Determination of Double Bond Position in Unsaturated Triglycerides by Analysis of the Oxidation Products by Gas Liquid Chromatography[1]

A. P. TULLOCH and B. M. CRAIG, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan

Abstract

Oleic, linoleic, and linolenic esters and olive, sunflower, linseed, rapeseed, hydrogenated rapeseed, and coriander oils have been oxidized by the permanganate-periodate reagent. The relative amounts of the dibasic acids and of the monobasic acids produced were determined by gas liquid chromatography (GLC). These results were used in combination with the compositions of the oils, also found by GLC, to calculate the positions of the double bonds in the unsaturated fatty acids of the oils. Evidence for the presence of small amounts of 11-octadecenoic acid and other unusual isomers was obtained.

Introduction

THE POSITIONS of the double bonds in unsaturated fatty acids is usually determined by identification of the fragments produced by oxidative cleavage. Since many acids that have the double bonds in unusual positions are now being found in small amounts in natural oils it is important that the oxidation step should be almost completely quantitative. The oxidizing agents usually employed are potassium permanganate and ozone. In almost all recent work GLC has been used to analyze the fission products. Oxidation with permanganate in acetic acid has been employed in structural determination (13,10) but overoxidation occurs to the extent of ca. 10%. Both oxidative ozonolysis (22,3,1) and reductive ozonolysis (18,17) have been used, mainly with individual fatty acids. Ozonolysis has the advantage that the oxidant leaves no non-volatile residue and consequently recovery of the oxidation products, particularly of the short chain dibasic acids, is quite easy. However, at the outset of this work it appeared that non-specific products of ozonolysis might present considerable difficulties (2) when mixtures of fatty acids were oxidized. This point has been emphasized in a recent review (12).

The aim of the present investigation was to work out a technique in which the maximum amount of information about double bond positions could be obtained from a single oxidation of a whole oil, instead of carrying out a number of oxidations of single purified fatty acids. The mild method of oxidation, using a mixture of potassium permanganate and sodium periodate, which was devised by Lemieux and von Rudloff (15) appeared to be the most promising. von Rudloff obtained good results with the permanganate-periodate oxidation of unsaturated fatty acids (19), though the products were analyzed by silicic acid column chromatography which would not detect minor by-products as readily as the GLC methods. Later von Rudloff showed that aqueous tertiary butyl alcohol was a good solvent for the permanganate-periodate oxidation of fatty acids esters and triglycerides (20). This procedure was also used in the determination of glyceride structure, only the dibasic acids being analyzed by GLC (25).

In the present study the permanganate-periodate reagent was used to oxidize either whole oils in aqueous tertiary butyl alcohol or the mixed fatty acids in water. The relative amounts of the oxidation products were then determined by GLC analysis. As it was not possible to estimate all the products quantitatively in one GLC analysis, the volatile monobasic acids were analyzed separately from the dibasic acids.

Before oxidation the composition of the oil was determined by GLC analysis and the relative amounts of dibasic and monobasic acids which should be produced on oxidation were calculated. Oxidation of linseed oil, for example, was expected to yield azelaic as the only dibasic acid and C_3,C_6 and C_9 monobasic acids. The molar percentages of these three acids should be the same as the molar percentage of the linolenic, linoleic, and oleic acids calculated from the GLC analysis by omitting the figures for the saturated acids. If the composition of the oxidation products differed from that expected then isomeric acids might be present. Sometimes the structure of the isomer and the amount present could be worked out by matching the monobasic acids with the dibasic acids. This method has been used in the examination of fungus spore oils for the presence of unusual acids (23,24). Particular attention was paid to the effect of overoxidation on the determination of isomeric acids.

The saturated acids of the oils, usually palmitic and stearic acids, were not affected by the oxidation and were recovered with the dibasic oxidation products. The relative molar percentages of the saturated acids and the dibasic acids were determined, and the figures for the former acids should be the same as those obtained in the analysis of the original oil if only one molecule of dibasic acid (other than malonic acid) was produced from each molecule of unsaturated acid. This analysis would be useful in the investigation of oils containing acids in which the double bonds are widely separated, since two molecules of dibasic acid could arise from one molecule of unsaturated acid.

Materials and Methods

Oleic acid and methyl oleate were isolated from olive oil by fractional distillation and urea fractionation. After 18 crystallizations they were at least 99% pure according to GLC. Methyl linoleate, at least 99% pure by GLC, was prepared by debromination of tetrabromostearic acid. The ester was also prepared, as a concentrate containing 9% oleate, by fractional crystallization of sunflower oil esters. Methyl linolenate, at least 99% pure by GLC, was prepared by debromination of hexabromostearic acid.

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Olive and sunflower oils were commercial oils. Linseed oil was obtained by grinding seed just before use. Rapeseed oil was prepared from seed of *Brassica napus*, variety Golden, obtained from the Canada Agriculture Research Laboratory, Saskatoon. Hydrogenated rapeseed oil was prepared by hydrogenation at 200C for 1 hr at 20 psi, and using 0.1% Raney nickel catalyst. The coriander oil was extracted from seed obtained from the R. T. French Company, Rochester, N.Y.; the oil content was 20%. Seed was grown in the greenhouse, and M. E. Mathias of the University of California, Los Angeles, kindly identified the plant as *Coriandrum sativum* L.

Method of Oxidation. The oxidation conditions were almost the same as those previously described (20). The stock oxidant was 0.0975 M in sodium metaperiodate and 0.0025 M in potassium permanganate. A standard oxidizing medium was used and the quantity of material to be oxidized was calculated. To simplify the calculation it was assumed that the malonic aldehyde or acid produced from polyenoic acids is completely oxidized (19). Therefore, 1 molecule oleic acid requires 4 atoms oxygen, or 1 mg requires 0.1418 ml M/10 oxidant solution; 1 molecule linoleic acid requires 12 atoms oxygen, or 1 mg requires 0.4286 ml M/10 oxidant; and 1 molecule linolenic acid requires 20 atoms oxygen, or 1 mg requires 0.7194 ml M/10 oxidant. In this way it was calculated that 140 mg methyl oleate, 46 mg methyl linoleate, 28 mg methyl linolenate, 137 mg olive oil, 65 mg sunflower oil, 40 mg linseed oil, 90 mg rapeseed oil, and 120 mg hydrogenated rapeseed oil or coriander seed oil required ca. 20 ml oxidant for complete oxidation. In practice a 100% excess of oxidant, or 40 ml, is used.

The oxidations were carried out in 60% tertiary butanol and in water. When tertiary butanol is used the stock oxidant (40 ml) is mixed with potassium carbonate solution (0.5%, 20 ml), water (20 ml) and tertiary butanol (100 ml). The substance to be oxidized is dissolved in tertiary butanol (20 ml) and added drop by drop to the other reagents with stirring over 1 hr. When the substance consists mainly of monoenes, such as oleate, olive, coriander and hydrogenated rapeseed oils, the solution can be added all at once. When the oxidation is carried out in water, the stock oxidant (40 ml) is mixed with potassium carbonate (2%, 15 ml) and water (115 ml). The free acids of the oil are obtained by saponification and dissolved in potassium carbonate (2%, 5 ml) and diluted with water (15 ml). This solution is then added to the other reagents over 1 hr. Using tertiary butanol the pH of the oxidation mixture is 8.4 and using water it is 8.0. The mixture is shaken for 18 hr, though oxidation is virtually complete in 6 hr for all the oils except rapeseed. Potassium hydroxide (0.1 g, pellet) is then added and ethylene passed in to destroy the oxidant (25) and the mixture evaporated to 40 ml on a steam bath in a current of air. One half of this solution is transferred to a steam distillation apparatus, acidified with 4 N sulfuric acid, steam distilled, and the volatile monobasic acids (C_3–C_{12}) estimated as decyl esters as previously described (9).

The other half is placed in a liquid-liquid extraction apparatus, acidified with 20 ml 4 N H_2SO_4 acid, and extracted with ether for 18 hr. The ether is removed from the extract and the dibasic and long chain monocarboxylic acids are converted to methyl esters with diazomethane.

Gas Liquid Chromatographic Analysis. The composition of the oils was determined by GLC analysis of the methyl esters (7,8). The GLC unit was of conventional design using thermal conductivity cells for detection. The columns used were a 30 in. x ¼ in. copper column packed with silicone on 60–80 mesh celite (1:6 w/w), operated at 220C and a flow rate of 40 ml helium/ min, and a 8 ft x $^3/_{16}$ in. copper column packed with ethylene glycol phthalate (6) on 40–60 mesh firebrick (1:4.5 w/w), operated at 205C and a flow rate of 60 ml helium/min. The polyester column also separated the mixture of dibasic acids and long chain saturated acids obtained from the oxidations. The emergence times of the methyl esters relative to methyl palmitate were: dibasic acids; C_6, 0.43; C_7, 0.63; C_8, 0.84; C_9, 1.12; C_{10}, 1.51; C_{11}, 2.04; C_{12}, 2.75; C_{13}, 3.54; C_{14}, 4.73; C_{15}, 6.30; monobasic acids: C_{16}, 1.00 (12 min); C_{18}, 1.90; C_{20}, 3.61; C_{22}, 6.99. As normally used this column does not resolve the esters of the C_{13} dibasic and arachidic acids. However, when the column is freshly prepared a separation is obtained, the arachidate emerging just before the C_{13} dibasic ester. Such a column was used to separate these two components in the oxidation products of hydrogenated rapeseed oil. A number of mixtures of methyl esters of dibasic acids and methyl palmitate and stearate were analyzed and it was found that factors were necessary to obtain good agreement, on a weight basis, between the observed and calculated results. Factors were also used in the analysis of the fatty acids of the oils. The factors were as follows: dibasic acids; C_6, 0.980; C_7, 0.985; C_8, 0.990; C_9, 1.013; C_{10}, 1.035; C_{11}, 1.057; C_{12}, 1.080; C_{13}, 1.105; C_{14}, 1.130; C_{15}, 1.145; palmitic, 1.000; palmitoleic, 1.010; stearic, 1.045; oleic, 1.055; linoleic, 1.065; linolenic, 1.080; arachidic, 1.095; eicosenoic, 1.100; behenic, 1.140; and erucic, 1.150. The results so obtained are considered accurate, on a weight basis, to within one unit percent and were converted to molar percentages for the purpose of this paper. Estimations of malonic, succinic, and glutaric acids by the present procedure were not reliable, probably because the acids were less readily extracted from aqueous media (10) and because the methyl esters are relatively volatile.

Results and Discussion

Before carrying out the oxidation of complete oils the oxidation of the individual fatty acids, oleic, linoleic, and linolenic was investigated. The permanganate-periodate oxidation of these acids has been reported previously (16) but quantitative results were not obtained.

Oxidation of Oleic Acid. On oxidation, oleic acid should yield only C_9 dibasic and monobasic acids; if other acids are found they may arise because isomers of oleic acid, with the double bond in other positions, are present or because overoxidation or, less probably, double bond shifting has taken place during the oxidation. Oleic acid was oxidized under three different sets of conditions. The first, in water at pH 8.0 as described earlier, gave the dibasic acids C_8, 1.5; C_9, 98.5; and the monobasic acids C_8, 1.7; C_9, 98.3 mol %. If the C_8 dibasic acid was produced from an isomer with an 8,9 double bond a C_{10} monobasic acid should have been found, which was not the case. The detection of a C_8 monobasic acid however, strongly suggests that the C_8 fragments are produced during the oxidation. The other two experiments support this view.

The second oxidation was in 60% tertiary butanol using 2% potassium carbonate solution (20 ml) which gives a pH of 9.2. This method gave the dibasic acids C_7, 0.6; C_8, 3.3; C_9, 96.1; and the monobasic acids C_7, 1.0; C_8, 1.6; C_9, 97.4 mol %. The third method was a modification, described by Sreenivasan et al. (21) for the oxidation of linoleic acid, in which a mixture of sodium hydroxide and sodium carbonate is used as alkali and the final pH is 9.2. This gave the dibasic acids C_7, 0.7; C_8, 4.0; C_9, 95.3; and monobasic acids C_7, 2.3; C_8, 4.4; C_9, 93.3 mol %. It is likely that the larger amounts of shorter chain fragments, found in the second and third methods, are produced at the higher pH by overoxidation by the permanganate during the initial hydroxylation step. No shorter chain acids were detected when azelaic and pelargonic acids were submitted to the oxidation procedure.

Previous workers (14) have reported that permanganate-periodate oxidation of oleic acid gave only 75% yield of dibasic acid. This point was investigated by oxidizing a mixture of methyl oleate (69.0) and methyl stearate (31.0 mol %) where the latter serves as an internal standard. Relative to the stearate the recovery of the dibasic acids was almost quantitative, the products were dibasic acids C_8, 0.6; C_9, 68.9; stearate, 30.5 mol %. Similar results were obtained when whole oils were oxidized.

Oxidation of Linoleic Acid. Linoleic acid or linoleate ester was oxidized under four different sets of conditions. The first was in water at pH 8.0, as described in the methods section, and yielded dibasic acids C_7, 0.9; C_8, 3.3; C_9, 95.8; and monobasic acids C_4, 0.6; C_5, 3.6; C_6, 95.8%. The second was in aqueous tertiary butanol at pH 8.4, as described in the methods section, it gave dibasic acids C_7, 0.7; C_8, 3.1; C_9, 96.2; and monobasic acids C_4, 0.2; C_5, 3.7; C_6, 96.1%. The third was in aqueous tertiary butanol at pH 9.2, as described for the oleate oxidation, dibasic acids were C_7, 1.0; C_8, 4.3; C_9, 94.7; and monobasic acids C_4, 0.8; C_5, 3.3; C_6, 95.9%. The fourth used the procedure of Sreenivasan et al. (21), dibasic acids were C_7, 2.6; C_8, 6.7; C_9, 90.7; and monobasic acids C_4, 1.8; C_5, 6.7; C_6, 91.5%. Again fragments shorter than those expected were found, about 4% in the first two oxidations and, as with oleate, appreciably more in the last two. Essentially the same results were obtained with both linoleate concentrated from sunflower oil esters by physical methods and linoleate prepared from tetrabromostearate. The possible presence of isomers with 8,13 double bonds cannot be ruled out since the small amount of glutaric acid which would be produced from carbons 9,10,11,12 and 13 could not be detected with certainty by the present method. An 8,11 isomer, on the other hand, cannot be present as no C_7 monobasic acid was found. But since overoxidation apparently occurred with oleate and would be most unlikely to be less extensive with linoleate, the amount of isomeric acids present probably does not exceed 1–1.5%. The results of the last two oxidations show that overoxidation certainly occurs at higher pH.

In examining overoxidation possibilities the reaction was carried out in stages using a reagent other than permanganate for the hydroxylation step. Linoleic acid was oxidized with iodine/silver acetate/wet acetic acid as described by Gunstone and Morris (11) and, after extraction of the crude material with 30% acetic acid, gave a 20% yield of a mixture of the two *dierythro* tetrahydroxystearic acids. The rest of the product was gummy by-products other than tetrahydroxy acids. Oxidation of the mixed tetrahydroxy acids in aqueous tertiary butanol at pH 8.4 gave the dibasic acids C_7, 1.0; C_8, 2.1; C_9, 96.9%; and the monobasic acids C_4, 0.9; C_5, 2.0; C_6, 97.1%. The decrease in the percentage of C_8 dibasic acid and C_5 monobasic acid provides support for the theory that overoxidation occurs during the initial attack of permanganate on the double bond.

Sreenivasan et al. (21) found 5–6% of C_8 dibasic acid when they oxidized linoleic acid and concluded that this arose from a dienoic acid having a double bond at the 8,9-position. However, the present findings show clearly that much of the C_8 acid found was actually produced by overoxidation. On the other hand, their suggestion that nonconjugatable dienes such as the 8,13 diene might be present to the extent of 1–2% may well be correct.

Oxidation of Linolenate. Linolenate was oxidized in aqueous tertiary butanol at pH 8.4 and 9.2 and the results were very similar to those obtained for linoleate. At pH 8.2 2.5% C_8 dibasic acid was found and at pH 9.2 1.4% C_7 and 5.3% C_8 dibasic acids were found. No monobasic acids of chain length longer than C_3 were found. Acetic and formic acids which might have been produced by overoxidation cannot be detected by the decyl ester procedure. Again it can be concluded that if isomers were present the amount was very small and that overoxidation probably occurred to a small extent.

Estimation of the Amount of Isomers in an Oil. The amounts of isomeric acids in an oil can be estimated after taking into account the amounts of overoxidation products which would be expected from the composition of the oil. Thus 1–2% of the apparent oleic acid would appear as C_8 fragments, 3–4% of the apparent linoleic acid as C_7 and C_8 dibasic acids and C_4–C_6 monobasic acids, and 3–4% of the apparent linolenic acid as C_7 and C_8 dibasic acids. Amounts of shorter chain fragments in excess of these would be derived from isomers, when longer chain fragments are found it is assumed that they are entirely derived from isomers. When the composition of the dibasic oxidation products and the original saturated acids is calculated in molar per cent the percentage of longer chain fragments is taken as the percentage of the isomers in the oil. The descriptions of the oxidations of the oil contain some examples.

Oxidation of Olive Oil. Olive oil was oxidized in aqueous tertiary butanol and the results show in Table I. The second section of the table shows the relative molar percentages of the dibasic oxidation products and the long chain saturated acids present in the original oil. For all the oils the calculated results were obtained by assuming that palmitoleic acid has a 9,10 double bond and that the unsaturated C_{18} acids consist entirely of oleic, linoleic, and linolenic acids. There was good agreement between the percentages of palmitate and stearate in the oxidation products and those in the original oil. It is most likely that the C_8 acids were the results of overoxidation. However, the C_{11} dibasic acid must be derived from an isomeric acid, 1.4% C_7 monobasic acid was expected from the palmitoleic acid but 4.1% was found. Thus it is probable that 11-octadecenoic acid is present in olive oil to the extent of 2–3%. This monoene has previously been found in the oil of an *Asclepias* species (4).

Oxidation of Sunflower Oil. A number of oxida-

TABLE I

Oil Composition and Oxidation Results

		Olive oil		Sunflower oil		Linseed oil		Rapeseed oil		Hydrogenated rapeseed oil †		Coriander seed oil	
Fatty acid composition (Mol %)	Palmitic	12.9		7.1		6.8		3.7		4.5		3.2	
	Palmitoleic	1.2		.....		.....		0.5		0.4		0.6	
	Stearic	2.5		4.1		3.7		1.1		7.9		1.1	
	Oleic	75.8		18.7		16.8		19.9		33.3		82.5	
	Linoleic	6.9		69.6		15.0		15.8		6.6		12.6	
	Linolenic	0.7		0.5		57.7		8.9		.....		.....	
	Arachidic	.....		.....		.....		0.2		2.8		.....	
	11-Eicosenoic	.....		.....		.....		12.8		10.6		.....	
	Behenic	.....		.....		.....		.....		5.1		.....	
	Erucic	.....		.....		.....		36.4		28.8		.....	
Dibasic oxidation products + saturated acids (Mol %)	C_6	.....		.....		.....		.....		1.3		74.6	
	C_7	.....		1.2		1.2		0.9		1.7		.....	
	C_8	1.4		3.3		2.7		1.1		5.0		.....	
	C_9	79.7		81.4		83.1		39.3		13.9		19.9	
	C_{10}	.....		.....		.....		.....		8.4		.....	
	C_{11}	3.0		1.4		1.5		13.4		12.3		1.0	
	C_{12}	.....		.....		.....		1.9		10.0		.....	
	C_{13}	.....		.....		.....		38.1		19.0		.....	
	C_{14}	.....		.....		.....		.....		6.0		.....	
	C_{15}	.....		.....		.....		.....		2.3		.....	
	Palmitic	13.3		7.9		7.2		3.9		3.8		3.5	
	Stearic	2.6		4.8		4.3		1.4		7.3		1.0	
	Arachidic	.....		.....		.....		*		3.0		.....	
	Behenic	.....		.....		.....		.....		6.0		.....	
Oxidation products alone		Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.
Dibasic acids (Mol %)	C_6	.....	.....	.....	.....	.....	.....	.....	.....	1.6	.....	78.1	.....
	C_7	.....	.....	1.4	.....	1.4	.....	0.9	.....	2.1	.....	.....	.....
	C_8	1.7	.....	3.8	.....	3.0	.....	1.2	.....	6.3	.....	.....	.....
	C_9	94.8	100	93.2	100	93.9	100	41.5	47.8	17.4	50.6	20.8	100
	C_{10}	.....	.....	.....	.....	.....	.....	.....	.....	10.5	.....	.....	.....
	C_{11}	3.5	.....	1.6	.....	1.7	.....	14.2	13.6	15.4	13.3	1.1	.....
	C_{12}	.....	.....	.....	.....	.....	.....	2.0	.....	12.5	.....	.....	.....
	C_{13}	.....	.....	.....	.....	.....	.....	40.2	38.6	23.8	36.1	.....	.....
	C_{14}	.....	.....	.....	.....	.....	.....	.....	.....	7.5	.....	.....	.....
	C_{15}	.....	.....	.....	.....	.....	.....	.....	.....	2.9	.....	.....	.....
Monobasic acids (Mol %)	C_3	0.8	0.8	0.4	0.6	65.0	64.0	6.9	9.4	2.1	.....	0.7	.....
	C_4	.....	.....	1.4	.....	0.3	.....	0.3	.....	2.1	.....	0.1	.....
	C_5	.....	.....	3.7	.....	0.7	.....	0.7	.....	3.1	.....	0.3	.....
	C_6	7.0	8.2	70.5	78.4	15.1	16.8	15.0	16.7	5.9	8.3	11.4	13.2
	C_7	4.1	1.4	0.9	.....	0.6	.....	3.6	0.6	8.6	0.5	1.2	0.6
	C_8	1.5	.....	.....	.....	.....	.....	2.0	.....	17.6	.....	0.3	.....
	C_9	86.6	89.6	23.1	21.0	19.3	19.2	71.5	73.3	45.3	91.2	8.6	86.3
	C_{10}	.....	.....	.....	.....	.....	.....	.....	.....	12.8	.....	0.5	.....
	C_{11}	.....	.....	.....	.....	.....	.....	.....	.....	2.5	.....	1.5	.....
	C_{12}	....	....	.....	.....	.....	.....	.....	.....	.....	.....	75.4	.....

† The unsaturated acids were mixtures of isomers.
* The arachidic peak was obscured by the C_{13} dibasic acid in this case.

tions of sunflower oil were carried out in aqueous tertiary butanol. However some samples of tertiary butanol appeared to contain traces of impurities which interfered with the estimation of decyl propionate. This effect was found only with the more unsaturated oils which, since smaller amounts were oxidized, gave proportionately smaller quantities of oxidation products. To avoid this difficulty the free acids of the oil can be oxidized in water. The results shown were obtained in this way but very similar results were obtained from the oxidations in aqueous tertiary butanol.

One feature of this oxidation which was difficult to explain was the low figure (75.6%) for the total of C_4,C_5, and C_6 monobasic acids compared to the expected figure for C_6 of 78.4%. This result was consistently obtained from a number of different oxidations both when the monobasic acids were analyzed as decyl esters and as phenacyl esters (9). Since the C_9 monobasic acid was 2% high a 6,9 diene could have been present, but no corresponding amount of C_6 dibasic acid was found. The discrepancies might be due to the presence of a number of isomers each in quite small amount. The C_{11} dibasic and the C_7 monobasic acids found indicate the presence of about 1% of 11-octadecenoic acid.

Oxidation of Linseed Oil. Again, as with sunflower oil, linseed oil was oxidized in aqueous tertiary butanol and also in water as the free acids. Both methods gave almost the same results and those in the table refer to an oxidation in water. The found and calculated results were in good agreement but ca. 0.5% of 11-octadecenoic acid might have been present.

Oxidation of Rapeseed Oil. The results of an oxidation, in water, of rapeseed acids from the variety Golden are shown in the table. The oil of this particular variety was not oxidized in aqueous tertiary butanol but the oils of several other varieties have been satisfactorily oxidized in this medium. The results showed that the structures usually assigned to the fatty acids of this oil were largely correct. The total of C_7,C_8, and C_9 dibasic acids is ca. 4% lower than the calculated figure. At least part of this can be accounted for by assuming the presence of 11-octadecenoic acid. The C_{11} dibasic acid percentage is not increased appreciably perhaps because some 13-eicosenoic acid is present; the amounts of C_7 monobasic acid and C_{13} dibasic acid found support this suggestion.

Oxidation of Partially Hydrogenated Rapeseed Oil. This experiment was carried out to indicate the application of the oxidation procedure to reactions in which double bond shifting occurs. The oil was oxidized in aqueous tertiary butanol. The calculated percentages were obtained by assuming that the unsaturation commenced at C_9 in all cases. The results were not sufficient for calculation of the structures of the component acids, but they agreed with the theory that isomerization mainly involves a shift of the double bonds to the neighboring carbon atoms.

Oxidation of Coriander Seed Oil. This oil was included to show how the oxidation could be used to determine the structure of the major fatty acid components of new oils. The oil was oxidized in aqueous tertiary butanol. In calculating the percentages of products it was assumed that all the C_{18} monoene was

oleic acid. However, the results showed that the monoene consisted of ca. 75% petroselinic acid and only 8% oleic acid. When the dibasic oxidation products are analyzed as methyl esters the methyl ester of the C_{12} monobasic acid also appears (between C_5 and C_6 dibasic esters) and if the amount is calculated relative to the C_6 dibasic ester it gives an additional check on the petroselinic acid present. The average molar ratio of the C_6 dibasic acid to the C_{12} monobasic acid was 50.5 to 49.5. Christian and Hilditch (5) reported that the oil of *Coriandrum sativum* L. contained 53% petroselinic acid and 32% oleic acid but they were using a much less accurate procedure and also they may have been working with a different variety of coriander seed. The variety of seed used in this work would be a good source of petroselinic acid. The percentage of C_6 monobasic acid agreed quite well with the calculated value and the percentage of C_9 dibasic acid agreed with the sum of the percentages of the C_6 and C_9 monobasic acids, both of which are derived from acids with unsaturation commencing at carbon 9. Thus there was no evidence for the presence of any appreciable amount of acids, other than petroselinic acid, with the first double bond in the 6,7 position. Again ca. 0.5–1% 11-octadecenoic acid was probably present.

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REFERENCES

1. Ackman, R. G., M. E. Retson, L. R. Gallay, and F. A. Vandenheuvel, Can. J. Chem. *39*, 1956 (1961).
2. Benton, F. L., A. A. Kiess, and H. J. Harwood, JAOCS *36*, 457 (1959).
3. Cason, J., and P. Tavs, J. Biol. Chem. *234*, 1401 (1959).
4. Chisholm, M. J., and C. Y. Hopkins, Can. J. Chem, *38*, 805 (1960).
5. Christian, B. C., and T. P. Hilditch, Biochem. J. *23*, 327 (1929).
6. Craig, B. M., Chem. Ind. (London), 1442 (1960).
7. Craig, B. M., and N. L. Murty, Can. J. Chem. *36*, 1297 (1958).
8. Craig, B. M., and N. L. Murty, JAOCS *36*, 549 (1959).
9. Craig, B. M., A. P. Tulloch, and N. L. Murty, *Ibid.* *40*, 61 (1963).
10. Fulco, A. J. and J. F. Mead, J. Biol. Chem. *234*, 1411 (1959).
11. Gunstone, F. D., and L. J. Morris, J. Chem. Soc. 487 (1957).
12. Harwood, H. J., Chem. Rev. *62*, 99 (1962).
13. James, A. T., and J. Webb, Biochem. J. *66*, 515 (1957).
14. Jones, E. P., and J. A. Stolp, JAOCS *35*, 71 (1958).
15. Lemieux, R. U., and E. von Rudloff, Can. J. Chem. *33*, 1701 (1955).
16. Nowakowska, J., E. H. Melvin, and R. Wiebe, JAOCS *34*, 411 (1957).
17. Privett, O. S., and C. Nickell, *Ibid.* *39*, 414 (1962).
18. Pryde, E. H., D. E. Anders, H. M. Teeter, and J. C. Cowan, J. Org. Chem. *25*, 618 (1960).
19. von Rudloff, E., JAOCS *33*, 126 (1956).
20. von Rudloff, E., Can. J. Chem., *34*, 1413 (1956).
21. Sreenivasan, B., J. B. Brown, E. P. Jones, V. L. Davison, and J. Nowakowska, JAOCS *39*, 255 (1962).
22. Stoffel, W., and E. H. Ahrens, J. Am. Chem. Soc. *80*, 6604 (1958).
23. Tulloch, A. P., and G. A. Ledingham, Can. J. Microbiol. *6*, 425 (1960).
24. Tulloch, A. P., and G. A. Ledingham, Can. J. Microbiol. *8*, 379 (1962).
25. Youngs, C. G., JAOCS *38*, 62 (1961).

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Relationship Between Molecular Structure and Flavor Perceptibility of Aliphatic Aldehydes

P. W. MEIJBOOM, Unilever Research Laboratory, Vlaardingen, The Netherlands

Abstract

The mean threshold values for odor and taste of some aliphatic aldehydes are determined in solutions of paraffin oil. These threshold values lie at low concn and display a distinct alternating effect in the same homologous series. Moreover it appears from experiments with mixtures of various aldehydes that in the case of certain ratios a masking effect in odor and taste occurs.

Introduction

OILS AND FATS, which are present in our daily diet, may develop during the induction period of the autoxidation the so-called reversion flavors (1,2). These flavor carriers formed from the odor- and tasteless-hydroperoxides (3) are mainly aldehydes. An investigation on odors is difficult, because the odor carriers and their precursors are present in very low concn. Although they can be clearly observed organoleptically, the picture of the composition of the volatile substances can be obtained only with very sensitive gas chromatographic methods (head space analysis).

Since it appears that this volatile part contains a large number of components, the question arises how and to what extent the type of reversion flavors is determined by these components. Does a single component with a "top fragrance" with respect to the other components in the same system play a predominant role? Are there other factors such as synergism and/or antagonism (intensifying respectively masking effects) which influence the taste of the fat in question? As aldehydes are important as flavor carriers, an investigation has been initiated into the relationship between the molecular structure and taste and odor of saturated and unsaturated aldehydes.

Results of a similar investigation in the field of perfumes have been published by Beets (4). Lea and Swoboda (5) have determined the flavor threshold value in water, in groundnut oil, and in paraffin oil, of a number of saturated and of two unsaturated aldehydes.

Our experiments were carried out with aldehydes of various homologous series and the mean threshold values for odor and taste in paraffin oil were determined in order to investigate the influence of chain length, of number and position of the double bonds and of stereo-isomerism (*cis-trans*).

Moreover, by means of experiments with mixtures of various aldehydes the mutual influence in some cases was demonstrated

Experimental

Saturated aldehydes. Saturated unbranched aliphatic aldehydes (C_3–C_{12}) were purified by distilling commercially available products respeatedly; if necessary the purification took place via their sodium bisulphite addition product. The purity (according to the GLC analysis) was increased to 98% or more. The substances dosed gravimetrically were dissolved in paraffin oil with concn of 0.0001, 0.001 etc. up to 1000 ppm inclusive, and to guarantee a homogeneous