

detection limit of 0.5%, thus showing the product to be enantiomerically pure.

Solubility Determination. Ten milliliters of hot (65 ± 2 °C) distilled water was rapidly added to 10.2 mg of dry L-1-HCl while rapidly magnetically stirring in a 50-mL beaker. The homogeneous solution thus obtained was diluted with hot water to 50 mL in a warm volumetric flask. This hot solution was filtered through a 0.45- μ m Type HA Millipore filter into a warm 50-mL volumetric flask. The concentration of L-1-HCl in the filtrate, which was maintained at 65 ± 2 °C, was then determined by HPLC analysis (2.0 mL/min 60% CH₃OH in 0.03 M KH₂PO₄; 286 nm) to be 191 mg/L. No crystallization or precipitation of L-1 was observed on cooling. After 4 h, HPLC analysis indicated the concentration of L-1-HCl to be 183 mg/L. Crystallization of L-1 then began to occur slowly. After 94 h (22 °C), the concentration of L-1 was determined to be 33 mg/L. The pH of this solution was 3.65.

An identical procedure employing 10.3 mg of DL-1-HCl yielded a solution having a DL-1-HCl concentration of 205 mg/L. After the solution was cooled over 4 h, the concentration had dropped to 186 mg/L, and after 94 h (22 °C) a concentration of 18 mg/L was determined. The pH of this solution was 3.57.

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Volatile Constituents of the Muscadine Grape (*Vitis rotundifolia*)

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The volatile constituents of the muscadine grape have been studied by the combined technique of gas chromatography and mass spectroscopy. Forty-nine compounds have been identified and confirmed. A gas chromatogram of a muscadine extract and a table of compounds and their associated mass spectral data are presented.

The muscadine grape grows abundantly throughout the southeastern United States. It is a large, dark, thick-skinned grape that ripens in late August and continues through September. The muscadine, which is cultivated and grows wild, can vary in flavor, color, and size, thereby resulting in several varieties of the grape. Because muscadines are rich in flavor, they are widely used by local residents to produce homemade foods and beverages (Wigginton, 1975).

Foods and beverages produced from muscadines have been studied extensively. The characteristics of muscadine wines (Carroll et al., 1975), preserves (Rizley et al., 1977), and juices and jellies (Flora, 1977a) have been reported. Additional research has included the cultivation (Lane, 1972), harvesting (Balerdi and Mortensen, 1973), processing (Flora, 1977b), and storage (Smit et al., 1971) of muscadine grapes.

Analytical research has included sugar and organic acid concentration (Carroll et al., 1971) as well as the relationship of anthocyanins to color (Nesbitt et al., 1974).

Table I. Volatile Constituents of Muscadines Previously Reported

Kepner and Webb (1956)	
methyl alcohol	biacetyl
ethyl alcohol	1-hexanal
<i>n</i> -butyl alcohol	2-hexenal
isoamyl alcohol	ethyl acetate
1-hexanol	caproate ester
2-phenylethanol	caprylate ester
acetaldehyde	caprate ester
isobutyraldehyde	laurate ester
acetal ^a	methyl ethyl ketone ^a
Berry et al. (1979)	
methanol	ethyl acetate
ethanol	ethyl propionate
butanol	propyl acetate
2-methylbutanol	butyl acetate
hexanol	benzyl acetate
<i>trans</i> -2-hexen-1-ol	ethyl caprate
2-phenylethanol	<i>d</i> -limonene
<i>trans</i> -2-hexenal	

^a Tentatively assigned.

The volatile constituents of the grape have been investigated by Kepner and Webb (1956) and Berry et al. (1979). These two investigations resulted in the identification of the major constituents of the grape. This paper presents

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Table II. Identity of Volatile Components of Muscadines

peak no. ^a	compound	retention ^b index (I_R) (CBW20M)	characteristic MS data, ^c m/e (rel intensity)
1	acetone	0.44	solvent
2	ethyl acetate	2.00	M^+ = 88, 43 (100), 29 (45), 45 (30), 61 (29), 70 (16), 73 (9), 88 (8), 56 (3)
3	methylene chloride		solvent
4	chloroform		solvent
5	toluene		solvent
6	butyl acetate	4.34	M^+ = 116, 43 (100), 56 (55), 73 (24), 41 (20), 61 (19), 55 (9), 29 (8), 87 (3)
7	isobutyl alcohol	4.36	M^+ = 74, 43 (100), 42 (60), 41 (58), 33 (55), 31 (45), 27 (25), 74 (15), 39 (10)
8	diethyl carbonate	4.75	M^+ = 118, 45 (100), 91 (36), 63 (18), 43 (9), 59 (6), 44 (5), 46 (4), 47 (4)
9	isoamyl acetate	4.86	M^+ = 130, 43 (100), 70 (80), 55 (62), 73 (60), 41 (50), 42 (43), 57 (33), 72 (27)
10	butanol	4.91	M^+ = 74, 56 (100), 31 (98), 41 (90), 43 (81), 42 (54), 27 (31), 29 (28), 55 (26)
11	ethyl crotonate	5.30	M^+ = 114, 69 (100), 41 (52), 99 (43), 86 (16), 68 (12), 39 (10), 29 (8), 114 (4)
12	1,4-cineole	5.52	M^+ = 154, 43 (100), 71 (90), 111 (85), 55 (50), 93 (31), 69 (26), 121 (19), 125 (10)
13	isoamyl alcohol ^d	5.55	M^+ = 88, 55 (100), 42 (94), 43 (76), 70 (69), 41 (57), 29 (42), 57 (41), 31 (30)
14	limonene	5.79	M^+ = 136, 68 (100), 93 (65), 67 (55), 79 (39), 94 (35), 136 (28), 121 (25), 107 (20)
15	1,8-cineole	5.97	M^+ = 154, 43 (100), 81 (81), 71 (69), 84 (50), 108 (32), 69 (25), 154 (10), 111 (8)
16	ethyl hexanoate	6.00	M^+ = 144, 88 (100), 43 (79), 99 (56), 60 (48), 29 (47), 71 (31), 61 (28), 73 (27)
17	isopropyl hexanoate	6.05	M^+ = 158, 43 (100), 99 (51), 60 (49), 117 (22), 59 (20), 71 (19), 73 (14), 102 (12)
18	styrene	6.08	M^+ = 104, 104 (100), 103 (50), 51 (30), 78 (21), 77 (12), 105 (8), 50 (4), 52 (2)
19	hexyl acetate	6.37	M^+ = 144, 43 (100), 56 (64), 84 (39), 55 (30), 61 (25), 42 (28), 69 (20), 73 (12)
20	<i>p</i> -cymene	6.48	M^+ = 134, 119 (100), 134 (25), 91 (17), 120 (10), 117 (9), 77 (6), 41 (5)
21	acetoin	6.54	M^+ = 88, 45 (100), 43 (76), 88 (16), 27 (15), 42 (7.5), 46 (5), 73 (4), 86 (2)
22	2-heptanol	6.66	M^+ = 116, 45 (100), 55 (19), 43 (12), 83 (10), 41 (9), 70 (7), 101 (5), 29 (3)
23	ethyl heptanoate	7.00	M^+ = 158, 88 (100), 43 (78), 70 (60), 60 (51), 101 (49), 73 (46), 61 (44), 113 (39)
24	hexanol	7.00	M^+ = 102, 56 (100), 43 (76), 55 (65), 42 (61), 41 (43), 69 (37), 31 (28), 84 (14)
25	<i>cis</i> -3-hexen-1-ol	7.35	M^+ = 100, 41 (100), 67 (83), 55 (48), 82 (40), 42 (23), 69 (22), 31 (19), 100 (3)
26	2-hexen-1-ol	7.71	M^+ = 100, 57 (100), 41 (43), 27 (29), 29 (25), 44 (19), 43 (19), 39 (15), 31 (13)
27	ethyl octanoate	8.00	M^+ = 172, 88 (100), 57 (62), 101 (57), 60 (53), 43 (45), 61 (41), 127 (40), 73 (38)
28	2-ethyl-1-hexanol	8.37	M^+ = 130, 57 (100), 41 (40), 43 (38), 29 (27), 55 (25), 27 (20), 56 (19), 70 (17)
29	ethyl 3-hydroxybutyrate	8.81	M^+ = 132, 43 (100), 45 (80), 60 (57), 71 (46), 88 (44), 87 (36), 61 (28), 118 (26)
30	benzaldehyde	8.91	M^+ = 106, 77 (100), 106 (83), 105 (78), 78 (46), 51 (32), 52 (22), 107 (20), 39 (8)
31	linalool	8.97	M^+ = 154, 71 (100), 41 (76), 93 (69), 55 (59), 43 (49), 69 (48), 80 (30), 121 (17), 136 (9)
32	1-octanol	9.04	M^+ = 130, 41 (100), 56 (92), 55 (85), 43 (84), 26 (66), 42 (63), 70 (57), 27 (53)
33	2-fenchyl alcohol	9.44	M^+ = 154, 81 (100), 41 (63), 80 (54), 43 (52), 69 (46), 55 (44), 57 (30), 71 (29), 111 (17)
34	terpinen-4-ol	9.64	M^+ = 154, 71 (100), 43 (85), 57 (82), 41 (62), 55 (47), 69 (46), 111 (39)
35	hexadecane	9.72	M^+ = 226, 57 (100), 71 (88), 43 (87), 85 (75), 41 (55), 55 (30), 99 (23)
36	methyl benzoate	10.00	M^+ = 136, 77 (100), 105 (77), 51 (47), 136 (40), 106 (14), 78 (11), 92 (7), 65 (3)
37	ethyl decanoate	10.00	M^+ = 200, 88 (100), 101 (46), 60 (20), 73 (19), 43 (19), 155 (13), 115 (6), 200 (2)
38	<i>p</i> -allylanisole	10.34	M^+ = 148, 148 (100), 147 (56), 117 (55), 121 (52), 77 (50), 91 (39), 105 (38), 133 (31)
39	1,2-dimethoxy-1-phenylethane	10.45	M^+ = 166, 121 (100), 77 (53), 91 (38), 122 (24), 105 (12)
40	α -terpineol	10.54	M^+ = 154, 59 (100), 93 (65), 121 (44), 136 (27), 81 (35), 43 (35), 68 (28), 67 (24)

Table II (Continued)

peak no. ^a	compound	retention ^b index (I_E) (CBW20M)	characteristic MS data, ^c m/e (rel intensity)
41	borneol	10.63	M^+ = 154, 95 (100), 121 (30), 93 (12), 110 (10), 136 (8), 43 (5), 60 (3)
42	benzyl acetate	10.92	M^+ = 150, 108 (100), 91 (83), 43 (62), 90 (58), 79 (34), 150 (30), 107 (22), 65 (11)
43	ethyl phenylacetate	11.49	M^+ = 164, 91 (100), 164 (40), 29 (30), 92 (27), 65 (16), 119 (6), 105 (6), 51 (3)
44	2-phenylethyl acetate	11.77	M^+ = 164, 43 (100), 104 (90), 91 (23), 105 (18), 77 (14)
45	geraniol	11.85	M^+ = 154, 41 (100), 69 (65), 39 (29), 93 (26), 27 (23), 29 (20), 68 (11)
46	ethyl laurate	12.00	M^+ = 228, 88 (100), 101 (88), 43 (73), 70 (71), 55 (70), 73 (69), 41 (68), 183 (62), 185 (50)
47	benzyl alcohol	12.19	M^+ = 108, 79 (100), 108 (72), 107 (52), 77 (43), 91 (12), 58 (10), 65 (6)
48	2-phenylethanol	12.55	M^+ = 122, 91 (100), 92 (61), 122 (25), 65 (18), 57 (11), 39 (9)
49	2-methoxy-1-phenyl-2-hydroxyethane	13.00	M^+ = 152, 121 (100), 77 (69), 91 (66), 122 (39), 51 (29), 105 (24), 78 (17), 65 (17)

^a Refers to the peaks in Figure 1. ^b Authentic I_E values were determined on packed columns (van den Dool and Kratz, 1963). ^c Determined with a Hitachi RMU-6L; the mass peak (M^+) was not observed for all compounds. ^d The major peak where overlapping occurred.

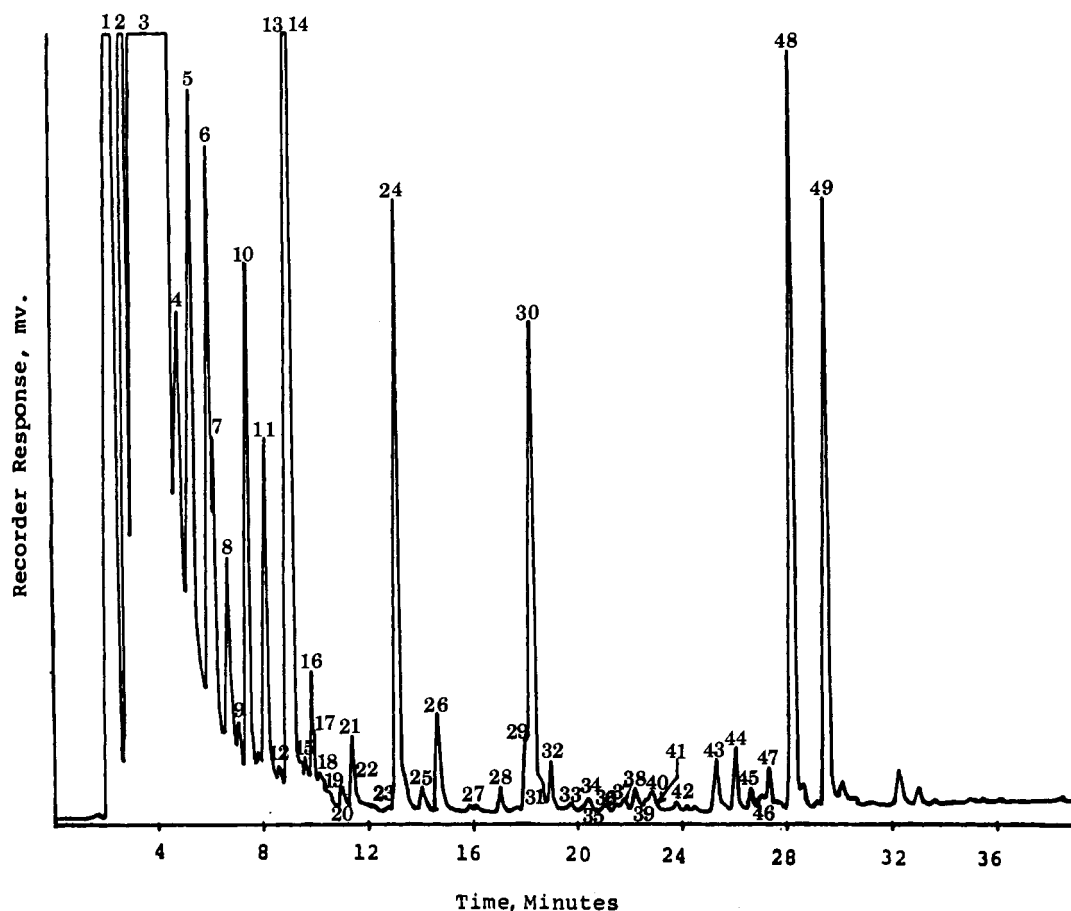


Figure 1. Typical gas chromatogram of concentrated muscadine extract.

an in-depth analysis of the volatile flavoring compounds found in ripe muscadine grapes grown in north central Georgia.

EXPERIMENTAL SECTION

Isolation of Volatiles. Eleven kilograms of fresh, black-skinned muscadines was pulverized in a Waring blender for 2 min. The resulting 4.09 L of juice and pulp was divided into three equal parts and vacuum steam distilled by using a Buchi rotor evaporator. The resulting

distillates were collected in dry ice-acetone cold traps, thawed, and combined. A total of 800 mL of distillate was saturated with sodium chloride, extracted with methylene chloride, and dried over anhydrous sodium sulfate. The extract was then concentrated through a 13-cm Vigreux column to ca. 1 mL. The concentrate had the typical odor associated with muscadines in general.

Identification of Volatiles by Combined GC/MS. The volatiles were separated and identified by using a Varian Aerograph Series 1200 gas chromatograph fitted

with a flame ionization detector and coupled to a Hitachi RMU-6L single-focusing, magnetic-sector mass spectrometer. A fraction of the column effluent was bypassed through a metering valve into a single-stage glass jet separator leading into the ion source. The jet separator and the metering valve were maintained at 200 °C within a convection-circulated air oven.

A 2 mm (i.d.) by 3 m glass column packed with 5% Carbowax 20M on 60–80-mesh acid-washed DMCS Chromosorb W was used throughout the analyses with a helium flow rate of 25 mL/min. The oven temperature was programmed at 2 °C/min from 70 to 220 °C. The injector and detector temperatures were maintained at 200 and 230 °C, respectively. All mass spectra were obtained at 90 eV with a filament current of 80 μ A while maintaining the ion source at 200 °C.

Gas chromatographic retention indices (I_R) were determined by using a mixture of ethyl esters (C_1 – C_{18}) as standards according to the method of van den Dool and Kratz (1963).

RESULTS AND DISCUSSION

A chromatogram of the concentrated extract is shown in Figure 1. Compound identification was based on its mass spectrum and calculated retention index. Table I lists the compounds which were reported in the earlier investigations. Table II lists 49 compounds, their retention indices, and mass spectral data.

The compounds which have been identified in the present analysis are consistent with the previous investigations with the exception of ethyl alcohol, methyl alcohol, acetaldehyde, isobutyraldehyde, biacetyl, 1-hexanal, 2-hexenal, ethyl propionate, and propyl acetate. The absence of these compounds in the present analysis is possibly due to differences in grape variety, climatic conditions, or geographical location. The grapes which were analyzed in the earlier investigations were cultivated in California and Florida, whereas the grapes used in the present analysis were cultivated in Georgia. The absence of these compounds may also be attributed to isolation techniques or the relative concentration of the compounds to the rest of the extract. Kepner and Webb (1956) reported that biacetyl was a byproduct of fermentation. Since the isolation procedure used in this analysis would preclude fermentation, biacetyl would not be found.

The remaining compounds identified in the present analysis, with the exception of 1,8- and 1,4-cineol and

1-methoxy-1-phenyl-2-hydroxyethane, have been reported in grapes. 1-Methoxy-1-phenyl-2-hydroxyethane was synthesized unequivocally by the reaction of methyl alcohol and styrene oxide in the presence of HCl. Identification was confirmed by GC retention time, NMR, and mass spectral data.

The aroma and flavor of the muscadine grape are attributable to the presence of isoamyl alcohol, hexanol, benzaldehyde, and 2-phenylethanol and its derivatives. For verification of this premise, the major volatile constituents were blended synthetically in the proper proportions and gave an aroma reminiscent of the muscadine grape. The aromatic compounds found in trace amounts undoubtedly contribute to the overall aroma and flavor. Methyl anthranilate, which is known to be an important aromatic compound found in a variety of grapes, was not found in muscadines. Several compounds remain unidentified; obviously their contribution to the flavor and aroma of the muscadine is unknown. It is possible these compounds do contribute to the unique character of the grape's flavor and aroma.

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