layer and does not react with the fat. No matter how much excess glycerol is used, there will be very little change in monoglyceride yield.

In the present work the presence of 2-monoglycerides in the reaction product has been neglected because it is generally assumed that the amount of 2-monoglycerides formed in this reaction is small. If the presence of 2-monoglycerides had been taken into account, it would have resulted in slightly more monoglyceride than shown by the standard method and less diglyceride than calculated.

Conclusions

The yield of monoglyceride is not dependent on the fatty acid composition of the oil but on the solubility of glycerol in oil, which is more or less dependent upon temperature.

No matter how much excess glycerol is used, the ultimate composition of the reaction product in a glycerolysis reaction is fixed; and this depends on the amount of glycerol miscible with the oil.

At 180°C. no more than 45% monoglyceride can be obtained by glycerolysis.

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A Kinetic Study of the Autoxidation of Methyl Linoleate and Linoleic Acid Emulsions in the Presence of Sodium Chloride[1, 2]

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Autoxidation of linoleic acid and methyl linoleate emulsions in aqueous buffer solutions was studied by the rate of oxygen uptake. The oxidation rates of methyl linoleate emulsions increased with an increase in the pH of the buffer solution. With linoleic acid, oxidation rates rose until the increase reached its peak at pH 5.50 and then decreased gradually to a minimum at pH 8.00.

Oxidation rates of methyl linoleate and linoleic acid emulsions decreased with increased concentration of NaCl in the system. The effect of variation of pH of the emulsion in the range investigated was similar to that in emulsions without NaCl. There was no evidence that NaCl accelerated the oxidation rates in the system.

The observed inhibitory effect of NaCl may result from the decreased solubility of oxygen in the emulsion with the increased concentration of NaCl. Consequently the availability of oxygen would be a limiting factor in oxidation rates.

The activation energy for the monomolecular and bimolecular reactions of methyl linoleate and linoleic acid autoxidation was found to be independent of the pH value and sodium chloride concentration of the system. The energy of activations for the monomolecular and bimolecular reactions of methyl linoleate and of linoleic acid are 22,000, 18,200, 19,600, and 16,400 cal./mol., respectively.

Spectrophotometric studies of the autoxidized emulsions of linoleic acid and its methyl ester indicate that the magnitude of the absorption at 2325Å is the same at different pH values. On the contrary, the secondary products showing absorption at 2775Å are to some extent dependent on the pH value of the emulsion.

DEVELOPMENT OF RANCIDITY in foods often involves an aqueous fat system. Comparatively little work has been done in such systems, and it is often difficult to draw any general conclusion as moisture may influence the solubility of accelerators or inhibitors. Lea (22), in reviewing the literature, reported that the fat in most powdered foods, crackers, and other cereal foods becomes more rancid as moisture content is lowered to extremely low values. On the contrary, moisture was found to have an accelerating effect on the oxidation of lard. Chang and Watts (7) observed that the effect of NaCl on fat oxidation depended greatly on the amount of moisture in the system. Later Spetsig (30) reported that methyl linoleate oxidized faster in the dry form than in the presence of water.

No common meat additive has a more profound or more puzzling effect on oxidative changes than sodium chloride. In 1934 Lea (20) reported that sodium chloride accelerated oxidation of pork fat. Later, in 1936, he (21) cited several studies in which NaCl solutions either had no effect or actually inhibited rancidity in various foods.

Sodium chloride has been shown to have no accelerating effect on oxidative rancidity in mayonnaise made from cottonseed oil (18), in herring oil (1), and in butterfat, lard, and pumpkin-seed oil (27). On the contrary, it was found to have an accelerating effect on oxidative rancidity in frozen pork (8,35,38), in cured pork (37), in bacon (9), in butter (4,5,10, 17,25,28,34), and in fish (1,6).

From a study on five series of butter consisting of 192 samples made and held under carefully controlled conditions for six years at 0 to −10°C., the relationship between the spoilage factors of NaCl, acid, both NaCl and acid, and natural milk spoilage enzymes was found to be 1:3:4:6, respectively (29). Commercial salt, when dispersed in the solid state in butterfat, exerted a catalytic effect on fat oxidation (12). This catalytic effect on fat oxidation by commercial

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salt in acid solution is attributed to the combined effects of halide and hydrogen ions and is a direct one between acid salt solution and fat (13). Using the filter paper technique, Chang and Watts (7) concluded that dry NaCl and NaCl solutions of high concentrations behaved as pro-oxidants in fat oxidation. Swartling and Mattson (31) reported that the rate of oxidation of methyl linoleate in buffer solutions depends on the pH of the aqueous solution irrespective of whether NaCl has been added to the reaction mixture or not.

Controversy regarding the effect of NaCl on fats has been chiefly directed to deciding whether NaCl accelerates oxidation, inhibits oxidation, or has no effect whatsoever on oxidative rancidity of fats. These questions have been reviewed by Lea (22) and Watts (36), and it must be admitted that the situation is still unclear.

The concepts stated in the literature depend upon evidence obtained from the study of salt on bacon during the manufacture of lard or ground meat. Thus one finds that the studied effect is not solely of NaCl but of other ingredients participating in the oxidation of fat.

The complexity of the problem suggests that the use of pure starting-material would be helpful. For this purpose pure samples of linoleic acid and methyl linoleate were used in this investigation to study the effect of NaCl on their oxidation rates. The rate of oxygen absorption is a good measure of the rate of oxidation of unsaturated fatty acids or esters and thus of the over-all reaction.

Materials and Methods

Linoleic Acid and Methyl Linoleate. Pure linoleic acid and methyl linoleate were obtained from the Hormel Institute, Austin, Minn. The chemical analysis of these two compounds showed iodine values of 181.0 and 172.5, respectively, with only a trace of conjugated diene in each. As different batches of linoleic acid and methyl linoleate sometimes behave differently, in each series of experiments, a single batch was used during the entire series.

Water. Freshly distilled, deionized CO_2-free water was used.

Phosphate Buffer Solutions. Potassium dihydrogen phosphate and sodium phosphate dibasic, Merck, reagent grade, were used in preparing 0.1M buffer solutions. The buffers were prepared by mixing 0.1M KH_2PO_4 solution with 0.1M Na_2HPO_4 solution until the desired pH was reached. Measurements of pH were determined with a Beckman Model G pH meter.

Phosphate buffer made it possible to cover pH 4.50 to 8.00 without changing the nature of the concentration of the acid radicals which might affect the rate of oxidation. The buffers were transferred to polyethylene bottles immediately after preparation. Nitrogen was bubbled through the buffer solutions during preparation. The pH value of each buffer solution was checked periodically during this work. No change in the pH value occurred in the buffer solutions after preparation.

Sodium Chloride Solutions. Reagent grade NaCl was used in preparing 1, 2, 5, 10, 12.5, 15, 20, and 25% solutions. The salt was dissolved in 0.1M phosphate buffers. Sodium chloride solutions were transferred to polyethylene bottles after preparation and kept under a blanket of nitrogen.

Preparation of Emulsions. Polyethylene bottles which had been carefully cleaned were used in preparing the emulsions to prevent metal contamination and to permit keeping the preparations under an atmosphere of nitrogen. Linoleic acid or methyl linoleate to the amount of 0.3 g. was added to 10 ml. of 0.1M phosphate buffer solution in a polyethylene bottle. The bottle was placed in the cup of a 10-KC Raytheon sonic oscillator, and 30 ml. distilled water were poured into the cup around the bottle for transferring the sonic waves. The output current used was 1.25 to 1.30 amp. for 30 min. A finely dispersed emulsion in which the diameter of the oil phase particles ranged between 2 and 4 microns was prepared by this method. This resulted in an enormous surface area of oil phase. All preparations were carried out in an atmosphere of nitrogen. Samples were used immediately after preparation.

Preliminary experiments, in which linoleic acid emulsion or methyl linoleate emulsion was used, disclosed almost incomplete stability on the addition of NaCl solution. The globules of the oil phase clumped and resulted in an emulsion which could not be used.

Emulsions stable for more than 3 hrs. were obtained by sonic vibrations, using starch as a stabilizing agent. By using 25 mg. of starch/10 ml. of 0.1M buffer solution containing 0.3 g. linoleic acid or methyl linoleate, it was possible to overcome the instability that resulted from the addition of sodium chloride solutions in concentrations up to 25% NaCl. Diameters of 2–4 microns were produced uniformly under a wide range of conditions of pH and NaCl concentration.

Starch was found to have no effect on the oxygen uptake or on oxidation-rate constants. A sample of starch solution showed absorption of only 1 microliter of oxygen over a 10-hour period at 50°C.

Method of Studying the Oxygen Uptake. Oxygen absorption measurements were made with the conventional technique by Umbreit, Burris, and Stauffer (33), using a circular, 18-place Warburg apparatus. The shaking speed was 120 strokes per minute with amplitude of 1.8 cm. Flasks with a capacity of 16 ml. were loaded with a total volume of 4 ml. Three ml. of linoleic acid or of methyl linoleate emulsion were placed in the main compartment, and 1.0 ml. of NaCl solution or buffer solution was placed in the side-arm. The flasks were placed in the water bath, and the contents of the side-arm were mixed with the emulsion after temperature equilibration. Manometric readings were corrected for atmospheric pressure changes by use of a thermobarometer.

Measurements of O_2 uptake required periods from 3 to 120 hrs.; readings were taken at intervals from 15 min. to 1 hr., depending upon the reactivity of the system. The resetting of the manometers was accomplished when necessary by opening the stopcock to air and adjusting the Brodie solution to its original level in the manometer. All experiments were carried out in an atmosphere of air.

Each determination was made at least in triplicate with the usual thermobarometric control. The flasks and manometers were calibrated by the method described in detail by Lazarow (19).

Ultraviolet Spectrophotometric Determinations. The ultraviolet absorptions of the autoxidized emulsions in the presence and absence of NaCl were determined with a Beckman DU spectrophotometer on suitable dilutions with "spectro-grade" methanol.

Experimental Results and Discussion

The Effect of pH of Buffer Solution and Temperature on Oxidation Rates. This was investigated for both linoleic acid and methyl linoleate at 50°C. The results are shown in Figure 1 for methyl linoleate and in Figure 2 for linoleic acid. The oxidation rate constants are plotted against the pH values of the buffer solutions used in preparing the emulsions.

It is quite clear from Figure 1 that, as the pH of the emulsion increases, the oxidation rates of methyl linoleate markedly increase.

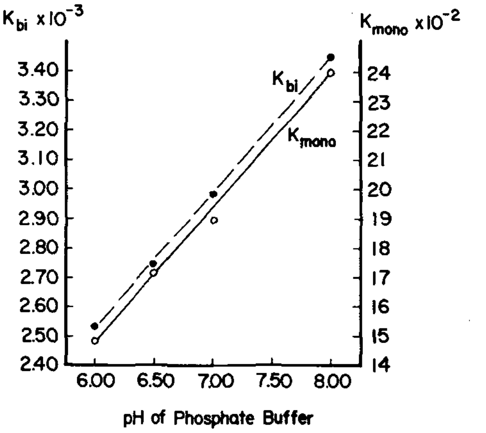

FIG. 1. Influence of pH on oxidation rates of methyl linoleate emulsions at 50°C.

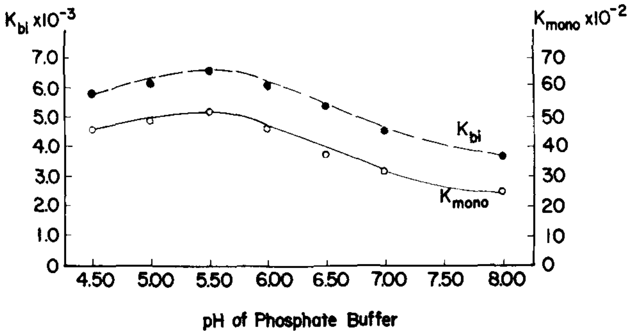

FIG. 2. Influence of pH on oxidation rates of linoleic acid emulsions at 50°C.

Figure 2 shows that the oxidation rates of linoleic acid emulsion increase gradually up to pH 5.50 and then decrease until minimum values are reached at pH 8.00. The acceleration is most pronounced at pH 5.50, where the oxidation rates are the highest. Also linoleic acid oxidizes more rapidly than its methyl ester. At pH 6.00, K_{mono} of linoleic acid is approximately twice that of its ester while at pH 8.00 it is only 1.09 times as great. The ratios of K_{bi} for linoleic acid to those of its ester at pH 6.00 and pH 8.00 are 1.76 and 1.13, respectively. This effect is probably due to the participation of the carboxyl groups in the decomposition of the peroxides. This effect is shown by the work of Privett *et al.* (26), in which the addition of linoleic acid to methyl linoleate peroxide accelerated its decomposition, and also by the work of Holman and Elmer (14).

Table I shows the oxidation rates of methyl linoleate and linoleic acid emulsions at different temperatures.

TABLE I

Oxidation Rates of Methyl Linoleate Emulsions of pH 8.00 and Linoleic Acid Emulsions of pH 6.00 at Different Temperatures

Temperature °C.	Methyl linoleate, pH 8.00		Linoleic acid, pH 6.00	
	K_{mono} X10^{-2}	K_{bi} X10^{-3}	K_{mono} X10^{-2}	K_{bi} X10^{-3}
	ul.$^{1/2}$ *min.*$^{-1}$ *m.m.M.L.*$^{-1/2}$	*min.*$^{-1}$	*ul.*$^{1/2}$ *min.*$^{-1}$ *m.m.L.A.*$^{-1/2}$	*min.*$^{-1}$
30	......	......	6.45	1.13
40	8.09	1.42	17.67	2.70
45	14.02	2.21	29.00	4.04
50	23.91	3.45	47.08	6.08
55	40.36	5.21	75.01	8.96

The oxidation rates of methyl linoleate emulsions and of linoleic acid emulsions were found to increase steadily with temperatures over the range 30 to 55°C.

K_{mono} and K_{bi} for linoleic acid emulsions are approximately 2.75 and 2.40 times greater for each 10°C. rise in temperature while those for methyl linoleate are 3.00 and 2.40 times, respectively. As temperature increases, the temperature coefficients decrease.

The energy of activation for the monomolecular and the bimolecular reactions of methyl linoleate and of linoleic acid autoxidations were calculated. The following values were obtained:

for methyl linoleate	
a) monomolecular reaction	22,000 cal./mol.
b) bimolecular reaction	18,200 cal./mol.
for linoleic acid	
a) monomolecular reaction	19,600 cal./mol.
b) bimolecular reaction	16,400 cal./mol.

These values are in close agreement with those reported in the literature (3, 15, 16) but 20% higher than the values reported by Tappel (32).

Effect of Sodium Chloride on Oxidation Rates of Methyl Linoleate Emulsions and Linoleic Acid Emulsions. To elucidate the effect of NaCl on the oxidation rates of methyl linoleate emulsions and linoleic acid emulsions, numerous experiments were carried out by using NaCl solutions of different concentrations (Tables II and III).

From the data presented in Tables II and III, it is quite clear that the oxidation rates decreased with the increasing concentration of NaCl in the system. Variation of pH in the range investigated, *i.e.*, 5.50 to 8.00, did not alter the decreasing effects of increasing NaCl concentration on the oxidation rates. Inhibition reached its maximum at a concentration of

TABLE II

Rate Constants of Linoleic Acid Autoxidation in Aqueous Emulsions of Different pH Values in the Presence of NaCl at 50°C.

NaCl concn. mol. NaCl/ mol. L.A.	pH 5.50		pH 7.00		pH 8.00	
	[a] K_{mono} X10^{-2}	K_{bi} X10^{-3}	K_{mono} X10^{-2}	K_{bi} X10^{-3}	K_{mono} X10^{-2}	K_{bi} X10^{-3}
0	52.61	6.63	32.58	4.57	26.02	3.87
0.54	44.32	6.11	27.49	3.77	23.92	3.65
1.08	40.71	5.55	25.87	3.26	20.20	3.39
2.69	39.22	5.30	24.27	2.93	19.05	2.81
5.38	37.98	4.99	22.35	2.67	17.18	2.78
10.75	36.21	4.87	19.95	2.36	16.36	2.39
13.44	34.19	4.82	19.38	2.29	16.27	2.22

[a] Units for K_{mono} = ul.$^{1/2}$ min.$^{-1}$ m.m. linoleic acid$^{-1/2}$
K_{bi} = min.$^{-1}$

TABLE III

Rate Constants of Methyl Linoleate Autoxidation in Aqueous Emulsions of Different pH Values in the Presence of NaCl at 50°C.

NaCl concn. mol. NaCl/ mol. M.L.	pH 6.50		pH 7.00		pH 8.00	
	[a] K_{mono} X10^{-2}	K_{bi} X10^{-3}	K_{mono} X10^{-2}	K_{bi} X10^{-3}	K_{mono} X10^{-2}	K_{bi} X10^{-3}
0	17.12	2.74	18.84	2.97	23.91	3.44
0.58	16.70	2.59	18.32	2.86	22.95	3.28
1.17	15.91	2.56	17.60	2.77	22.09	3.20
5.85	15.68	2.46	17.27	2.67	21.40	3.07
11.70	14.92	2.31	16.10	2.50	20.93	2.92
14.62	14.46	2.22	15.45	2.41	20.70	2.86

[a] Units for K_{mono} = ul.$^{1/2}$ min.$^{-1}$ m.m. methyl linoleate$^{-1/2}$
K_{bi} = min.$^{-1}$

5.38 mole NaCl/mole linoleic acid or 5.85 mole NaCl/mole methyl linoleate, and after that the inhibition increased at a reduced rate. There was no evidence that NaCl accelerated the oxidation rates of linoleic acid or methyl linoleate in this system.

The inhibitory effect of NaCl may result from decreasing the amount of oxygen dissolved in the emulsion with increasing concentrations of NaCl. Consequently the availability of oxygen would be a limiting factor in the reaction rates. Bolland (2) stated, that at oxygen pressure above 200 m.m., the oxidation rate of ethyl linoleate is independent of oxygen pressure. In the present work the oxygen pressure is above the critical pressure, but the rate of oxygen diffusion is not the same because the solubility of oxygen decreases with the increasing of the concentration of NaCl in the system. The solubility of oxygen in aqueous solutions decreases as the temperature and/or the sodium chloride concentration increases (24).

Effect of Temperature on the Oxidation Rates of Linoleic Acid and Methyl Linoleate in the Presence of NaCl. In order to determine the effect of temperature upon the oxidation rates of linoleic acid emulsions and methyl linoleate emulsions, a series of experiments was conducted at 55, 50, 45, and 40°C. The data obtained at 40 and 50°C. are tabulated in Tables IV and V.

Increasing the temperature from 40 to 55°C. caused an increase in the oxidation rates. The energies of activation were calculated by plotting in K *versus* the reciprocal of the absolute temperature. The values in the presence of NaCl were the same as those in its absence. The following values were obtained:

methyl linoleate
a) monomolecular reaction............... 22,000 ± 200 cal./mol.
b) bimolecular reaction..................... 18,200 ± 250 cal./mol.
linoleic acid
a) monomolecular reaction............... 19,500 ± 200 cal./mol.
b) bimolecular reaction..................... 16,200 ± 200 cal./mol.

TABLE IV

Oxidation Rates of Linoleic Acid Emulsions of pH 6.00 at 40 and 50°C.

NaCl concn. mol. NaCl/ mol. L.A.	40°C.		50°C.	
	[a] K_{mono} X10^{-2}	K_{bi} X10^{-3}	K_{mono} X10^{-2}	K_{bi} X10^{-3}
0	17.67	2.70	47.08	6.08
0.54	16.56	2.50	40.78	5.27
1.08	15.99	2.49	36.77	4.70
2.69	14.86	2.42	32.58	4.26
5.38	14.17	2.25	31.11	4.13
10.75	13.85	1.85	28.67	3.93
13.44	13.68	1.83	28.63	3.88

[a] Units for K_{mono} = ul.$^{1/2}$ min.$^{-1}$ m.m. linoleic acid$^{-1/2}$
K_{bi} = min.$^{-1}$

TABLE V

Oxidation Rates of Methyl Linoleate Emulsions of pH 8.00 at 40 and 50°C.

NaCl concn. mol. NaCl/ mol. M.L.	40°C.		50°C.	
	[a] K_{mono} X10^{-2}	K_{bi} X10^{-3}	K_{mono} X10^{-2}	K_{bi} X10^{-3}
0	8.09	1.42	23.91	3.44
0.58	7.83	1.33	22.95	3.28
1.17	7.58	1.28	22.09	3.20
5.85	7.33	1.20	21.40	3.07
11.70	7.04	1.12	20.93	2.93
14.62	6.85	1.10	20.70	2.86

[a] Units for K_{mono} = ul.$^{1/2}$ min.$^{-1}$ m.m. methyl linoleate$^{-1/2}$
K_{bi} = min.$^{-1}$

Spectral Characteristics of Linoleic Acid in Emulsion. Spectrophotometric studies of the autoxidized emulsions of linoleic acid and its methyl ester at different pH values indicate the development of conjugated diene unsaturation parallel to the oxygen uptake. In addition, secondary products showing absorption at 2775Å are formed in proportion to the oxygen uptake. This is in agreement with the work reported on dry methyl linoleate by Gunstone and Hilditch (11) and Lundberg and Chipault (23).

The rate of chromophore accumulation is the same at different pH values. The character of the secondary products is to some extent dependent on the pH of the emulsion. On the addition of alkali the absorption at 2775Å increases by approximately 6 to 8 times at pH 6.00 but only by 2–2.5 times at pH 8.00. This increase is caused by secondary products which yield conjugated triene structure on alkali treatment. These chromophores may well be formed in the chain-terminating reactions. At pH 6.00 the number of reaction chains is increased, and the rate of termination is increased with a consequent decrease of the over-all chain-length.

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Preparation of Petroselinic Acid

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Petroselinic acid of a grade which is suitable for most laboratory applications has been prepared by a single crystallization of the mixed fatty acids of parsley seed oil from 90% ethanol. A product of higher purity has been prepared from this acid by application of urea segregation techniques for the removal of saturated materials. No evidence of the presence of unsaturated fatty acids other than petroselinic acid was found in either of these samples when their ozonolysis products were examined chromatographically. On the basis of iodine values, assuming the absence of other unsaturated materials, the purities of the two preparations are 96.0 and 99.4%.

IN CONNECTION WITH RESEARCH now in progress in this laboratory on the chemistry of petroselinic (*cis*-6-octadecenoic) acid, it was necessary to prepare moderate quantities of this material. The methods generally used for the preparation of petroselinic acid are tedious, employing lithium or lead salt separations in conjunction with solvent crystallization of the free acids or fractional distillation of their methyl esters (1,2,3). Recently a different approach has been applied to the problem by Shenolikar and Subbaram (4), who used urea segregation for the isolation of petroselinic acid. Although the procedure utilized 5 crystallizations of the urea complexes, their final product was rather impure as judged from its iodine value (84.1).

One of the chief difficulties in the isolation of pure petroselinic acid is effecting its separation from its isomer oleic acid. Solubility data for oleic and petroselinic acids presented by Kolb and Brown (5) suggested the possibility of separating these acids by a direct crystallization procedure.

Parsley seed oil was selected as the source of petroselinic acid because its component acids have been reported to contain a high percentage (70–76%) of petroselinic acid (1,2). The oil employed was found to contain approximately 25% of unsaponifiable material. Since it was first thought that the presence of such a large amount of unsaponifiables would unduly complicate the isolation of petroselinic acid, the initial experiments were conducted using fatty acids which had been freed of unsaponifiable material. In exploratory experiments, a number of solvents and various temperatures were employed in attempting to separate petroselinic acid from the other component acids. It was found that a satisfactory product could be obtained by a single crystallization either from ethanol or petroleum ether (b.p. 60–70°C.) at a temperature of −25°C. Further work showed that a comparable product could be obtained under the same conditions from fatty acids which still contained the unsaponifiable material. This product upon ozonolysis and examination of its degradation products showed no evidence of the presence of oleic or linoleic acid, and on the basis of its iodine value was considered to be approximately 96% pure. An acid of this purity is suitable as a starting material for many types of chemical modification.

It was found that an acid of higher purity could be prepared from the above-described product by application of urea segregation techniques for the removal of saturated materials. The petroselinic acid prepared in this manner had a melting point corresponding to that reported in the literature (6) and a purity of 99.4% based on iodine value determination. It contained no *trans*-isomers and, like its precursor, appeared to be free of other unsaturated acids.

Experimental

Parsley Seed Oil. Parsley seed which had been ground in a Bauer mill was extracted with petroleum ether (b.p. 35–60°C.) using a Soxhlet type extractor. In a typical preparation, 1,000.0 g. of the seed yielded 189.3 g. of a dark green oil having an iodine value of 115.7, a saponification number of 135.3 and containing 24.8% of unsaponifiable material. The mixed fatty acids, which had been freed of unsaponifiable material, had the following composition as determined by A.O.C.S. Method Cd 7-58 (7): monounsaturated acids, 84.6%; linoleic acid, 10.6%; and saturated acids, 4.6%. Ozonolysis of 500 mg. (approximately 1.8 millimoles) of the mixed fatty acids by the procedure described below yielded 0.27 millimole of azelaic and 1.27 millimoles of adipic acid. It is evident from the foregoing that this sample of parsley seed oil is similar in its fatty acid composition to those examined by other workers (1,2).

Ozonolysis Procedure. A half gram of the acid, accurately weighed, was dissolved in 35 ml. of methanol and ozonized at 0°C. The methanol was removed in a rotary dryer under reduced pressure, at room temperature or lower. To the residue were added 5.0 ml. of 90% fomic acid and 2.5 ml. of 30% hydrogen peroxide. The flask was fitted with a condenser and immersed in a glycerine bath. The temperature was raised slowly until reaction started at 80–90°C.

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