

### Summary

Steam-distillation of dry whole milk was found to be an effective means of separating the products of fat oxidation preparatory to reacting them with 2-thiobarbituric acid (TBA). The milk powder samples were reconstituted and acidified prior to steam distillation. A fraction of the distillate was reacted with the reagent to produce the red-colored product, which was then estimated spectrophotometrically at 530 m $\mu$ . Factors which influence the sensitivity and reliability of the method were investigated. The method is rapid and reproducible, and the test solutions are free of turbidity. The reaction is a measure of the oxidation of dry whole milk, and when it was applied to samples from storage, the results had validity in terms of flavor.

### Acknowledgment

The authors express their appreciation to Francis J. Pilgrim of this Institute for the flavor evaluation of the mixture of oxidized and non-oxidized milk powders.

### REFERENCES

1. Biggs, D. A., and Bryant, L. R., *Can. J. Technol.*, **31**, 138-145 (1953).
2. Coulter, S. T., Jenness, Robert, and Geddes, W. F., "Advances in Food Research," vol. 3, p. 95, New York, Academic Press Inc., 1951.
3. Dunkley, W. L., and Jennings, W. G., *J. Dairy Sci.*, **34**, 1064-1069 (1951).
4. Dutton, H. J., Schwab, A. W., Moser, Helen A., and Cowan, J. C., *J. Am. Oil Chemists' Soc.*, **25**, 385-388 (1948).
5. Guilford, J. P., "Psychometric Measurements," p. 207, New York, McGraw-Hill Book Company Inc., 1936.
6. Henick, A. S., *Food Technol.*, **5**, 145-147 (1951).
7. Patton, S., and Kurtz, G. W., *J. Dairy Sci.*, **34**, 669-674 (1951).
8. Sidwell, C. G., Salwin, Harold, Benca, Milada, and Mitchell, J. H. Jr., *J. Am. Oil Chemists' Soc.*, **31**, 603-606 (1954).

[Received March 29, 1954]

## Some Investigations with Long Chain Compounds

DONALD A. SUTTON, National Chemical Research Laboratory, South African Council for Scientific and Industrial Research, Pretoria, South Africa

THE Organic Chemistry Division of the National Chemical Research Laboratory has devoted a large proportion of its effort during the past five years to the study of a variety of natural and manufactured products containing long chain carbon compounds. This choice of subject has followed from the importance which these substances have in relation to South Africa's economy. Fortunately, during these years, a growing interest in long chain compounds has emerged in almost every country of the world so that there has been the constant stimulus of important new publications.

It is the author's privilege to present this report on behalf of those colleagues who, at various times, have been concerned with the program: A. L. Clingman, J. P. de Villiers, H. H. Hahn, D. A. Harris, C. A. J. Hoeve, D. H. S. Horn, F. W. Hougen, D. Ilse, F. J. Joubert, S. P. Ligthelm, M. Matic, J. R. Nunn, D. E. A. Rivett, S. D. Rossouw, H. M. Schwartz, H. H. Sephton, M. H. Silk, M. M. von Holdt, and E. M. von Rudloff.

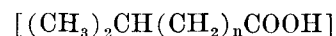
In the following paragraphs this laboratory's research on the individual topics studied is reviewed. In order to keep this review reasonably short, the background of literature given in each case is brief.

### Wool Wax

Wool wax, which is likely to become increasingly important in South Africa, is one of the most complex natural mixtures known. It is the sebaceous secretion of the sheep, which is recovered as crude "wool grease" from commercial wool-washing processes. The pure wax is essentially a mixture of esters together with a small proportion of hydrocarbons (19). The percentage of free uncombined carboxyl and hydroxyl groups is small (77). Separation of some individual esters by laborious repetitive crystallization has been claimed recently by Tiedt and Truter (71) in England. These are cholesteryl 24-methylhexacosanoate, cholesteryl 26-methyloctacosanoate, cholesteryl 28-

methyltriacontanoate, and a less pure ester of a hydroxy-acid. In view of the large number of individual acids and alcohols present, it is to be expected that the percentage of any given ester will be small if anything approaching a random distribution obtains, but there is very little information available on this point (74). Nevertheless crude fractionation of wool grease to yield a series of materials with graded physical properties is likely to become commercially important. The hydrocarbons of wool wax are of unknown constitution although their physical properties suggest that they are not solely normal unbranched paraffins.

The major contribution to our knowledge of wool wax acids was made by Weitkamp (79), who showed that members of four homologous series were present, *viz.*: a) normal (even-numbered) acids from C<sub>10</sub> through C<sub>26</sub>; b) *iso*-branched



(even-numbered) acids from C<sub>10</sub> through C<sub>28</sub>; c) optically active (dextrorotatory) *anteiso*-branched



(odd-numbered) acids from C<sub>9</sub> through C<sub>27</sub>, and C<sub>31</sub>; d) normal optically active  $\alpha$ -hydroxy-(even numbered) acids of C<sub>14</sub> and C<sub>16</sub> chain length. The percentage of d) was small, but this was contrary to the later publication of Bertram (8), who presented analytical values for the hydroxyl content of wool wax derivatives. It seemed probable that the preliminary chromatography and subsequent lengthy precision distillation used by Weitkamp may have resulted in some loss or decomposition of hydroxy-acid components. These components were therefore isolated from total wool wax acids, using the mild Craig countercurrent solvent distribution procedure. It was found that nearly 30% of  $\alpha$ -hydroxy-acids were present, including those isolated by Weitkamp together with the normal C<sub>12</sub> and C<sub>18</sub> homologues and 2-hydroxy-16-methylheptadecanoic acid (36). An additional method of study-

ing these compounds was to reduce the total wool wax acid mixture with lithium aluminum hydride and separate the derived 1:2 diols from the rest of the product by chromatography on alumina; the diols were then fractionally distilled in an efficient column as their diacetates (36). A further advantage of using the Craig method was that it enabled the wool wax acids mixture to be studied as a whole, and it was found that about 10% of it was separable from the rest as an ill-defined group of highly polar "resin acids," which are now under investigation. By a novel application of the Kolbé anodic synthesis a number of optically active homologous normal  $\alpha$ -hydroxy-acids were prepared, starting from (–)-malic acid (37); these proved to be enantiomorphous with the wool wax series, which is thereby correlated with (+)-malic acid. Although Weitkamp's structural assignments in the *iso*- and *anteiso*-series were fully accepted, there remained some discrepancies in certain cases between the properties which he reported and those recorded for the synthetic compounds in the older literature. Partly for this reason, but chiefly because a number of very pure long chain acids was required as synthetic intermediates, a series of *anteiso*- (53) and *iso*-acids (39) were prepared by several modern methods including the Bowman ketone synthesis, Grignard addition to *cyclo*-alkanone rings, followed by Fieser oxidative fission, chain lengthening by the dihydropyran method, the use of acetylenic intermediates, and anodic synthesis. Since that time the latter method has been used exclusively for the purpose of making saturated long chain compounds in this laboratory. The *anteiso*-acids prepared were the racemic mixtures, but Velick and English (76) had previously shown that (+)-14-methylhexadecanoic acid obtained by chain lengthening "active" amyl alcohol [(–)-2-methylbutanol] was identical with the naturally occurring acid; the correlation of these acids with glyceraldehyde has been discussed by Crombie and Harper (18) and by Klyne (46). It would seem therefore that knowledge of the wool wax acids is now virtually complete, with the exception of the "resin acids" and possibly some higher molecular weight acids.

Total wool wax alcohols are a mixture of cholesterol and  $\beta$ -cholestanol, "isocholesterol" (mixed alcohols including lanostenol, lanostadienol, agnostadienol, agnostatrienol) together with aliphatic monohydric alcohols and 1:2 dihydric alcohols. Cholesterol is present to the extent of about 30% and "isocholesterol" to about 20% (19). The structure of lanostenol has recently been fully elucidated by Fridrichsons and Mathieson in Australia (23). The aliphatic monohydric alcohols had never been studied as a group although there was some rather unsatisfactory evidence in the older literature for the identity of a few components (*e.g.*, cetyl alcohol). This group of compounds was therefore obtained free from the cyclic compounds by urea complex formation in a yield of 21% (78). Properties of the group indicated that a complex mixture including branched chain compounds was present, but further investigation was dropped when it was learned that Hatt's team in Australia had been working with this material for some time. Conversely, these Australian workers ceased work on the 1:2 glycols and hydroxy-acids of wool wax when they learned of our interest. The Australians isolated the following (52): a) *iso*-branched (even-numbered)

alcohols from  $C_{20}$  through  $C_{26}$  and b) optically active *anteiso*-branched (odd-numbered) alcohols from  $C_{17}$  through  $C_{27}$ . They oxidized the latter to the corresponding acids, which were evidently identical with the wool wax *anteiso*-acids and therefore of the same configuration. No unbranched aliphatic monohydric alcohols were isolated, and it was concluded that these could only be present in small amounts. Tiedt and Truter (72) however claimed to have isolated, in about 10% yield, a series of normal alcohols (even-numbered,  $C_{18}$  through  $C_{26}$ ), which were segregated by repeated fractional crystallization of their urea adducts after they had been separated as a group from the branched chain alcohols and dihydric alcohols present. The presence of normal alcohols has since been confirmed by the Australian workers (47), who have also added the  $C_{28}$  and  $C_{30}$  members to the list. The dihydric alcohols were isolated in this laboratory (4-5% yield) in the form of a concentrate which was adsorbed on alumina more strongly than the bulk of the wool wax alcohols. They were then fractionally distilled as their diacetates to give the *iso*-branched (even-numbered) 1:2 diols from  $C_{18}$  through  $C_{24}$  together with the normal  $C_{16}$  compound (38). Reduction of the normal  $C_{16}$   $\alpha$ -hydroxy acid of wool wax gave a 1:2 diol which was identical with that derived from the  $C_{16}$  diol, so that it is clear that the hydroxy acid and 1:2 diol series are configurationally related, at least in this case. By no means the whole of total wool wax alcohols are accounted for by the compounds discussed above, and there is therefore scope for further investigation. With the exception of the cholesterol and "isocholesterol" components, the wool wax alcohols represent exact counterparts of the wool wax acids in which the  $-\text{COOH}$  is reduced to  $-\text{CH}_2\text{OH}$ .

The laboratory has also been engaged in some technological studies, including a process for the saponification of wool wax with lime (58), and the use of synthetic detergents in wool scouring (59).

### Human Hair Wax

The wax which exudes from the sebaceous glands at the roots of hairs is of considerable biochemical interest. The free fatty acids (of total surface skin fat) were studied by Weitkamp, Smiljanic, and Rothman (80), who found the following to be present: 14 normal (odd- and even-numbered) acids in the range  $C_7$ - $C_{22}$  and a variety of mono- and di-unsaturated (odd- and even-numbered) acids in the range of  $C_{11}$ - $C_{18}$ . The non-saponifiables obtained from the neutral fat have now been examined (40), using methods similar to those employed for wool wax. That fraction of the (non-paraffinic) non-saponifiables which formed a urea complex contains six (even-numbered) primary alcohols from  $C_{14}$  through  $C_{24}$ , three *iso*-branched  $[(\text{CH}_3)_2\text{CH}(\text{CH}_2)_n\text{CH}_2\text{OH}]$  (even-numbered) primary alcohols from  $C_{20}$  through  $C_{24}$  and two (even-numbered) mono-unsaturated (*cis*) primary alcohols (*n*-docos-12-en-ol, *n*-tetracos-14-en-ol). A third mono-unsaturated (*cis*) primary alcohol (*n*-eicos-10-en-ol) was obtained from the non-complex-forming fraction.

Non-saponifiables of human hair wax were shown to contain polycyclic aromatic hydrocarbons (41), among which are probably phenanthrene, pyrene, fluoranthrene, anthracene, and chrysene. If such hydrocarbons were true metabolic products, these observations

would be of profound significance in connection with the production of experimental carcinogenesis. It is however more than possible that they arise from atmospheric soot (17), which may be expected to collect on greasy hair surfaces.

### South African Pilchard Oil

There are many published papers which give approximate gross over-all compositions of fish body oils according to the traditional methods of analysis (30), but the literature on their precise structural chemistry has been confused. These oils contain large proportions of highly unsaturated fatty acids which are liable to structural alteration during column distillation even at fairly low pressures (*e.g.*, 1-10 mm.), and this fact has been a main stumbling block in accepting unreservedly the majority of the older literature and also those papers which still continue to appear, describing the use of such distillations. However it is possible that highly unsaturated acids of relatively short chain length (*e.g.*,  $C_{16}$ ) may escape serious alteration during rapid vacuum distillation as they will be the first to distil and will therefore be subjected to heating for only a short time. Toyama and Yamamoto (73) have recently described a hexadecatetraenoic acid, isolated from sardine oil, which may possibly have escaped heat alteration, but there is no published information on this point. Indeed it may be remarked that our knowledge of the degree of purity attained in the majority of preparations of highly unsaturated acids is still extremely deficient. In some of the recent literature this situation has been improved by critical examination of the purity of the saturated (hydrogenated) counterparts together with exact measurement of hydrogen uptake. Even these criteria are incomplete because they do not exclude the possibility of the existence of isomers having different double bond positions. Mercifully, nature usually omits this latter complication although it has been observed in human hair wax by Weitkamp (80). The danger of heat alteration was early recognized by Farmer who, with Vandenheuvel (20, 21), used falling film molecular distillation to fractionate the esters of cod liver oil acids and claimed to have isolated a pure docosohexaenoic acid. Since that time there has been increasing use of milder separation methods, with the result that almost the whole gamut has now been applied in this and other laboratories. Thus, for example, Holman (35) in America has applied urea complex fractionation and elution chromatography and has isolated docosopentanoic, docosohexaenoic, and eicosopentanoic acids from cod liver oil in purities of 90% and over. Herb and Riemenschneider (28, 29) had previously used elution chromatography for the isolation of arachidonic, eicosopentanoic, and docosopentanoic acids. Hammond and Lundberg (24) used the same procedure for the isolation of a docosohexaenoic acid. Vandenheuvel (75) in Canada has been exploring the use of efficient molecular stills and chromatography. Our facilities for the structural diagnosis of highly unsaturated acids have been extended by the wider application of spectroscopy and by the extension of ozonolytic degradation methods due to Klenk and Bongard (45).

Pilchard oil, which is of considerable economic importance in South Africa, has been under study here for the past two years, following earlier work in which

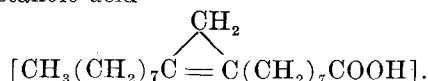
the traditional methods of oil analysis were applied (9, 10). It has become clear that the highly unsaturated acids are an exceedingly complex mixture, and we have formed the opinion that success in achieving worthwhile separations is most likely to result from the application of several purification procedures in appropriate sequence. It was first decided to evaluate the limitations of certain separation methods in order to obtain a rational approach. Thus (62) the classical lithium salt separation of Tsujimoto (depending on the high solubility in aqueous acetone of the lithium salts of highly unsaturated acids as a group) was compared with the use of repetitive urea complex fractionation and with the result of using the two procedures in sequence. A fourth variant was the application of the urea technique to the mixed alcohols resulting from lithium aluminium hydride reduction of total pilchard oil acids. Chain length compositions of the various concentrates were determined (after hydrogenation) by the reversed phase chromatographic method of Howard and Martin which had already been extended to include mixtures of saturated acids up to  $C_{24}$  (63). The reversed phase method was then used with the highly unsaturated acids themselves, and it was shown that a combination of lithium salt:acetone segregation, three-stage molecular distillation, and urea complex fractionation gave sub-fractions with a comparatively small number of components (64). The molecular distillation step was accomplished by the use of a three-stage refractionating falling film molecular still, which was designed and constructed for the purpose (69). The final resolution of the sub-fractions by reversed phase partition chromatography was not perfect in all cases, but it has already resulted in the isolation of one new acid, which has been shown by spectroscopic and degradative methods to be the all *cis* form of hexadeca-6-9-12-15-tetraenoic acid (65). A second new acid has been isolated in a less pure state. It was found that the limitations in resolving power of these chromatograms were similar to those noted by Ahrens and Craig (2) in their use of the Craig countercurrent distribution method with fatty acid mixtures. Thus negligible resolution is obtainable when an acid is mixed with a homologue containing two more carbon atoms and one more double bond; resolution between *cis* and *trans* isomers is only partial (60). In general, the columns used had a separative power equivalent to 600 Craig transfers or more. The scale of operation has been progressively stepped up by the construction of larger columns until quantities of about 5 g. (51) can now be separated in a single run, and there is no reason to believe that the limit of practical operation has yet been reached. It also seems probable that columns equivalent to considerably more than 600 transfers could be designed.

### Some Unusual Vegetable Oil Fatty Acids

Two of the vegetable seed oils examined in this laboratory have proved to contain acids of unusual structures, *viz.*, the seed oils of *Sterculia foetida* and those of certain *Ximenia* species.

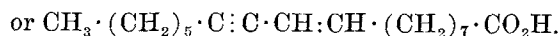
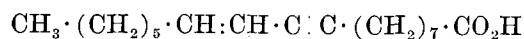
*Sterculia foetida*. The seed oil was known to polymerize rapidly and even explosively on heating (67). The acid component (sterculic acid) responsible for the polymerization had not been isolated in a pure state although it was considered to be a methyl sub-

stituted conjugated diene acid (31). The acid (54) ( $C_{19}H_{34}O_2$ ) was purified by low temperature crystallization after the removal of the non-polymerizing acids of the oil as their urea complexes. It polymerized rapidly on standing with a progressive loss of free carboxyl and a gain of combined carboxyl function. On partial hydrogenation a dihydrosterculic acid was obtained which gave no color with tetranitromethane, but which, on further hydrogenation, yielded a mixture of  $C_{19}$  acids. Oxidation of sterculic acid with permanganate gave azelaic and pelargonic acids so that one carbon atom was lost in the process. Ozonolysis at low temperature followed by hydrogenation of the product yielded an acid ( $C_{19}H_{34}O_4$ ) which was a 1:3 diketone. Further oxidation of the latter again gave pelargonic and azelaic acids. It was therefore 9:13 diketononadecanoic acid. Dihydrosterculic acid had a band in the infrared in the same position ( $9.85 \mu$ ) as that of the disubstituted saturated cyclopropane acid of *Lactobacillus arabinosis* (33). The latter acid cleaves in the three possible ways on hydrogenation, thus providing an explanation of the formation of a mixture on complete hydrogenation of dihydrosterculic acid. From these observations sterculic acid was formulated as  $-(2-n\text{-octyl-cyclo-prop-1-enyl) octanoic acid}$



Loss of carboxyl function on storage is probably due to reaction of carboxyl groups with cyclo-propene rings whereas the thermal polymerization of the parent seed oil must be by a different mechanism, probably direct linkage between cyclo-propene rings. Confirmatory evidence of the presence of a cyclo-propene ring in dihydro-sterculic acid has since been obtained by Hofmann *et al.* (34), who have also considered the stereochemistry of the ring.

*Ximenia* species. Certain indigenous *Ximenia* species seed oils were found to contain a band in the ultraviolet at  $229 m\mu$ , and the acid (ximenynic acid,  $C_{18}H_{30}O_2$ ) responsible for this feature was isolated by various procedures from the total fatty acids of the oils (48). On catalytic hydrogenation the acid took up three moles of hydrogen to give stearic acid; the position and intensity of its ultraviolet absorption band indicated it to be a conjugated enyne. Permanganate oxidation of the acid gave n-heptanoic and azelaic acids. Ximenynyl alcohol was prepared by lithium aluminum hydride reduction of the acid and had an ultraviolet absorption spectrum similar to that of the acid itself. From these observations it was clear that ximenynic acid was either



Ozonolysis followed by reductive fission of the ozonide gave n-heptanaldehyde and azelaic acid, thus establishing the structure as that of the former alternative (heptadec-10-en-9-yne-1-carboxylic acid) since the other possible structure has no hydrogen attached to the 12th carbon atom.

One of the ximenia oils was further investigated (49) and found to contain a small amount of a mono-hydroxy-enyne acid ( $C_{18}H_{30}O_3$ ) with an ultraviolet spectrum very similar to that of ximenynic acid. The

infrared spectra of the esters of the two acids were also very similar except in the  $2.9 \mu$  region ( $-\text{OH}$  group). Lithium aluminum hydride reduction gave a dihydroxy-diene. For such a triple bond reduction to occur it appears to be necessary that an  $-\text{OH}$  group be in the  $\alpha$ -position to the triple bond (13, 3); the system  $-\text{CH}=\text{CH}-\text{C}\equiv\text{C}-\text{CHOH}-$  was therefore probably present. Oxidative cleavage with ozone gave a small amount of an aldehyde, probably n-heptaldehyde. On hydrogenation three moles of hydrogen were taken up, and a monohydroxy stearic acid was formed. The corresponding methyl ketostearate was identical with methyl 8-ketostearate. Schmidt degradation of the free keto acid gave suberic acid. From the above evidence it appears probable that the acid is heptadec-10-en-8-yne-7-hydroxy-1-carboxylic acid [ $\text{CH}_3 \cdot (\text{CH}_2)_5 \cdot \text{CH} : \text{CH} \cdot \text{C} : \text{C} \cdot \text{CHOH} \cdot (\text{CH}_2)_6 \cdot \text{COOH}$ ].

The ximenia oils studied also contain (50) acids of high molecular weight, including an interesting group of six mono-unsaturated normal acids of general formula  $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH} \cdot (\text{CH}_2)_n\text{COOH}$ , *viz.*: oleic ( $n=7$ ), docos-13-enoic ( $n=11$ ), tetracos-15-enoic ( $n=13$ ), hexacos-17-enoic ( $n=15$ ), octacos-19-enoic ( $n=17$ ), and triacont-21-enoic ( $n=19$ ).

#### Thermal Polymerization of Triglyceride Oils

Thermal treatment of unsaturated triglyceride oils results in the formation of covalent links between fatty acid chains so that polymers of the original triglycerides are built up. Saponification of the polymeric triglyceride mixtures yields monomeric acids, dimeric acids, and smaller amounts of trimeric (and higher) acids. Apart from their importance as an existing building block unit in polymerized oils, the dimeric acids are utilized (after isolation) in the industrial synthesis of surface coatings. In this laboratory the structures of the dimeric acids and the molecular weight distribution of the polymeric triglyceride oils have been studied and are discussed below.

a) *Structural Chemistry of Dimeric Acids.* Those triglyceride oils which are used industrially for conversion to "stand" (*i.e.*, thermally polymerized) oils contain dienes or trienes either of the conjugated or methylene-interrupted type. According to one widely accepted hypothesis (11), the conjugated type dimerizes on heating by 1:4 addition to a double bond in another molecule (in Diels-Alder fashion) whereas the unconjugated type first isomerizes to conjugated form on heating and thereafter undergoes the same 1:4 addition. Thus the linkage formed consists of a cyclo-hexene ring substituted in four neighboring positions. A second hypothesis, due to Sunderland (68), proposes that a hydrogen separation reaction is responsible for linkage. A double bond in one molecule opens to receive at one end a hydrogen atom from a methylene group in another molecule and becomes joined at the other end to the methylene group site. Thus the linkage formed is a single carbon-carbon bond. Circumstantial evidence existed for both hypotheses, but neither had been formally proven. Using the maleic anhydride adduct of methyl  $\beta$ -elaeostearate as a model, experimental conditions were established for a substitutive bromination: dehydrobromination (*i.e.*, dehydrogenation): oxidation sequence, which resulted in a 35% yield of the expected prehnitic (benzene 1:2:3:4 tetracarboxylic acid (14)); the

corresponding triol gave a 13% yield and the thermal dimer of methyl  $\beta$ -elaeostearate gave 9% (15). This and other supporting evidence confirmed the Diels-Alder type of addition in the case of the conjugated oils. Application of the same experimental conditions to the dimer of methyl linoleate gave only 3% of prehnitic acid (15), but this was not surprising in view of the drastic heat treatment (12 hours at 300°C.) to which the linoleate had been subjected. However a linoleate dimer prepared by milder heating (1 hour at 300°C.) gave a similar yield of prehnitic acid (57). Thus, although the existence of some Diels-Alder type of addition was proven for the non-conjugated oils, it seemed possible that another mode of polymerization, such as that proposed by Sunderland, was taking place simultaneously. For this reason attempts are now being made to separate linoleate dimer into structurally different types.

b) *Molecular Weight Distribution*. Evidence has accumulated over the last 10 years that fairly large molecules (*e.g.*, decamers and upwards) exist as important constituents of typical polymeric triglyceride oils, but knowledge of molecular weight distributions is relatively incomplete. Broadly speaking, there are two ways in which the molecular weight distribution problem may be approached. The "classical" method depends upon fractionation into fractions of different polymer size. This method can never give better than a very approximate distribution curve unless fractionation is pursued to the logical conclusion where every final fraction consists of one (or at most two) polymeric species. Such a final conclusion is unlikely to be reached with existing techniques even after considerable labor. However such fractionations (7, 42) of stand oils as have been reported in the literature are of value in that they indicate the approximate range of species present. The second way of attacking the problem is to use probability statistics following the Flory-Stockmayer methods. For this it is necessary to know the extent of reaction and the degree of randomness in respect to attachment of fatty acid chains to glycerol. Adams and Powers (1) had already attempted to apply a method of this kind. The extent of reaction was based on the observed iodine value decrease and certain simplifying assumptions about the functionality of the component acids and their distribution were made. A recent approach in this laboratory (32) has been concerned with using the Flory-Stockmayer mathematics; as many assumptions as possible have been eliminated. The extent of reaction is obtained experimentally from the found relative proportions of monomeric, dimeric, etc., acid chains in a given stand oil, and the system is then considered as one in which these proportions of acids are completely and randomly esterified with glycerol. Allowance is made for the presence of the free acids which arise by thermal breakdown in stand oils. The weight average molecular weight and the complete molecular weight distribution of the stand oil can be calculated from the found extent of reaction. Experimental measurement of the weight average molecular weight by the light scattering method provides a check by which inaccuracies arising from experimental technique and simplifying assumptions can be assessed. Included in such inaccuracies is that arising from the assumption in the Flory theory of polycondensation reactions with respect to the lack of intramolecular

reaction; this however is probably of small magnitude. The most important assumption, which will lead to a large error if it is incorrect, is that ester-interchange to a random state occurs at polymerizing temperature. Preliminary experiments have indicated that agreement between the calculated and observed weight average molecular weights is fair. This agreement can probably be interpreted to mean that any disturbances arising from imperfect compliance with the ester-interchange assumption are not of serious dimensions. However it would obviously be more satisfactory if quantitative experimental data were available on the ester-interchange reaction itself, and experiments designed to provide such data are in hand. In this connection the work of Barker, Crawford, and Hilditch (4) showed that conversion from a completely ordered state (as represented by the pure triglyceride  $\beta$ -oleodistearin) to a completely random state takes about 10 hours at 300°C.; this time is comparable with the total polymerization times often employed in stand oil preparation. However in the case of actual stand oils it is only necessary to go from a partially ordered to a completely random state.

#### Dehydro-polymerization of Long Chain Compounds with di-*tert.*-butyl Peroxide

Farmer and Moore (22) studied the reaction of di-*tert.*-butyl peroxide with various hydrocarbons (*e.g.*, *cyclo*-hexene, *cyclo*-hexane, hept-1-ene) at temperatures near 140°C. They found that the main characteristic of the reaction was the abstraction of hydrogen atoms from active centers in the hydrocarbon molecules with the formation of dehydro-polymers and *tert.*-butyl alcohol. In general, one complete linkage between two molecules was formed by the expenditure of one peroxide molecule, but in the case of 1-enes a double bond polymerization was catalyzed so that a higher ratio of linkages was formed and a proportion of the original double bonds was used up. Kharasch and co-workers (43, 44) had previously studied the reaction of diacetyl peroxide with saturated fatty acids and found that dehydro-dimers were formed by junction at sites in the  $\alpha$ -position to carboxyl groups. Harrison and Wheeler (25) had investigated the reaction of di-*tert.*-butyl peroxide with vinyl and with allyl linoleate, and it seemed that their results could be explained by dehydro-linking involving the pentadiene system in addition to double bond polymerization at the vinyl or allyl group analogous to the 1-ene type of Farmer and Moore. The formation of dimeric acids from monomeric drying oil acids and di-*tert.*-butyl peroxide appeared technically attractive since the original unsaturation should be retained to subsequent advantage in the surface-coating drying step. The expected absence of side reactions would be a desirable feature. A third possible advantage was the likelihood that the saturated and mono-unsaturated acids present in commercial drying oil acid mixtures would also dimerize. It is to be noted that none of these desirable features obtains in the purely thermal polymerization of drying oil esters by which stand oils and dimeric acids are produced. The reactions of methyl linoleate and of methyl stearate with di-*tert.*-butyl peroxide were studied to make the necessary fundamental information available (16). It was later discovered that Harrison and Wheeler in Amer-

ica had been investigating methyl linoleate (26, 27) at about the same time with the same results. The formation of methyl linoleate mixed dehydro-dimer isomers was accompanied by rearrangement of a substantial proportion of the pentadiene groups with the formation of conjugated dienes. Higher dehydro-polymers were also formed and these, too, contained conjugated diene. The formation of conjugated dienes occurred at a rapid rate from the beginning of the reaction; the concentration of these groups eventually reached a maximum and then slowly decreased, presumably owing to their loss by thermal polymerization. Use of a high proportion of peroxide to linoleate resulted in the formation of gels containing rubbery ether-insoluble fractions presumably consisting of cross-linked networks. The rearrangement of double bonds which occurred was expected in view of the free radical nature of the reaction [Farmer and Moore (22)] and the similar behavior of linoleate during autoxidation; it indicated that many of the linkages were formed near the original double bond sites. The dehydro-dimer fraction was isolated and found to have the expected number (four) of double bonds. *Tert.*-butyl alcohol was the main product arising from the expended peroxide. Evidence on the stoichiometry of the over-all reaction was incomplete but was in favor of the expectation that the formation of one linkage between molecules occurred for the expenditure of one peroxide molecule. Harrison and Wheeler (27) found that the conjugated diene formed was of the *trans-trans* type and some isolated *trans* double bonds were also present. Under the conditions of reaction used for methyl linoleate, methyl stearate also reacted to form dehydro-dimers and higher dehydro-polymers. This provided a formal demonstration of the ability of saturated long chain esters to link readily under the conditions used and indicated the probability that, in the linoleate molecule, linkage may occur at other sites in addition to those which involve the pentadiene system. The use of di-*tert.*-butyl peroxide as a polymerization catalyst for triglyceride oils has been patented by the Shell Development Company (81), who anticipated our South African application (66). The author is not aware of the present extent of the commercial use of such a process, but presumably this will be conditioned by the price at which di-*tert.*-butyl peroxide can be produced.

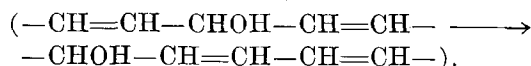
#### Autoxidation of Methyl Linoleate

Linoleic acid is important because of its widespread occurrence in food and paint oils. It is also a convenient simple model substance containing the methylene-interrupted type of unsaturation which is found in very many other useful and biologically important fatty acids.

It has been known since 1943 that the pentadiene system ( $-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$ ) present in linoleic acid autoxidizes to give monohydroperoxides, some of which contain a conjugated double bond system ( $-\text{CHOOH}-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$ ). From the ultraviolet extinction coefficient of the product, in comparison with that of pure conjugated *trans-trans* linoleate, it was believed that the conjugated dienes made up some two-thirds of the total (*i.e.*, as the 9- and 13-hydroperoxide esters) while the remaining one-third was the unconjugated 11-hydroperoxide

( $-\text{CH}=\text{CH}-\text{CHOOH}-\text{CH}=\text{CH}-$ ). The existence of the 9- and 13-hydroperoxides was demonstrated by Bergström (5), who isolated the corresponding hydroxy esters after complete hydrogenation. Recently the geometrical configuration of the peroxides was studied in America, and it was shown that the double bonds of debromination linoleate (largely all *cis* together with some *trans*) become converted to *cis-trans* during low temperature (0°C.) autoxidation together with *trans-trans* at higher temperatures (24°C.) (12, 55, 56). In addition, the peroxide mixture was obtained in a purer form than hitherto by the use of countercurrent solvent distribution procedures. The proportion of the conjugated diene forms in the purified monohydroperoxide mixture was recalculated on the basis of the extinction coefficient of pure *cis-trans* linoleate, with the result that some 90% was thought to consist of the conjugated diene forms, leaving only 10% as the unconjugated 11-hydroperoxide. In general, it can be said that literature estimates of the proportion of the 11-isomer are unsatisfactory in that they depend for their accuracy on the purity of the peroxide preparation. If the latter contains other compounds which are unconjugated, then these are estimated as 11-peroxide.

The spectroscopic data of the American workers were confirmed, using linoleate peroxide concentrates prepared by their method after autoxidation of linoleate at 4°C. and 23°C. (61). The two peroxide concentrates were reduced with sodium borohydride to the corresponding hydroxy-linoleates, which were further studied. Countercurrent solvent distribution of the hydroxy-esters revealed the presence of an impurity, the removal of which yielded purified preparations of higher ultraviolet extinction coefficients and therefore of lower than 10% "calculated" 11-isomer content. Further the purified hydroxy-ester preparations failed to show any signs of anionotropic rearrangement when treated with sulphuric acid under conditions which are known to cause rearrangement of similar systems



It was therefore concluded that the 11-isomer, if present at all, exists only in very small amount. The existence of *trans-trans* forms was demonstrated directly by separating these (as hydroxy-esters) from the *cis-trans*, using urea complex formation (70); hydrogenation of the *trans-trans* preparation yielded the expected methyl 9- and 13-hydroxy-stearates in approximately equal amounts. Hydrogenation of the *cis-trans* material gave the same result (60). Attention is now being directed to the mechanism of formation of the *trans-trans* forms during autoxidation. Do these arise directly during higher temperature autoxidation or is the primary product always the *cis-trans* which then rearranges? It is to be assumed, on the grounds of the generally accepted mechanism, that the *trans* double bond in the *cis-trans* forms must always be nearest to the hydroperoxide group, but this has never yet been formally proven.

An advance in technique has been the application of reversed phase partition chromatography to the further purification of peroxide concentrates; in this way the equivalent of more than 600 Craig transfers can readily be applied. One result has been the preparation of a substantially pure *trans-trans* linoleate



peroxide; this partial resolution of geometrical isomers parallels the incomplete separation by Ahrens and Craig (2) of *cis*- and *trans*-isomers of linoleic acid itself. It seems very probable that the advantages of this separation method will lead to its wide application in organic autoxidation research. On the biochemical side, the small scale of operation may well be important; thus, for example, it may now be possible to study autoxidized sebaceous secretions in connection with the cancer problem (6).

### Acknowledgment

It is a pleasure to record the interest of W. S. Rapon in this work and his never-failing encouragement.

This paper is published by permission of the South African Council for Scientific and Industrial Research.

### REFERENCES

- Adams, H. E., and Powers, P. O., "High Polymers Physics," pp. 417-439. H. A. Robinson, editor, Remsen Press (1948).
- Ahrens, E. H., and Craig, L. C., *J. Biol. Chem.*, **195**, 299 (1952).
- Attenburrow, J., Cameron, A. F. B., Chapman, J. H., Evans, R. M., Hems, B. A., Jansen, A. B. A., and Walker, T., *J. Chem. Soc.*, 1094 (1952).
- Barker, C., Crawford, R. V., and Hilditch, T. P., *J. Chem. Soc.*, 1194 (1951).
- Bergström, S., *Arkiv för Kemi, Mineralogi och Geologi* **214**, No. 14 (1945).
- Bernheim, M. L. C., Bernheim, F., and Wilbur, K. M., *Nature*, **172**, 306 (1953).
- Bernstein, I. M., *J. Oil and Colour Chem. Asso.*, **32**, 447 (1949).
- Bertram, S. H., *J. Am. Oil Chemists' Soc.*, **26**, 454 (1949).
- Black, M. M., and Schwartz, H. M., *J. Sci. Food Agric.*, **1**, 182 (1950).
- Black, M. M., and Schwartz, H. M., *J. Sci. Food Agric.*, **1**, 248 (1950).
- Bradley, T. F., *J. Oil and Colour Chem. Asso.*, **30**, 225 (1947).
- Cannon, J. A., Zilch, K. T., Burket, S. C., and Sutton, H. J., *J. Am. Oil Chemists' Soc.*, **29**, 447 (1952).
- Chanley, J. D., and Sobotka, H., *J. Am. Chem. Soc.*, **71**, 4140 (1949).
- Clingman, A. L., Rivett, D. E. A., and Sutton, D. A., *Chemistry and Industry*, 798 (1953).
- Clingman, A. L., Rivett, D. E. A., and Sutton, D. A., *J. Chem. Soc.*, 1088 (1954).
- Clingman, A. L., and Sutton, D. A., *J. Am. Oil Chemists' Soc.*, **30**, 53 (1953).
- Cooper, R. L., and Lindsey, A. J., *Chemistry and Industry*, 1177 (1953).
- Crombie, L., and Harper, S. H., *Chemistry and Industry*, 757 (1950).
- Daniel, D., Lederer, E., and Velluz, L., *Bull. Soc. Chim. biol.*, **27**, 218 (1945); Lederer, E., *J. Chem. Soc.*, 2115 (1949).
- Farmer, E. H., and Vandenheuvel, F. A., *J. Soc. Chem. Ind. (London)*, **57**, 24 (1937).
- Farmer, E. H., and Vandenheuvel, F. A., *J. Chem. Soc.*, 427 (1938).
- Farmer, E. H., and Moore, C. G., *J. Chem. Soc.*, 131-153 (1951).
- Fridrichsons, J., and Mathieson, A. McL., *J. Chem. Soc.*, 2159 (1953).
- Hammond, E. G., and Lundberg, W. O., *J. Am. Oil Chemists' Soc.*, **30**, 438 (1953).
- Harrison, S. A., and Wheeler, D. H., *J. Am. Chem. Soc.*, **73**, 839 (1951).
- Harrison, S. A., and Wheeler, D. H., *Minnesota Chemist*, **4**, No. 5, 17 (1952).
- Harrison, S. A., and Wheeler, D. H., *J. Am. Chem. Soc.*, **76**, 2379 (1954).
- Herb, S. F., Riemenschneider, R. W., and Donaldson, J., *J. Am. Oil Chemists' Soc.*, **28**, 55 (1951).
- Herb, S. F., Witnauer, L. P., and Riemenschneider, R. W., *J. Am. Oil Chemists' Soc.*, **28**, 505 (1951).
- Hilditch, T. P., "The Chemical Constitution of Natural Fats," ch. 2, 2nd ed., Chapman and Hall, London (1947).
- Hilditch, T. P., Meara, M. L., and Zaky, Y. A. H., *J. Soc. Chem. Ind. (London)*, **60**, 198T (1941).
- Hoeve, C. A. J. (unpublished).
- Hofmann, K., and Lucas, R. A., *J. Am. Chem. Soc.*, **72**, 4328 (1950).
- Hofmann, K., Jucker, O., Miller, W. R., Young, A. C., and Tausig, F., *J. Am. Chem. Soc.*, **76**, 1799 (1954).
- Holman, E. T., and Abu Nasr, A. M., *J. Am. Oil Chemists' Soc.*, **31**, 41 (1954).
- Horn, D. H. S., Hougen, F. W., von Rudloff, E. M., and Sutton, D. A., *J. Chem. Soc.*, 177 (1954).
- Horn, D. H. S., and Pretorius, Y. Y., *J. Chem. Soc.*, 1460 (1954).
- Horn, D. H. S., and Hougen, F. W., *J. Chem. Soc.*, 3533 (1953).
- Hougen, F. W., Ilse, D., Sutton, D. A., and deVilliers, J. P., *J. Chem. Soc.*, 98 (1953).
- Hougen, F. W. (to be published).
- Hougen, F. W., *Chemistry and Industry*, 192 (1954).
- Joubert, F. J., and Sutton, D. A., *J. Am. Oil Chemists' Soc.*, **29**, 287 (1952).
- Kharasch, M. S., and Gladstone, M. T., *J. Am. Chem. Soc.*, **65**, 15 (1943).
- Kharasch, M. S., Jensen, E. V., and Urry, W. H., *J. Org. Chem.*, **10**, 386 (1945).
- Klenk, E., and Bongard, W., *Z. physiol. Chem.*, **290**, 181 (1952).
- Klyne, W., *Biochem. J.*, **53**, 378 (1953).
- Knol, H. W., *J. Am. Oil Chemists' Soc.*, **31**, 59 (1954) (referring to unpublished work of Murray).
- Ligthelm, S. P., Schwartz, H. M., and von Holdt, M. M., *J. Chem. Soc.*, 1088 (1952).
- Ligthelm, S. P., *Chemistry and Industry*, 249 (1954).
- Ligthelm, S. P., Horn, D. H. S., Schwartz, H. M., and von Holdt, M. M., *J. Sci. Food Agric.*, **5**, 281 (1954).
- Matic, M. (unpublished).
- Murray, K. E., and Schoenfeld, R., *J. Am. Oil Chemists' Soc.*, **29**, 416 (1952).
- Nunn, J. R., *J. Chem. Soc.*, 1740 (1951).
- Nunn, J. R., *J. Chem. Soc.*, 313 (1952).
- Privett, O. S., Lundberg, W. O., and Nickell, C., *J. Am. Oil Chemists' Soc.*, **30**, 17 (1953).
- Privett, O. S., Lundberg, W. O., Khan, N. A., Tolberg, W. E., and Wheeler, D. H., *J. Am. Oil Chemists' Soc.*, **30**, 61 (1953).
- Rivett, D. E. A. (unpublished).
- Rossouw, S. D., and von Rudloff, E. M., *J. Appl. Chem.*, **2**, 335 (1952).
- Rossouw, S. D., *South African Industrial Chemist*, **6**, 90 (1952).
- Septon, H. H. (unpublished).
- Septon, H. H., and Sutton, D. A., *Chemistry and Industry*, 667 (1953).
- Silk, M. H., Septon, H. H., and Hahn, H. H., "South African Pilchard Oil, Part II," *Biochem. J.*, **57**, 574 (1954).
- Silk, M. H., and Hahn, H. H., "South African Pilchard Oil, Part I," *Biochem. J.*, **56**, 406 (1954).
- Silk, M. H., and Hahn, H. H., "South African Pilchard Oil, Part III," *Biochem. J.*, **57**, 577 (1954).
- Silk, M. H., and Hahn, H. H., "South African Pilchard Oil, Part IV," *Biochem. J.*, **57**, 582 (1954).
- South African Council for Scientific and Industrial Research, *South African Provisional Patent Specification* 3098 (1951).
- Steger, A., and van Loon, J., *Fette u. Seifen*, **50**, 305 (1943).
- Sunderland, E., *J. Oil and Colour Chem. Asso.*, **28**, 137 (1945).
- Sutton, D. A., *Chemistry and Industry*, 1383 (1953).
- Sutton, D. A., *South African Industrial Chemist* 196 (1953) (quoting unpublished work of H. H. Septon).
- Tiedt, J., and Truter, E. V., *Chemistry and Industry*, 403 (1952).
- Tiedt, J., and Truter, E. V., *J. Chem. Soc.*, 4628 (1952).
- Toyama, Y., and Yamamoto, T., *J. Oil Chemists' Soc. (Japan)*, **2**, 108, 147, 193 (1953); [*Chem. Abs.* **48**, 1710 (1954)].
- Truter, E. V., *Quarterly Reviews of the Chemical Society*, **5**, 390 (1951).
- Vandenheuvel, F. A., *Annual Report of the Fisheries Research Board of Canada*, 181 (1952).
- Velick, S. F., and English, J., *J. Biol. Chem.*, **160**, 473 (1945).
- von Rudloff, E. M., "A Study of the Saponifiable and Non-saponifiable Components of Wool Wax." Thesis, University of Pretoria, December 1952.
- von Rudloff, E. M., *Chemistry and Industry*, 338 (1951).
- Weitkamp, A. W., *J. Am. Chem. Soc.*, **67**, 447 (1945).
- Weitkamp, A. W., Smiljanic, A. M., and Rothman, S., *J. Am. Chem. Soc.*, **69**, 1936 (1947).
- Wiebe, L. P., assignor to Shell Development Company, *United States Patent No. 2,588,902* (1952); application date, 1950.

[Received August 30, 1954]