

Volatile from Grapes

Identification of Volatiles from Concord Essence

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The volatiles of Concord grape (*Vitis labrusca*) were examined by capillary gas chromatography-mass spectral combination. Sixty components were

identified; of particular interest were a series of crotonate esters and a possible ethyl alkylthioester.

The extensive use of Concord grape (*Vitis labrusca*) products in the food industry has prompted an investigation of the volatile constituents in the essence. The present communication is an extension of previous work on Concord grape volatiles in which 16 constituents were identified (Stevens *et al.*, 1965). These components along with those identified by Holley *et al.* (1955) are summarized in Table I. Also included are compounds tentatively identified by Neudoerffer *et al.* (1965), using relative retention times.

Although methyl anthranilate is known to be the major contributor to the typical aroma of the fruit (Power and Chesnut, 1921), the anthranilate odor is not the full aroma of the Concord grape (Holley *et al.*, 1955; Neudoerffer *et al.*, 1965). Methyl anthranilate was first isolated from Concord grapes and identified by Power and Chesnut using classical methods; however, this type of investigation is extremely difficult because of the low concentration of volatile organic material in the fresh fruit.

Holley *et al.* (1955) were also limited by the low sensitivity of the classical methods of analysis. In addition to the problem of trace components, the identification of esters was difficult, if not impossible. Ester structures could not be assigned, since only acid and alcohol components could be identified following hydrolysis of the ester mixture.

In recent years, the advent of gas chromatography and mass spectrometry has greatly simplified the analysis of trace components, especially the technique of capillary gas chromatography-mass spectral combination described by McFadden and Teranishi (1963). This technique was used in this investigation along with separate chromatographic runs using a flame ionization detector.

EXPERIMENTAL

Concord essence (13 liters, 150-fold) was obtained from the Seneca Grape Juice Corp., Prosser, Wash. Isopentane,

purified by chromatography over alumina and distillation through a 10-plate Oldershaw column, was used in three 1-liter portions to extract the essence. The isopentane extracts were combined, dried over sodium sulfate, and concentrated to approximately 75 ml. The resulting extract contained 5% oil, and no further concentration was carried out.

Table I. Components of Concord Essence Previously Reported

Holley <i>et al.</i> (1955)	
Ethanol	Acetone
Methanol	Acetaldehyde
Ethyl acetate	Methyl anthranilate
Methyl acetate	Acetic acid
Stevens <i>et al.</i> (1965)	
Ethyl acetate	2-Methyl-3-buten-2-ol
Isopropyl acetate	Ethyl <i>n</i> -butyrate
Ethanol	Isobutanol
2-Propanol	1-Butanol
Ethyl propionate	2-Methyl-1-butanol
<i>n</i> -Propyl acetate	3-Methyl-1-butanol
Chloroform	Styrene
1-Propanol	Ethyl hexanoate
Neudoerffer <i>et al.</i> (1965)	
Tentative Identification by Relative Retention Times	
Acetaldehyde	Ethanol
Ethyl formate	Pentanone
Ethyl chloride	Acetyl acetone
Propionaldehyde	Crotonal
Isobutyraldehyde	3-Heptanone
Acetoin	Ethyl propionate
Acrolein	Diacetyl
Acetone	Propanol
Butyraldehyde	Ethyl butyrate
2-Butanone	Isobutyl isobutyrate
Methyl acetate	Phenyl ethyl butyrate
Isovaleraldehyde	Isoamyl acetate
Ethyl acetate	2-Butanol
Methanol	Amyl propionate
2-Propanol	Benzyl formate
Valeraldehyde	Isoamyl alcohol

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Separation of components was performed on a column (500 feet \times 0.02-inch I.D.) coated with GE SF-96(50) silicone oil containing a trace of Igepal. The injector was held at 150° C. and the column programmed from 30° to 186° C. at approximately 1.5° per minute. The average linear velocity was 30 cm. per second at 30° C. A Biemann separator (Watson and Biemann, 1964) was placed between the gas chromatograph effluent and a Bendix Time-of-Flight Model 12 mass spectrometer to increase the concentration of organic material entering the mass spectrometer. The separator was heated to 150° C. and selectively removed approximately 90% of the helium thus giving a 5- to 10-fold increase in dynamic range.

The mass spectra of separated components emerging from the gas chromatograph were monitored on an oscilloscope, and a chromatographic record was obtained on a strip chart recorder by monitoring the ion current at mass 43. Mass spectra were recorded by a high speed Visicorder scanning from 20 to 200 mass units in 2.5 seconds. The charts were compared with mass spectral patterns of known compounds, and assignments were made. The mass spectral analysis was confirmed by enriching the grape oil with the suggested compounds, rechromatographing with a flame ionization detector, and obtaining an increase in response at the assigned retention time. A number of compounds were identified by

relative retention times using known standards or by comparing their mass spectra with those of known samples. The known compounds were either synthesized by standard methods (Connell, 1964; Haagen-Smit *et al.*, 1946; Rodin *et al.*, 1966) or obtained from commercial sources.

RESULTS

Figure 1 is a chromatograph of the Concord grape oil using the flame ionization detector. The numbers above the peaks correspond to the compounds listed in Table II, along with references if previously reported, in grapes.

The hydrocarbons, isopentane, 2-methylpentane, 3-methylpentane, hexane, methylcyclopentane, cyclohexane, and toluene (peaks 1, 3, 4, 5, 8, 10, and 18, respectively) are known contaminants from the solvent. Although the higher boiling hydrocarbons such as the xylenes (peaks 23 and 28), ethyltoluenes (33, 34, and 37), trimethylbenzenes (35, 39, and 43) etc., were not detected in a control experiment, the possibility of solvent contamination cannot be eliminated.

Ethyl acetate (peak 6) is the largest component in the grape oil extract. The preponderance of this ester was previously reported (Stevens *et al.*, 1965) but in contrast to the prior investigation only a small amount of 2-methyl-3-buten-2-ol (peak 7) was found. A number of possibilities

Table II. Components of Concord Essence Found in This Investigation

Peak No.	Compound	Reference	Peak No.	Compound	Reference
1	Isopentane	Stevens <i>et al.</i> (1966)	29	Hex-2-en-1-ol	Stevens <i>et al.</i> (1966)
2	Ethanol	^a	30	Ethyl pentanoate	
3	2-Methylpentane		31	Isopropylbenzene	
4	3-Methylpentane	Stevens <i>et al.</i> (1966)	32	<i>n</i> -Propylbenzene	
5	Hexane ^b		33	<i>m</i> -Ethyltoluene	
6	Ethyl acetate	^c	34	<i>p</i> -Ethyl toluene	
7	2-Methyl-3-buten-2-ol		35	1,3,5-Trimethylbenzene	Stevens <i>et al.</i> (1966)
8	Methylcyclopentane	Stevens <i>et al.</i> (1966)	36	Benzaldehyde	Stevens <i>et al.</i> (1966)
9	Isopropyl acetate	Stevens <i>et al.</i> (1965)	37	<i>o</i> -Ethyltoluene	Stevens <i>et al.</i> (1966)
10	Cyclohexane	Stevens <i>et al.</i> (1966)	38	Ethyl hexanoate	Stevens <i>et al.</i> (1965)
11	Ethyl propionate	^d	39	1,2,4-Trimethylbenzene	Stevens <i>et al.</i> (1966)
12	<i>n</i> -Propyl acetate	Chaudhary <i>et al.</i> (1964)	40	Isobutyl crotonate	
		Stevens <i>et al.</i> (1965)	41	Isobutylbenzene	
13	Methyl butyrate	Chaudhary <i>et al.</i> (1964)	42	Methylpropylbenzene ^e	
14	Diethoxyethane	Kepner and Webb (1956)	43	1,2,3-Trimethylbenzene	
		Stevens <i>et al.</i> (1957)	44	Ethyl hex-2-enoate	
15	4-Methyl-2-pentanone	Webb and Kepner (1957)	45	Alkylbenzene ^b	
16	Ethyl isobutyrate	Neudoerffer <i>et al.</i> (1965)	46	Alkylbenzene ^b	
17	Methyl crotonate		47	Acetophenone	Stevens <i>et al.</i> (1966)
18	Toluene	Stevens <i>et al.</i> (1966)	48	Alkylbenzene ^b	
19	Hexanal	^e	49	Methoxyphenol ^g	
20	Ethyl butyrate		50	Linalool	Stevens <i>et al.</i> (1966)
21	Ethyl crotonate		51	Ethyl alkylthioester	
22	2-Hexenal	^f	52	Ethyl β -hydroxyhexanoate	
23	<i>p</i> - and/or <i>m</i> -xylene	Stevens <i>et al.</i> (1966)	53	Ethyl benzoate	Stevens <i>et al.</i> (1966)
24	Furfural ^g		54	Indole	
25	Isopropyl crotonate		55	Terpinene-4-ol	Stevens <i>et al.</i> (1966)
26	Hex-3-ene-1-ol	Kepner and Webb (1956)	56	α -Terpineol	Stevens <i>et al.</i> (1966)
27	Hexanol	Chaudhary <i>et al.</i> (1964)	57	Methyl benzyl acetate ^g	
		Stevens <i>et al.</i> (1966)	58	Methyl anthranilate	Holley <i>et al.</i> (1955)
28	<i>o</i> -Xylene	Stevens <i>et al.</i> (1966)	59	Ethyl anthranilate	Power and Chesnut (1921)

^a Chaudhary *et al.*, 1964; Haagen-Smit *et al.*, 1946; Holley *et al.*, 1955; Kepner and Webb, 1956; Neudoerffer *et al.*, 1965; Stevens *et al.*, 1965; Webb and Kepner, 1957. ^b Indicates no retention data. ^c Chaudhary *et al.*, 1964; Holley *et al.*, 1955; Kepner and Webb, 1956; Neudoerffer *et al.*, 1965; Stevens *et al.*, 1965, 1966. ^d Chaudhary *et al.*, 1964; Neudoerffer *et al.*, 1965; Stevens *et al.*, 1965. ^e Kepner and Webb, 1956; Stevens *et al.*, 1966; Webb and Kepner, 1957. ^f Chaudhary *et al.*, 1964; Haagen-Smit *et al.*, 1949; Kepner and Webb, 1956; Stevens *et al.*, 1966; Webb and Kepner, 1957. ^g Indicates relative retention time only.

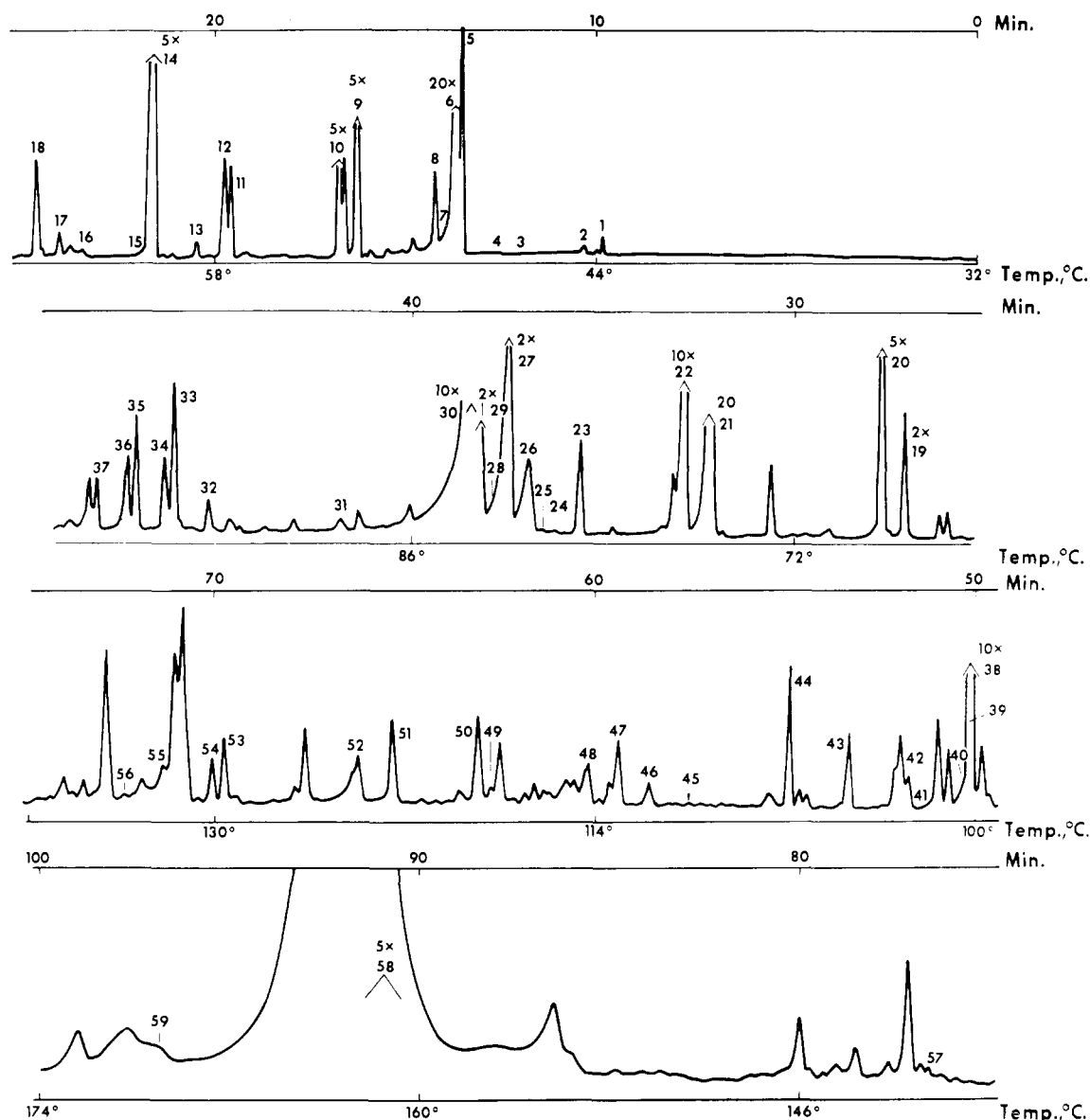


Figure 1. Capillary gas chromatographic analysis of Concord grape essence by SF 96(50) column (500 feet \times 0.02-inch I.D.)

might account for this discrepancy: In this investigation isopentane was used as a solvent rather than ether; the extracted oil was not distilled in this investigation; or the sources of grape essence are different.

In addition to ethyl acetate, a large number of other esters has been identified. Methyl butyrate, ethyl isobutyrate, ethyl 2-hexenoate, ethyl benzoate, and methyl benzyl acetate (peaks 13, 16, 44, 53, and 57) have not previously been reported in Concord grapes. A series of crotonates not previously detected, methyl, ethyl, isopropyl, and possibly isobutyl (peaks 17, 21, 25, and 40), made up a large percentage of the oil with ethyl crotonate most predominant.

Peak 51 appears to be an ethyl alkylthioester. The mass spectrum shows a parent peak at m/e 162 with an intense 88 and 75. The analogs, ethyl *n*-propylthioacetate, ethyl 3-ethylthiopropoate, ethyl 4-methylthio-

butanoate—i.e., $\text{EtOOCH}_2\text{SCH}_2\text{CH}_2\text{CH}_3$, etc.—were synthesized, and their retention times were close to that of peak 51. Distinct similarities were observed in the mass spectra but none was identical. Accurate mass measurements on a CEC 21-110 mass spectrometer showed that the ion peaks at masses 88 and 75 contained one sulfur and no oxygen, in agreement with measurement on the authentic compound, ethyl *n*-propylthioacetate. Two other isomers were prepared, ethyl isopropylthioacetate and ethyl 2-ethylthiopropoate; their retention times were shorter than that of peak 51. Work is still in progress to elucidate the structure of this compound.

Methyl anthranilate (peak 58) appears as a large peak at the end of the chromatogram. This component was first identified by Power and Chesnut (1921) and has since been confirmed by a number of workers. An interesting analog, ethyl anthranilate (peak 59), has been determined,

and this also has an aroma similar to the methyl ester.

In the authors' previous work (Stevens *et al.*, 1965) and that of Holley *et al.* (1955), ethanol was the major constituent of the essence. In this analysis, ethanol was not a very large constituent, probably because of its low solubility in isopentane.

CONCLUSION

Esters represent the largest percentage of components in this analysis of Concord grape essence with ethyl acetate and methyl anthranilate predominating. A number of other esters were found, not previously reported, among them ethyl crotonate in relatively large amount. A possible ethyl alkylthioester was observed, but the structure has not yet been fully elucidated. These esters are pleasant in aroma and undoubtedly contribute to the over-all pleasant aroma of the Concord grape essence.

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