

**Table I.  $R_f$ 's of Isolated Sulfonium Salt and Authentic S-Methyl Methionine Bromide<sup>a</sup>**

Compound	Butanol-Acetic Acid-Water		Sec-Butyl alcohol- tert-Butyl alcohol-Water	
	Whatman No. 1	S & S 589 Blue Ribbon	Whatman No. 1	S & S 589 Blue Ribbon
	Isolated sulfonium salt	0.09	0.10	0.12
S-Methyl methionine sulfonium bromide	0.09	0.10	0.12	0.11
Homoserine	0.28	0.28	0.47	0.40
Degradation product of isolated sulfonium	0.29	0.28	0.46	0.40

<sup>a</sup>  $R_f$ 's calculated with respect to methionine.

sulfonium bromide and chloride were compared in an automatic amino acid analyzer (24). Authentic methyl methionine sulfonium chloride was obtained from the California Corporation for Biochemical Research and converted to the bromide using the procedure described for the isolation of the natural occurring salt. In the tomato juice, a prominent peak was found that coincided with the peak of the authentic material. When an aliquot of the tomato sample was heated at length in a water bath, the sulfonium peak disappeared, and an almost equimolar increase in homoserine, a known degradation product of methyl methionine sulfonium, took place (9, 10, 11).

Calculations based on the amino acid analyses indicate the concentration of the methyl methionine sulfonium salts in the tomato to be on the order of 16 to 35 p.p.m. Miers (12) found that exhaustive collection of the volatile materials from tomato preparations

indicate that dimethyl sulfide occurs within this range. These findings show that methyl methionine sulfonium salt plays an important role in the formation of flavor in heated tomato products.

#### Literature Cited

- (1) Bywood, R., Challenger, F., Leaver, D., Whitaker, M. I., *Biochem. J.* **48**, XXX (1951).
- (2) Carson, J. F., Wong, F. F., *J. Agr. Food Chem.* **9**, 140 (1961).
- (3) Carson, J. F., Wong, F. F., *J. Org. Chem.* **26**, 4997 (1961).
- (4) Cavallito, C. J., Bailey, J. H., Buck, J. S., *J. Am. Chem. Soc.* **67**, 1032 (1945).
- (5) Challenger, F., Haywood, B. J., *Chem. Ind. (London)* **1954**, p. 729.
- (6) Challenger, F., Simpson, M. I., *J. Chem. Soc.* **1948**, p. 1591.
- (7) Gumbmann, M. R., Burr, H. K., *J. Agr. Food Chem.* **12**, 404 (1964).
- (8) Jensen, K. A., Conti, J., Kjaer, A., *Acta Chem. Scand.* **7**, 1267 (1953).
- (9) Kiribuchi, T., Yamanishi, T., *Agr. Biol. Chem. (Japan)* **27**, 56 (1963).

- (10) Lavine, T. F., Floyd, N. F., Cammaroti, M. S., *J. Biol. Chem.* **207**, 107 (1954).
- (11) McRorie, R. A., Sutherland, G. L., Davis, M. S., Barton, A. D., Glazener, M. R., Shive, W., *J. Am. Chem. Soc.* **76**, 115 (1954).
- (12) Miers, J. C., *J. Agr. Food Chem.* **14**, in press (1966).
- (13) Morris, C. J., Thompson, J. F., *J. Am. Chem. Soc.* **78**, 1605 (1956).
- (14) Nakajima, S., Okuyama, G., *Japan. Patent* **161** (1958).
- (15) Schwimmer, S., Carson, J. F., Makower, R., Mazelis, M., Wong, F. F., *Experientia* **16**, 449 (1960).
- (16) Stoll, A., Seebeck, E., *Adv. Enzymol.* **11**, 377 (1951); *Experientia* **3**, 114 (1947).
- (17) Synge, R. L. M., Wood, J. C., *Biochem. J.* **64**, 252 (1956).
- (18) Toennies, G., Kolb, J. J., *J. Am. Chem. Soc.* **67**, 849 (1945).
- (19) Virtanen, A. I., *Angew. Chem.* **74**, 374 (1962); *Intern. Ed. Engl.* **1**, 229 (1962).
- (20) Virtanen, A. I., Matikkala, E. J., *Acta. Chem. Scand.* **13**, 1898 (1959).
- (21) *Ibid.*, p. 693.
- (22) Woolley, D. W., *Nature* **171**, 327 (1953).
- (23) Jacobs, M. R., "The Chemistry and Technology of Food and Food Products," second ed., Vol. II, Interscience, New York, 1951.
- (24) Skodak, F., Wong, F. F., White, L., *Anal. Biochem.* **13**, 568-71 (1965).

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## MUSCAT VOLATILES

### Volatiles from Grapes. Muscat of Alexandria

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The volatile constituents found in Muscat of Alexandria have been determined with a capillary gas chromatograph attached to a mass spectrometer. Some 60 components were identified; the most abundant were 1-hexanol, geraniol, and linalool.

INVESTIGATION of fruit volatiles by the combination of capillary GLC and rapid scan mass spectrometry (4) has yielded a tremendous amount of information which, only a few years ago, would have been very difficult if not impossible to obtain. In the present investigation, analysis of the volatile constituents of Muscat of Alexandria (*Vitis vinifera*) has yielded the identity of some 60 components and has revealed the presence of many unknown components.

Muscat of Alexandria has been previously investigated by Cordonnier

(7) and Webb (6). By using liquid/solid adsorption chromatography, Cordonnier identified geraniol, terpineol, limonene, and linalool. Webb, employing distillation, derivative formation, and liquid/solid chromatography, identified 13 compounds along with several C<sub>4</sub> to C<sub>12</sub> carboxylic acid esters.

#### Experimental

Mature muscat grapes (*Vitis vinifera* var. Muscat of Alexandria) were harvested near Fresno, Calif., on the afternoon of October 6, 1964. From these grapes, 1000 gallons of free-run juice

(23° Brix) was obtained on the morning of October 7, 1964. The operation was carried out by a commercial winery with precautions to prevent contamination. All crushers, conveyors, pipes, tanks, etc.; were flushed with water before processing. After the pulp, seeds, skins, and stems had been filtered off, the juice was cooled to 2° C., then pumped into twenty 55-gallon drums lined with polyethylene liners. One pound of USP sodium benzoate was added to each barrel as a preservative. The juice was shipped to Albany, Calif., in a refrigerated van at 0° C., and was stored at the same temperature until stripped.

Stripping began on October 14, 1964, and continued through October 20, 1964. The operation was performed with a Kontro wiped-film evaporator to which a fractionating column was attached. Juice was fed to the evaporator at a rate of 350 pounds per hour, and essence was removed from the top of the fractionating column at 3.5 pounds per hour, thus giving 100-fold essence. The evaporation rate was 27% of the feed rate. The evaporation temperature was held at 71° C. by employing a vacuum. The fractionating column contained 10 stainless steel sieve trays each 6 inches in diameter containing 1/8-inch holes on 21/64-inch staggered centers. Each plate was 12 inches above the next with a 1-inch diameter downcomer having a 1/4-inch wire. The distillate from the Kontro evaporator was added between the fourth and fifth plate from the bottom. A reboiler attached to the bottom had a reboil rate of 75 pounds per hour. Undoubtedly, volatile material was lost with the uncondensable gases leaving the vacuum system.

The grape essence thus obtained (approximately 10 gallons) was extracted in batches with isopentane which had been purified by distillation through a 10-plate Oldershaw column. The isopentane extract was dried over anhydrous sodium sulfate, and the pentane was removed by distillation through a 10-plate Oldershaw column. The resulting yellow oil (6.0 grams) contained traces of isopentane.

After the essence had been extracted with isopentane, freshly distilled ether

was used to remove any polar compounds remaining in the aqueous phase. Ethanol and methanol, with smaller amounts of polar compounds, were shown by gas chromatography to be the two main components removed by the ether.

The muscat oil was analyzed by coupling a capillary gas chromatography column directly to a mass spectrometer. In this investigation, a 500-foot  $\times$  0.02-inch ID stainless steel tube coated with GE SF-96(50) was employed to separate the compounds. The temperature was programmed from 25° to 200° C. over a period of two hours. Helium flow rate was set at 15 cm. per second at 125° C. The mass spectrometer was a Bendix time-of-flight Model 12. The column effluent was allowed to enter directly into the ionization chamber. Emerging components were monitored by watching the mass spectral pattern on an oscilloscope and by recording the ionization due to mass 41 ( $C_3H_5^+$ ) on a strip-chart recorder. At the appearance of a compound on the recorder and/or on the oscilloscope, the spectral range from 24 to 200 mass units was scanned over a period of 2 1/2 seconds.

The mass spectral data were analyzed by comparing the charts with the mass spectral patterns of known compounds. The compounds were then secured either by purchase from a commercial supplier or by synthesis, and the retention times were determined by running the compounds under identical conditions (same flow rate and temperature program) with two internal standards,

toluene and linalool. Confirmation of identity was defined as agreement between retention time and the mass spectral assignment. Often the mass spectral data can place a compound in a certain class (e.g., terpene), but cannot specify its identity. In these cases, no retention data could be obtained. In some other cases, the compound was not readily available, and no attempt was made to synthesize it. The identity of such compounds must consequently be considered tentative.

## Results

A GLC chromatogram of the muscat oil obtained with a thermistor detector is shown in Figure 1. The temperature program was identical with the program used in the GLC-MS hookup, and the pressure was adjusted to make the linear velocities of the carrier gas identical. The numbers above the peaks are for identification and refer to Tables I and II and the ensuing discussion.

The hydrocarbons isopentane, *n*-pentane, 3-methylpentane, and methylcyclopentane (peaks 1, 2, 5, and 10) arise from the solvent (detected in a control experiment). Cyclohexane (peak 13) and *n*-hexane (peak 6) were also found in the solvent but in much smaller amounts than in the oil. The origin of these two hydrocarbons is uncertain; they may come from solvents used in spraying the fruit prior to harvesting. Several other hydrocarbons (Table I)

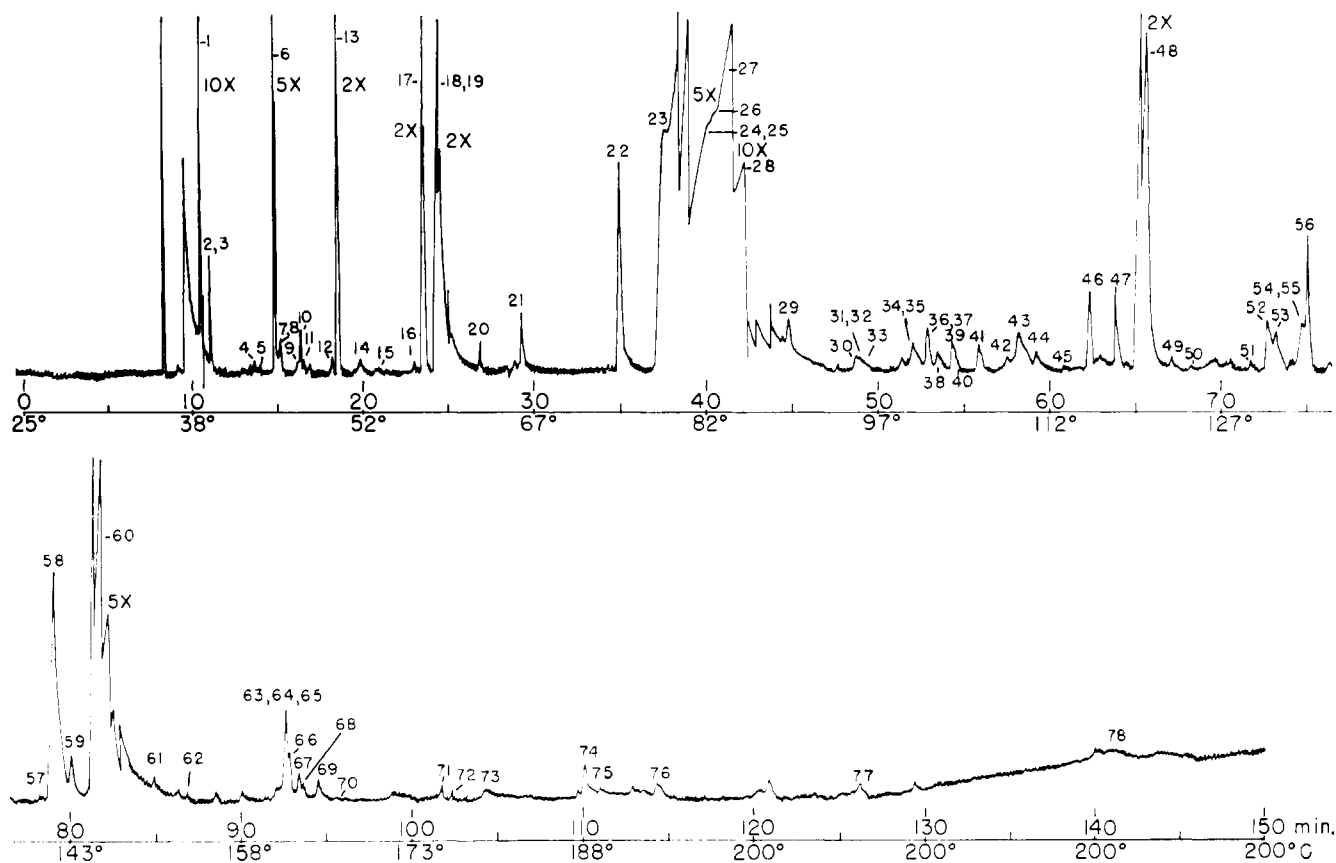


Figure 1. GLC chromatogram of muscat oil using 500-foot  $\times$  0.02-inch tube coated with SF-96(50)

have been identified that may be from insecticides, fungicides, etc., but biosynthesis cannot be entirely disregarded. A considerable amount of biphenyl (peak 63) is present. Schultz *et al.* (5) reported biphenyl in orange juice and attributed its presence to packaging material which was impregnated with the fungistat biphenyl. The grapes used in the present investigation were handled in bulk, and no fungistat could have come from packaging. Two terpene

hydrocarbons, myrcene (peak 36) and limonene (peak 42), have also been identified. Limonene was found by Cordonnier (7).

The alcohols (Table I) comprise the bulk of material found in the muscat oil. By employing isopentane as the solvent, methanol and ethanol were selectively discarded with the aqueous phase and consequently were not detected in the muscat oil by mass spectrometry. 1-Hexanol (peak 28) was the most

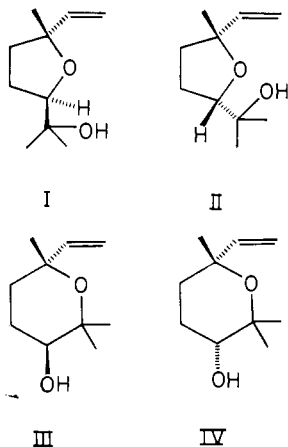
abundant of the alcohols extracted. Considerable quantities of iso- and active-amyl alcohols (peaks 18 and 19) were also found, as was the leaf alcohol, *cis*-3-hexen-1-ol (peak 23). Webb (6) reported the presence of 1-hexanol, 3-methyl-1-butanol, and *cis*-3-hexen-1-ol in Muscat of Alexandria. Of particular interest were the terpene alcohols which comprise a large percentage of the oil and have a pleasant sweet aroma. Linaloöl (peak 48) and geraniol (peak

Table I. Compounds Identified in Muscat Oil

Peak No.	Hydrocarbons	Alcohols	Esters	Aldehydes and Ketones	Misc.
1	Isopentane				
2	<i>n</i> -Pentane				
3					
4				<i>n</i> -Butanal	Ether
5	3-Methylpentane				
6	<i>n</i> -Hexane				
7			Ethyl acetate		
8					Chloroform
9		2-Methyl-1-propanol			
10	Methylcyclopentane	1,1-Dimethylethanol			
11					1-Ethoxy-1-methoxyethane
12					
13	Cyclohexane				
14				3-Pentanone	
15		3-Pentanol			
17					1,1-Diethoxyethane
18		3-Methyl-1-butanol			
19		2-Methyl-1-butanol			
20	Toluene				
21				Hexanal	
22				2-Hexenal	
23		<i>cis</i> -3-Hexen-1-ol			
24	<i>m</i> -Xylene } and/or				
25	<i>p</i> -Xylene }				
26	<i>o</i> -Xylene }				
27		<i>trans</i> -3-Hexen-1-ol <sup>a</sup>			
28		1-Hexanol			
30				Benzaldehyde	
31	Mesitylene				
32	<i>o</i> -Ethyltoluene				
33	Pseudocumene				
34		<i>trans</i> -2-Hexen-1-ol <sup>a</sup>			
36	Myrcene				
39			Hexyl acetate		
42	Limonene				
43	<i>n</i> -Propylbenzene				
45			Acetophenone		
46		I <sup>b</sup>			
47		II <sup>b</sup>			
48		Linaloöl			
49					1-Ethoxy-1-(2-hexenoxy)-ethane <sup>a</sup>
50		2-Phenylethanol			
51			Ethyl benzoate		
52		III <sup>b</sup>			
53		IV <sup>b</sup>			
56		$\alpha$ -Terpineol			
57		Citronellol			
58		Nerol			
59			2-Phenylethyl acetate		
60		Geraniol			
61	2-Methylnaphthalene				
62			Methyl geranate		
63	Biphenyl				
64			Heptyl valerate <sup>a</sup>		
65			2-Hexenyl-1-hexanoate <sup>a</sup>		
66			Hexyl hexanoate		
68			Ethyl decanoate		
69					Diphenyl ether
70			Methyl phthalate <sup>a</sup>		
72	3-Methylbiphenyl		Hexyl octanoate		
74					
76				Benzophenone	
77			Hexyl decanoate		

<sup>a</sup> Compound not confirmed by retention data. <sup>b</sup> See text for structural formula.

60), previously reported by Cordonnier (7), are the two largest peaks of the terpene alcohols. Nerol,  $\alpha$ -terpineol, and citronellol are present in smaller quantities. Peaks numbered 46, 47, 52, and 53 have been identified as cyclic ethers I, II, III, and IV, respectively.



Synthesis by a modified procedure of Felix *et al.* (2) allowed us to identify peak 46 as the *trans* isomer (I), and peak 47 as the *cis* isomer (II). The relative configuration of the tetrahydropyrans (III and IV) has been determined by Klein *et al.* (3). The liquid isomer (III) was shown to have the *trans* configuration, and the crystalline isomer (IV) to have the *cis* configuration. These four compounds probably arise from linalool by either air or enzymatic oxidation with subsequent rearrangement.

The esters (Table I) comprise a small percentage of the total oil, but a fair number are present. Surprisingly, only a small amount of ethyl acetate (peak 7) was detected. Webb (6) reported the presence of ethyl hexanoate, but no trace of this compound could be detected. Ethyl benzoate (peak 51) may have

arisen from the preservative, sodium benzoate.

Table I also lists the carbonyl components identified in the muscat oil. The largest component in this group was the "leaf aldehyde," 2-hexenal (peak 22). A fair amount of 1-hexanal (peak 21) was also identified. Webb (6) identified both  $C_6$  aldehydes in Muscat of Alexandria. Smaller amounts of 1-1-butanal, 3-pentanone, benzaldehyde, acetophenone, and benzophenone were identified in the oil. The presence of benzaldehyde may be an artifact in that reduction of sodium benzoate would lead to the aromatic aldehyde.

A relatively large amount of 1,1-diethoxyethane (peak 17) is present in the oil, and is tabulated in Table I along with several other miscellaneous compounds. A very small amount of ether (peak 3), chloroform (peak 8), and 1-methoxy-1-ethoxyethane (peak 12) was detected. Larger amounts of 1-ethoxy-1-(2-hexenoxy)ethane and diphenyl ether were identified, although the identification of the acetal must be held as tentative since an authentic sample was not available.

Table II is a list of peaks which have not been completely identified, and work is now in progress to elucidate their exact chemical structures.

#### Discussion

An examination of the chromatogram shows that linalool and geraniol are the major components with the exception of hexanol. The rather sweet floral aroma of these two compounds undoubtedly contribute to the over-all pleasant aroma of muscat grapes. In general, the unknown components listed in Table II are present only in trace amounts but, nevertheless, may be important in the aroma of the fruit. Sensory evaluations must be employed to determine the

**Table II. Some Unknown Components in Muscat Oil**

Peak No.	Comment
16	A secondary hexenol
29	Aromatic
35	Aldehyde or ketone
37	Aromatic
38	An octenol
40	A furan
41	An octenol
44	Terpene
54	Ethyl ester
55	Terpene
67	A hexenyl hexanoate
71	Unknown
73	Hexenyl ester
75	Ethyl ester
78	Phthalate ester

relative importance of each of the components. If the minor components are necessary to give the material its "true character," then these constituents must be identified, a task made possible by columns with high resolving power.

#### Literature Cited

- (1) Cordonnier, R., *Ann. Inst. Natl. Recherches Argon.*, Ser. E., *Ann. Technol. Agr.* **5**, 75 (1956).
- (2) Felix, D., Melera, A., Seibl, J., Kovats, E., *Helv. Chim. Acta* **46**, 1513 (1963).
- (3) Klein, E., Farnow, H., Rojahn, W., *Ann.* **675**, 73 (1964).
- (4) McFadden, W. H., Teranishi, R., *Nature* **200**, 329 (1963).
- (5) Schultz, T. H., Teranishi, R., McFadden, W. H., Kilpatrick, P. W., Corse, J., *J. Food Sci.* **29**, 790 (1964).
- (6) Webb, A. D., Kepner, R. E., *Food Research* **22**, 384 (1957).

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