Table II. Alkaloid and NNN Levels in High Nornicotine Tobacco Varieties (ppm)

tobacco	nicotine	nornicotine	NNN
TI-1112	4 800	31 400	1000
V8	13 300	3 700	70
V18	43 800	6 700	142
V48	25 600	600	169
V445	4 000	2 700	100
V446	13 900	10 800	ND[a]

[a] ND = none detected.

nicotine to nornicotine and thus have ratios of nornicotine:nicotine significantly larger. These results are given in Table II. Again, there appeared to be no correlation of nornicotine levels with NNN levels. In the case of TI-1112, where the nornicotine level was very high, the NNN level was also high. However, for variety V446, containing nornicotine at about 10 times a normal level, there was no NNN in the tobacco. Consequently, there appears to be no correlation between alkaloid contents of tobacco leaf and levels of NNN. We will be examining the smoke from these tobaccos to determine any effects of varying alkaloid levels on the formation of NNN in smoke.

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Registry No. *N*-Nitrosonornicotine, 16543-55-8; nicotine, 54-11-5; nornicotine, 494-97-3.

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COMMUNICATIONS

Identification of Two New Volatile Amines in Wine

Two amines, 1-pyrroline and 4,5-dimethyl-1,3-dioxolane-2-propanamine, were identified in wine. This was accomplished by comparison of retention times and mass spectra of trifluoroacetamide (TFA) derivatives of the wine components with those of synthetic TFA derivatives.

Schreier (1979) reviewed previous amine work in wines. In an earlier paper Ough et al. (1981) reported the identification of and mass spectra for 10 amines in grapes and wines. Gas chromatographic analysis revealed a number of other unidentified amines in wine. This paper reports the structures of two such amines which were not previously characterized.

MATERIALS AND METHODS

Grapes and Wine. Pinot noir wine, the same made for a previous study (Ough et al., 1981; Daudt, 1980), and sherries, both baked and flor, were used for analysis by gas chromatography–mass spectrometry (GC–MS).

Separation and Derivatization of Amines in Wine. The procedure for isolation of amines and their derivatization with trifluoroacetic anhydride (TFAA) has been reported (Ough et al., 1981). In addition, the amines studied here were also obtained from the wine, made basic, by distillation (30 °C at 4 mmHg) under N_2 gas, and then derivatized (Daudt, 1980).

An alternative procedure, not involving distillation, gave the same dioxolaneamine TFA that had been produced by the procedure described above: The wine sample (750 mL) was acidified (pH 1.5) with HCl, concentrated (40 °C and 22 mmHg) in a rotary evaporator to 100 mL, made basic (pH 11), and extracted with 100 mL of 1-butanol. The solvent layer was washed with 1 M NaOH and then with two portions of 1 M HCl. The HCl solution was extracted twice with butanol and then concentrated at reduced pressure to a dry salt.

The salt was derivatized as before with 8 mL (TFAA) and washed with 30 mL of saturated aqueous $NaHCO_3$. Solid $NaHCO_3$ was added to make the solution basic and the mixture was extracted with 15 mL of ether. The ether was washed with saturated $NaHCO_3$ and then with water. The ether solution was dried, filtered, and concentrated under a stream of N_2 to ca. 0.1 mL of liquid which was analyzed immediately by GC–MS.

1-Pyrroline, 1, and Its Hydrochloride Salt, 1a. The procedure of Amoore (1979) was scaled up by a factor of 20 for the preparation of aqueous 1-pyrroline. The hydrochloride salt of 1 was prepared under conditions similar to the isolation of salts from wine (Ough et al., 1981) to yield 150 mg of a white solid.

Reaction Product, 1b, of 1a with TFAA. The salt **1a** was combined with 1 mL of TFAA. The reaction condi-

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Table I. Comparative GC-MS[a] Data for 1b (from Authentic 1-Pyrroline), the Wine Sample Similarly Prepared and Treated with TFAA, and 3-Pyrroline TFA, 3[b]

sample	Rt, min	m/e (abundance)
1b	3.7	165 (100), 69 (51), 96 (37), 68 (28), 164 (27), 94 (25), 67 (14), 166 (7)
wine	3.9	165 (100), 69 (57), 96 (44), 68 (31), 164 (27), 94 (25), 67 (24), 166 (6)
3	4.3	69 (100), 165 (95), 67 (43), 96 (41), 94 (37), 68 (29), 78 (11), 116 (7)

[a] Methyl silicone column maintained at 60 °C. See Materials and Methods for details. [b] Daudt (1980).

tions and product isolation procedure used for the TFA derivatives of amines in wine (vide supra) were followed. The product, **1b**, was then subjected to GC–MS analysis.

4,5-Dimethyl-1,3-dioxolane-2-propanamine TFA, 2. Dry HCl was bubbled through a mixture of 4-aminobutyraldehyde diethyl acetal (1.5 mmol) and 2,3-butanediol (4.5 mmol, meso and D(−) steriosomers) for 5 min. The solution was placed under 25-mmHg pressure at 30 °C on a rotary evaporator for 20 min, more HCl was added, and the mixture was placed under 4-mmHg pressure at 65 °C for 30 min to remove excess 2,3-butanediol. The residue was treated with 1 mL of TFAA and the preparation for GC–MS was as described above. Pure D(−)-2,3-butanediol was used to prepare a single stereoisomeric product.

GC–MS Analysis. All comparative analyses were made with a Hewlett-Packard 5992A system using 25 m × 0.20 mm i.d. Carbowax 20M or methyl silicone fused silica columns. Both were operated with a 1:60 sample inlet split and 1.0 mL/min He carrier gas flow with the injection port maintained at 250 °C. Mass spectra were taken by using electron ionization (EI) at 70 eV. Other operating conditions varied according to the separation made (Tables I and II). Chemical ionization (CI) and exact mass EI measurements were performed on a VG Micromass, Ltd., Model 7070F mass spectrometer.

RESULTS AND DISCUSSION

Identification of 1-Pyrroline, 1, in Wine. Daudt (1980) observed that a component of wine which had been derivatized with TFAA and 3-pyrroline TFA, **3**, gave mass spectra which resembled one another but had distinctly different GC retention times. Table I provides positive identification of this component as 1-pyrroline; the retention times and mass spectra of the wine component, treated with TFAA, are virtually identical with those of 1-pyrroline, similarly treated.

By use of Daudt's data, the concentration of 1-pyrroline in wine is estimated to be in the 0.01–0.5 mg/L range. Poisel (1978) reported that 1-pyrroline is stable in solutions of up to 10% by volume in methanol but trimerizes at higher concentrations.

1-Pyrroline is a tertiary amine and therefore TFA derivative formation cannot be considered normal. However, the resemblance of the mass spectrum of this product (with a parent ion at *m/e* 165) to that of **3** suggests that it is 2-pyrroline TFA. Whereas 2-pyrrolineacetamide has been characterized (Stille and Becker, 1980), according to Katritsky and Lagowski (1968) unsubstituted 2-pyrroline spontaneously tautomerizes to 1-pyrroline.

While proline is present in wine in concentrations of up to 3000 mg/L (Ough, 1968), it cannot be considered to be a source of 1-pyrroline during the isolation procedure. In a control experiment, 2.5 g of proline and 0.1 g of isobutyl alcohol were dissolved in mixtures of 1 L of water and alcohol (8:1 by volume). This solution was treated in the same way as the wine samples (Materials and Methods). No peak corresponding to 1-pyrroline was found by GC–MS analysis.

Identification of 4,5-Dimethyl-1,3-dioxolane-2-propanamine in Wine. Daudt (1980) noted an amine component in Pinot noir wine and in both baked and flor sherries. Its concentration increased during Pinot noir juice fermentation. In the present study, isobutane CI analysis gave the following *m/e* (rel intensity) data: 166 (100), 184 (67), 256 (38), 101 (15), 167 (8), 113 (7), 185 (5), and 257 (4). The intensity of the *m/e* 256 ion strongly suggested a parent ion of 255 for the compound. Exact mass experiments on EI using perfluorokerosene as a standard gave 254.1011, 101.0609, 73.0651, and 166.0485 for the significant peaks which indicated $C_{10}H_{15}F_3NO_3^+$, $C_5H_9O_2^+$, $C_4H_9O^+$, and $C_6H_7F_3NO^+$ as the most likely formulas for these fragments. The fragments $C_5H_9O_2^+$ and $C_4H_9O^+$ suggested a butyl ester function in the compound of interest. However, published mass spectra of a number of amino acid butyl esters (Lawless and Chadha, 1971; Leimer et al., 1977) exhibit low intensities for the *m/e* 101 peak. Welch and Hunter (1980) reported high-intensity peaks at *m/e* 87, 59, and 41 for a series of 4-methyl-1,3-dioxolane derivatives. These peaks are homologous to the series 101, 73, and 55 in the present study.

2,3-Butanediol is present in red wine at concentrations of up to 1610 mg/L (Amerine and Ough, 1980). It was, therefore, considered as a strong possibility for the acetal segment of the compound; the aldehyde segment chosen was 4-aminobutyraldehyde because 4-aminobutyric acid is present in wine (Ough and Tabacman, 1979) and because its Schiff base, 1-pyrroline, is present as well.

Positive identification of the compound of interest as 4,5-dimethyl-1,3-dioxolane-2-propanamine was made by retention time and mass spectrometric comparison of the

Table II. Retention Time and Mass Spectral Comparisons of Trifluoroacetamides from Pinot noir (Wine), 2, and Synthetic 4,5-Dimethyl-1,3-dioxolane-2-propanamine TFA from D(−)-2,3-Butanediol, 2a

sample	peak	Rt, min[a]	% stereoisomer[b]	abundance at m/e							
				55	69	73	101	126	142	166	182
wine	main	24.06	90	48	22	46	100	17	11	6	5
	2nd	25.32	8	53	51	63	100				
	3rd	26.18	2								
2	main	24.26	77	52	22	49	100	17	10	6	5
	2nd	25.44	17	46	23	59	100	21	10	9	7
	3rd	26.32	6	51	19	61	100	14	9	<1	<1
2a	main	24.32	99	52	22	50	100	18	9	6	5
	2nd	25.40	1	62		71	100				
	3rd										

[a] Carbowax 20 M. Using a methyl silicone column the retention times for the main peaks for wine and **2a** were 15.38 and 15.41 min, respectively. Both columns were programmed initially at 70 °C for 5 min and then raised 4 °C/min. [b] Percent of each stereoisomer within each sample.

TFA derivatives on separate runs (see Table II).

Structural Assignment with Respect to Stereoisomers. Immediately following the main fraction of the wine sample, two smaller fractions were eluted which were also present in synthetic **2** derived from a mixture of the diastereomeric 2,3-butanediols. Mass spectra for the three components differed in their abundances at *m/e* 55, 73, 126, and 182 (see Table II). GC–MS analysis of 4,5-dimethyl-1,3-dioxolane-2-propanamine TFA, **2a**, which was synthesized by using essentially pure D(−)-2,3-butanediol, gave only the first (main) fraction. It can therefore be concluded that the main fraction of 4,5-dimethyl-1,3-dioxolane-2-propanamine in wine is an acetal of *threo*-2,3-butanediol and that the two minor fractions are acetals of *erythro*- (*meso*-) 2,3-butanediol.

Stevens (1969) reported the presence of both 2,4,5-trimethyldioxolane and 2,4-dimethyl-5-ethyldioxolane in wine. Muller et al. (1979) also identified a number of cyclic acetals in sherry. This and the present work suggest that cyclic acetals of other aldehydes may be present in wine.

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Registry No. 1-Pyrroline, 5724-81-2; 4,5-dimethyl-1,3-dioxolane-2-propanamine isomer 1, 85236-72-2; 2-pyrroline trifluoroacetamide, 85236-73-3; 4,5-dimethyl-1,3-dioxolane-2-propane trifluoroacetamide, 85236-74-4; 4-aminobutyraldehyde diethyl acetal, 6346-09-4; [*R*(*R**,*R**)]-2,3-butanediol, 24347-58-8; 1-pyrroline·HCl, 85236-75-5; (*R**,*R**)-butanediol, 35007-63-7; (*R**,*S**)-butanediol, 5341-95-7; 4,5-dimethyl-1,3-dioxolane-2-propanamine isomer 2, 85236-76-6; 4,5-dimethyl-1,3-dioxolane-2-propanamine isomer 3, 85236-77-7.

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Elucidation of the Nitrogen Forms in Melanoidins and Humic Acid by Nitrogen-15 Cross Polarization–Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy

Nitrogen-15 labeled melanoidins were synthesized by reaction of xylose and glucose with ^{15}N-labeled glycine and ammonium sulfate and compared by cross polarization–magic angle spinning (CP–MAS) ^{15}N NMR to a ^{15}N-labeled humic acid. The nitrogen in the melanoidins obtained with glycine, like in the humic acid, was mainly in the secondary amide form with some present as aliphatic amines and/or ammonium ions and some as pyrrole and/or indole nitrogen. The pyrrole- and pyridine-like nitrogen appears to exceed the amide nitrogen in the melanoidins obtained from xylose and ammonium sulfate.

Melanoidins, the dark brown nitrogenous polymers resulting from the interaction of carbohydrates and amino acids, have been part of man's diet since fire was first used for food preparation (Mauron, 1981). These polymers may also be at the origin of humic substances, as suggested by Maillard (1912, 1916). Until recently, however, this hypothesis was difficult to evaluate. A recent study (Benzing-Purdie and Ripmeester, 1983) using solid-state ^{13}C NMR revealed striking similarities between synthetic melanoidins and a humidified soil. These results, which lend support to Maillard's hypothesis, led us to pursue this comparison further. In spite of the importance of nitrogen to soil fertility (Cooke, 1981), almost half of the soil nitrogenous components remain unidentified (Greenfield, 1979; Ivarson and Schnitzer, 1979), mainly because when chemical degradation is attempted, some are unhydrolyzable and others may be decomposed as a result of the necessary strong hydrolytic conditions. Since melanoidins also contain nitrogen, and as no direct information about the nitrogen in these substances can be obtained from ^{13}C CP–MAS NMR, the ^{15}N solid-state NMR technique was applied. Solid-state ^{15}N NMR spectroscopy offers several advantages over solution NMR. No solubilization of material is necessary, thereby eliminating possible errors due to partial solubility of products. Also, as in ^{13}C CP–MAS NMR, cross polarization and magic angle spinning ^{15}N NMR increases the sensitivity and gave, for example, a line width of 0.5 ppm in the case of polycrystalline glycine (Opella et al., 1981). ^{15}N CP–MAS NMR has been used recently in studies of protein turno-

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