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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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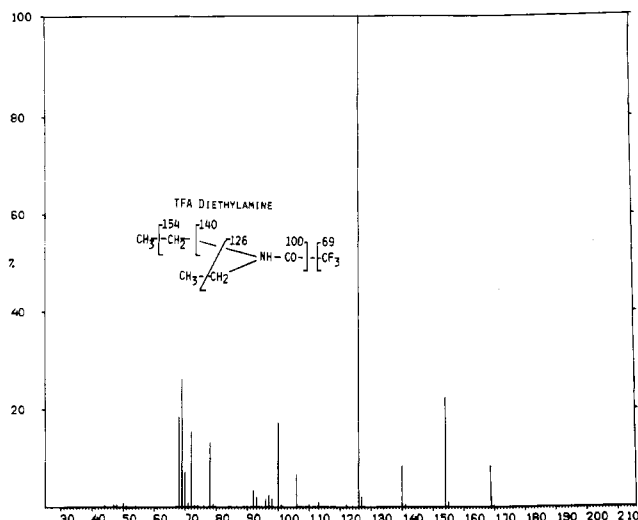


Figure 1. Mass spectrum of the trifluoroacetamide derivative of diethylamine and its fragment pattern. The x axis is the *m/e* values and the y axis the percent of each fragment compared to the base peak.

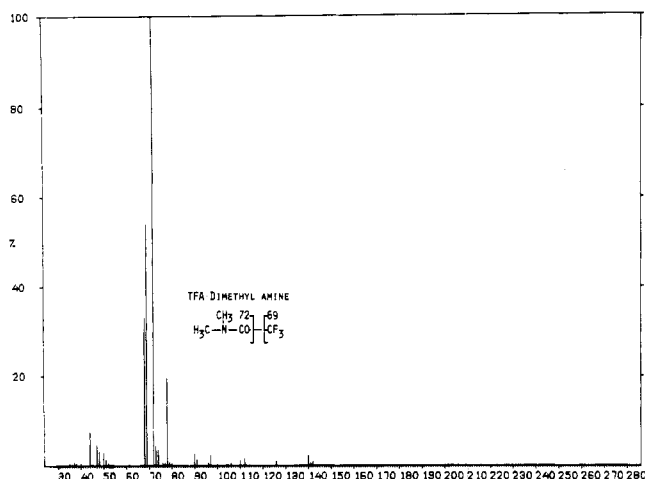


Figure 2. Mass spectrum of the TFA derivative of dimethylamine and its fragment pattern. See the legend to Figure 1 for further details.

Reference Amines. Pure amines were purchased from Aldrich Chemical Co. and the J. T. Baker Co. All had minimum of 97% purity and, when made into TFA derivatives, only showed one peak on the GC chromatograms.

Mass Spectral Data. Concentrated trifluoroacetamide derivatives were seen on two different mass spectrographs. Pure samples were analyzed on a Hewlett-Packard 5930A GC-MS with a System Industries 150 data system by using the above-mentioned fused silica column. Grape and wine concentrated derivatives were determined on a Hewlett-Packard 5992A GC-MS with a Hewlett-Packard 5992A data system. The above-mentioned fused silica Carbowax column and a 30 m × 30 mm i.d. fused silica column coated with SE-54 were used. Both were operated with a 1:60 split ratio with various temperature-programmed conditions. Carrier gas flows were 0.5 mL/min. Generally, 3-μL injections were used.

Amine Verification. Pure amine derivatives were compared with the grape and wine derivatives by retention times on the two columns plus the comparison of the mass spectral fragments.

RESULTS AND DISCUSSION

Working with wines, Ough and Daudt (1981) indicated that amines were present in grape juice prior to fermentation. This led to further research on the grapes them-

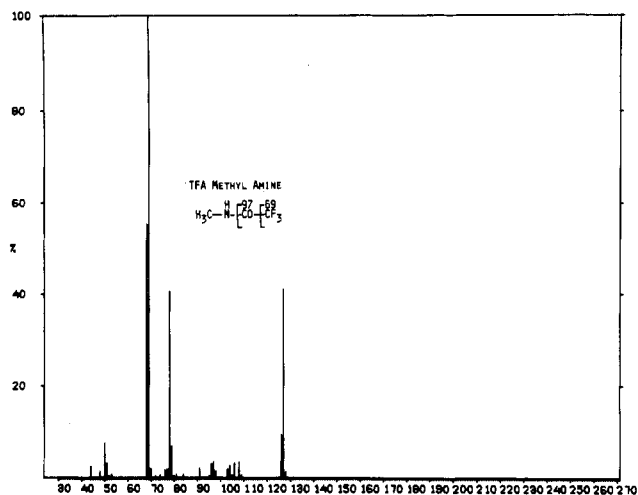


Figure 3. Mass spectrum of the TFA derivative of methylamine and its fragment pattern. See the legend to Figure 1 for further details.

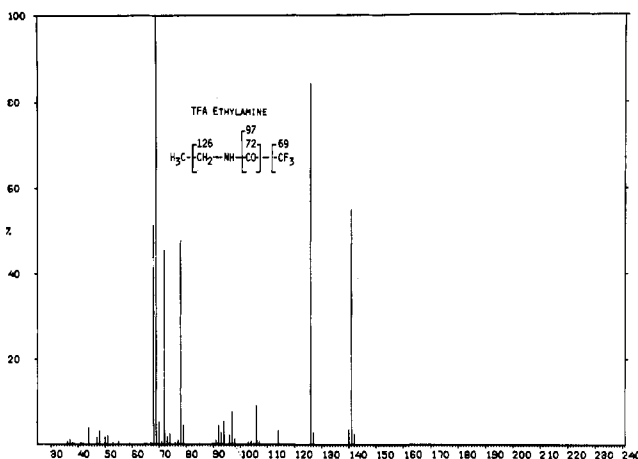


Figure 4. Mass spectrum of the TFA derivative of ethylamine and its fragment pattern. See the legend to Figure 1 for further details.

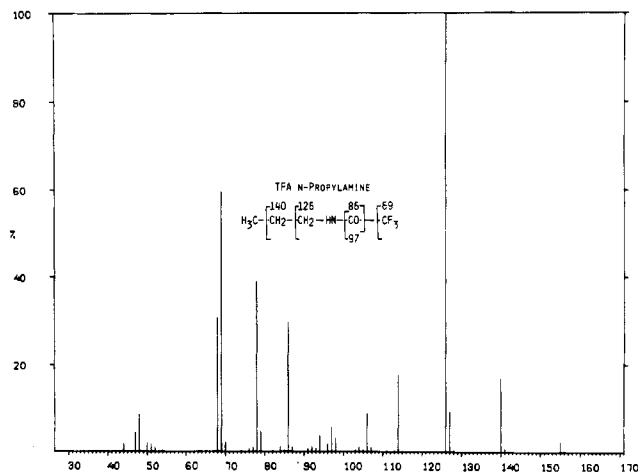


Figure 5. Mass spectrum of the TFA derivative of *n*-propylamine and its fragment pattern. See the legend to Figure 1 for further details.

selves (Daudt and Ough, 1981). As this work advanced, it became increasingly evident that a great number of amines yet unidentified in grapes were present. Also, several amines heretofore unreported were positively identified in wines.

Table II lists the amines identified in grapes and wines along with their retention time on the Carbowax 20M column using the N/P detector. In addition to this, the wine amine TFA derivatives were verified on an SE-54

Table I

volatile amine ^a	range, $\mu\text{g/L}$	reference
methylamine	70	Singer and Lijinsky (1976)
pyrrolidine	60	Singer and Lijinsky (1976)
morpholine	<700	Singer and Lijinsky (1976)
isoamylamine	1000-28 000	Puputti and Suomalainen (1969); Mayer and Pause (1973); Neurath et al. (1977)
2-phenethylamine	0-9000	Mayer and Pause (1973); Neurath et al. (1977)
ethylamine	500-2000	Puputti and Suomalainen (1969)
hexylamine	400-700	Puputti and Suomalainen (1969)
isopropylamine	≈ 50	Puputti and Suomalainen (1969)
isobutylamine	≈ 50	Puputti and Suomalainen (1969)
<i>n</i> -butylamine	trace, 10	Puputti and Suomalainen (1969)
<i>n</i> -amylamine	trace, 10	Puputti and Suomalainen (1969)
dimethylamine	+ ^b	Puputti and Suomalainen (1969); Schreier and Drawert (1975)
<i>n</i> -propylamine	+	Drawert (1965); Schreier and Drawert (1975)
ethanolamine	+	Schreier and Drawert (1975)
3-(methylthio)propylamine	+	Schreier and Drawert (1975)

^a Other investigators have also qualitatively established the presence of some of the amines which show quantitative ranges. ^b (+) indicates identification only.

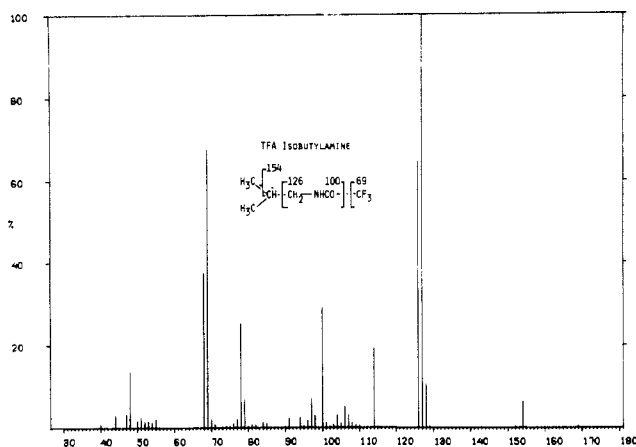


Figure 6. Mass spectrum of the TFA derivative of isobutylamine and its fragment pattern. See the legend to Figure 1 for further details.

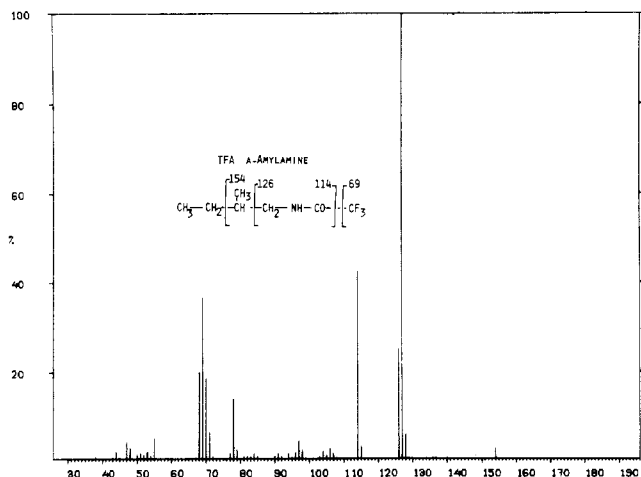


Figure 7. Mass spectrum of the TFA derivative of α -amylamine and its fragment pattern. See the legend to Figure 1 for further details.

column on the mass spectra as well as on the Carbowax 20M column on the mass spectra.

The pure prepared amine TFA derivatives are given in Figures 1-10. The major fragments are identified. The base peak of α -amylamine is m/e 127 as is due to $[\text{CF}_3\text{CONHCH}_3]^+$. Most of the other amines usually show a fragment at m/e 126 due to $[\text{CF}_3\text{CON}=\text{CH}_2]^+$. When

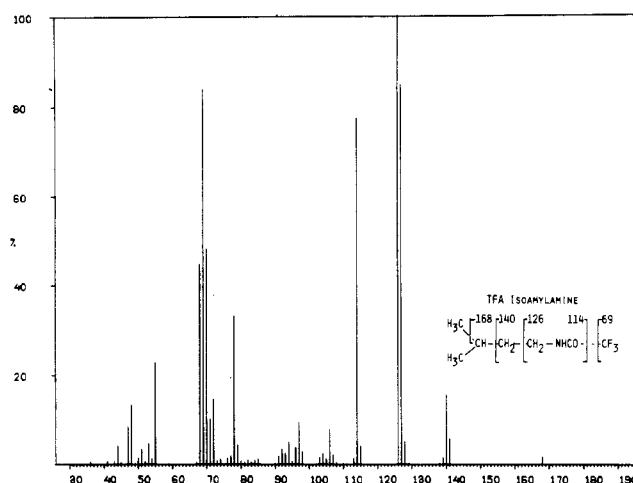


Figure 8. Mass spectrum of the TFA derivative of isoamylamine and its fragment pattern. See the legend to Figure 1 for further details.

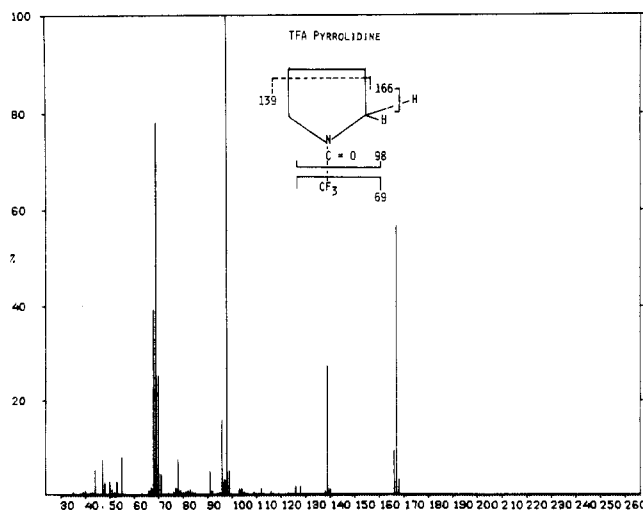


Figure 9. Mass spectrum of the TFA derivative of pyrrolidine and its fragment pattern. See the legend to Figure 1 for further details.

first electron is removed from the nitrogen atom. In the case of the α -amylamine, it is proposed that there is a most aliphatic amines are subjected to bombardment, the hydride shift from the methyl carbon (attached to the

Table II. Volatile Amines Identified in Grapes and Wines

amine ^a	retention time data, min, Carbowax 20M	mass spectra data
methyl	16.9	69 (100), 78 (30), 126 (12), 127 (40)
dimethyl	4.1	69 (53), 72 (100), 78 (13), 90 (4), 97 (5), 131 (5), 141 (40)
ethyl	14.2	69 (100), 72 (75), 78 (48), 106 (10), 126 (87), 141 (53)
diethyl	4.7	69 (52), 72 (32), 78 (20), 100 (24), 106 (10), 126 (100), 140 (12), 154 (53), 169 (21)
<i>n</i> -propyl	17.5	69 (80), 78 (48), 86 (31), 114 (11), 126 (100), 127 (15), 140 (20)
α -amyl	24.1	69 (68), 70 (30), 71 (10), 73 (20), 78 (12), 114 (48), 115 (44), 126 (20), 127 (100), 154 (7)
isoamyl	28.75	69 (88), 70 (70), 71 (14), 72 (26), 78 (32), 114 (74), 126 (100), 127 (90), 140 (18), 168 (5)
2-phenethyl	87.7	64 (18), 69 (18), 78 (14), 91 (72), 92 (5), 104 (100), 105 (14), 126 (10), 148 (3), 217 (5)
pyrrolidine	16.1	69 (100), 70 (37), 78 (6), 96 (12), 98 (85), 139 (37), 166 (19), 167 (87), 168 (7)
isobutyl	16.6	69 (60), 73 (14), 78 (26), 95 (10), 100 (20), 114 (24), 126 (65), 127 (100), 128 (9), 154 (8)

^a These were identified in more than one sample of both grapes and wine as their TFA derivative.

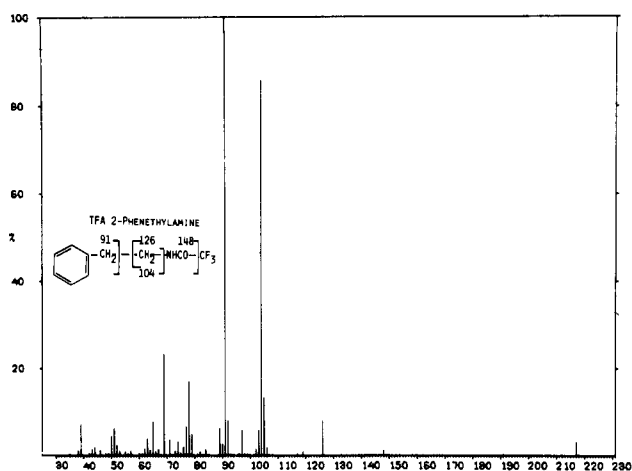
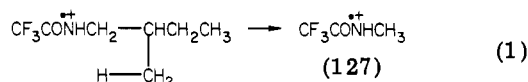
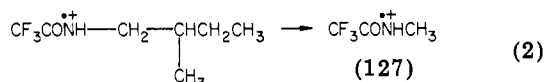


Figure 10. Mass spectrum of the TFA derivative of 2-phenethylamine and its fragment pattern. See the legend to Figure 1 for further details.

carbon) to the α -carbon where cleavage occurs preferentially.



It is possible that a methyl shift occurs, but this seems less likely due to the fact that cleavage between the α - and β -carbons adjacent to the nitrogen atom is preferred.



The base peak at m/e 127, which is also the base peak of isobutylamine TFA, accounts for the main mass spectra differences between α -amyl- and isoamylamines (and *n*-amyl also). The differences in the two amines are due mainly in the proportions of the fragments.

It is noteworthy to mention that dimethylamine does not give a significant peak at m/e 126. Its base peak is m/e 72, probably due to a loss of CF_3 and the formation of $[\text{OCN}(\text{CH}_3)_2]^+$. The peaks m/e 69, 78, and 97 show up on all the spectra of these amine TFA derivatives and are CF_3 , $[\text{CF}_2\text{C}\equiv\text{O}]^+$, and $[\text{CF}_3\text{C}\equiv\text{O}]^+$, respectively.

The diethylamine was the other new amine reported in

wine (besides the α -amylamine). This amine had a base peak of m/e 126 but had several unique fragments (m/e 100, 154, and 169) which made the verification fairly simple.

Several other amines were also detected, but further work needs to be done for positive identification.

The amine formed in the grapes can conceivably come either from the reductive amination of the corresponding aldehyde or the transamination of the aldehyde from an amino acid. Hartmann (1967) could not demonstrate any amines resulting from decarboxylation of amino acids in apples but could show aldehydes were converted to amines. The transamination pathway mechanism has been proposed by Lowe and Ingraham (1974). By this mechanism the secondary amines can also be formed. In wines the amines may be assimilated partially by the yeast (Ough and Daudt, 1981). All of the amines discussed in this report are in the grape prior to fermentation.

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