The Decomposition of Monocarbonyl Precursors

Monomeric hydroperoxides, the primary products of autoxidation, are considered the precursors of the monocarbonyl compounds in autoxidized fat. The aldehydes isolated and identified are those predicted by the scission of parent hydroperoxide isomers (9,10). Hydroperoxides are labile and highly reactive, and direct measurement of free aldehydes in their presence has been considered questionable (17). Schwartz et al. (26) showed that, in the dilute phosphoric acid-celite-2,4-dinitrophenylhydrazine reaction method, hydroperoxides disappeared. These investigators, on the basis of experiments with methyl linoleate hydroperoxides, concluded that no monocarbonyls were produced in the Schwartz column reaction. However, significant differences related to autoxidation have been shown in this investigation to occur between the Schwartz and volatile monocarbonyl compounds during the early stages. The observed differences may be due to the decomposition of other hydroperoxides or precursors. Also it should be considered that a pure preparation of hydroperoxides may not be the same as an oxidized fat system. A change occurred with advanced oxidation since volatile monocarbonyls equaled and frequently exceeded those isolated by the Schwartz method.

Heating at 165C breaks down primary oxidation products, greatly increases the monocarbonyl compounds, and modifies their composition. Heating autoxidized lard under N_2 (15 min at 165C) produced the results shown in Figure 6 (as compared with Figure 4). It is evident that the treatment narrowed differences. This would have to be due to the decomposition or decrease of precursors.

The evidence presented shows interesting and significant differences between the monocarbonyl isolation methods examined. This is unquestionably influenced by the carbonyl precursors present, which in turn might be related to the degree and course of autoxidation. It should be considered that the range of autoxidation conditions used in these experiments was purposely narrow; and other conditions, environments and substrates might give other relationship patterns.

ACKNOWLEDGMENT

Joe N. Boyd, Biometrician, Biometrical Services, performed the statistical analysis, and Francis E. Thornton of this Laboratory gave technical assistance.

REFERENCES

1. Begemann, P. H., and K. de Jong., Rec. Trav. Chim. *78*, 275 (1959).
2. Ellis, R., A. M. Gaddis and G. T. Currie., Anal. Chem. *30*, 475 (1959).
3. Ellis, R., and A. M. Gaddis, *Ibid. 31*, 1997 (1959).
4. Ellis, R. A., A. M. Gaddis and G. T. Currie, J. Food Sci., *26*, 131 (1961).
5. Fritsch, C. W., and F. E. Deatherage, JAOCS *33*, 109 (1956).
6. Gaddis, A. M., R. Ellis, and G. T. Currie, Food Research *24*, 283 (1959).
7. Gaddis, A. M., and R. Ellis, Anal. Chem. *31*, 870 (1959).
8. Gaddis, A. M., and R. Ellis, Food Research *24*, 392 (1959).
9. Gaddis, A. M., R. Ellis, and G. T. Currie, *Ibid. 25*, 495 (1960).
10. Gaddis, A. M., R. Ellis, and G. T. Currie, JAOCS 38, 371 (1961).
11. Gaddis, A. M., R. Ellis, and G. T. Currie, J. Food Sci. *29*, 6 (1964).
12. Henick, A. S., M. F. Benca, and J. H. Mitchell, JAOCS *31*, 88 (1954).
13. Hornstein, I., and P. F. Crowe, Anal. Chem. *34*, 1037 (1962).
14. Keeney, M., in "Symposium on Foods: Lipids and Their Oxidation." eds, H. W. Schultz, E. A. Day, and R. O. Shinnhuber, The AVI Publ. Co., Westport, Conn., 1962. p. 79.
15. Kenaston, C. B., K. M. Wilbur, A. Ottolenghi and F. Bernheim, JAOCS *32*, 33 (1955).
16. Keith, R. W., and E. A. Day, *Ibid. 40*, 121 (1963).
17. Knight, H. B., and D. Swern, *Ibid. 26*, 366 (1949).
18. Lea, C. H., and P. A. T. Swoboda, J. Sci. Food Agr. *13*, 148 (1962).
19. Mookherjee, B. D., and S. S. Chang, JAOCS *40*, 232 (1963).
20. Parks, O. W., M. Keeney, and D. P. Schwartz, J. Dairy Sci. *44*, 1940 (1961).
21. Parks, O. W., M. Keeney, and D. P. Schwartz, *Ibid. 46*, 295 (1963).
22. Pool, M. F., and A. A. Klose, JAOCS *28*, 215 (1951).
23. Schogt, J. C. M., P. H. Begemann, and J. Koster, J. Lipid Res. *1*, 446 (1960).
24. Schwartz, D. P., and O. W. Parks, Anal. Chem. *33*, 1396 (1961).
25. Schwartz, D. P., A. R. Johnson, and O. W. Parks, Microchem. J. *VI*, 37 (1962).
26. Schwartz, D. P., H. S. Haller, and M. Keeney, Anal. Chem. *35*, 2191 (1963).
27. Sulzbacher, W. L., A. M. Gaddis and R. Ellis, Proceedings American Meat Institute Foundation Research Conference, Chicago, Ill., 111–119 (1963).
28. Wyatt, C. J., and E. A. Day, J. Food Sci. *28*, 305 (1963).

[Received November 17, 1964—Accepted January 29, 1965]

The Triglycerides of Sable Fish *(Anaplopoma fimbria)*. I. Quantitative Fractionation by Column Chromatography on Silica Gel Impregnated with Silver Nitrate

AMI DOLEV and H. S. OLCOTT, Department of Nutritional Sciences, Institute of Marine Resources, University of California, Berkeley, California

Abstract

The triglycerides of sable fish muscle oil were eluted from a silver-nitrate-impregnated silica gel column with petroleum ether containing increasing amounts of ethyl ether as solvents. Eighteen peak fractions were analyzed for fatty acid composition. Calculated iodine values progressed regularly from 38 to 115. The detailed data show that the fractionation did not depend upon the presence of individual fatty acids but on total unsaturation. Not all of the triglycerides containing polyunsaturated fatty acids were eluted.

Introduction

SATISFACTORY METHODS for the separation of individual triglycerides are not yet available. Several investigators have attempted to apply the procedures of fatty acid fractionation, e.g., fractional crystallization, countercurrent distribution, thermal gradient fractionation, thin-layer chromatography, and others (1–8). More recently attempts have been made to separate triglycerides by gas chromatography (9–11) and by liquid-liquid column partition chromatography (12). Separate lipid components have been fractionated by silicic acid gel chromatography (13–16). The discovery by deVries (17,18) that silver nitrate-impregnated silicic acid successfully separated fatty acids with different degrees of saturation suggested that such a technique might be useful in the separation of triglycerides. Wurster et al. (19) have described the separation of methyl esters on such a column and Barrett et al. (20) used a silver nitrate-impregnated silicic acid gel for thin-layer chromatography. We have now applied this method to the fractionation of the triglycerides of sable fish oil. The oil of the sable fish [black cod (*Anaplopomo fimbria*)]

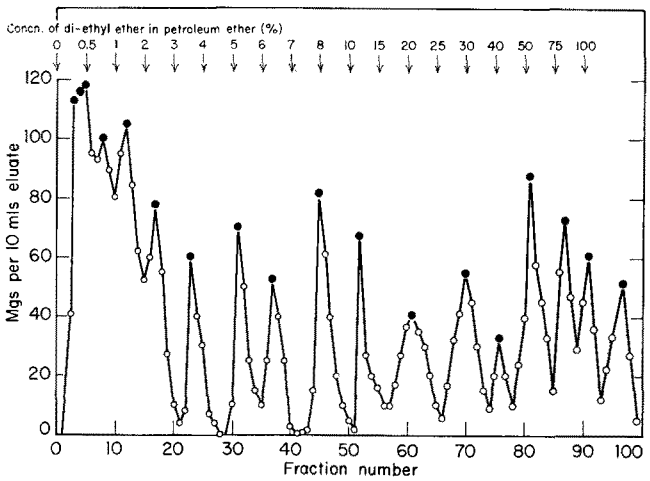

FIG. 1. Sable fish triglycerides fractionation on silica gel $-AgNo_3$ column. Solvent gradient started with 100% petroleum ether and ended with 100% diethyl ether.

is much more stable than other fish oils, an advantage to this type of study. Since the completion of the work described in this paper, Jurriens et al. (21) have published results on a study of triglycerides of other oils based on the same method.

Experimental

Material

Three sable fish weighing 25, 17 and 11 lb, were caught at the mouth of the Columbia River, immediately frozen, shipped to this laboratory with dry ice packaging, and held at −18C until used.

Extraction

After the fish were thawed, muscle tissue was excised and extracted as follows: 50 g of tissue and 200 ml of chloroform:methanol (95:5) were mixed in a Waring blendor for 4 minutes, then filtered. The residue was re-extracted with 200 ml of the same solvent, again filtered, and discarded. The filtrates were combined, the aqueous layer was removed, and the solvent was removed from the chloroform layer in a rotary evaporator. The residual oil was stored under nitrogen at −18C. Yield, 9.8%.

Separation of Neutral Lipids

Neutral lipids were separated from the rest of the lipid components by column chromatography on silicic acid gel as described by Shuster et al. (16). The oil was dissolved in chloroform containing 0.5% ethanol and washed through the column with the same solvent. Subsequent work showed that about 6% of the total extract remained on the column.

Column Chromatography

The silica gel for the fractionation of the triglycerides was prepared by a modification of the de Vries (18) procedure as follows: 50 g of silicic acid (Silica Gel G, according to Stahl, 100–200 mesh) were suspended in 100 ml of 40% silver nitrate, brought to boiling with stirring, allowed to cool, filtered, and dried overnight in an oven at 130C. The cooled powder was ground in a ball-mill and stored in a desiccator. All operations were protected from light.

A water-jacketed column, 10 mm I.D., 40 cm long, was packed as described by de Vries (18) for the fractionation of fatty acids. The column was held at 10C. Five hundred milligrams of triglycerides were dissolved in 5 ml petroleum ether and applied to the column. The eluting solvents consisted of petroleum ether (boiling point range, 40–45C) with increasing amounts of redistilled ethyl ether, as shown in Figure 1. Pressure at the top of the column was maintained at 1 lb/sq in. with nitrogen and the flow was about 65 ml/hr. One hundred 10 ml fractions were collected. A 1 ml aliquot of each fraction was evaporated to dryness and weighed.

TABLE I

Fatty Acid Composition of the Total Triglycerides of Sable Fish (Black Cod—*Anaplopoma fimbria*)

Carbon number	Fatty Acids of triglycerides %		Fatty Acids from Hydrogenated triglycerides %
C_{14}	4.1		4.1
C_{15}	0.7		0.6
C_{16}	14.8		
$C_{16:1}$	11.6	28.4	28.4
$C_{16:3}$	2.0		
C_{17}	1.0		1.0
C_{18}	3.8		
$C_{18:1}$	38.0		
$C_{18:2}$	2.1	45.2	45.9
$C_{18:3}$	1.3		
C_{19}	1.0		1.4
C_{20}	6.6		
$C_{20:4}$	1.0	12.2	11.7
$C_{20:5}$	4.6		
C_{22}	4.1		
$C_{22:6}$	2.2	6.3	6.0
C_{24}	1.4		1.1
	100.3		100.2

Analytical Methods

Peroxides were determined by a modification of the AOCS standard procedure (22). Iodine values were obtained by the Wijs method as described by Mehlenbacher (23). Oil stability was assessed by the weighing method described by Olcott (24). A 200 mg sample of oil in a 10 ml beaker was held in a draft oven at 50C. Beakers were weighed daily and peroxide

TABLE II

Percentage Fatty Acid Composition of Triglyceride Fractions of Sable Fish Oil

Carbon No.	Fraction No.																	
	3	4	5	8	12	17	23	31	37	45	52	61	70	76	81	87	91	97
C_{12}	0.9	T	T		T								T					
C_{14}	9.7	8.2	8.5	8.2	8.6	6.0	5.0	4.8	5.0	3.2	1.0	0.5	0.4	0.5	0.8	2.2	2.5	2.9
$C_{14:1}$		T	T	T	T	T	T	T	0.4	0.4	0.2	0.2	0.3	0.4	0.6	T	T	T
C_{15}	0.8	0.7	0.6	0.7	0.6	0.5	0.4	0.3	0.4	0.2	T	T	T	T	T		T	0.4
C_{16}	30.5	26.9	27.9	29.2	29.5	22.0	19.4	18.5	17.8	10.6	3.5	1.7	1.6	1.6	2.9	10.0	10.1	13.1
$C_{16:1}$	7.0	8.3	7.2	8.7	8.2	9.3	10.5	13.3	14.6	13.9	12.5	14.5	18.8	20.5	23.1	17.1	15.6	12.1
C_{17}	1.8	T	1.0	1.4	1.4	1.5	1.0	T	T	1.2	1.0	T						
C_{18}	5.9	5.1	5.5	5.0	5.4	3.8	3.4	2.9	2.2	1.0							T	2.7
$C_{18:1}$	30.0	36.6	34.7	34.8	34.2	41.1	46.2	48.4	47.0	50.1	55.0	59.1	58.4	62.3	58.4	44.9	42.5	37.8
C_{20}	6.9	7.4	7.9	6.4	6.6	8.4	8.1	7.1	7.8	10.6	14.5	14.3	12.6	9.7	8.5	7.6	6.9	6.9
C_{22}	4.9	5.1	4.8	4.2	4.5	5.9	4.4	3.5	3.6	6.7	8.8	7.0	4.5	2.8	2.3	2.7	3.1	3.0
C_{24}	1.6	2.0	1.6	1.4	1.0	1.5	0.8	0.6	0.8	1.9	1.5	0.7	0.4	0.4	0.3	0.4	0.8	0.9
$C_{22:6}$											0.9	0.9	1.3	1.3	1.5	0.8	0.7	0.3

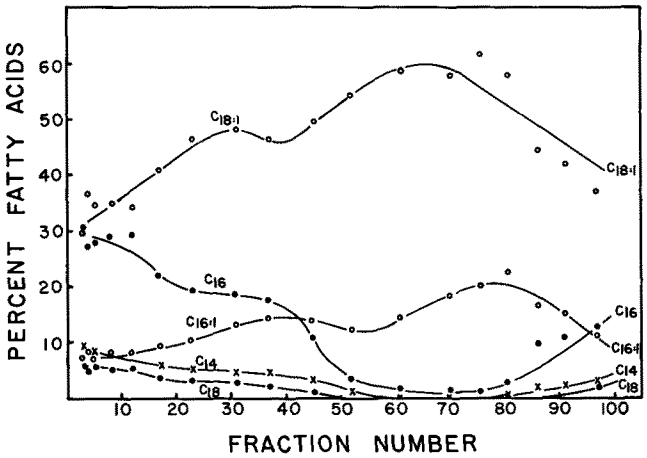

FIG. 2. Distribution of C_{14}, C_{16}, $C_{16:1}$, C_{18}, $C_{18:1}$ fatty acids in sable fish triglyceride fractions (cf. Fig. 1).

values were determined at the end of the induction period. Tocopherol content was determined as described by the Analytical Methods Committee 1959 (25).

Fatty Acid Analysis

Fatty acid composition of the total triglycerides and of selected fractions was determined by gas chromatography following methylation by transesterification with methanol containing 1% sulfuric acid. A Wilkens Aerograph HY-FI Model 600 was used. A siliconized coiled glass column (213 cm long, 2 mm I.D.) was packed with 1% EGSS-X (siliconized ethylene glycol succinate, Applied Science (26) on siliconized chromosorb P (mesh 100–200). Samples were run three times each at 150C and at 190C. Peak areas were estimated by the method described by Carroll (27), and the fatty acid esters were identified by comparison with those of NIH Standard Mixes (28). In addition, all samples were hydrogenated according to Farquhar et al. (29) and rerun.

Results and Discussion

The stability of sable fish oil is unusual. The iodine number of 80 for the triglyceride fraction is far less than that of most marine oils. The iodine number calculated from the fatty acid composition of the triglyceride fraction as shown in Table I was 83. The peroxide value of the whole extract after being stored for

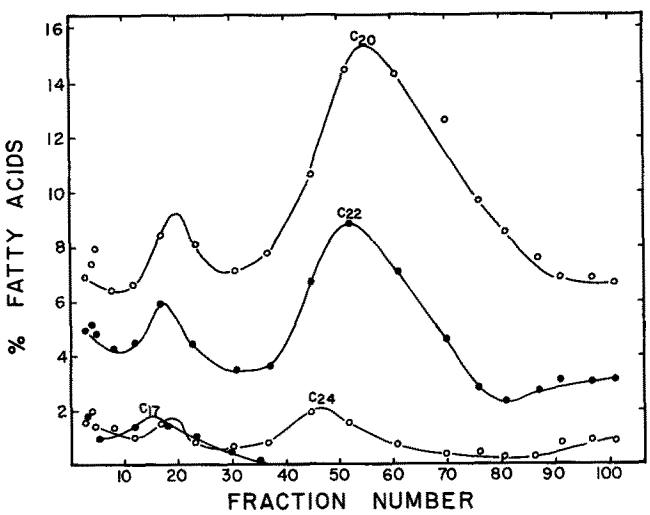

FIG. 3. Distribution of C_{17}, C_{20}, C_{22}, C_{24} fatty acids in sable fish triglyceride fractions (cf. Fig. 1).

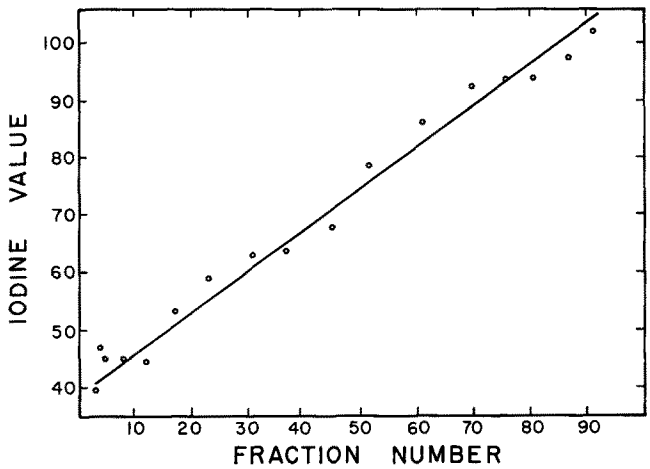

FIG. 4. The iodine value (calculated) of sable fish triglyceride fractions plotted against fraction number (cf. Fig. 1).

one year at −18C was less than 5. The induction period at 50C was over 5 months during which there was no weight gain, no peroxide formation and no detectable rancidity. This stability is probably ascribable to the tocopherols which were present in this sable oil at a level of 224 mg/kg, and to the phospholipids (3). A different sample of sable fish oil was reported to contain 628 mg/kg of tocopherol (31). The separated triglycerides, presumably phospholipid-free, had an induction period of 5 days at 50C.

The total triglyceride fraction contained much less polyunsaturated fatty acids than do most marine oils (32) and a high content of oleic acid (Table I). Composition may vary with season, feed, etc. (31,33).

Results of the fractionation are shown in Figure 1. Recovery was 94%. Eighteen peaks were chosen for fatty acid analysis. The fatty acid composition of these fractions is presented in Table II. As shown in Figures 2 and 3, there was no indication that fractionation was dependent on any single fatty acid. Oleic and palmitoleic acids followed the same elution patterns and so did the C_{20}, C_{22} and C_{24} saturated fatty acids. A clear picture of the column behavior emerged when the theoretical iodine number obtained from the fatty acid analyses of each fraction was plotted against fraction number (Fig. 4). The oil with original iodine number 80 was separated into fractions with iodine numbers ranging from 38 to 115. Since the polyunsaturated fatty acids in the original oil were not quantitatively recovered in the fractions, the losses during chromatography are probably due to failure to elute glycerides containing them.

Based on the assumption that all possible variations of fatty acid distribution might occur, 17 different fatty acids could lead to 2602 different triglycerides $[(n^3 + n^2)/2]$ (34). Even if it proves not possible to separate isomers, the 969 separate triglycerides $[(n^3 + 3n^2 + 2n)/6]$ would be extremely difficult to separate and identify (29). In all likelihood, however, the biosynthetic mechanisms involved will have sufficient specificity so that the triglyceride picture will eventually be considerably simplified. The positional distribution of fatty acids within the separate fractions is now being studied by the pancreatic lipase technique (35).

ACKNOWLEDGMENTS

M. E. Stansby of the Bureau of Commercial Fisheries, Seattle, Washington, arranged for the catch and shipment of the sable fish. J. Van der Veen assisted in the preparation of this paper.

REFERENCES

1. Youngs, C. G., JAOCS *38*, 62 (1961).
2. Hilditch, T. P., "The Chemical Constitution of Natural Fats," 3rd ed., John Wiley & Sons, Inc., New York, 1956.
3. Kartha, A. R. S., JAOCS *30*, 280 (1953).
4. Scholfield, C. R., JAOCS *38*, 562 (1961).
5. Baker, C. A., and R. J. P. Williams, J. Chem. Soc. (London) 2352 (1956).
6. Jones, G. V., and E. G. Hammond, JAOCS *38*, 69 (1961).
7. Privett, O. S., and M. L. Blank, J. Lipid Res. *2*, 37 (1961).
8. Van der Wal, R. J., JAOCS *40*, 242 (1963).
9. Kuksis, A., M. J. McCarthy and J. M. R. Beveridge, JAOCS *41*, 201 (1964).
10. Trowbridge, J. R., A. B. Herrick and R. A. Bauman, JAOCS *41*, 306 (1964).
11. Kuksis, A., M. J. McCarthy and J. F. R. Beveridge, JAOCS *40*, 530 (1963).
12. Black, B. C., and E. G. Hammond, JAOCS *40*, 575 (1963).
13. Nelson, J. H., R. L. Glass and W. F. Geddes, Cereal Chem. *40*, 337 (1963).
14. Krell, K., and S. A. Hashim, J. Lipid Res. *4*, 407 (1963).
15. Kaufmann, H. P., and T. H. Khoe, Fette, Seifen, Anstrichsmittel *64*, 81 (1962).
16. Shuster, C. Y., J. R. Froines and H. S. Olcott, JAOCS *41*, 36 (1964).
17. De Vries, B., Chem. Ind. p. 1049 (1962).
18. De Vries, B., JAOCS *40*, 184 (1963).
19. Wurster, C. F., J. H. Copenhaver and P. R. Shafer, JAOCS *40*, 513 (1963).
20. Barrett, C. B., M. S. Dallas and F. B. Padley, JAOCS *40*, 580 (1963).
21. Jurriens, G., B. de Vries, and L. Schouten, J. Lipid Research *5*, 366 (1964); Jurriens, G. and A. C. J. Kroesen, JAOCS *42*, 9 (1965).
22. Olcott, H. S., and Ami Dolev, Proc. Soc. Exp. Biol. Med. *114*, 820 (1963).
23. Mehlenbacher, V. C., "The Analysis of Fats and Oils," The Garrard Press, Champaign, Ill., 1962, p. 319, 353.
24. Olcott, H. S., and Einset, JAOCS *35*, 161 (1958).
25. Analytical Method Committee 1959, Analyst *4*, 356 (1959).
26. Horning, E. C., Anal. Chem. *35*, 526 (1963).
27. Carroll, K. K., Nature *191*, 377 (1961).
28. Horning, E. C., E. H. Ahrens, Jr., S. R. Lipsky, F. H. Mattson, J. F. Mead, D. A. Turner and W. H. Goldwater, J. Lipid Res. 5, 20 (1964).
29. Farquhar, J. W., W. Insull, P. Rosen, W. Stoffel, E. H. Ahrens, Nutr. Rev. (Suppl.) *17*, No. 8, part II.
30. Olcott, H. S., and J. Van der Veen, J. Food Sci. *28*, 313 (1963).
31. Einset, E., H. S. Olcott and M. E. Stansby, Comm. Fisheries Rev. *19*, 35 (1957).
32. Lovern, J. A., in "Fish in Nutrition," E. Heen and R. Kreuzer, Eds., Fishing News (Books) Ltd., London, 1962, p. 83.
33. De Witt, K. W., J. Sci. Fd. Agric. *14*, 92 (1963).
34. Deuel, H. J., "The Lipids," Interscience Publishers, Inc., Vol. I, 1951, pp. 46, 229.
35. Desnuelle, P., and P. Savary, J. Lipid Res. *4*, 369 (1963).

[Received September 15, 1964—Accepted March 18, 1965]

Degradation of Linear Alkylate Sulfonates in Sewage

J. P. SIMKO, JR., E. M. EMERY and E. W. BLANK, Colgate-Palmolive Research Center, New Brunswick, New Jersey

Abstract

Residual linear alkylate sulfonates isolated from sewage effluents during a field test of biologically soft detergents reveal chain-length and phenyl isomer distributions similar to those in the influent stream. The data suggest no preferential degradation of linear alkylate sulfonate isomers under the field test conditions. The effluent is, however, characterized by apparent changes in branching content.

Introduction

AN INTENSIVE STUDY has been conducted in this laboratory of the alkyl benzene sulfonates isolated from sewage during a field test of the biodegradation of linear alkylate sulfonates in a sewage treatment plant located at Elm Farm Mobile Home Park community near Woodbridge, Virginia (1). At this locality the sanitary waste is treated in an extended aeration activated sludge system. Conditions for control of the test were particularly favorable at this site and the satisfactory analytical results obtained reflect this factor.

To complement the more extended study (1) information was sought regarding the nature of residual linear alkylate sulfonate species present in the effluent stream from the plant subsequent to introduction of products containing these materials. To obtain this additional information samples of raw sewage and treated effluent were taken periodically and the residual linear alkylate sulfonate species isolated as the methyl heptyl ammonium salts. These isolated materials were examined by infrared spectroscopy, then desulfonated and the alkylates analyzed by mass spectrometry to determine chain length distribution and phenyl position isomer distribution.

Experimental

Sewage samples were taken over a five-week period after the introduction of products containing linear alkylate sulfonate (LAS). Three raw sewage influent samples were taken during the study, one prior to the introduction of products containing LAS and two during the interval these were being used. The influent designated A in Table I was taken approximately one month after the introduction of LAS products and influent B one month later. The influent control sample shown in Table I was taken prior to the introduction of LAS material. Influent studies were made to monitor the changeover to linear material and to permit comparison with materials isolated from the treated effluent leaving the plant. The standard sample listed in Table I is the base linear alkylate sulfonate with which the test products were formulated.

A total of four treated waste samples were studied, the first (Effluent A, Table I) taken approximately six weeks after the introduction of LAS materials and the remaining three, namely B, C, and D, at successive weekly intervals. Details of the sampling methods are outlined elsewhere (1). All sewage samples in this work were preserved with formaldehyde and, as an additional precaution, maintained at a temperature of 4C or less, prior to analysis.

Methylene blue active species (MBAS) were determined by the method described in *Standard Methods for the Examination of Water and Waste Water*

TABLE I

Mass Spectrometric and Infrared Characterization of ABS Isolated from Sewage

Sample	Date taken	Na ABS ppm		Est. % branching		Mol. wt.	Est. % unsat.
		as MBAS	by IR	IR	m.s.		
Influent "Control"	8-26-63	27.0	14.0	90	88.2	250.0	21.5
Standard	.....	.....	.....	0	3.1	256.0	4.9
Influent A	9-27-63	24.5	20.7	11	14.0	256.0	7.2
Influent B	10-28-63	28.0	17.6	2	6.4	257.2	10.6
Effluent A	10-14-63	3.7	3.8	51	34.5	258.3	22.9
Effluent B	10-21-63	2.4	2.4	79	62.9	255.6	32.6
Effluent C	10-28-63	1.1	0.85	53	22.5	261.5	35.2
Effluent D	11- 4-63	0.8	0.59	60	21.0	259.2	32.8