono- and di-*O*- β -D-glucopyranosides containing a hydrophobic aglycon consisting of aliphatic α,ω -glycols of the 3C, 4C, 6C, and 8C series were prepared and tasted. 2-Hydroxyethyl mono-*O*- α - and - β -D-glucopyranosides and allyl mono-*O*- α -D-glucoside were definitely sweet; however, 2-hydroxyethyl mono-*O*- β -D- and allyl mono-*O*- α -D-glucosides gave a bitter aftertaste. The hydroxyalkyl mono-*O*- β -D-glucosides with extended alkylene chains and allyl mono-*O*- β -D-glucopyranoside were bitter with no sweetness. The crystalline di-*O*- β -D-glucosides with extended alkylene chains (4C, 6C, and 8C) and the noncrystalline 3-hydroxypropyl mono-*O*- β -D-glucoside were water soluble but tasteless. 1,4-Anhydroerythrityl mono-*O*- β -D-glucoside was bitter and not sweet.

The search for stereochemical and structural requirements for a compound to elicit an intensely sweet taste has produced recognition of the importance of a hydrophobic or lipophilic site in the sweet molecule in addition to specifically oriented hydrophilic sites (Deutsch and Hansch, 1966; Kier, 1972). The binary hydrogen-bonding theory of sweet taste induction (Shallenberger and Acree, 1969, 1971) has been extended to include a third hydrophobic bonding area spaced away from the hydrogen-bonding sites (Shallenberger and Birch, 1975; Shallenberger and Lindley, 1977; Van Der Heijden et al., 1978; Shallenberger, 1980). Hodge and Inglett (1974) correlated the structures of five intensely sweet glycosides of botanical origin and pointed to a widely extended hydrophobic area between polar hydrophilic end groups that was common to all five sweet glycosides. Whether the dispersed hydrophobic area requires dimensional and spatial specificity needs to be examined more fully.

The objective of this investigation was to synthesize mono- and diglucosides of a series of alkanediols which contain hydrophilic-hydrophobic-hydrophilic arrangements of the functional groups for structure-taste correlations. Several new diol glucosides were prepared for this purpose.

EXPERIMENTAL SECTION

Preparative reactions were monitored by thin-layer chromatography (TLC). Purity of the compounds was established by TLC, gas-liquid chromatography (GLC), melting point (mp), and elemental analyses. TLC was conducted on 0.25-mm layers of EM Reagent silica gel G

(Brinkmann Instruments, Inc.) with air-dried plates. The spots were detected by spraying with 5% ethanolic sulfuric acid and charring. TLC was performed with 50% ethyl acetate-benzene (v/v) for acetylated compounds and 23:10:2 methyl ethyl ketone-water-absolute ethanol (v/v) for deacetylated compounds. The acetylated glucosides were isolated by dry column chromatography on silica gel G (type 60, EM Reagents, EM Laboratories, Inc., Elmsford, NY) (5% water), using a 2.5 \times 85 cm column, with 50% ethyl acetate-benzene and 70% ethyl acetate-hexane (v/v) as the eluant. Deacetylation was performed with sodium methoxide in dry methanol solutions (Thompson and Wolfrom, 1963), and the solution was deionized by stirring with methanol-washed Amberlite IR-120 (H⁺) ion-exchange resin (Rohm and Haas Co., Philadelphia, PA.). GLC analyses of trimethylsilyl ethers (Sweeley et al., 1963) of the glucosides were recorded on an F and M Model 700 laboratory chromatograph with a flame-ionization detector, which was fitted with a 1/8 in. \times 6 ft stainless steel column containing 3% JXR silicone gum on 100-120-mesh Gas-Chrom Q support (Anspec, Ann Arbor, MI). Single symmetrical peaks were obtained. ¹H NMR spectra were recorded with a Varian Model HA-100 spectrometer: resonances were identified by spin-decoupling experiments and chemical shifts are referred to internal tetramethylsilane. Products were vacuum-dried in the presence of phosphorus pentoxide for 24-48 h at room temperature before analyses. Melting points, measured in capillary tubes, are not corrected.

Mono- and di-*O*- β -D-glucopyranosides (4, 6, 8, 13, 15, 17, and 19) were prepared by a modification of the procedure of Schroeder et al. (1974). Modification involved a prolonged reaction time (18 h at 25 °C) in purified 1,4-dioxane (Wiberg, 1960) containing Drierite (W. A. Hammond Drierite Co., Xenia, OH). Mono- and di-*O*-tetra-*O*-acetyl- β -D-glucopyranosides (3, 5, 7, 12, 14, 16, and 18) were

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isolated by dry-column chromatography and deacetylated as described above.

1,2-Ethanediol Mono-*O*- α -D-glucopyranoside and Mono-*O*- β -D-glucopyranoside (1 and 2). Compounds 1 and 2 were obtained from the work of Otey et al. (1965); however, ^1H NMR data and taste testing are original. The ^1H NMR data are as follows: for 1 (α) ($\text{C}_5\text{D}_5\text{N}$), δ 5.35 (d, H-1, $J = 4.0$ Hz); for 2 (β) ($\text{C}_5\text{D}_5\text{N}$), δ 4.54 (d, H-1, $J = 7.0$ Hz).

1,3-Propanediol 2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranoside (3). An 11.2% yield of 3; crystallized from ethyl acetate-hexane; mp 96.5–98 °C; $[\alpha]_{\text{D}}^{20} -26^\circ$ (c 0.5, chloroform); ^1H NMR data (CDCl_3) δ 4.54 (d, H-1, $J = 7.0$ Hz), 1.82 (two methylene protons). Anal. Calcd for $\text{C}_{17}\text{H}_{26}\text{O}_{11}$: C, 50.3; H, 6.45. Found: C, 50.1; H, 6.55.

1,3-Propanediol Mono-*O*- β -D-glucopyranoside (4). 84% yield of 4 (syrup); $[\alpha]_{\text{D}}^{20} -37.5^\circ$ (c 1, methanol); ^1H NMR data ($\text{C}_5\text{D}_5\text{N}$) δ 7.20 (d, H-1, $J = 7.0$ Hz), 2.04 (two methylene protons). Anal. Calcd for $\text{C}_9\text{H}_{18}\text{O}_7$: C, 45.4; H, 7.62. Found: C, 45.6; H, 7.53.

1,4-Butanediol 2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranoside (5). A 17.5% yield of 5; recrystallized from ethyl acetate-petroleum ether; mp 78–79 °C; $[\alpha]_{\text{D}}^{20} -25^\circ$ (c 0.5, chloroform); reported (Bhattacharyya et al., 1976) yield 12.5%; mp 71–73 °C; $[\alpha]_{\text{D}}^{24} -22.2^\circ$ (c 0.95, chloroform); ^1H NMR data (CDCl_3) δ 4.48 (d, H-1, $J = 7.0$ Hz), 1.63 (four methylene protons). Anal. Calcd for $\text{C}_{18}\text{H}_{28}\text{O}_{11}$: C, 51.20; H, 6.71. Found: C, 51.35; H, 6.88.

1,4-Butanediol Mono-*O*- β -D-glucopyranoside (6). A 73% yield of 6, recrystallization from methanol-ethyl acetate; mp 101–102 °C; $[\alpha]_{\text{D}}^{20} -39^\circ$ (c 0.5, water); reported (Bhattacharyya et al., 1976) mp 99–100 °C; $[\alpha]_{\text{D}}^{24} -37^\circ$ (c 0.85, methanol); ^1H NMR data ($\text{Me}_2\text{SO}-d_6$) δ 4.35 (d, H-1, $J = 7.0$ Hz), 1.73 (four methylene protons). Anal. Calcd for $\text{C}_{10}\text{H}_{20}\text{O}_7$: C, 47.62; H, 7.93. Found: C, 47.49; H, 7.95.

1,6-Hexanediol 2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranoside (7). A 14.2% yield of 7; recrystallized from ethyl acetate-hexane; mp 72–73 °C; $[\alpha]_{\text{D}}^{20} -18^\circ$ (c 0.5, chloroform); reported (Bhattacharyya et al., 1976) yield 11.2%; mp 58–60 °C; $[\alpha]_{\text{D}}^{24} -20.5^\circ$ (c 0.95, chloroform); ^1H NMR data (CDCl_3) δ 4.44 (d, H-1, $J = 7.0$ Hz), 1.40 (eight methylene protons). Anal. Calcd for $\text{C}_{20}\text{H}_{32}\text{O}_{11}$: C, 53.56; H, 7.19. Found: C, 53.69; H, 7.34.

1,6-Hexanediol Mono-*O*- β -D-glucopyranoside (8). A 58% yield of 8; recrystallization from methanol-anhydrous ether; mp 109.5–110.5 °C; $[\alpha]_{\text{D}}^{20} -32^\circ$ (c 0.5, water); reported (Bhattacharyya et al., 1976) mp 105–107 °C; $[\alpha]_{\text{D}}^{24} -35^\circ$ (c 0.85, methanol); ^1H NMR data ($\text{Me}_2\text{SO}-d_6$) δ 4.08 (d, H-1, $J = 7.0$ Hz), 1.40 (eight methylene protons). Anal. Calcd for $\text{C}_{12}\text{H}_{24}\text{O}_7$: C, 51.42; H, 8.83. Found: C, 51.34; H, 8.87.

Allyl Mono-*O*- α -D-glucopyranoside and Mono-*O*- β -D-glucopyranoside (9 and 10). Compounds 9 and 10 were obtained from the work of Otey et al. (1972); however, the ^1H NMR data and taste testing are original. ^1H NMR data are as follows: for 9 (α) ($\text{C}_5\text{D}_5\text{N}$), δ 5.30 (d, H-1, $J = 3.0$ Hz), 5.98 (vinyl methine proton), 5.45, 5.30, 5.15, and 5.04 (two vinyl methylene protons); for 10 (β) ($\text{C}_5\text{D}_5\text{N}$), δ 4.82 (d, H-1, $J = 7.0$ Hz), 6.03 (vinyl methine proton), 5.45, 5.30, 5.18, and 5.08 (two vinyl methylene protons).

Glycerol Mono-*O*- β -D-glucopyranoside (11). Bourquelot et al. (1915, 1917) reported 11, a crystalline compound found to possess a sweet taste, which was followed by a bitter aftertaste.

1,4-Anhydroerythrityl 2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranoside (12). A 13.6% yield of 12; crystallized from ethyl acetate-petroleum ether; mp 127–129 °C; $[\alpha]_{\text{D}}^{20} -4^\circ$ (c 0.5, chloroform); ^1H NMR data (CDCl_3) δ 4.57 (d,

H-1, $J = 7.0$ Hz). Anal. Calcd for $\text{C}_{18}\text{H}_{26}\text{O}_{12}$: C, 49.65; H, 6.25. Found: C, 49.78; H, 6.11.

1,4-Anhydroerythrityl Mono-*O*- β -D-glucopyranoside (13). A 78.3% yield of 13; crystallization from methanol-anhydrous ether; mp 172–174 °C; $[\alpha]_{\text{D}}^{20} -17^\circ$ (c 0.5, water); ^1H NMR data ($\text{C}_5\text{D}_5\text{N}$) δ 5.05 (d, H-1, $J = 7.0$ Hz). Anal. Calcd for $\text{C}_{10}\text{H}_{18}\text{O}_8$: C, 45.32; H, 6.79. Found: C, 45.22; H, 6.91.

1,4-Butanediol Di-*O*-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside (14). A 11.2% yield of 14; recrystallization from ethyl acetate-petroleum ether; mp 137.5–139 °C; $[\alpha]_{\text{D}}^{20} -28^\circ$ (c 0.5, chloroform); reported (Bhattacharyya et al., 1976) yield 10%; mp 139–140 °C; $[\alpha]_{\text{D}}^{24} -30^\circ$ (c 0.95, chloroform); ^1H NMR data (CDCl_3) δ 4.44 (d, H-1, $J = 7.0$ Hz), 1.60 (four methylene protons). Anal. Calcd for $\text{C}_{32}\text{H}_{46}\text{O}_{20}$: C, 51.20; H, 6.18. Found: C, 51.23; H, 6.38.

1,4-Butanediol Di-*O*- β -D-glucopyranoside (15). A 63% yield of 15; recrystallization from methanol-anhydrous ether; mp 182–183 °C; $[\alpha]_{\text{D}}^{20} -31.4^\circ$ (c 0.35, water); reported (Bhattacharyya et al., 1976) mp 173–175 °C; $[\alpha]_{\text{D}}^{24} -68.5^\circ$ (c 0.85, methanol); ^1H NMR data ($\text{Me}_2\text{SO}-d_6$) δ 4.54 (d, H-1, $J = 7.0$ Hz), 1.75 (four methylene protons). Anal. Calcd for $\text{C}_{16}\text{H}_{30}\text{O}_{12}$: C, 46.37; H, 7.30. Found: C, 46.26; H, 7.42.

1,6-Hexanediol Di-*O*-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside (16). A 10.7% yield of 16; recrystallization from ethyl acetate-hexane; mp 143–144 °C; $[\alpha]_{\text{D}}^{20} -24^\circ$ (c 1, chloroform); reported (Bhattacharyya et al., 1976) 9.5% yield; mp 118–120 °C; $[\alpha]_{\text{D}}^{24} -27^\circ$ (c 0.95, chloroform); ^1H NMR data (CDCl_3) δ 4.45 (d, H-1, $J = 7.0$ Hz), 1.44 (eight methylene protons). Anal. Calcd for $\text{C}_{34}\text{H}_{50}\text{O}_{20}$: C, 52.44; H, 6.47. Found: C, 52.56; H, 6.34.

1,6-Hexanediol Di-*O*- β -D-glucopyranoside (17). A 74.1% yield of 17; recrystallization from methanol-anhydrous ether; mp 149.5–150 °C; $[\alpha]_{\text{D}}^{20} -69^\circ$ (c 0.5, water); reported (Bhattacharyya et al., 1976) mp 138–140 °C; $[\alpha]_{\text{D}}^{24} -54.5^\circ$ (c 0.85, methanol); ^1H NMR data ($\text{Me}_2\text{SO}-d_6$) δ 4.07 (d, H-1, $J = 7.0$ Hz), 1.40 (eight methylene protons). Anal. Calcd for $\text{C}_{18}\text{H}_{34}\text{O}_{12}$: C, 48.86; H, 7.75. Found: C, 48.79; H, 7.86.

1,8-Octanediol Di-*O*-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosides (18). A 13.2% yield; crystallization from ethyl acetate-petroleum ether; mp 98–99 °C; $[\alpha]_{\text{D}}^{20} -16^\circ$ (c 0.5, chloroform); ^1H NMR data (CDCl_3) δ 4.45 (d, H-1, $J = 7.0$ Hz), 1.45 (12 methylene protons). Anal. Calcd for $\text{C}_{36}\text{H}_{54}\text{O}_{20}$: C, 53.59; H, 6.75. Found: C, 53.51; H, 6.76.

1,8-Octanediol Di-*O*- β -D-glucopyranoside (19). A 68% yield of 19; crystallization from methanol-anhydrous ether; mp 127.5–128.5 °C; $[\alpha]_{\text{D}}^{20} -13^\circ$ (c 0.5, water); ^1H NMR data ($\text{C}_5\text{D}_5\text{N}$) δ 4.82 (d, H-1, $J = 7.0$ Hz), 1.45 (12 methylene protons). Anal. Calcd for $\text{C}_{20}\text{H}_{38}\text{O}_{12}$: C, 51.06; H, 8.14. Found: C, 51.09; H, 8.18.

RESULTS AND DISCUSSION

Several simple molecules and some glycosides of botanical origin (Hodge and Inglett, 1974) that are much sweeter than sucrose show an important hydrophobic section centered between two polar hydrophilic functional groups. Therefore, roughly similar mono- and di-*O*- β -D-glucopyranosides of aliphatic α,ω -glycols of the 3C, 4C, 6C, and 8C series were prepared to test the structural requirements for a hydrophobic glycoside to elicit a sweet taste response. The alkanediols were limited to those whose glucosides will retain water solubility with gradually increased chain length of the aglycon. Other aglycons surveyed were derived from allyl alcohol, glycerol, and 1,4-anhydroerythritol.

Structural features of the compounds tested are shown in Figure 1. The relative sweetness and bitterness of the

Table I. Structural Features of Mono- and Di-*O*- β -D-Glucopyranosides

compounds	hydrophilic radical	hydrophobic radical	hydrophilic radical of aglycon	taste ^a
mono-<i>O</i>-D-glucosides				
1, C ₈ H ₁₆ O ₇	α -D-glucopyranosyloxy	2-hydroxyethyl ^b	OH	S
2, C ₈ H ₁₆ O ₇	β -D-glucopyranosyloxy	2-hydroxyethyl ^b	OH	SB
4, C ₉ H ₁₈ O ₇	β -D-glucopyranosyloxy	3-hydroxypropyl ^c	OH	T
6, C ₁₀ H ₂₀ O ₇	β -D-glucopyranosyloxy	4-hydroxybutyl	OH	BB
8, C ₁₂ H ₂₄ O ₇	β -D-glucopyranosyloxy	6-hydroxyhexyl	OH	BB
9, C ₉ H ₁₆ O ₆	α -D-glucopyranosyloxy	allyl ^d		SB
10, C ₉ H ₁₆ O ₆	β -D-glucopyranosyloxy	allyl ^d		B
11, C ₉ H ₁₈ O ₈	β -D-glucopyranosyloxy	1,2-hydroxyglyceryl ^e	OH	SB ^e
13, C ₁₀ H ₁₈ O ₈	β -D-glucopyranosyloxy	1,4-anhydro-3-hydroxyerythrityl	OH	B
di-<i>O</i>-D-glucosides				
15, C ₁₆ H ₃₀ O ₁₂	β -D-glucopyranosyloxy	tetramethylene	β -D-glucopyranosyloxy	T
17, C ₁₈ H ₃₄ O ₁₂	β -D-glucopyranosyloxy	hexamethylene	β -D-glucopyranosyloxy	T
19, C ₂₀ H ₃₈ O ₁₂	β -D-glucopyranosyloxy	octamethylene	β -D-glucopyranosyloxy	T

^a B = bitter; BB = intensely bitter; S = sweet; SB = sweet, bitter after taste; T = tasteless. ^b Reference is Otey et al. (1965); tasting is original. ^c This compound is noncrystalline. ^d Reference is Otey et al. (1972); tasting is original. ^e Reference is Bourquelot et al. (1915, 1917).

R	R'	n	Taste ^f	
R—O—(CH ₂) _n —O—R'				
1	α -gluc	H	2	S
2	β -gluc	H	2	SB
4	β -gluc	H	3	T
6	β -gluc	H	4	BB
8	β -gluc	H	6	BB
15	β -gluc	β -gluc	4	T
17	β -gluc	β -gluc	6	T
19	β -gluc	β -gluc	8	T
R—O—CH ₂ —CH=CH ₂				
9	α -gluc			SB
10	β -gluc			B
R—O—CH ₂ —CHOH—CH ₂ OH				
11	β -gluc			SB ^g

13 β -gluc B

^f B=bitter; BB=intensely bitter; S=sweet; SB=sweet, bitter after taste; T=tasteless.
^g Ref., Bourquelot et al. (1915, 1917).

Figure 1. Structural features and taste results of compounds with increased chain length or allyl, glyceryl, and 1,4-anhydroerythrityl aglycons.

mono- and di-*O*- β -D-glucopyranosides having a hydrophobic aglycon center terminated with one or two hydrophilic centers are reported in Table I. The informal taste testing was accomplished by placing a few milligrams of each glucoside on the tip of the tongue; however, the sweetness of 2-hydroxyethyl mono-*O*- β -D-glucosides and allyl α -D-glucosides were compared with equilibrated D-glucose and methyl α -D-glucoside at 4% concentrations by the standard method of Swartz and Furia (1977). Taste responses were recorded as sweet (S), bitter (B), intensely bitter (BB), and tasteless (T) (Table I). Although methyl α -D- and β -D-glucosides are well-known to be both sweet and bitter, 2-hydroxyethyl α -D-glucoside 1 was found to be sweet with no bitterness. 2-Hydroxyethyl β -D-glucoside 2 was found to be as sweet as equilibrated D-glucose and methyl α -D-glucoside solutions at 4% concentrations with bitter aftertaste. Here a flexible hydrophobic ethyl group stands between a hydrophilic center. On the other hand, compounds 6 and 8 with longer hydroxyalkyl chains were intensely bitter and not sweet. With the addition of more than two methylene groups in the hydrophobic aglycon, sweetness is lost. However, the noncrystalline water-sol-

uble 1,3-propanediol mono-*O*- β -D-glucopyranoside (4) with three methylene groups in the hydrophobic aglycon was tasteless. The monoglucoside of 1,4-anhydroerythritol (13) contains two inflexible methylene groups between hydroxyl groups in the aglycon (Figure 1). It was not sweet, but it was less bitter than compounds 6 and 8. Bourquelot et al. (1915, 1917) reported a crystalline mono-*O*- β -D-glucoside of glycerol (11) (Figure 1), which possessed a sweet taste followed by a bitter aftertaste. However, compound 11 does not possess a hydrophobic aglycon, which limits its solubility in water.

The mono-*O*- α -D-glucoside of allyl alcohol (9) (Figure 1) having a hydrophobic aglycon terminated with a vinyl group instead of a hydroxyl group was sweet with a bitter aftertaste, whereas the β anomer (10) (Figure 1) was bitter with no sweetness. Allyl α -D-glucoside was found to be half as sweet as equilibrated D-glucose and methyl α -D-glucoside solutions at 4% concentrations despite the bitter aftertaste in the allyl α -D-glucoside solution. The carbon-carbon double bond of the vinylic group of 9 and 10 may serve as a B in the AH,B system of Shallenberger and Acree (1969) to induce a sweet taste response (Shallenberger and Acree, 1969; Acton et al., 1970; Kier, 1972). The vinylic group may also induce a bitter taste response. Apparently, the hydrophobic three-carbon aglycon in allyl α -D-glucoside resulted in a decrease in sweetness and increased bitterness relative to an equilibrated D-glucose solution at 4% concentration. The water-soluble di-*O*- β -D-glucosides 15, 17, and 19 (Figure 1) were tasteless (Table I).

Although melting points for our compounds 5-8 and 15-17 are much higher than those reported by Bhattacharyya et al. (1976), specific rotations for our compounds 5-8 and 14-17 differ slightly. Compounds 3, 4, 12, 13, 18, and 19 are new. The series of glycol glucosides does not include the α anomers beyond $n = 2$. These should be prepared in future work, because the α anomer of the allyl glucosides was found to be sweet with bitter aftertaste whereas the β anomer was not sweet.

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Identification of New Volatile Amines in Grapes and Wines

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A number of amines were identified for the first time in grapes. These include methylamine, dimethylamine, ethylamine, diethylamine, *n*-propylamine, isobutylamine, α -amylamine, isoamylamine, pyrrolidine, and 2-phenethylamine. The trifluoroacetamides of the isolated amines were separated on Carbowax 20M or SE-54 fused silica capillary columns and identified by retention times and mass spectra. Two amines, diethyl and α -amyl, were identified in wine for the first time. Mass spectra of the pure TFA derivatives of these amines are given.

In a recent review, Schreier (1979) compiled a list of the volatile amines found in wines. The volatile amines he summarized plus others detected in wines are given in Table I.

An extensive literature search showed no reports of volatile amines being present in grapes. Maga (1978), in his extensive review of amines in foods, including fruits, did not reference any amines in grapes. Smith (1980), in summarizing the volatile amines found in plants, made no reference to the *Vitis* species.

Numerous methods for isolation and determination of amines have been proposed and reported on by Singer and Lijinsky (1976). They prepared the tosylamide derivatives and used GC-MS for separation and identification. Puputti and Suomalainen (1969) extracted with organic solvent and chromatographed on thin layer and used GC for further identification of the volatile amines. Neurath et al. (1977) steam distilled the samples from basic solution and trapped the distillate in acid solution. Derivatization was by trifluoroacetic anhydride (TFA). This system was first reported by Pailer and Hübsch (1966). Neurath et al. (1977) went on to trap the amine TFA derivatives on ion-exchange columns to purify and separate.

The purpose of this research was to isolate and identify volatile amines in grapes and wines.

MATERIALS AND METHODS

Grapes. The grapes for these experiments were obtained from the experimental vineyards at Davis and at

Oakville of the Department of Viticulture and Enology, University of California. Grapes were harvested from 20 to 26 °Brix and crushed, and the juice was separated and quickly frozen prior to analysis.

Wines. Crushed grapes and wines were treated in the normal accepted manner. Sulfur dioxide additions and additions of a pure yeast starter of *Saccharomyces cerevisiae* were made at the usual times with the standard amounts. Wines were cellared at 11 °C for up to periods of 1 year. Juice samples taken were frozen prior to analysis [see Ramey and Ough (1980) for the usual fermentation treatment conditions].

Separation and Derivatization. One-liter samples of grape juice or wine were used for each analysis. The samples were treated as described by Daudt and Ough (1980). The method involves vacuum distillation of the volatile amines from the sample (made basic) into a trapping solution of HCl, vacuum concentration on a rotary evaporator of the amine salts, derivatization of the salts with trifluoroacetic anhydride, extraction of the HCl and trifluoroacetic acid with bicarbonate solution, extraction of the amine TFA derivatives into ethyl ether, drying the ethyl ether, and concentration of the extract on a micro-Kontes evaporator to 1 mL.

Detection. One to three microliters of the concentrated sample was injected onto a 25 m \times 0.20 mm i.d. fused silica column coated with Carbowax 20M with a 1:80 split ratio with an appropriate 90-min temperature program. Equipment used was a Hewlett-Packard 5710A GC with a N/P detector. The chromatograms showed only the amine derivatives and the solvent peaks. Sensitivity was excellent and background was minimal.

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