the usual pure-culture bacteria strains used for determination of bacteriostatic and bactericidal effects of quaternary ammonium compounds (Clausen—*Medd. Norsk Farm. Selskap 17*, 124). In this work new test methods were devised. Tomcsik (*Proc. Soc. Exptl. Soc. Biol. Med. 89*, 459) has visually demonstrated that cationic and anionic detergent action as bactericidal agent occurs through damage and denaturation of the cytoplasmic membrane and the cytoplasm. Ito *et al.* (*J. Pharm. Soc. Japan 75*, 310) also demonstrated damage to cell membrane by detergent and also measured inhibition of respiration caused by the detergent. Garvie & Clark (*J. Appl. Bacteriol. 18*, 90, 107) have evaluated the effect of factors such as humidity, light, carrier, and contaminants on disinfectant properties of quaternary ammonium compounds. Nonionic detergents may stimulate growth of certain bacteria, and they may be inert, or inhibit growth depending on the type of anionic (Kidder *et al.*—*Exptl. Cell. Res. 7*, 256). Ozonized nonionic detergents have bactericidal activity which starts high but decreases rapidly with time (Ferlin & Karabinos—*Trans. Ill. State Acad. Sci. 47*, 86). Some nonionic detergents are decomposed by the action of fungi (Okabayashi—*J. Fermentation Technol. Japan, 32*, 482).

In disinfection processes in which precleaning is with soap followed by a disinfecting detergent, the latter if not compatible, may be ineffective when residual soap is present on the surfaces (Ortenzizo *et al.*—*Proc. Chem. Specialties Mfrs. Assoc. 1954*, 82).

The influence of numerous surface-active agents on hemolysis was recorded (Morikawa—*Folia Pharmacol., Japan, 50*, 193–204). Cationic surface-active agents were much stronger than anionic and nonionic agents. Hutchinson & Bean (*Arch. Biochem. & Biophys. 58*, 81) have shown that hemolysis of human red cells by sodium alkyl sulfates does not occur at an arbitrary three-minute endpoint unless lysin concentration exceeds a certain critical value.

The effectiveness of various anionic detergents for inhibition of trysin has been determined (Viswanatha *et al.*—*J. Biol. Chem. 212*, 301). The theoretical implications of these data were discussed in relation to protein-detergent action. At pH 7.8 and ionic strength, 0.2, it was possible to separate certain complexes of human serum albumin with surface-active compounds by precipitation and electrophoretic methods (Ardry—*Bull. soc. chim. biol. 36*, 595, 603). After addition of anionic detergent to the serum it became impossible to extract lipides therefrom, and cationic detergents render extraction of phosphoaminolipides with ether incomplete.

The effects of alkali washing agents on the pH and acid coat of skin have been measured (Jacobi—*Fette u. Seifen 56*, 928; Ramsay & Jones—*Brit. J. Dermatol. 67*, 1). It is suggested that soap is absorbed on the skin and hydrolyzed by the skin acidity. The four-hour sebaceous secretion on forehead skin was practically the same during periods when soap was used for washing as when sodium alkyl sulfate was used (Kirk & Effersoe—*J. Invest. Dermatol. 22*, 257). Eczema resulting from use of certain commercial washing agents was traced to nickel and chromium which these washing agents contained (Kroepfli & Schuppli—*Dermatalogica 110*, 1).

As uncommonly known applications, soaps and/or detergents were demonstrated for extraction of certain chemicals from hydrocarbon mixtures (Grekel & Hujsak—*U. S. 2,710,831*), for the volumetric determination of metals (Siggia *et al.*—*Anal. Chem. 27*, 1745; Gwilt—*J. Appl. Chem. 5*, 471) and sulfate (Davey & Gwilt—*Ibid.* 474), for improvement of hydraulic stability of soils (Grossi & Woolsey—*Ind. Eng. Chem. 47*, 2253), to reduce caking tendency of stored fertilizer (Tucker—*J. Agr. & Food Chem. 3*, 669), as ingredient of buffing compounds (Larsen—*U. S. 2,699,990*), for accelerating the growth of chicks (Ely—*U. S. 2,717,208;* Ely & Schott—*U. S. 2,717,209*), for improving the digestion of various starches (Yokozawa & Sakurai—*Rept. Food Res. Inst. Tokyo, 9*, 145), for stimulating the growth of calves (Lassiter *et al.*—*J. Dairy Sci. 38*, 407), for improving the wettabilities of insecticidal solutions (Kuwada & Hirota—*Rept. Takamine Lab. 4*, 150, 156; *5*, 143), for impregnating porous soft board with polyvinyl acetate (Austin *et al.*—*U. S. 2,716,617*), for preparing solutions of polyvinylformal and polyvinyl butral (Isemura & Kimura—*J. Polymer Sci. 16*, 92), for degumming of silk fibers (Veneroso—*Ann. sper. agrar, Rome, 8*, 1701), for retting of vegetable textiles (Lourd—*U. S. 2,725,289*), and for removing insecticidal chemicals from fresh vegetables (van Middelem *et al.*—*Proc. Am. Soc. Hort. Sci. 65*, 357, 365). In most of the above communications suitable detergents for the various applications were suggested. Several communications were on experiences on dust-wetting agents in dedusting mines, their testing, method of use, and efficiencies (Sosnovskii—*Bor'bas Silikozom, Akad. Nauk S.S.S.R. Sbornik Statei 1953*, 69, 126; Berkovich *et al.*—*Ibid.* 134; Rebinder *et al.*—*Ibid.* 57; Prokopova & Novakova—*Pracovni Lekarstvi 6*).

Methods were outlined for overcoming difficulties in primary sedimentation and from foam formation due to detergents at sewage treatment plants (Pilpel—*Research, London, 8*, 62). The effect of synthetic detergents on the settling of suspended solids (Degens *et al.*—*Sewage & Ind. Waste 26*, 1081), on frothing and oxygen transfer in sewage (Lynch & Sawyer—*Ibid.* 1193) and observations on decay of detergents (Bogan & Sawyer—*Ibid.* 1069; Hammerton—*J. Appl. Chem. 5*, 517) were published as data pertinent to handling sewage treatment problems caused by detergents.

The Chemistry of Polymerized Oils. V. The Autoxidation of Methyl Linoleate

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EVER SINCE THE DISCOVERY (10) that autoxidation of methylene-interrupted polyene systems results in double bond rearrangement to conjugated forms, there has been uncertainty as to the quantitative amount of such rearrangement. This uncertainty has been linked together with an uncertainty as to the positional isomers formed and, in particular, as to the presence or absence of the 11-isomer in the case of linoleate. Bolland and Koch (4) concluded that some 70% of methyl linoleate hydroperoxide mixed isomers were conjugated double bond forms, leaving some 30% of unconjugated diene isomer(s). These relative proportions were thought to be caused by isomerism in intermediate radical forms so that the conjugated peroxides were the 9-peroxido $\Delta^{10:12}$ and the 13-peroxido $\Delta^{9:11}$, whereas the non-conjugated isomer was the 11-peroxido $\Delta^{9:12}$.

It is unnecessary to repeat here the generally accepted reaction formulae which fit this interpretation. The extensive studies of Bolland clarified the kinetic pathway which occurs, and it was evident that mesomerism in the radicals (R′ and RO'_2) was the likely explanation for the formation of conjugated dienes. However the great technical difficulties inherent in analyzing mixtures of unstable and closely related isomers have, so far, proved insurmountable, and no complete direct experimental demonstration of the exact composition has ever been published. Bergström (3) showed that hydrogenated methyl linoleate hydroperoxide isomers gave methyl 9- and 13-hydroxystearates. Methyl 11-hydroxy stearate was not found, but its total absence could not be rigidly proven. The possibility that the 11-peroxido $\Delta^{9:12}$ form might have rearranged during hydrogenation is not great since such rearrangement does not occur in the case of similarly constituted hydroxy polyenes

(7). Later Cannon *et al.* (9) and Privett and Wheeler and their collaborators (24, 25) reopened the subject and made two important advances, *viz.* a) the mixed peroxide isomers were obtained purer than hitherto by the use of countercurrent solvent distribution; b) application of infrared spectroscopy demonstrated the presence of both *cis-trans* and *trans-trans* dienes. As a result of these two advances and of a critical examination of the supporting ultraviolet spectroscopic evidence, it was concluded by both groups of workers that some 90% of the mixed isomers were conjugated dienes, leaving some 10% as the non-conjugated (presumably 11-peroxido $\Delta^{9:12}$) form(s).

At this stage in the development of the problem all the evidence bearing upon the presence or absence of the 11-isomer was indirect. Supporting evidence for the substantial or complete absence of the 11-isomer was presented by Sephton and Sutton (27), who attempted to rearrange anionotropically the mixed methyl hydroxylinoleate isomers resulting from sodium borohydride reduction of the peroxide. It was found that no such rearrangement could be detected under conditions which should have effected rearrangement of the 11-hydroxy $\Delta^{9:12}$ form. Khan, Lundberg, and Holman (14) found evidence for the formation of the 11-isomer when linoleate was autoxidized in the presence of chlorophyll, but no rigid proof of identification was adduced. In our present state of knowledge it is not clear whether this catalyzed reaction is at all related to straight self-catalyzed autoxidation; again, lipoxidase-catalyzed linoleate autoxidation may proceed by a different mechanism since the hydroperoxide formed is optically active (26). Thus evidence bearing upon the fine structure of the products in these catalyzed reactions may have no direct bearing upon the self-catalyzed situation.

Experimental

Ultraviolet spectral absorption measurements were made in 96% ethanol, using a Beckman model D.U. spectrophotometer calibrated against standard dichromate solution (11). Infrared absorption data were obtained on CS_2 solutions, using either the Perkin-Elmer single beam model 12C or double beam model 21 spectrophotometers fitted with rock salt prisms.

Autoxidations were conducted in the dark at 4°C. and in diffused daylight at 25°C. and 45°C. by shaking in oxygen at approximately atmospheric pressure. Reactions were discontinued after 0.1 mole of oxygen per mole linoleate had been absorbed so as to minimize secondary reactions.

Peroxides and hydroxyesters contained in aqueous alcoholic media from solvent partition separations were recovered by extraction with pentane, and the latter was then removed at the pump. Samples were stored at −80°C. in glass ampoules, which had been evacuated to 10^{-4} mm. and then sealed.

Hydroperoxide and hydroxylinoleate preparations rapidly deteriorated in air at room temperature, and correct elementary analyses could only be obtained on

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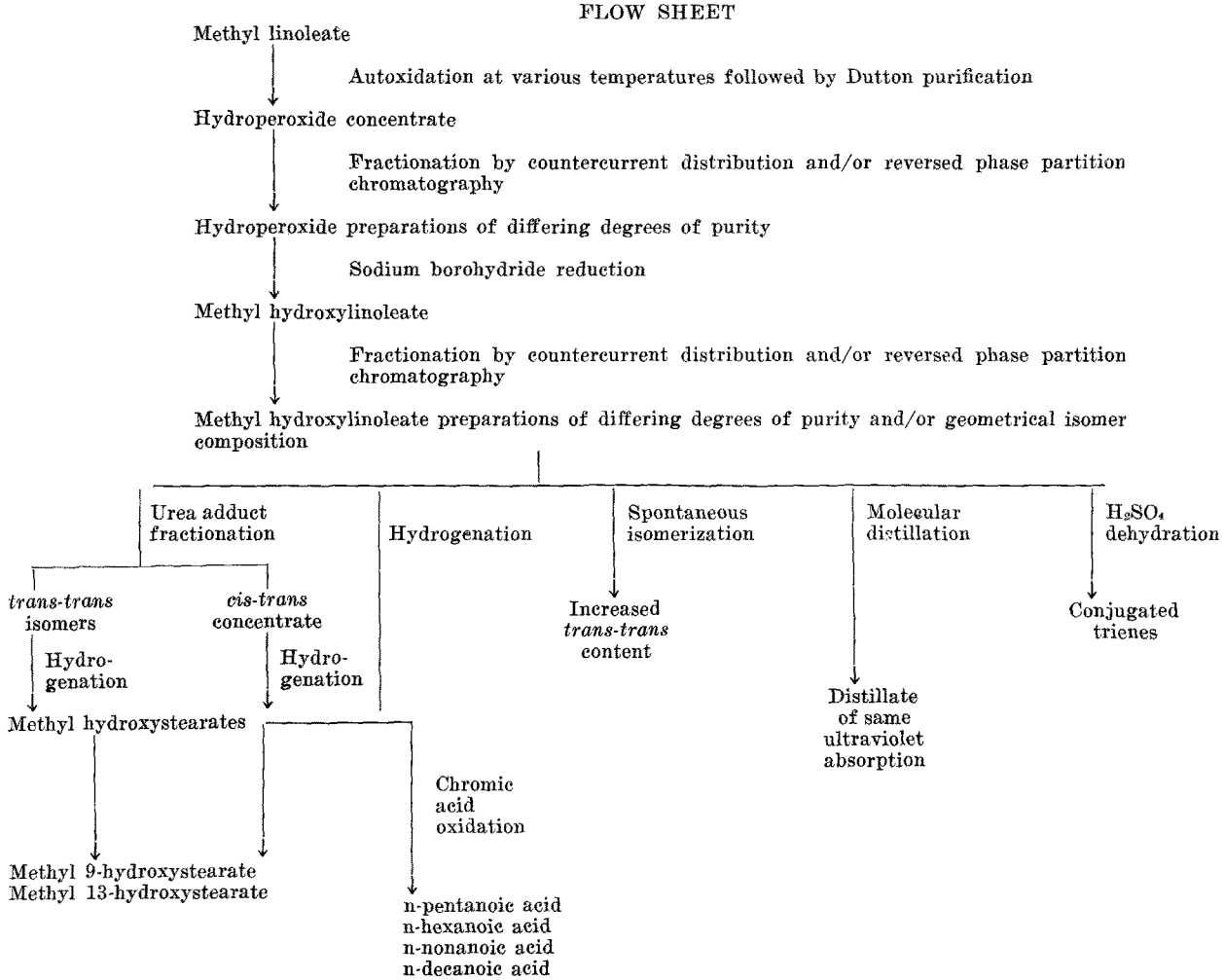


samples stored under the above rigorous conditions.

The operations carried out are summarized below:

Preparation of Methyl Hydroperoxidolinoleate Concentrates. Methyl linoleate was prepared from propane-segregated sunflower seed oil (Sonnol from Marine Oil Refiners of Africa Ltd.) by bromination-debromination. It contained about 4% of its double bonds in the isolated *trans* form. Oxygenated linoleate was concentrated by a simple solvent partition method (31); hydroperoxide concentrates thus obtained were further purified by applying Craig countercurrent solvent distribution in an all-glass apparatus. Alternatively, they were further purfiied by reversed phase partition chromatography, using n-heptane (on hydrophobic celite) as stationary phase and 65% aqueous methanol as eluting phase. Up to 5 g. of peroxide concentrate could be processed at a time. The preparative chromatographic procedure was based on the Howard and Martin (12) analytical technique, which had been scaled up in this laboratory (28, 18).

Methyl linoleate (94 g.) was autoxidized at 23°C. in diffused daylight by shaking continuously in gaseous oxygen. After an induction period of 6 hrs. the reaction rate increased steadily; the experiment was discontinued after 100 hrs. when 1.03 g. of oxygen (*i.e.*, 0.1 mol./mole of linoleate) had been absorbed.

Concentration of the hydroperoxide by the Dutton procedure gave three fractions weighing 0.939 g., 5.338 g., and 88 g. in decreasing order of polarity. The middle fraction contained 88% of peroxide by the stannous chloride method (1) and took up 3.01 moles of H_2 per mole on hydrogenation in the presence of Adams' catalyst. It was further purified by 42 transfers in a 30-tube Craig countercurrent solvent distribution apparatus, using 2 parts of 80% aqueous methanol against a blend of 1 part of pentane and 1 part of "isohexane." Analytical data are in Table I. Ultraviolet and infrared absorption data on these purified products were comparable to those of previous workers (24, 25, 9).

TABLE I

Data Obtained on Craig Countercurrent Purified Hydroperoxides[a] (23°C. Autoxidation)

Tube Nos.	9–10	11–12	13–14	15–16	17–18	19–20
Weight (g.)	0.20	0.50	0.71	0.69	0.49	0.25
ϵ value (232 mμ)	22,200	25,600	25,900	26,000	26,200	26,400
Peroxide value[b]	113%	113%	114%	114%	113%	—
% C[c]	—	69.2	69.6	69.8	—	—
% H[c]	—	10.5	10.6	10.6	—	—
% O[c] (Diff.)	—	20.3	19.8	19.6	—	—

[a] Apparatus: 30-tube glass model filled with N_2. Solvents: 100 ml. 80% aqueous methanol per tube plus 100 ml. of a 1:1 mixture of pentane and "isohexane"; 42 transfers.
[b] Expressed as % of theoretical; iodometric method (24).
[c] $C_{19}H_{34}O_4$ requires: C, 70.0; H, 10.4; O, 19.6.

In the reversed phase partition chromatographic method a n-heptane/hydrophobic celite column (12) was packed in a 3 cm. x 100 cm., water-jacketed tube (23°C.). The eluate was collected as 3.5-ml. aliquots in a syphon; elution of peroxide from the column was followed by measuring the intensity of absorption at 232 mμ of individual aliquots. The peroxide contents of these aliquots were determined by using the colorimetric ferrous thiocyanate method (5). Aliquots were combined after consideration of the analytical data, and the solutes were recovered. In Figure 1 is shown the distribution of a (23°C.) peroxide on such a chromatogram: the peroxide value curve a) has been superimposed upon the diene absorption curve b) by arbitrarily fitting the maximum point of peroxide value to the maximum point of diene value. A trace of a peroxide of low diene absorption and high polarity was eluted before the monohydroperoxide.

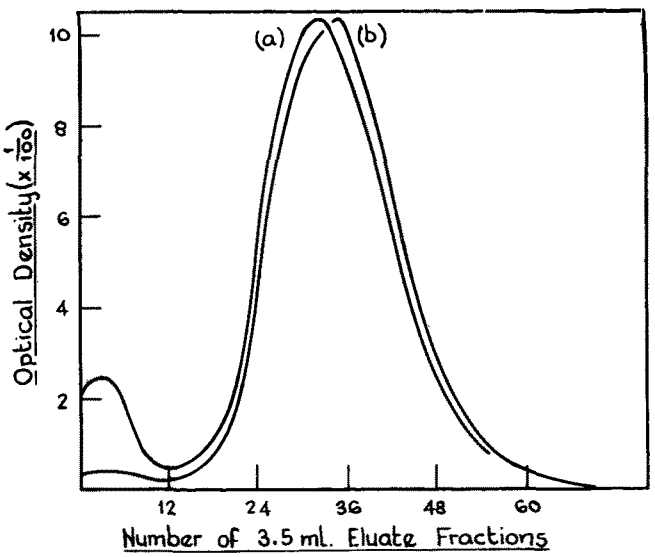


FIG. 1. Reversed phase partition chromatogram of methyl linoleate hydroperoxide; 23°C. autoxidation.
(a) Peroxide value.
(b) 232 mμ absorption.

Similar methods of autoxidation and solvent partition purification methods were applied as necessary to the 4°C. and 45°C. autoxidation products.

Analytical results were in conformity with expectations; thus, for example, the peak fraction from the reversed phase partition chromatography of a hydroperoxide (4°C. autoxidation) gave the following:

found: C, 69.6; H, 10.6; on hydrogenation in acetic acid with Adams' catalyst it took up 3.09 moles H_2/mole and with lithium aluminium hydride in tetrahydrofuran 1.93 moles H_2/ mole were evolved. $C_{19}H_{34}O_4$ requires: C, 70.0; H, 10.4; theoretical H_2 uptake, 3 H_2/mole and theoretical H_2 evolution with lithium aluminium hydride, 2 H_2/mole.

Preparation of Methyl Hydroxylinoleate Concentrates. Hydroperoxide concentrates were reduced to the corresponding methyl hydroxylinoleates by adding sodium borohydride in methanol solution (19) and leaving to stand at room temperature for 2 hrs. The proportion of sodium borohydride used was based on an experiment in which it was found that 90 mg. reduced 80 mg. of peroxide in 5 ml. of methanol. Use of increasing amounts of borohydride resulted in the reduction of increasing proportions of the ester groups. In trial experiments infrared and ultraviolet absorption measurements on the products showed that the diene structures remained unaltered during reduction. Methyl hydroxylinoleates were purified by countercurrent solvent distribution (30 or more transfers) and reversed phase partition chromatographic procedures as appropriate. The infrared and ultraviolet data on countercurrent purified products revealed that some relatively polar material of low conjugated diene content was easily removed after reduction since the ϵ values of the purified reduction products were slightly higher than those of the parent peroxides.

TABLE II

Reversed Phase Partition Chromatography of Methyl Hydroxylinoleate[a] (4°C. Autoxidation, Figure 2)

Fraction No.[b]	Weight (g.)	ε value (232 mμ)	λ max. (mμ)	% *cis-trans*[c]	% *trans-trans*[c]	ε value calculated[d] from % *cis-trans* and % *trans-trans*
2	0.1360	—	—	—	—	—
3						
4	0.0523	23,800	233.5			
5	0.1506	23,800	233.0	55	20	21,700
6	0.1920	25,200	233.0	59	21	23,100
7	0.0776	26,900	232.5	54	27	23,700
8	0.0965	27,600	232.0	34	48	24,700
9	0.0410	22,100	231.5	10	58	21,100

[a] Column (3 cm. x 90 cm.) packed with hydrophobic celite impregnated with n-heptane. Elution, using 65% aqueous methanol, was followed by ultraviolet absorption measurements on 3.5-ml. portions of eluate.

[b] Fractions are numbered according to cut points indicated by arrows on Figure 2; thus fraction 5 was collected between cut points 4 and 5.

[c] Determined with double beam instrument calibrated for *cis-trans* and *trans-trans* absorption against the reference standards described.

[d] Assuming *cis-trans* has ε = 28,000 and *trans-trans* ε = 31,600.

Table II gives the results obtained when 0.84 g. of hydroxylinoleate was processed by this method. The material used contained a considerable percentage of compounds other than monohydroxylinoleates. It was obtained by applying the Dutton procedure to autoxidized linoleate (4°C.; 0.1 mole oxygen/mole) and then reducing with sodium borohydride (1 g. to 0.9 g. of Dutton concentrate). The distribution curve as indicated by absorption measurements at 232 mμ on the eluates is presented in Figure 2. The first sharp peak probably represents diols produced by reduction of the ester groups in some molecules. Infrared absorption data (Figure 3) were recorded on the double

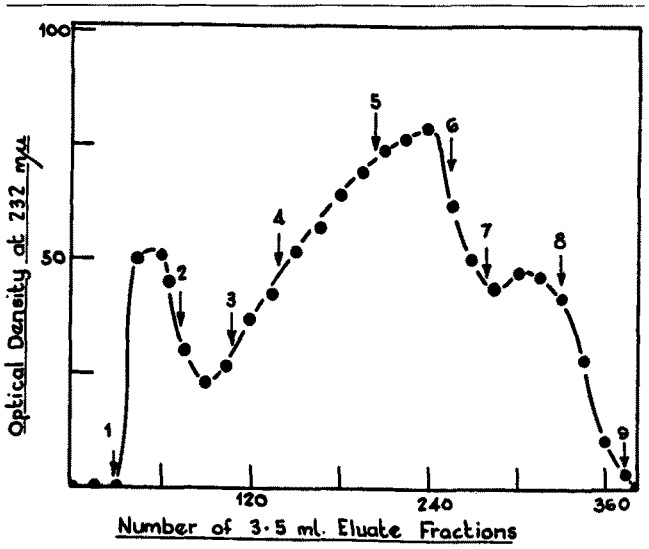


FIG. 2. Reversed phase partition chromatogram of methyl hydroxylinoleates; 4°C. autoxidation.

beam instrument, and calculations of *cis-trans* and *trans-trans* conjugated forms were carried out after calibration with the *cis-trans* and *trans-trans* standards described later.

Urea Complex Formation of Methyl Hydroxylinoleates. The *trans-trans* conjugated diene forms were separated by urea complex fractionation.

A Craig countercurrent purified methyl hydroxylinoleate mixture (1.901 g.) (45°C. autoxidation) gave:

found: C, 73.3; H, 11.1; on hydrogenation in ethanol with Adams' catalyst it took up 1.99 moles H_2/mole, and with lithium aluminium hydride in tetrahydrofuran 1.03 moles H_2/mole were evolved. $C_{19}H_{34}O_3$ requires: C, 73.5; H, 11.0; theoretical H_2 uptake 2 H_2/mole and theoretical H_2 evolution with lithium aluminium hydride 1 H_2/mole.

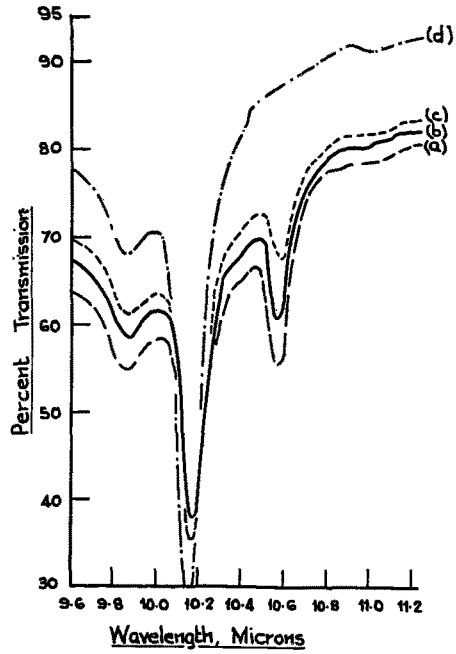


FIG. 3. Infrared spectra of methyl hydroxylinoleates, double beam instrument, CS_2 solutions, 0.5 mm. cell.
(a) Fraction 6, Table II; 1.095% w/w
(b) Fraction 7, Table II; 0.92 % w/w
(c) Fraction 8, Table II; 0.785% w/w
(d) *trans-trans* hydroxylinoleate (ε = 31,400 at 231 mμ); 0.755% w/w
Where (a) and (c) are not shown they coincide with (b) except that the 10.16 μ peak of (c) is superimposed upon that of (a).

This was dissolved in methanol (100 ml.), and the solution was saturated with urea at 35°C. under anaerobic conditions. It was left to crystallize at 4°C.; the crystals were filtered and washed with cold (—50°C.) methanol. The washings and mother liquors were combined and evaporated under vacuum to the saturation point at 35°C. The second and third crops of crystals were obtained and added to the first crop. From the combined crops the *trans-trans* concentrate was obtained by pentane extraction from aqueous solution (yield 0.6411 g.). The latter product was crystalline (m.p. *ca.* 30°C.) and had a molecular diene extinction coefficient (ε) of 30,600 at 231 mμ:

found: C, 73.1; H, 11.2. $C_{19}H_{34}O_3$ requires: C, 73.5; H, 11.0.

The infrared spectrum indicated that approximately 10% of *cis-trans* forms were still present. By recrystallizing the urea complex twice from a minimum of methanol at low temperature, all but a trace of the *cis-trans* forms (10.54 μ band) were removed. This final product (Figure 3) was used as a standard conjugated *trans-trans* methyl hydroxylinoleate for spectroscopic measurements; it had ε = 31,400 at 231 mμ. The remaining material (1.208 g.) obtained from the combined mother liquors had an ε value of 27,000 at 232 mμ, and infrared spectroscopy showed that it still contained more *trans-trans* than *cis-trans*.

A *cis-trans* concentrate containing about 10% of *trans-trans* dienes was obtained in bulk from methyl hydroxylinoleates, originating from a 4°C. autoxidation after precipitation of the *trans-trans* forms as their urea complexes: it had ε = 24,000 at 233 mμ.

In an alternative procedure a methanol solution of the *cis-trans/trans-trans* mixture was passed slowly through a short column (25 cm. x 1 cm.), packed with finely ground urea; most of the *trans-trans* forms were retained on the column as complex, and the *cis-trans* dienes were concentrated in the eluate.

Infrared Absorption Data. Early infrared spectral absorption data were obtained on a Perkin Elmer Model 12C instrument. The solvent background absorption (CS_2) is included in Figures 4 and 6 which were obtained on this instrument.

In these early experiments the bands at 10.12 μ and 10.54 μ, resulting from *trans-trans* and *cis-trans* conjugated diene absorption, respectively, were compared with the 10.33 μ band of methyl elaidate to assess the approximate concentration of each component. In mixtures of the two components the 10.12 μ band due to *trans-trans* shifts to 10.16 μ because of superimposition with the the 10.18 μ band of *cis-trans* conjugated dienes. Results were qualitatively significant only because of the use of an indirect standard. At a later stage better standards were used, and it was found that the above method analyzed the *cis-trans* concentrations approximately 20% too low and the *trans-trans* too high by about a similar factor.

A reference standard for *trans-trans* conjugated dienes was prepared by urea adduct separation as described above.

For the preparation of a *cis-trans* standard, methyl linoleate (10 g.), potassium hydroxide (6.1 g.), water (3 g.), and ethanol (10 ml.) were sealed up in an evacuated glass bulb and heated at 80°C. for six days. The recovered acids had $E^{1\%}_{1\,cm.} = 324$ at 323 mμ; they were esterified with diazomethane. The methyl esters were crystallized from methanol at −80° C. until an $E^{1\%}_{1\,cm.}$ value of 390 was reached, *i.e.*, the preparation contained 41% of *cis-trans* linoleate (assuming $\epsilon = 28{,}000$ for pure *cis-trans* linoleate). This mixture was used as an infrared standard since the presence of unconjugated linoleate does not interfere with the bands at 10.18 μ and 10.55 μ. A check on the *cis-trans* content was provided by isomerizing the mixed acids with iodine in iso-octane solution (2) so that the *cis-trans* became *trans-trans*; based on the observed increase in absorption at 232 mμ there was 40% of *cis-trans* (assuming $\epsilon = 31{,}600$ for pure *trans-trans* linoleate).

The two standards were used to calibrate the double beam instrument (Perkin Elmer Model 21) for *cis-trans* and *trans-trans* absorption.

Isomerization of Cis-Trans Forms to Trans-Trans. a) *With Iodine.* In attempts to induce isomerization purified methyl hydroxylinoleates were treated with iodine in "iso-octane" solution, following Nichols *et al.* (21). No increases in ultraviolet absorption at 232 mμ were observed, but a slow decrease accompanied by an increase in absorption at 270 mμ occurred. This was probably caused by dehydration with the formation of conjugated trienes.

b) *Spontaneous Isomerization.* It was observed from infrared and ultraviolet spectral data that a change occurs in the *cis-trans* to *trans-trans* ratios of purified autoxidation products after storage for a number of months. During storage samples were kept sealed in vacuum at −80°C. in the dark, but they were occasionally allowed to warm up to room temperature and opened for sampling purposes. The results of semiquantitative (single beam) infrared analyses on some of the samples are given in Table III.

TABLE III

Changes in Geometrical Isomer Content With Time

Description of material	Date of analysis	% *trans-trans*[a]	% *cis-trans*[a]
(1) Purified hydroxy-linoleate from 23°C. autoxidation	2/26/53		
	4/28/54	56	23
		64	20
(2) Purified hydroperoxide from 4°C. autoxidation	4/30/53		
	4/28/54	14	55
		29	31
(3) Purified hydroxylinoleate from 4°C. autoxidation	5/ 4/53		
	4/28/54	26	59
		40	32
(4) Urea complex concentrated *cis-trans* hydroxylinoleate from 23°C. autoxidation[b]	7/27/53		
	4/22/54	17	53
		51	25
after a further 4 weeks at 25°C.	7/26/54	88	10

[a] Single beam instrument, based on methyl elaidate.
[b] See Figure 4.

The changes which occurred in sample No. 4 of Table III are shown in Figure 4.

Dehydration of Methyl Hydroxylinoleates with Dilute Sulphuric Acid. Methyl hydroxylinoleate (55 mg.) (Fraction 8, in Table II) was dissolved in purified ethanol (10 ml.), and a solution of sulphuric acid (15%) in ethanol (500 ml.) was added. The optical density of the solution was measured at 232 mμ and 270 mμ at short intervals (5 min.) after suitable dilution (*e.g.* 60 λ in 2.5 ml.). A gradual decrease in absorption at 232 mμ with a larger increase at 270 mμ was noted; the latter attained a maximum value 15 min. after mixing the reagents. The product was poured into distilled water (1 l.) covered with n-pentane (500 ml.) in a separating funnel. The pentane-soluble material was extracted and washed with water (2 x 100 ml.). The product (± 50 mg.) was subjected to reversed phase partition chromatography on a n-heptane celite column, using aqueous

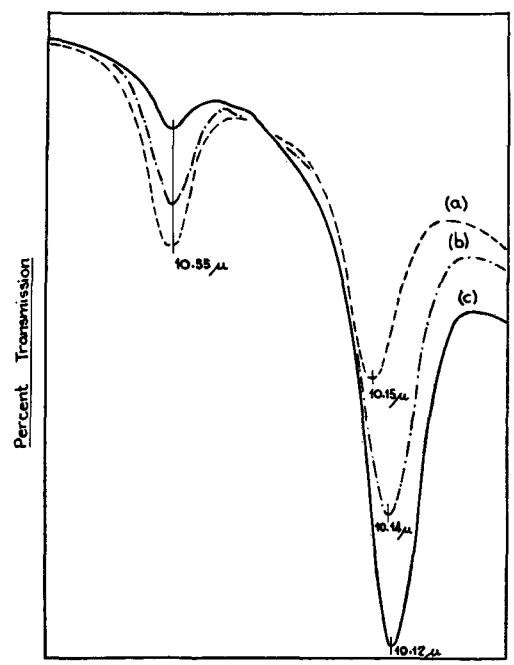


FIG. 4. Infrared spectra of methyl hydroxylinoleates (sample 4, Table III) showing isomerization of *cis-trans* to *trans-trans*; single beam instrument in CS_2, curves superimposed at 10·4μ.
(a) At 7/27/53; 0.8 % w/w
(b) At 4/22/54; 1.04% w/w
(c) At 7/26/54; 0.8 % w/w

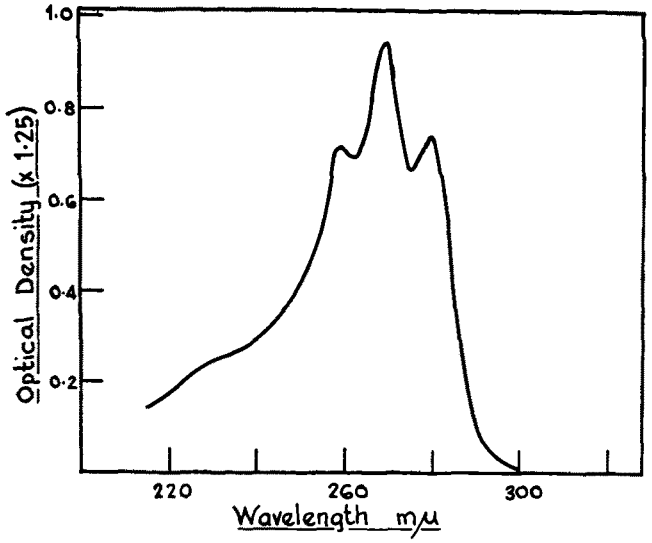


FIG. 5. Ultraviolet absorption curve of triene obtained by dehydration of methyl hydroxylinoleates (Fraction 8, Table II).

methanol (80%) saturated with n-heptane as the moving phase. Development was followed by measuring the optical density of the eluate at 232 mμ and 270 mμ. The unreacted methyl hydroxylinoleate and some polar degradation products were removed from the column by this procedure. The conjugated triene (39 mg.) was recovered from the column by stripping it with n-pentane (500 ml). Its ultraviolet absorption curve (Figure 5) shows three peaks with $\epsilon = 32{,}400$ at 268.5 mμ (methyl β-elaeostearate has $\epsilon = 54{,}000$ at 268 mμ). The infrared absorption curve over the 10 μ region is given in Figure 6.

Molecular Distillation of Methyl Hydroxylinoleates. A single stage falling film molecular still was used at 96°C. and three microns pressure. One half of the charge (200 mg., countercurrent purified, 23°C. autoxidation) volatilized and was collected. It had $\epsilon = 27{,}100$ at 232 mμ, which was the same figure as that of the starting material.

Hydrogenation of Methyl Hydroxylinoleates to Methyl Hydroxystearates. Methyl hydroxylinoleate (100 mg. of combined fractions 4 and 5, Table II) was quantitatively hydrogenated (Adams' catalyst), taking up 1.94 moles H_2/mole. The product was subjected to a 30 transfer Craig purification between

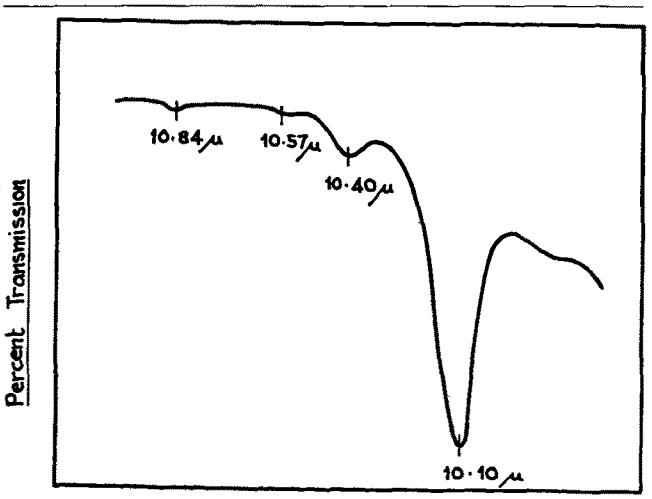


FIG. 6. Infrared spectrum of triene obtained by dehydration of methyl hydroxylinoleates (Fraction 8, Table II); single beam instrument, CS_2 solution, 0.5 mm. cell, qualitative measurement with approximately 1% solution.

80% aqueous methanol and pentane-"isohexane" (1:1). All of the material applied was recovered in a single peak, indicating that only material of the same polarity as the methyl monohydroxystearates was present. A sample of the corresponding acids was further examined by the paper partition method of Micheel and Schweppe (20); it gave only a single spot having a Rf value identical with that of authentic dl-9-hydroxystearic acid.

Chromic Acid Oxidation of Monohydroxystearic Acids. Monohydroxystearic acids (obtained by hydrogenation of methyl hydroxylinoleate fractions 6, 7, and 8, Table II) were oxidized with chromic/sulphuric acid and simultaneously steam-distilled so as to remove monocarboxylic acid fragments on formation (17). Usually 20–40 mg. of hydroxystearic acid mixtures were added to 25% sulfuric acid (2.5 ml.) containing 8% chromic acid and steam was passed through the mixture for about 45 min. The volume was kept constant by heating with a microburner. The distillate was neutralized with NaOH and concentrated; after acidification with sodium bisulfate the free fatty acids were extracted with n-butanol and analyzed by paper partition chromatography (Lindqvist and Storgärds [15]). Three well-defined intense spots were obtained, two of which had Rf values identical with those of n-pentanoic and n-hexanoic acids; the third spot appeared in a position corresponding to acids of $>C_8$ chain length which are not resolved by this method. Three very faint spots occurred with Rf values identical with those of n-butanoic, n-propanoic, and acetic acids. When authentic 12-hydroxystearic acid was oxidized, it yielded n-heptanoic and n-hexanoic acids with traces of n-pentanoic, n-butanoic, n-propanoic, and acetic acids. The method described by Micheel and Schweppe (20) was used for detecting steam-volatile acids of chain lengths in the range C_5–C_{18}. The butanol extracts were esterified with diazomethane, and the corresponding hydroxamic acids were prepared; n-pentanoic, n-hexanoic, n-nonanoic, and n-decanoic acids were found. The presence of traces of acids of chain length C_4 and shorter was indicated by a faint diffuse spot, and there was an acid present with a Rf value equal to that of 9-ketostearic acid.

No indication of the presence of n-heptanoic, n-octanoic, or n-undecanoic acids was obtained by oxidation of any of the mixed hydroxystearic acids.

Tests for the Presence of 2-Hydroxystearic Acid. The possible presence of a small concentration (<2.5%) of 2-hydroxystearic acid in fraction 6, Table II, was indicated by 1,2-glycol determinations, using potassium paraperiodate (13), on the diol mixture produced by lithium aluminium hydride reduction.

The presence of 2-hydroxystearic acid (<2.5%) in fraction 6, Table II, was also indicated by iso-propylidene formation with acetone according to the procedure of Bourne *et al.* (6). The small yield of recovered material precluded its positive identification.

Alumina Chromatography. The following methyl hydroxystearate preparations were resolved:

a) obtained by hydrogenation of *trans-trans* methyl hydroxylinoleate of $\epsilon = 30{,}600$ at 231 mμ (described above; 45°C. autoxidation);

b) obtained by hydrogenation of a *cis-trans* concentrate after substantial removal of the *trans-trans* isomers by urea complex formation (described above; 4°C. autoxidation);

c) from a *cis-trans* concentrate prepared by partial separation of the geometrical isomers on a reversed phase partition chromatographic column (Fractions 4 and 5, Table II; 4°C. autoxidation).

Alumina used for chromatography was washed with HCl (2N), then with water until neutral, and finally with ethanol. It was activated at 150°C. for 5 hrs. and had Brockman and Schodder (8) grade II activity. The alumina was poured into a tube (0.7 cm. x 100 cm.) filled with pentane. Elution was effected with pentane containing 0–10% chloroform. Aliquots of fractions were evaporated. Suitable composite fractions were made up by combining selected residues, which were weighed and the melting points of which were determined.

Material of type (a) above (65 mg.) was chromatographed and resolved into two fractions (yield, 97% of that applied) of about equal weight. The melting point curve of the fractions is given in Figure 7 as b), and the weight distribution as a). From the middle fractions of the two peaks (collected between consecutive pairs of arrows) the pure methyl hydroxystearates were obtained in about 80% yield by recrystallizing repeatedly from pentane at —10°C. These had melting points of 52.4–52.9°C. (found: C, 72.5; H, 12.1. $C_{19}H_{33}O_3$ requires: C, 72.6; H, 12.1) (ex first peak) and 50.6–50.8°C. (found: C, 72.35; H, 12.0) (ex second peak). They were identified as methyl dl-13-hydroxystearate and methyl-dl-9-hydroxystearate, respectively, by mixed melting point determinations with authentic compounds. An equimolar mixture of the 9- and 13-compounds had a melting point depressed by 10°C.

Material of type (b) above was similarly treated to give methyl 13-hydroxystearate (m.p. 52.1–52.8°C.) (found: C, 72.35; H, 12.0) and methyl 9-hydroxystearate (m.p. 50.2–50.6°C.) (found: C, 72.4; H, 12.4).

Material of type (c) above, which had already been shown to be of homogeneous polarity (p. 268), was similarly treated and gave two chromatographic peaks (recovery 96.5% of original) of nearly equal size which yielded, after crystallization, pure methyl 13- and 9-hydroxystearates as in the other cases. Material between the two chromatographic peaks appeared, from melting point and mixed melting point tests, to be an approximately equal mixture of the 9- and 13-isomers.

Discussion

Prior to the start of the present work a re-investigation into the geometrical and positional isomerism problems appeared desirable since the available evidence was inconsistent. On the one hand, recent American work (9, 24, 25) had considerably raised the estimate of the proportion of conjugated 9- and 13-isomers, and this, taken together with the evidence of Sephton and Sutton (27) as to the probable absence of the (unconjugated) 11-isomer, had raised serious doubts as to the existence of any but conjugated forms in methyl hydroperoxidolinoleate. On the other hand, the ultraviolet extinction coefficients at 232 mμ observed both by the American workers and by ourselves were too low for mixtures consisting only of conjugated *cis-trans* and *trans-trans* dienes; indeed the observed coefficients were even lower than that of the pure *cis-trans* form.

The possibility that the observed low coefficients are caused by admixture with non-hydroperoxide impurities also exists and is not easy to dispose of.

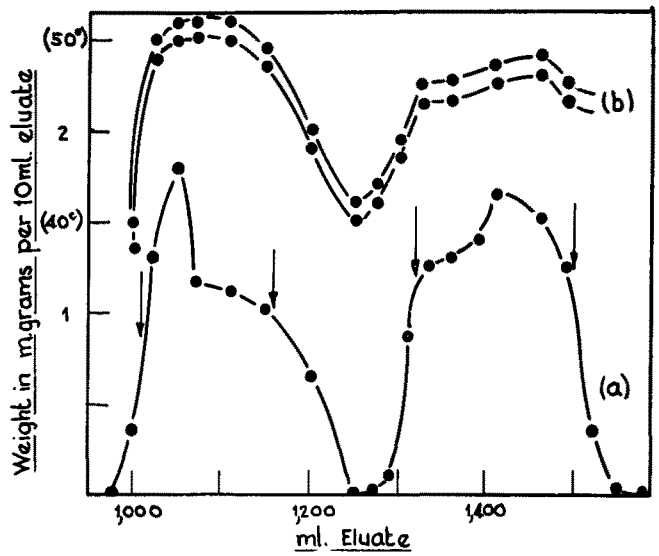
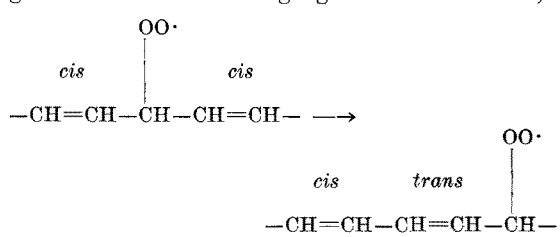


FIG. 7. Alumina chromatography of methyl hydroxystearates; from nearly pure *trans-trans* methyl hydroxylinoleates, 45°C. autoxidation.
(a) Weight curve.
(b) Melting point range curve.

However we have disproved the possibility that polymers are present by the molecular distillation experiment recorded above. We have reduced the overall possibility of impurities being present in quantity by applying elaborate separation methods. The ultraviolet extinction coefficients used in the present work are $\epsilon = 28{,}000$ and $\epsilon = 31{,}600$ for pure *cis-trans* and pure *trans-trans* dienes, respectively; and substantial revision of these figures would necessitate revision of our calculations.

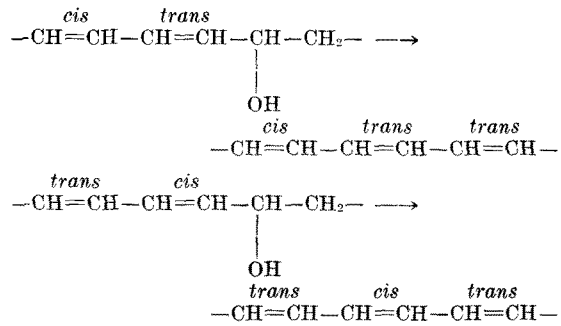
Geometrical isomerism. The American work cited (9, 24, 25) showed that hydroperoxide formed near 0°C. was predominantly *cis-trans* whereas that formed at higher temperatures contained more *trans-trans*.

In low temperature autoxidation it can be expected that rearrangement of the intermediate radical forms (R′ and RO'_2) to conjugation will result in the positioning of the *trans* double bond nearest to the –OOH group since it is the moving double bond which has the greater chance of changing from *cis* to *trans, e.g.:*



On this interpretation (29) only two conjugated forms exist in low temperature autoxidation, *viz.*, 9-peroxido 10-*trans*, 12-*cis* and 13-peroxido 9-*cis*, 11-*trans*. There are two primary influences which may, in actual practice, modify complete adherence to this behavior. First, debromination linoleate is not exclusively unconjugated *cis-cis*, and, secondly, the moving double bond might appear in the *cis* form. Thus, even at low temperatures, there may be formed, by the primary reaction, small amounts of *trans-trans* and *cis-cis*. Additionally, secondary thermal changes (*e.g.*, *cis-cis* ⟶ *trans-cis*) may occur. Evidence for the placing of at least some proportion of *trans* double bonds next to the –OOH groups is afforded by the experiment in which dehydration of hydroxy-linole-

ate gave a conjugated *cis-trans-trans* triene as shown by its infrared spectrum, having a band near 10.4 μ characteristic of α-elaeostearic acid (23); there was also a large amount of *trans-trans-trans* material in in the product. The triene preparation contained by-products as evidenced by its low ϵ value at 268.5 mμ, but this should not affect the argument. If the *trans* double bond were remote from the –OOH group, then a *trans-cis-trans* triene should result:



Cis-cis forms could give rise to *cis-cis-trans* forms on dehydration, but these were not identified.

The preponderance of *trans-trans* forms in higher temperature autoxidation can be explained in two ways. Purely thermal rearrangement of *cis-trans* peroxide to *trans-trans* could occur (*cf.* carotenoids [30]) or rearrangement from unconjugated *cis-cis* to conjugated *trans-trans* could take place in the radical state. That the former alternative can play some role is evidenced by the spontaneous rearrangement from *cis-trans* to *trans-trans* which we have observed both in the case of hydroperoxidolinoleate and hydroxylinoleate. A complete solution of this aspect of the problem must await kinetic study.

The possibility has been raised above that *cis-cis* conjugated diene can be formed, but we have not been successful in showing whether this is the case or not. However our efforts to this end may be of interest. It should be possible to deduce the amounts of *cis-trans* and *trans-trans* forms in mixtures (*e.g.*, in our various reversed phase chromatographic fractions of methyl hydroxylinoleate) from infrared data. The contributions of these amounts to the ultraviolet extinction coefficient at 232 mμ could be calculated, and the calculated coefficient could be compared with the experimental figure. The difference between the calculated and experimental coefficients would represent the contribution of any remaining components which are neither *cis-trans* nor *trans-trans* dienes. This method is open to errors inherent in the quantitative infrared measurements themselves, and there is the further handicap that the necessary *cis-trans* and *trans-trans* hydroxylinoleate reference standards are difficult of access. We have reduced the latter handicap by preparing undoubted conjugated methyl *trans-trans* hydroxylinoleate (actually a mixture of 9-hydroxy, 10-*trans*, 12-*trans*- and 13-hydroxy 9-*trans* 11-*trans*-linoleates) by urea complex fractionation of total methyl hydroxylinoleates, but we have had to resort to the use of non-hydroxylated conjugated *cis-trans* linoleate (prepared by alkali isomerization of linoleic acid) as a standard for the *cis-trans* type. We have applied the suggested calculations for what they are worth (see Table II). The fractions around the main peak of the hydroxylinoleate reversed phase partition chromatogram (Figure 2) give a sum of about 80% of *cis-trans* plus *trans-trans* forms, leaving some 20% unaccounted for. It is calculated that this 20% has a specific molecular ultraviolet extinction coefficient (ϵ) of 10,500–16,800 at 232 mμ. This possibly indicates that the third type of conjugated diene (*viz.*, *cis-cis*) is present.

Literature bearing upon the spectroscopic characteristics of pure *cis-cis* conjugated dienes uncomplicated by other resonating groups is virtually nonexistent.[1] However in the recent paper of Lunde and Zechmeister (16) *cis-cis*, *cis-trans*, and *trans-trans* diphenylbutadienes are described, and there appears to be no prominent band in the 10–11 μ region characteristic of the *cis-cis* structure. The latter structure is, in the one instance studied by these authors, characterized by a band at 7.31 μ, which is unfortunately a position already occupied by a portion of the 7.4 μ band common to all our methyl esters.

Our reversed phase partition fractions from methyl hydroxylinoleate all had a very weak band near 10.9 μ; a band at this wavelength occurs both in a) methyl linoleate (unconjugated *cis-cis*) and b) in impurities in most peroxide preparations (27, 25). In a) this band is of low intensity and, by comparison, our fractions 6–8, Table II, could contain about 20% of unconjugated *cis-cis* unsaturation. However our pure *trans-trans* hydroxylinoleate preparation also had a band of similar intensity at 10.9 μ (Figure 3). In b) it is of high intensity and if the band in our case is due to this impurity, only a trace of it can be present.

Further points which have some bearing on the geometrical isomerism problem are a) a partial separation of *cis-trans* and *trans-trans* forms of both total methyl peroxidolinoleate and total methyl hydroxylinoleate can be accomplished by reversed phase partition chromatography—the infrared results in Table II illustrate this partial separation, which is also in accord with Figure 1 where the peroxide value curve a) precedes the diene value curve b); b) all our attempts to accomplish the *cis-trans* ⟶ *trans-trans* rearrangement, using iodine on the methyl hydroxylinoleates, have failed (*cf.* Oroshnik and Mebane [22]).

With regard to the presence of isomers which are neither conjugated *cis-trans* nor *trans-trans* we consider that *cis-cis* conjugated dienes could be present; the evidence that they are is however based on a tortuous argument and their existence has not been directly confirmed. Again there is some unconfirmed and less strong evidence pointing to the possible presence of unconjugated *cis-cis* (?) dienes. The synthesis or preparation of close analogues to serve as model reference standards is a necessity before this aspect of the problem can be further advanced by infrared methods. Another possibility is that a decision in this matter might be arrived at on the results of spectra recorded in the far ultraviolet and near infrared.

Positional Isomerism

Bergström (3) showed by direct isolation that 9- and 13-hydroxystearates were present in hydrogenated autoxidized linoleate, and this has now been confirmed. We have extended Bergström's observations by examining the completely hydrogenated products from a) *trans-trans* hydroxylinoleate obtained by

[1] Since this paper was first prepared, a *cis-cis* conjugated methyl linoleate has been synthesized by R. R. Allen (private communication), having ϵ = 26,800 and with no bands in the infrared between 10 μ and 11 μ.

urea complex fractionation, b) the *cis-trans* concentrate obtained as a urea complex raffinate, and c) a *cis-trans* concentrate of hydroxylinoleate obtained from a reversed phase partition column. In each case separation of the mixed methyl hydroxystearates on neutral alumina yielded methyl 9-hydroxystearate and methyl 13-hydroxystearate in approximately equal amounts; we have not been able to detect other isomers. It is, of course, not possible on this evidence to state with certainty that other isomers do not exist in these mixtures, and we have had to resort to means other than direct chromatographic isolation in order to make progress.

Possible points of primary attack in the linoleate molecule, in addition to carbon atom No. 11, are at Nos. 2, 8, and 14 since these are activated by the proximity of either the carboxyl group or a double bond. There also exists, but with less likelihood, the possibility that a degree of random attack on other methylene groups can occur as exists in the case of n-decane (2).

We endeavored to estimate attack at position 2 by reducing total methyl hydroxystearate (obtained from a 4°C. autoxidation) with lithium aluminium hydride to the corresponding mixed diols, which were then reacted with potassium para-periodate. Under such conditions only 1,2-glycols should react and be estimated. Traces only of 1,2-glycols were present (<2.5%). As an additional check the total mixed hydroxystearic acids (from a 23°C. autoxidation) were reacted with acetone under conditions which are known to lead to the formation of an isopropylidene derivative from 2-hydroxystearic acid (6). Again the presence of a very small proportion of the 2-hydroxy compound was indicated (<2.5%), but the very small amount of derivative formed precluded its positive identification.

Primary attack on positions 8 and 14 could lead to the corresponding hydroperoxides or (after allylic rearrangement) to the 10- and 12-hydroperoxides. It was thus of importance to seek the 8-, 10-, 12-, and 14-hydroxystearates in the fully hydrogenated products. Chromic acid oxidation of each monohydroxystearic acid should yield only two monocarboxylic acids and two dicarboxylic acids, provided that further degradation of the primarily produced fragments can be prevented, *e.g.*:

$$\underset{\text{12-hydroxystearic acid}}{CH_3(CH_2)_5CHOH(CH_2)_{10}COOH} \longrightarrow \begin{matrix} CH_3(CH_2)_5COOH + CH_3(CH_2)_4COOH \\ COOH(CH_2)_{10}COOH + COOH(CH_2)_9COOH \end{matrix}$$

Thus from a mixture of 8-, 9-, 10-, 11-, 12-, 13-, and 14-monohydroxystearic acids there should result monocarboxylic acids from C_4 through C_{11}. Of these the C_5, C_6, C_9, and C_{10} will result from the 9- and 13-hydroxystearic acids known to be present. If 11-hydroxystearic acid is present, it will give rise also to C_7 and C_8 monocarboxylic acids. Obviously the formation of any of the other monocarboxylic acids would be significant for the presence of the appropriate hydroxystearic acids. Conditions were established for the chromic acid oxidation of 12-hydroxystearic acid, by which C_6 and C_7 monocarboxylic acids were obtained in bulk with traces only of lower acids arising from further degradation. When a total mixture of hydroxystearic acids was oxidized with chromic acid, we obtained C_5, C_6, C_9, and C_{10} monocarboxylic acids in large amounts; there were traces of C_4, C_3, and C_2, but C_7, C_8, and C_{11} could not be detected. Therefore no appreciable amount of 11- or 8-hydroxystearic acid was present in the mixture. Although 14-hydroxystearic acid would give rise to C_4 monocarboxylic acid, the traces of C_4 found are hardly significant since similar traces of further degradation products were also formed in our model oxidation of 12-hydroxystearic acid; further the diene system of linoleate is symmetrical, and it is inconceivable that attack could occur at C_{14} but not at C_8.

This work indicates again that the 9- and 13-position isomers predominate in linoleate monohydroperoxide. Traces of 2-peroxidolinoleate may be present, but efforts to find other position isomers have failed. This failure may not indicate the total absence of other position isomers; it would be very difficult, for example, to detect small amounts of each possible isomer that could result from a degree of random oxygen attack along the chain even though the sum of these amounts might be appreciable. A possible method of solving this problem may lie in the application of mass spectrometry to the mixed hydroxystearic acids resulting from complete hydrogenation of hydroperoxidolinoleate.

Summary

1. The geometrical forms obtained during autoxidation of methyl linoleate at ordinary temperatures are largely conjugated *cis-trans* and *trans-trans* as shown by previous workers; there is a possibility that conjugated *cis-cis* forms are also produced.

2. The *trans-trans* molecules arise partly, at least, by thermal rearrangement of already formed *cis-trans* peroxide.

3. Some proportion, at least, of the *cis-trans* molecules have their *trans* double bonds nearest to the hydroperoxide group.

4. A partial separation of the geometrical forms can be accomplished by reversed phase partition chromatography both on methyl linoleate hydroperoxides and on the corresponding mixed hydroxy compounds; isolation of the *trans-trans* forms can be accomplished in the latter case by urea complex fractionation.

5. No position isomers except the known 9- and 13-isomers have been positively identified; there is a possibility that very minor amounts of the 2-isomer are formed; the 9- and 13-isomers are present in about equal amounts; the 11-isomer was not detected by the methods applied.

6. Various ways in which the linoleate autoxidation problem might be advanced further are suggested.

Acknowledgments

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Filtration-Extraction of Flaxseed as Affected by Preparation Variables[1]

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THE VERSATILITY of the filtration-extraction process has been established previously by its adaptation to the extraction of oil from cottonseed (1, 4, 13), rice bran (7), soybeans (2, 6), peanuts (11), and sesame seed (15). The sequence of operations for the filtration-extraction process is outlined in flow sheet, Figure 1, using flaxseed as the oil-bearing material. The process is similar for all oleaginous materials, but the inherent characteristics of each specific oil-bearing material necessitate the determination of a set of optimum processing conditions (5) for meats preparation, rolling or flaking, cooking, and crisping. Unlike cottonseed and soybeans, flaxseed is not hulled before rolling. The efficiency of extraction of the prepared material is directly related to the particle size or degree of comminution, time and temperature of cooking, the moisture during cooking, the degree of crispness (incompressibility), time and temperature of slurrying, and the number and temperature of the washes. It is the purpose of this paper to present data showing the material preparation and extraction conditions required for the efficient removal of oil from flaxseed by the filtration-extraction process.

At the present time there are 32 commercial mills (9) located in 11 states engaged in the extraction of oil from flaxseed. Approximately 60% of the mills use screw-pressing, and 30% use screw-pressing followed by solvent extraction. The remainder use either hydraulic pressing or direct solvent-extraction.

Direct solvent-extraction of flaxseed has not proved successful in the past since the solvent tends to dissolve the connective mucilaginous material which holds the flakes together (10, 14), consequently the flakes disintegrate and become powdery and caky, making the process inefficient primarily because of channeling. Prepressing flaxseed overcomes this difficulty by imparting a rigid structure to the connective mucilaginous material and thus minimizing the inherent characteristic of this material to dissolve in the solvent. In filtration-extraction the particular method of preparation, *i.e.*, rolling, mild cooking, and crisping by evaporative cooling, yields a material in which the mucilaginous material becomes hardened, and the fines are minimized by agglomeration during the crisping operation. This preparation produces a material which can be extracted directly and efficiently by the filtration-extraction process.

Material and Equipment

The flaxseed used in this study was obtained from a commercial processor in Illinois and analyzed 6½% dockage, 35% oil, and 9.7% H_2O, and had a test weight of 49 lbs. per bushel.

Pilot-plant equipment used in the preparation of the material for this investigation consisted of: smooth dual flaking rolls; a five-high stand of rolls, the top two rolls of which are corrugated, and the bottom three rolls of which are smooth (12); and a jacketed Loomis [3] mixer-cooker (155 sq. in. heat transfer surface area), equipped with a spray nozzle and a steam ejector for the direct addition of moisture during cooking.

Bench-scale equipment shown in Figure 2 and previously described by Graci *et al.* (8) was used to evaluate the filtration-extraction characteristics of the prepared materials.

Experimental Procedure

The general procedure in preparing flaxseed for these experiments consisted of rolling by either the single-pass dual rolls, or through the five-high rolls, cooking in the mixer-type cooker, screening through a 4- or 8-mesh screen, and crisping by aeration on open trays for approximately 20 min., followed by an evaluation of the prepared material for filtration-extraction characteristics. Experiments were designed to investigate the effect of the two principal phases of material preparation, that is, rolling and cooking

[1] Presented at the fall meeting of the American Oil Chemists' Society, Philadelphia, Pa., Oct. 10–12, 1955.
[2] One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.
[3] The mention of trade names should not be construed as an endorsement by the Department of Agriculture over similar products.