

carbamate formed by the reaction of ammonia and DEDC in wine is variable with pH and the concentration of the reactants. The analyses of the 15 commercial wines indicate that under conditions of use, with monitored and adjusted pH and ammonia content, not enough ethylcarbamate is formed to be detected accurately even by this sensitive test. The test can detect added ethylcarbamate at 10 µg/l. with reasonable accuracy, allowing the detection of erroneous additions. The amounts formed are far less than that present naturally. It therefore seems plausible that the controlled use of DEDC as a yeast sterilant should be reconsidered.

LITERATURE CITED

Amerine, M. A., Ough, C. S., "Wine and Must Analysis", Wiley, New York, N.Y., 1974, pp 16-17, 76-77.

- Berg, H. W., Akiyoshi, M., *Am. J. Enol. Vitic.* **22**, 127 (1971).
 Du Plessis, C. S., *S. Afr. J. Agric. Sci.* **6**, 561 (1963).
 Fischer, E., *Z. Lebensm.-Unters.-Forsch.* **147-148**, 221 (1971-1972).
 Kissler, J. J., Ough, C. S., Alley, C. J., *Calif., Agric. Exp. Stn. Bull.* **No. 865**, 1 (1973).
 Lofroth, G., Gejvall, T., *Science* **174**, 1248 (1971).
 McWilliam, D. J., Ough, C. S., *Am. J. Enol. Vitic.* **25**, 67 (1974).
 Ough, C. S., *Wines Vines* **45**(5), 29 (1964).
 Ough, C. S., *Wines Vines* **47**(1), 17 (1966).
 Ough, C. S., *Am. J. Enol. Vitic.* **20**, 213 (1969).
 Ough, C. S., *J. Agric. Food Chem.*, preceding paper in this issue (1976).
 Zamorani, A., Asmundo, C. N., Maccarrone, A., *Riv. Viticolt. Enol.* **26**, 462 (1973).

Received for review July 21, 1975. Accepted November 18, 1975.

Identification of Volatile Constituents from Grapes

Peter Schreier, Friedrich Drawert,* and Albrecht Junker

The volatile constituents of grapes (varieties Riesling, Traminer, Ruländer, Müller-Thurgau, Scheurebe, Optima, and Rieslaner, harvested 1972-1974) were isolated under enzymic inhibition, enriched by liquid-liquid extraction (pentane-methylene chloride, 2:1), and fractionated on silica gel. Analysis by gas chromatography and coupled GLC-mass spectrometry led to the identification of 225 aroma compounds that included 81 hydrocarbons, 48 acids, 31 alcohols, 23 aldehydes, 18 ketones, 11 esters, and 13 constituents of miscellaneous structures. Eighty-one aroma substances were identified for the first time in a grape aroma. The mass spectral identifications were confirmed by matching retention indices.

It is well known that grapes of different varieties show quite characteristic aromas that are also distinctive of the sort-typical wines. But it must be considered that different biosynthetic pathways are interacting at the formation of the aroma of alcoholic beverages. As Drawert and Rapp (1966) and Drawert (1974) described, with the aid of the biotechnological sequence of wine, different factors play a part in the formation of the total aroma. It could be shown that the volatile aroma substances arise from components of the grapes, the activity of fermentation of sugars, and aroma compounds formed during storage. Furthermore, those reactions that are caused by the technology of winemaking are important, such as, for instance, the formation of C₆-aldehydes and -alcohols during crushing and at the moment of destruction of the cell structure (Drawert et al., 1973).

In our recent studies we identified more than 200 aroma substances in different wine varieties by means of adsorption, gas chromatography, and mass spectrometry (Drawert et al., 1974; Schreier and Drawert, 1974a,b; Schreier et al., 1974a, 1975). These methods are particularly qualified for a tracer analysis because of their selectivity and sensitivity. We found that numerous aroma compounds occurring only in traces (micrograms per liter) are important for the sort-typical aromas of the different

wines. We applied the same methods for the investigation of the grape aroma. The present study gives a survey of the volatile aroma substances identified by GLC-mass spectrometry in grapes.

EXPERIMENTAL SECTION

Materials. Samples of 30 kg of grapes (varieties Riesling, Traminer, Ruländer, Müller-Thurgau, Scheurebe, Optima, and Rieslaner, harvested 1972-1974 in Rheinpfalz and Franken, West Germany) were investigated.

Aroma Separation. Two different methods were used in order to inhibit the enzymes (Drawert et al., 1973). (1) The grapes were crushed by adding methanol (total concentration 66%) with a Stephan type crusher. The methanol-juice mixture was separated immediately in a Hafico type hydraulic press. (2) The grapes were stemmed and crushed with a horizontal type crusher-stemmer under carbon dioxide using a short-time heating procedure (5 sec at 100°C; heat exchanger). The juice was evaporated to a volume of about 3 l. using a thin film evaporator (Centritherm, Alfa-Laval).

Extraction and Fractionation. The grape volatiles were extracted from the methanolic solution (method 1) and from the aqueous distillate (method 2) with a pentane-methylene chloride (2:1) mixture. The solvent was removed by distillation through a Vigreux column (20 cm, 40°C) (Drawert and Rapp, 1968; Drawert et al., 1969). The aroma concentrate was then subjected to ascending column chromatography using pentane-ether mixtures as the migrating solvents (Schreier and Drawert, 1974b). A particular fraction (pentane elution) could be divided by preparative GLC separation. The column and gas chro-

Institut für Lebensmitteltechnologie und Analytische Chemie der Technischen Universität München, Lehrstuhl für Chemisch-technische Analyse und chemische Lebensmitteltechnologie, D-8050 Freising-Weißenstephan, West Germany.

Table I. Compounds Identified from Grapes by Gas Chromatography-Mass Spectrometry

Compound	Lit. cited	Compound	Lit. cited
(1) Hydrocarbons		(4) Aldehydes	
<i>n</i> -Alkanes, C ₁₀ -C ₃₂	<i>a-e</i>	3-Methylbutanal	<i>i, cc</i>
<i>n</i> -Alkenes, C ₁₀ , C ₁₂ , C ₁₄ , C ₁₆	<i>a-e</i>	2-Methyl-2-butenal	
C ₁₈ -C ₃₂	<i>a-e</i>	1-Pentanal	<i>i, cc</i>
Toluene	<i>a, b, g-i, k, l</i>	<i>cis</i> -2-Pentenal	
<i>m</i> -Xylene	<i>a, b, i, l</i>	<i>trans</i> -2-Pentenal	
<i>o</i> -Xylene	<i>a, b, g, h, l</i>	2-Methyl-2-pentenal	
<i>p</i> -Xylene	<i>a, b, g, h, l</i>	1-Hexanal	<i>g-i, k, l, o, bb</i>
Ethylbenzene	<i>a, b, i</i>	<i>cis</i> -2-Hexenal	<i>g, i</i>
3-Alkylbenzenes, <i>P</i> = 120	<i>a, b, g, h, l</i>	<i>trans</i> -2-Hexenal	<i>g-i, k, l, v, w, x</i>
5-Alkylbenzenes, <i>P</i> = 134	<i>a, b, g, h, l</i>	<i>cis</i> -3-Hexenal	<i>f</i>
5-Alkylbenzenes, <i>P</i> = 148	<i>a, b, h</i>	<i>trans,trans</i> -2,4-Hexadienal	
2-Methylnaphthalenes	<i>g, h</i>	<i>trans,cis</i> -2,4-Hexadienal	
Diphenyl		1-Heptanal	
3-Methyldiphenyl	<i>l</i>	<i>trans</i> -2-Heptenal	
Limonene	<i>g, i, k-o</i>	<i>trans,trans</i> -2,4-Heptadienal	
Myrcene	<i>l-o</i>	<i>trans,cis</i> -2,4-Heptadienal	
β -Caryophyllene		1-Octanal	
α -Humulene		<i>trans</i> -2-Octenal	
Calamenene		<i>trans,trans</i> -2,4-Octadienal	
δ -Cadinene		1-Nonanal	<i>g</i>
γ -Cadinene		1-Decanal	
β -Bourbonene		Benzaldehyde	<i>g, h, l</i>
α -Muurolene		Phenylacetaldehyde	
γ -Muurolene		(5) Ketones	
β -Selinene		3-Methyl-2-butanone	
α -Copaene		3-Methyl-3-buten-2-one	
β -Ylangene		3-Pentanone	<i>i, l</i>
α -Guaiene		3-Methyl-2-pentanone	<i>j</i>
Germacrene D		2-Methyl-3-pentanone	<i>i</i>
α -Farnesene		1-Penten-3-one	
(2) Esters		2-Hexanone	
Ethyl acetate	<i>g-i, w, x</i>	2-Heptanone	
Isobutyl acetate		3-Heptanone	<i>g, cc</i>
Hexyl acetate	<i>g, i, k, l, w</i>	2-Methyl-2-hepten-6-one	<i>g, c</i>
<i>cis</i> -3-Hexen-1-yl acetate	<i>g, i</i>	6-Methyl-3,5-heptadien-2-one	
<i>trans</i> -2-Hexen-1-yl acetate	<i>g</i>	2-Octanone	
Methyl hexanoate		2-Nonanone	
Ethyl hexanoate	<i>h, i, l, w</i>	2-Decanone	<i>g</i>
Methyl octanoate		6,10,14-Trimethyl-2-pentadecanone	
Ethyl octanoate	<i>w</i>	Acetophenone	<i>g, h, l</i>
<i>trans</i> -Methyl geranate	<i>l</i>	β -Ionone	<i>u</i>
Dimethyl phthalate	<i>l</i>	Damascenone	
(3) Alcohols		(6) Acids	
1-Butanol	<i>n, w</i>	2-Methylpropionic acid	<i>x</i>
2- and 3-methyl-1-butanol	<i>g, i, k, l, w, x</i>	Butyric acid	<i>v-x</i>
3-Methyl-2-buten-1-ol		2-Methylbutyric acid	<i>x</i>
1-Pentanol	<i>n, w, x</i>	3-Methylbutyric acid	<i>w, x</i>
<i>trans</i> -2-Penten-1-ol		Pentanoic acid	<i>w, x</i>
3-Pentanol	<i>l</i>	Hexanoic acid	<i>v, w</i>
1-Penten-3-ol		<i>cis</i> -3-Hexenoic acid	<i>w</i>
1-Hexanol	<i>g-i, k, l, n-p</i>	2-Ethylhexanoic acid	
<i>trans</i> -2-Hexen-1-ol	<i>g-i, k, l, o</i>	<i>n</i> -Heptanoic acid	<i>w, x</i>
<i>cis</i> -3-Hexen-1-ol	<i>g-i, k, e</i>	<i>n</i> -Octanoic acid	<i>w, x</i>
<i>trans</i> -3-Hexen-1-ol	<i>g, l</i>	<i>trans</i> -Geranic acid	
2-Ethyl-1-hexanol	<i>g</i>	<i>n</i> -Nonanoic acid	<i>x</i>
1-Heptanol	<i>g, n</i>	3-Nonenoic acid	
1-Octanol	<i>g, i, n</i>	<i>n</i> -Decanoic acid	<i>w, x</i>
<i>trans</i> -2-Octen-1-ol		<i>n</i> -Undecanoic acid	<i>x</i>
3-Octanol		Lauric acid, C _{12:0}	<i>x</i>
1-Octen-3-ol		<i>n</i> -Tridecanoic acid, C _{13:0}	
1-Nonanol	<i>g, n</i>	Myristic acid, C _{14:0}	
1-Decanol	<i>i, n</i>	C _{14:1}	
1-Undecanol		<i>n</i> -Pentadecanoic acid, C _{15:0}	
Benzyl alcohol	<i>i, n</i>	C _{15:0} (iso)	
1-Phenylethanol		C _{15:1}	
2-Phenylethanol	<i>l, n, s, w</i>	Palmitic acid, C _{16:0}	<i>y, z</i>
α -Cadinol		Palmitoleic acid, C _{16:1}	<i>y</i>
Citronellol	<i>g, l, p</i>	<i>n</i> -Heptadecanoic acid, C _{17:0}	
Linalool	<i>g, h, l-t, u, x, bb</i>	C _{17:1}	
ho-Trienol		Stearic acid, C _{18:0}	<i>y, z</i>
Geraniol	<i>g, l, m, n, p, s-u</i>	Oleic acid, C _{18:1}	<i>y, z</i>
Nerol	<i>g, l, m-p, s, t</i>	Linoleic acid, C _{18:2}	<i>y, z</i>
α -Terpineol	<i>g, h, l, m, o, p, s, t</i>	3 C _{18:2} (iso)	
4-Terpinenol	<i>h, i</i>	Linolenic acid, C _{18:3}	<i>y</i>
		Arachidic acid, C _{20:0}	

Table I (Continued)

Compound	Lit. cited	Compound	Lit. cited
C _{20:1}		<i>cis</i> -Roseoxide	
Behenic acid, C _{22:0}	<i>j, x, aa</i>	<i>trans</i> -Roseoxide	
Succinic acid	<i>aa</i>	Acetovanillone	
Glutaric acid		2,6,10,10-Tetramethyl-1-oxaspiro[4.5]-	
Suberic acid		dec-6-ene (Theaspirane)	
Azelaic acid	<i>aa</i>	2-Hydroxy-2,6,6-trimethylcyclohexyliden-	
Benzoic acid		acetic acid γ -lactone (dihydro-	
4-Methylbenzoic acid	<i>aa</i>	actinidiolide)	<i>l, s, t</i>
2-Hydroxybenzoic acid	<i>aa</i>	2-Vinyl-2-methyl-5-(1'-hydroxy-1'-methyl-	
4-Hydroxybenzoic acid		ethyl)tetrahydrofuran (<i>cis</i> -linalool oxide)	<i>l, s, t</i>
Phenylacetic acid		2-Vinyl-2-methyl-5-(1'-hydroxy-1'-methyl-	
3-Phenylpropionic acid	<i>aa</i>	ethyl)tetrahydrofuran (<i>trans</i> -linalool	
Cinnamic acid		oxide)	<i>l, s, t</i>
Phthalic acid		2,6,6-Trimethyl-2-vinyl-4-hydroxytetra-	
(7) Miscellaneous		hydropyran (liquid isomer)	<i>l, s, t</i>
Benzothiazole		2,6,6-Trimethyl-2-vinyl-4-hydroxytetra-	
2-Pentylfuran		hydropyran (crystallized isomer)	
Neroloxide		2-Vinyl-2-methyltetrahydrofuran-5-one	

^a Speck, 1971. ^b Drawert, 1970. ^c Radler, 1965a. ^d Radler, 1965b. ^e Radler and Horn, 1965. ^f Drawert et al., 1973. ^g Stevens et al., 1967. ^h Stern et al., 1967. ⁱ Stevens et al., 1969. ^j Kliewer, 1966. ^k Webb and Kepner, 1957. ^l Stevens et al., 1966. ^m Rodopulo et al., 1974a. ⁿ Rodopulo et al., 1974b. ^o Hardy, 1970. ^p Bayonove and Cordonnier, 1971a. ^q Bayonove and Cordonnier, 1971b. ^r Usseglio-Tomasset, 1966. ^s Terrier et al., 1972a. ^t Terrier et al., 1972b. ^u Mud-joyan et al., 1971. ^v Haagen-Smit et al., 1949. ^w Chaudhary et al., 1964. ^x Van Wyk et al., 1967. ^y Kinsella, 1974. ^z Hameed et al., 1972. ^{aa} Leupold, 1974. ^{bb} Webb et al., 1966. ^{cc} Neudoerffer et al., 1965.

matographic fractions were analyzed using the GLC-mass spectrometry system described below. Analytical grade solvents (99%) were redistilled before use.

Isolation of Acid Fraction and Derivatization. The free fatty acids were removed from the aroma extracts by washing the organic phase three times in a separating funnel with 50-ml portions of 5% aqueous Na₂CO₃ solution. The combined acid extract was washed three times with 50-ml portions of ether and the traces of water-soluble substances were stripped from the carbonate solution in a rotary evaporator. The aqueous concentrate (about 20 ml) was dissolved in 50 ml of H₂O, acidified to pH 2-3 with H₂SO₄ (10%), and extracted with three successive 50-ml portions of ether. The ether extract was washed with water, dried over anhydrous Na₂SO₄, and concentrated (20 ml) by distilling away the ether through a Vigreux column. The esterification was realized by using diazomethane in the following technique. An alcoholic KOH solution (10%) was added to a solution of 1 mM *N*-methyl-*N*-(tolylsulfonyl-(4)]nitrosamide in ether. The diazomethane formed was converted to the reaction funnel using N₂. In order to complete the esterification (persistence of a yellow color) the ether was removed by distillation through a Vigreux column and the methyl ester concentrate was analyzed by GLC and coupled GLC-mass spectrometry.

Gas-Liquid Chromatography Conditions. A Varian Aerograph series 1800 gas chromatograph equipped with a flame ionization detector was used. The instrument was fitted with a stainless steel column (5 m \times 1/8 in. o.d.) packed with 5% FFAP on Varaport 30 (80-100 mesh) (C1). The operating conditions were the following: injection port temperature, 220°C; detector temperature, 280°C; column temperature, programmed from 70 to 250°C at 2°C/min; carrier gas flow (N₂), 20 ml/min; hydrogen flow, 30 ml/min; air flow, 300 ml/min.

Preparative Gas Chromatography. The apolar aroma fractions obtained by column chromatography on silica gel (pentane elutions) were separated on a Hewlett-Packard Model 1075A gas chromatograph, equipped with a flame ionization detector. A stainless steel column (3 m \times 0.25 in. o.d.) packed with Carbowax 20M (20% by weight) on Varaport 30 (60-80 mesh) was employed. The operating parameters were the following: injection port temperature, 200°C; detector temperature, 250°C; column temperature

programmed from 120 to 190°C at 2°C/min; carrier gas flow (N₂), 120 ml/min.

Combined Gas Chromatography-Mass Spectrometry. A Varian Aerograph Model 1201 was coupled via a helium separator according to Watson and Bieman to a mass spectrometer (Varian-MAT, Bremen, West Germany, Model CH 7). The gas chromatograph was equipped with different types of columns: C2—150 ft \times 0.02 in. i.d. stainless steel SCOT column coated with Carbowax 20M; a helium flow of 1.65 ml/min was used; C3—"Micropak" stainless steel column (6 m \times 1/16 in. o.d., 0.75 mm i.d.), packed with 5% UCON LB 550X on Varaport 30 (100-120 mesh); a helium flow rate of 2.5 ml/min was used; C4—"Micropak" glass column (5 m \times 1/16 in. o.d., 1 mm i.d.), packed with 5% FFAP on Varaport 30 (100-120 mesh); helium flow 4.1 ml/min; C5—"Micropak" stainless steel column (6 m \times 1/16 in. o.d., 0.75 mm i.d.), packed with 5% FFAP on Varaport 30 (100-120 mesh); a helium flow of 5.3 ml/min was used. The columns C2-C5 were operated at a temperature programmed from 70 to 100°C at 1°C/min, 100 to 190°C (C2,C3) or 100 to 250°C (C4, C5) at 2°C/min. Injection volumes were: 0.1-0.5 μ l (C2) and 0.5-1.0 μ l (C3-C5). The total ionization current obtained from the ionization chamber operating at 70 eV was recorded as a gas chromatogram. The ion source temperature was 250°C. The mass spectra were recorded on a Siemens Oscillograph.

Reference Samples. Some compounds which could not be obtained from commercial sources were prepared as described elsewhere (Schreier and Drawert, 1974b; Tressl et al., 1970a).

RESULTS AND DISCUSSION

Some years ago only a few components were known and identified in fruit aromas. Modern and efficient methods such as gas chromatography and mass spectrometry led in the meantime to the identification of several hundred compounds in fruit. If we succeed in isolating the genuine aroma substances extensively unchanged by special steps (for instance by inhibiting the enzymes) we can be sure that the original primary aroma, which supplies the foundation for all biogenetic reflections, will be preserved. On the contrary, secondary aromas were obtained without those steps because of enzymic reactions that are typical

Table II. Mass Spectral Data^a of Compounds from the Neutral Aroma Fractions^{b,c} Identified for the First Time in Grapes

Compounds (mol wt)	m/e (%)
β -Caryophyllene (204)	41 (100), 69 (69), 79 (58), 91 (63), 93 (98), 105 (44), 107 (40), 133 (100)
α -Humulene (204)	41 (44), 53 (17), 55 (16), 67 (13), 80 (45), 93 (100), 121 (30), 204 (23)
Calamenene (202)	41 (7), 128 (9), 129 (10), 131 (8), 144 (7), 159 (100), 160 (13), 202 (11)
δ -Cadinene (204)	41 (35), 81 (32), 91 (36), 105 (50), 119 (56), 134 (60), 161 (100), 204 (48)
γ -Cadinene (204)	41 (41), 55 (29), 79 (30), 91 (29), 92 (27), 105 (26), 161 (100), 204 (45)
β -Bourbonene (204)	41 (20), 77 (11), 79 (23), 80 (62), 81 (100), 91 (11), 123 (50), 161 (22)
α -Muurolene (204)	41 (41), 81 (27), 93 (47), 94 (44), 105 (100), 119 (25), 161 (67), 204 (38)
γ -Muurolene (204)	41 (24), 79 (23), 91 (27), 93 (38), 105 (38), 119 (36), 161 (100), 204 (29)
β -Selinene (204)	41 (98), 55 (51), 67 (45), 81 (54), 83 (50), 107 (50), 107 (50), 204 (100)
α -Copaene (204)	41 (41), 55 (28), 81 (29), 91 (28), 93 (56), 105 (90), 119 (100), 161 (100)
β -Ylangene (204)	41 (79), 55 (34), 91 (29), 93 (29), 105 (30), 119 (31), 161 (100), 204 (46)
α -Guaiene (204)	41 (64), 91 (45), 105 (100), 107 (36), 119 (50), 147 (41), 161 (100), 204 (86)
Germacrene D (204)	41 (80), 79 (59), 81 (67), 91 (71), 93 (42), 105 (85), 119 (62), 161 (100)
α -Farnesene (204)	41 (98), 55 (70), 69 (76), 79 (44), 93 (100), 107 (44), 119 (40), 123 (36)
Isobutyl acetate (116)	29 (14), 39 (10), 41 (28), 43 (100), 55 (12), 56 (45), 57 (12), 73 (35)
<i>trans</i> -2-Penten-1-ol (86)	27 (14), 29 (18), 31 (14), 41 (33), 55 (17), 57 (100), 67 (17), 68 (28)
1-Penten-3-ol (86)	29 (40), 31 (18), 39 (8), 41 (16), 43 (12), 55 (6), 57 (100), 58 (14)
<i>trans</i> -2-Octen-1-ol (128)	41 (48), 42 (24), 43 (36), 55 (41), 57 (100), 67 (27), 68 (30), 81 (25)
3-Octanol (130)	29 (22), 31 (38), 41 (56), 43 (24), 55 (98), 59 (100), 83 (58), 102 (18)
1-Octen-3-ol (128)	29 (17), 41 (14), 43 (24), 55 (10), 57 (100), 59 (20), 71 (10), 72 (17)
1-Undecanol (172)	41 (67), 42 (35), 43 (98), 55 (100), 56 (93), 57 (56), 69 (83), 70 (79)
1-Phenylethanol (122)	43 (30), 50 (20), 51 (45), 77 (100), 79 (50), 105 (93), 107 (50), 122 (20)
α -Cadinol (222)	41 (73), 43 (100), 81 (45), 95 (98), 105 (44), 121 (79), 161 (74), 204 (43)
3,7-Dimethyl-1,5,7-octatrien-3-ol (ho-trienol) (152)	41 (14), 43 (52), 53 (7), 55 (9), 67 (48), 71 (100), 79 (8), 82 (70)
2-Methyl-2-butenal (84)	27 (40), 29 (61), 39 (13), 41 (11), 53 (15), 55 (98), 83 (9), 84 (100)
<i>trans</i> -2-Pentalal (84)	27 (61), 29 (60), 39 (60), 41 (61), 53 (21), 55 (100), 83 (42), 84 (64)
2-Methyl-2-pentalal (98)	29 (14), 39 (39), 41 (100), 43 (10), 55 (25), 69 (38), 83 (15), 98 (53)
<i>trans,trans</i> -2,4-Hexadienal (96)	27 (16), 39 (56), 41 (56), 53 (35), 67 (36), 81 (100), 95 (16), 96 (47)
1-Heptanal (114)	29 (43), 41 (64), 42 (50), 43 (68), 44 (100), 55 (46), 57 (43), 70 (61)
<i>trans</i> -2-Heptenal (112)	27 (46), 39 (49), 41 (100), 43 (37), 55 (70), 56 (45), 57 (47), 70 (61)
<i>trans,trans</i> -2,4-Heptadienal (110)	39 (23), 41 (26), 53 (25), 67 (13), 77 (13), 79 (19), 81 (100), 110 (19)
1-Octanal (128)	29 (85), 41 (92), 43 (100), 44 (79), 55 (50), 56 (51), 57 (69), 84 (40)
<i>trans</i> -2-Octenal (126)	29 (57), 39 (41), 41 (100), 42 (40), 55 (89), 57 (38), 70 (53), 83 (48)
<i>trans,trans</i> -2,4-Octadienal (124)	27 (20), 39 (30), 41 (24), 53 (19), 54 (16), 67 (21), 81 (100), 124 (15)
1-Decanal (156)	41 (75), 43 (92), 44 (56), 55 (61), 57 (100), 70 (53), 71 (52), 82 (49)
Phenylacetaldehyde (120)	39 (15), 41 (10), 43 (9), 51 (8), 65 (29), 91 (100), 92 (31), 120 (22)
3-Methyl-2-butanone (86)	27 (13), 29 (2), 39 (9), 41 (13), 42 (5), 43 (100), 71 (3), 86 (6)
3-Methyl-3-buten-2-one (84)	27 (9), 29 (6), 39 (43), 41 (100), 42 (9), 43 (95), 69 (39), 84 (39)
1-Penten-3-one (84)	27 (50), 29 (27), 55 (100), 56 (5), 57 (7), 59 (4), 74 (3), 84 (13)
2-Hexanone (100)	29 (16), 41 (22), 43 (100), 57 (18), 58 (47), 71 (6), 85 (8), 100 (8)
2-Methyl-2-hepten-6-one (126)	39 (15), 41 (55), 43 (100), 55 (38), 58 (18), 67 (16), 69 (33), 108 (34)
6-Methyl-3,5-heptadien-2-one (124)	39 (22), 41 (20), 43 (37), 53 (19), 79 (18), 81 (39), 109 (100), 124 (18)
2-Octanone (128)	41 (14), 43 (82), 57 (8), 58 (100), 59 (26), 71 (18), 72 (8), 128 (10)
2-Decanone (156)	41 (20), 43 (70), 55 (12), 57 (26), 58 (100), 59 (60), 71 (38), 96 (12)
6,10,14-Trimethyl-2-pentadecanone (250)	41 (30), 43 (80), 55 (34), 57 (43), 58 (100), 59 (47), 69 (23), 71 (37)
Damascenone (190)	39 (9), 41 (31), 69 (100), 77 (8), 91 (11), 105 (18), 121 (54), 190 (11)
Benzothiazole (135)	39 (7), 63 (10), 69 (21), 82 (10), 91 (10), 108 (37), 135 (100), 136 (8)
2-Pentylfuran (138)	27 (11), 29 (5), 39 (8), 41 (8), 53 (15), 81 (100), 82 (27), 138 (14)
Neroloxide (152)	41 (18), 53 (13), 55 (15), 67 (66), 68 (100), 69 (13), 83 (72), 96 (11)
Roseoxide (154)	39 (14), 41 (35), 55 (35), 69 (64), 83 (26), 85 (14), 139 (100), 154 (20)
Theaspirane (194)	41 (10), 43 (6), 55 (14), 82 (40), 83 (19), 96 (22), 109 (11), 138 (100)
Dihydroactinidiolide (180)	43 (50), 67 (30), 69 (15), 109 (60), 111 (100), 137 (45), 152 (14), 180 (27)
2-Vinyl-2-methyltetrahydrofuran-5-one (126)	27 (76), 41 (35), 43 (95), 55 (78), 56 (34), 67 (52), 71 (40), 111 (100)

^a The data were taken from the mass spectra of known substances. ^b Numbers in italics are the base peaks of the compounds. ^c The mass spectral data of acids (as methyl esters) are reported (Scherer, 1975).

for technological processes. In recent studies we found that grapes also form in the same manner C₆-aldehydes and -alcohols at the moment of destruction of cell structure (Drawert et al., 1973). As these components quantitatively exceed the other compounds, the aroma of grape juice could be determined in a high degree by these substances. Thus, Stevens et al. (1967) and Ramshaw and Hardy (1969) identified great amounts of *trans*-2-hexen-1-al and hexanal in grape juice from the varieties Grenache and Sultana.

In order to avoid and to reduce the formation of secondary aroma compounds that also included other groups

of sensitive aroma substances such as terpenes (Tressl et al., 1970b) we separated the grape aroma under enzyme inhibition. The inhibition was realized by heating as well as by adding methanol. Identifications were based on comparison with mass spectra and retention times of authentic compounds. After separation of the free fatty acids and fractionation of the neutral substances on silica gel the volatiles were analyzed by GLC-mass spectrometry. Table I lists the components that were identified in the different column chromatographic separated fractions. Table II shows the mass spectral data of compounds detected for the first time in grapes. The first group in

Table I consists of 81 hydrocarbons also including long-chain alkanes and alkenes, well-known constituents of the grape surface waxes. More details about this group can be found elsewhere (Schreier et al., 1976).

In the acid fraction most of the compounds found are fairly common fruit acids, with the exception of *trans*-geranic acid. This terpenic acid, recently detected in wine varieties of intense aromas (Drawert et al., 1974), was reported as a constituent of geranium oil (Ter Heide et al., 1975) and can be derived from citral (Joglekar and Dhavlikar, 1969). The amounts of *trans*-geranic acid in grapes depend on the varieties. A relative comparison of the different varieties (2,2-dimethylpentanoic acid as internal standard) showed that Morio-Muskat (78 $\mu\text{g}/\text{kg}$), Traminer (24 $\mu\text{g}/\text{kg}$), and Scheurebe (17 $\mu\text{g}/\text{kg}$) contained the highest concentrations of this acid and that in Ruländer only traces (<1 $\mu\text{g}/\text{kg}$) were present.

Some free acids from grapes that are not appreciably altered by fermentation appear in wines: 2-ethylhexanoic acid, *cis*-3-hexanoic acid, phenylacetic acid, salicylic acid, 3-phenylpropionic acid, cinnamic acid, suberic acid, 4-hydroxybenzoic acid, azelaic acid, and phthalic acid. Differences in the concentrations depending on the varieties could not be ascertained. Most of these acids were present only in traces. The structures of six other compounds (Scherer, 1975) could not be established because of the lack of a reference sample.

Only a few esters were identified in small concentrations in the grape aroma. We suppose that the esters are perhaps not genuine grape constituents because biochemical reactions can occur in a low degree under the partial semitechnological conditions of the aroma separation. The same problem became evident concerning the aldehydic and alcoholic fractions. When we applied inhibition with methanol, only traces of hexenals, hexanal, and hexanol could be detected, but after inhibition by heating the number of C_6 components was remarkably higher (Kuchenbauer, 1976).

Among the aldehydes the saturated compounds of chain length C_4 to C_6 and also the unsaturated aldehydes from 2-pentenal to 2-octenal were identified. At the experiments under enzyme inhibition 2,4-dienals could only be found in traces, but during tests without inhibition they were detected in high concentrations. In particular, the increase in the amounts of isomer 2,4-hexadienals was remarkable. The alkenals and alkadienals can be considered as degradation products from lipid precursors (Forss, 1969; Ohloff, 1973; Saijyo and Takeo, 1972; Hatanaka and Harada, 1973).

In the alcoholic fraction we found the saturated components of chain length C_4 to C_{10} . Sort-typical differences could be determined in the group of terpenic alcohols including linalool, α -terpineol (Schreier et al., 1974b), geraniol, nerol, α -terpineol, and citronellol. Some sesquiterpenic alcohols were also found, but until now only α -cadinol could be identified by mass spectrometry.

A total of 10 newly identified ketones are listed in Table I. 2- and 3-alkanones may be derived from fatty acid metabolism (Forss, 1969). Damascenone, a constituent of various aromas (Demole et al., 1970; Demole and Berthet, 1971, 1972; Winter and Enggist, 1971; Nurstew and Wolfe, 1972; Renold et al., 1974) originating during carotene metabolism (Ohloff et al., 1973), has already been described as a component of wine aroma (Schreier and Drawert, 1974a). 2,2,6-Trimethyl-7-oxabicyclo[4.3.0]non-9-en-8-one (dihydroactinidiolide), an oxidation product of β -ionone, has been identified for the first time in black tea (Bricout et al., 1967).

Other heterocyclic components such as neroloxide, roseoxide, and the linalool oxides (in their furanic and pyranic forms) are important representatives of grape and wine varieties showing intense aromas (Schreier and Drawert, 1974b). One of this group of aroma substances is 2-vinyl-2-methyltetrahydrofuran-5-one. Probable precursors for this lactone, also identified in Burley tobacco (Roberts and Rohde, 1972) and in tea aroma (Renold et al., 1974), are linalool or the linalool oxides (Okazaki et al., 1973).

Distinct differences in the amounts of terpenic alcohols and the furanic and pyranic derivatives depending on the varieties were found. The results of sort-typical differentiation by gas chromatography will be reported in detail in the near future.

ACKNOWLEDGMENT

We are very grateful to Dr. Ohloff, Firmenich SA, Geneva, Dr. Bauer, Haarmann & Reimer, Holzminden, and ORIL SA, Paris, for providing some aroma substances.

LITERATURE CITED

- Bayonove, C., Cordonnier, R., *Ann. Technol. Agric.* **20**, 347 (1971a).
 Bayonove, C., Cordonnier, R., *C. R. Seances Acad. Agric. Fr.* **57**, 1374 (1971b).
 Bricout, J., Viani, R., Muggler-Chavan, F., Marion, J. P., Reymond, D., Egli, R. H., *Helv. Chim. Acta* **50**, 1517 (1967).
 Chaudhary, S. S., Kepner, R. E., Webb, A. D., *Am. J. Enol. Vitic.* **15**, 190 (1964).
 Demole, E., Berthet, D., *Helv. Chim. Acta* **54**, 681 (1971).
 Demole, E., Berthet, D., *Helv. Chim. Acta* **55**, 1866 (1972).
 Demole, E., Enggist, P., Sauberli, U., Stoll, M., Kovats, E. sz., *Helv. Chim. Acta* **53**, 541 (1970).
 Drawert, F., *Chem. Landwirtschaftl. Produk.* **189** (1970).
 Drawert, F., *Adv. Chem. Ser. No.* **137**, 1 (1974).
 Drawert, F., Heimann, W., Emberger, R., Tressl, R., *Chromatographia* **2**, 57 (1969).
 Drawert, F., Rapp, A., *Vitis*, **5**, 351 (1966).
 Drawert, F., Rapp, A., *Chromatographia* **1**, 446 (1968).
 Drawert, F., Schreier, P., Scherer, W., *Z. Lebensm.-Unters.-Forsch.* **155**, 342 (1974).
 Drawert, F., Tressl, R., Heimann, W., Emberger, R., Speck, M., *Chem. Mikrobiol. Technol. Lebensm.* **2**, 10 (1973).
 Forss, D. A., *J. Agric. Food Chem.*, **17**, 681 (1969).
 Haagen-Smit, A. J., Hirosawa, F. N., Wang, T. H., *Food Res.* **14**, 249 (1949).
 Hameed, H., Patak, S. R., Zaidi, H. R., *J. Oil Technol. Assoc. India* **4**, 128 (1972).
 Hardy, P. J., *Phytochemistry* **9**, 709 (1970).
 Hatanaka, A., Harada, T., *Phytochemistry* **12**, 2341 (1973).
 Holley, R. W., Stoyla, B., Holley, A. D., *Food Res.* **20**, 326 (1955).
 Joglekar, S. S., Dhavlikar, R. S., *Appl. Microbiol.* **18**, 1084 (1969).
 Kepner, R. E., Webb, A. D., *Am. J. Enol. Vitic.* **7**, 8 (1956).
 Kinsella, J. E., *Food Technol.* **28**, 58 (1974).
 Kliewer, W. M., *Plant Physiol.* **41**, 923 (1966).
 Kuchenbauer, F., Doctoral Dissertation, Techn. Universität München, in preparation, 1976.
 Leupold, G., Doctoral Dissertation, Techn. Universität München, 1974.
 Mudjoyan, E. L., Saakyan, R. G., Saakyan, A. S., *Vinodel. Vinograd. SSSR* **31**, 18 (1971).
 Neudoerffer, T. S., Sandler, S., Zubeckis, E., Smith, M. D., *J. Agric. Food Chem.* **13**, 584 (1965).
 Nurstew, H. E., Wolfe, M. L., *J. Sci. Food Agric.* **23**, 803 (1972).
 Ohloff, G., in *Fette als funktionelle Bestandteile von Lebensmitteln*, Forster, Zürich, 1973, p. 119.
 Ohloff, G., Rautenstrauch, V., Schulte-Elte, K. H., *Helv. Chim. Acta* **56**, 1503 (1973).
 Okazaki, T., Ohsuka, A., Kotake, M., *Nippon Kagaku Kaishi* **355** (1973).
 Radler, F., *Aust. J. Biol. Sci.* **18**, 1045 (1965a).
 Radler, F., *Am. J. Enol. Vitic.* **16**, 159 (1965b).
 Radler, F., Horn, D. H. S., *Aust. J. Chem.* **18**, 1059 (1965).
 Ramshaw, E. H., Hardy, P. J., *J. Sci. Food Agric.*, **20**, 619 (1969).

- Renold, W., Näf-Müller, R., Keller, U., Willhalm, B., Ohloff, G., *Helv. Chim. Acta* **57**, 1301 (1974).
- Roberts, D. L., Rohde, A. A., *Tob. Sci.* **16**, 107 (1972).
- Rodopulo, A. K., Bezzubov, A. A., Egorov, I. A., Skouin, K. P., *Vinodel. Vinograd SSSR* **34**, 53 (1974a).
- Rodopulo, A. K., Egorov, I. A., Bezzubov, A. A., Skouin, K. P., *Prikl. Biokhim. Mikrobiol.* **10**, 280 (1974b).
- Saijyo, R., Takeo, T., *Plant Cell Physiol.* **13**, 991 (1972).
- Scherer, W., Doctoral Dissertation, Techn. Universität München, 1975.
- Schreier, P., Drawert, F., *Z. Lebensm.-Unters.-Forsch.* **154**, 273 (1974a).
- Schreier, P., Drawert, F., *Chem. Mikrobiol. Technol. Lebensm.* **3**, 154 (1974b).
- Schreier, P., Drawert, F., Junker, A., *Z. Lebensm.-Unters.-Forsch.* **154**, 279 (1974a).
- Schreier, P., Drawert, F., Junker, A., *Z. Lebensm.-Unters.-Forsch.* **155**, 98 (1974b).
- Schreier, P., Drawert, F., Junker, A., *Z. Lebensm.-Unters.-Forsch.* **157**, 34 (1975).
- Schreier, P., Drawert, F., Junker, A., *Z. Lebensm.-Unters.-Forsch.*, in press (1976).
- Speck, M., Doctoral Dissertation, Techn. Universität München, 1971.
- Stern, D. J., Lee, A., McFadden, W. H., Stevens, K. L., *J. Agric. Food Chem.* **15**, 1100 (1967).
- Stevens, K. L., Bomben, J. L., Lee, A., McFadden, W. H., *J. Agric. Food Chem.* **14**, 249 (1966).
- Stevens, K. L., Bomben, J. L., McFadden, W. H., *J. Agric. Food Chem.* **15**, 378 (1967).
- Stevens, K. L., Flath, R. A., Lee, A., Stern, D. J., *J. Agric. Food Chem.* **17**, 1102 (1969).
- Stevens, K. L., Lee, A., McFadden, W. H., Teranishi, R., *J. Food Sci.* **30**, 1006 (1965).
- Ter Heide, R., de Valois, P. J., Wobben, H. J., Timmer, R., *J. Agric. Food Chem.* **23**, 57 (1975).
- Terrier, A., Boidron, J. N., Ribèreau-Gayon, P., *C. R. Hebd. Seances Acad. Sci., Ser. D*, **275**, 495 (1972a).
- Terrier, A., Boidron, J. N., Ribèreau-Gayon, P., *C. R. Hebd. Seances Acad. Sci., Ser. D*, **275**, 941 (1972b).
- Tressl, R., Drawert, F., Heimann, W., *Z. Lebensm.-Unters.-Forsch.* **142**, 249 (1970a).
- Tressl, R., Drawert, F., Heimann, W., Emberger, R., *Phytochemistry* **9**, 2327 (1970b).
- Usseglio-Tomasset, L., *Ind. Agrar.*, **1** (1966).
- Van Wyk, C. J., Webb, A. D., Kepner, R. E., *J. Food Sci.* **32**, 660 (1967).
- Webb, A. D., Kepner, R. E., *Food Res.* **22**, 384 (1957).
- Webb, A. D., Kepner, R. E., Maggiora, L., *Am. J. Enol. Vitic.* **17**, 247 (1966).
- Winter, M., Enggist, P., *Helv. Chim. Acta* **54**, 1891 (1971).

Received for review July 31, 1975. Accepted October 28, 1975. A grant from the Bundesministerium für Ernährung, Landwirtschaft und Forsten is gratefully acknowledged.

Volatile Flavor Components of Leek

L. Schreyen,* P. Dirinck, F. Van Wassenhove, and N. Schamp

The flavor complex from steam-distilled leek (*Allium porrum* L.) was studied by a combination of capillary gas chromatography and mass spectrometry. A total of 67 compounds were characterized, of which 57 have not been previously reported in leek. Odor properties were evaluated by running aromagrams on a thermal conductivity detector.

Much classical work has been done on aroma analysis since the advent of gas-liquid chromatography, especially in combination with mass spectrometry. Among vegetables, the *Allium* genus is one of the best investigated objects as to constitution, formation, and origin of volatile compounds.

The constitution of *Allium* species has been the subject of many publications. *Allium cepa* L. (onion) is undoubtedly the most important representative because of its very strong and pungent flavor. It has been studied intensively by Carson (1967), Bernhard (1968), Brodnitz et al. (1969, 1971), Boelens et al. (1971), and by Dembele and Dubois (1973). Other *Allium* species however, such as *A. chinense* G. Don (rakkyo), *A. sativum* L. (garlic), *A. schoenoprasum* L. (chive), *A. porrum* L. (leek), and *A. victorialis* L. (caucas), received little attention. A general and recent review of *Allium* constituents was published by Johnson et al. (1971).

The mechanism of flavor formation in *Allium* species has been investigated by Stoll and Seebeck (1948, 1949a,b, 1951), Schwimmer and Guadagni (1968), Schwimmer and Weston (1961), Calvallito and Bailey (1950), and some other workers.

This paper deals with the isolation, separation, and characterization of the major volatile constituents of leek oil obtained by steam distillation. The components were analyzed using gas-liquid chromatography and mass spectrometry. The structure of these components was identified by direct comparison of their mass spectra and retention times with those of reference products. Many of those products, although known to occur in other *Allium* species, were not reported earlier in leek.

EXPERIMENTAL SECTION

Sample Preparation. About 30 kg of freshly harvested leek (Nov 1974) was chopped and placed in a 60-l. glass vessel of the pilot plant installation shown in Figure 1. Steam under atmospheric pressure was blown through the leek by means of a 6-kW steam generator. The volatile components were stripped and the vapor condensed and cooled, yielding a total distillate of about 25 l. in 8 h. This distillate was submitted to continuous ether extraction (Figure 2) during 24 h and the extract rectified over a column in order to remove the solvent. The yield was about 1.5 ml of a dark brown leek oil with a strong smell which was very similar to that of boiled leek soup.

Gas Chromatography. The components of the ethereal essence were analyzed on a Varian 2400 gas chromatograph, equipped with a flame ionization detector and a subambient temperature programmer. The subambient system was operated by connecting a liquid nitrogen Dewar

* State University of Ghent, Faculty of Agricultural Sciences, Department of Organic Chemistry, B-9000 Ghent, Belgium.