ABSTRACTS OF PAPERS MINNEAPOLIS FALL MEETING

— 1 —

A SYSTEM FOR DESIGNATING THE CONFIGURATION OF OPTICALLY ACTIVE GLYCERIDES

C. C. Litchfield and Henry Rakoff

There are four possible types of optically active glycerides: 1-monoglycerides; 1,2-diglycerides; 1,3-diglycerides; and triglycerides. Fischer and Baer (Chem. Revs.: 29: 287, 1941) have suggested rules for assigning D and L configurations to 1-monoglycerides and 1,2-diglycerides. No similar rules have been proposed for active triglycerides and 1,3-diglycerides.

The *rectus* and *sinister* (R and S) system of Cahn et al. (Experientia 12:81, 1956) can be used to designate the configurations of *all* active glycerides. Typical R and S assignments are shown. Since the R and S system has not yet achieved widespread use, it would be useful to make D and L assignments for all optically active glycerides. Suggestions are made for adopting the priority sequence rules of the R and S system to make D and L assignments for active triglycerides and 1,3-diglycerides. This assures maximum compatibility between the two systems. Simple rules are presented for assigning the configuration of optically active glycerides by either system.

— 2 —

OPTICALLY ACTIVE TRIGLYCERIDES

René Maier and R. T. Holman

The oil obtained from fruits of the Chinese tallow tree, *Sapium sebiferum*, commonly known as "Stillingia oil," is known to be optically active. The oil also contains a deca-2,4-dienoic acid which contributes strong ultraviolet absorption to the spectrum of the oil. The gas-liquid chromatographic analyses of the methyl esters of the total fatty acids revealed 8% unusual fatty acids of medium chain length including deca-2,4-dienoic acid. The oil itself had a specific rotation of −6° in chloroform but the methyl esters of the fatty acids had no activity. By thin-layer and column chromatography two triglyceride fractions have been separated. The less polar, with an R_f value of common triglycerides, was 73%, and the more polar one amounted to 22% of the total oil. The less polar fraction gave no evidence of optical activity, ultraviolet absorption or of the presence of any unusual fatty acids. The more polar fraction, on the contrary, had a specific rotation of −21°, a maximum at 268 mμ and all the unusual fatty acids of the total oil. The extinction of the U. V. absorption and the optical activity were both proportional to the content of the polar fraction in the oil. The triglycerides of the polar fraction were further separated by countercurrent distribution. The four fractions obtained showed the same specific rotation as the starting material, but a difference in the distribution of the fatty acids. An attempt was made to determine the position of the short-chain fatty acids by means of hydrolysis by pancreatic lipase. A few of the short-chain fatty acids were isolated by partition chromatography and other techniques, and their structure investigated by physical and chemical means. For comparative purposes the oil of *Sebastiana lingustrina* was also investigated. A similar fraction exhibiting optical activity was isolated and its fatty acid composition determined.

— 3 —

DETERMINATION OF THE GLYCERIDE STRUCTURE OF FATS: DISTRIBUTION OF INDIVIDUAL SATURATED AND UNSATURATED ACIDS

M. R. Subbaram and C. G. Youngs

A method which gives the distribution of saturated and unsaturated fatty acids, involves fractionation of the triglycerides into groups on the basis of total unsaturation by employing chromatography on a silicic acid-silver nitrate column according to the method of deVries. The glyceride composition of each fraction is then determined by GLC of oxidized glycerides.

Thus lard was fractionated on the silicic acid-silver nitrate column into five fractions (0-4 double bonds). Glyceride composition of each of these fractions was then determined, to give quantitative amounts of 24 glycerides in the following proportions: O_2L 6.0; ML_2 0.7; PL_2 2.4; SL_2 0.6; O_3 7.7; MLO 1.1; PLO 11.2; SLO 4.0; P_2Li 0.3; PSLi 0.3; MO_2 1.3; PO_2 24.3; SO_2 8.7; P_2L 1.2; PSL 2.2; M_2O 0.1; MPO 0.8; P_2O 5.4; PSO 15.6; S_2O 1.4; MP_2 0.2; P_3 0.5; P_2S 1.8; PS_2 2.2. (M, myristic; P, palmitic; S, stearic; O, oleic; L, linoleic; Li, linolenic). Duplicate analysis agreed to within ± 0.5%. The fatty acid composition calculated from the glyceride composition agreed to within ± 0.5% with that of the original lard.

This approach provides a new basis for the evaluation of the glyceride types in natural fats and for the first time permits the quantitative determination of glycerides of myristic, palmitic, stearic, oleic, linoleic and linolenic acids.

— 4 —

EXPLOITATION OF THE SELECTIVITY OF VARIOUS CHROMATOGRAPHIC TECHNIQUES FOR THE STUDY OF THE TRIGLYCERIDE STRUCTURE OF NATURAL FATS

M. J. McCarthy and A. Kuksis

The separation of naturally occurring triglyceride mixtures on the basis of either molecular weight, polarity, or adsorbent affinity by such methods as gas-liquid, liquid-liquid, and thin-layer chromatography, respectively, rarely results in fractions representing pure triglycerides. Furthermore, most of the fractions obtained are too complex for detailed structural analyses. An integrated system using all three of these techniques for a sequential fractionation and isolation of triglyceride groups of progressively decreasing complexity, however, while not yielding single glycerides, can provide practical information for the assignment of structures. Since the probability of some components overlapping in all three systems is very small, a prudent application of these techniques affords triglyceride groups that can be analyzed meaningfully by enzymatic methods, and, in most cases, can lead to a complete assessment of the triglyceride structure of the mixture.

For an examination of the scope of this integrated analytical system, selected synthetic and naturally occurring triglyceride mixtures were used as models. The success of the system rests upon accurate quantitative determination of the fatty acids and triglycerides.

— 5 —

USE OF RUBBER COLUMNS FOR THE CHROMATOGRAPHIC SEPARATION OF TRIGLYCERIDES AND OTHER NON-POLAR COMPOUNDS

J. R. Trowbridge, A. B. Herrick, and R. A. Bauman

Some applications of rubber powder as a stationary phase in the chromatographic separation of relatively non-polar compounds have been explored. Evidence of a substantial degree of separations of triglyceride components in coconut oil and linseed oil was obtained using a mixture of methanol and acetone as the mobile phase. Other separations made include a) trilaurin from mono and dilaurin, b) individual methyl esters of coconut fatty acids from a mixture of esters, and c) terpenes from other constituents of Oil of Petitgrain. A continuous recording differential refractometer was used to observe changes in composition of the eluent stream, providing an immediate record of the degree of separation achieved.

— 6 —

A NATURALLY OCCURRING ALL-CIS 6,9,12,15-OCTADECATETRAENOIC ACID IN PLANT OILS

B. M. Craig and M. K. Bhatty

Oil extracted from freshly ground seed of *Onosmodium occidentale* contains 9-oleic, 9,12-linoleic, 6,9,12-linolenic, 9,12,15-linolenic, 6,9,-12,15-octadecatetraenoic and 11-eicosenoic as unsaturated fatty acids. Column chromatography employing silicic acid-silver nitrate and oxidation with permanganate-periodate was used in conjunction with gas liquid chromatography to confirm the structures of the unsaturated acids. Oils extracted from other members of the Boraginaceae family also contain the 6,9,12-linolenic and octadecatetraenoic acids.

— 7 —

THE POLYUNSATURATED ODD-NUMBERED ACIDS OF MULLET (MUGIL CEPHALUS)

N. Sen and H. Schlenk

Mullet oil contains more than 25% straight-chain odd-numbered fatty acids. Odd- and even-numbered components of chain lengths C_{15} to C_{20} were isolated and their structure determined. The vinylmethane rhythm prevails in all polyunsaturated acids. Many of the homologous acids have their double bonds in identical positions when counting from the carboxyl group as, for example, $\Delta^{9,12}$— and $\Delta^{6,9,12}$—C_{18}, —C_{16}, —C_{17}, and —C_{18} acids. Comparison of double bond systems shows: that the proximal structure has greater influence than the terminal structure in the biosynthesis of the unsaturated odd-numbered acids; that chain length C_{17} and C_{18} with double bonds in position 9 are crucial for the synthesis of fatty acids by elongation and desaturation; that chain length C_{15} and C_{16} with double bonds in position 9 can be desaturated but that they are not a suitable precursor for the higher polyunsaturated fatty acids found in mullet.

It is not known which organisms participate in the synthesis of these acids. Therefore, a more general classification which brings all acids of mullet oil into a rational order is of value for working hypotheses but it can not claim the predictive value which the traditional classification of oleic, linoleic, etc., families has.

— 8 —

THE PREPARATION OF C^{14}-LABELED POLYUNSATURATED FATTY ACIDS FROM OCHROMONAS DANICA

J. L. Gellerman and H. Schlenk

Polyunsaturated fatty acids labeled with C^{14} are of interest for metabolic studies. The protozoa *Ochromonas danica* is rich in linoleic, linolenic, eicosatrienoic, arachidonic and docosapentaenoic acids and, therefore, was chosen as organism to incorporate C^{14} into the lipids. Preliminary work with *Ochromonas danica* showed that the percentage of polyunsaturated acids increases with age, that their specific activity is relatively highest by adding the C^{14}-acetate at the beginning of growth, and that both chemical and radioactive yields are affected by the atmospheric conditions imposed on the growing cultures. By choosing a proper combination of these variables we obtained 2.1 g fatty esters from 12 liters of media. The specific activity of the mixed esters was 1.2×10^7 cts/min/mg, which represented a 21% yield of the radioactivity fed. An additional 6% was recovered in the nonsaponifiables.

Polyunsaturates amounted to about 50% of the total fatty esters. For determining relative radioactivity of esters in the mixture, a convenient method was developed using 1 mg of sample for separation by GLC, collection and scintillation counting of the peaks. Results by this method showed that the level of radioactivity in different esters varied by a factor of not more than 3. Palmitate was the lowest, while the polyunsaturates were higher and similar to each other.

The esters can be separated on preparative scale by LLC and GLC. In using individual esters for studying metabolic inter-

(Continued on page 14)

Abstracts of Papers . . .

(Continued from page 9)

conversions one has to keep in mind that the distribution of radioactivity in the chain may be uneven. Work toward a micro method to determine this is underway.

— 9 —

SEPARATION OF CIS AND TRANS FATTY ACID ESTERS BY ARGENTATION WITH A CATION EXCHANGE RESIN

E. A. Emken, C. R. Scholfield, and H. J. Dutton

Methyl oleate and methyl elaidate, as well as other monoene *cis* and *trans* isomers of fatty esters, separate quickly and conveniently by preparative chromatography in which a silver-saturated ion-exchange resin is used. Separations are based on differences in stabilities of the silver-olefin complexes. Recoveries of better than 95% were made, and pure *trans* and *cis* monoene fractions were collected. This method was also used to separate saturates from *cis* and *trans* monoenes. In separating the *cis,trans-,cis,cis-*, and *trans,trans*-9,12-octadecadienoates, the *cis,trans-* and *trans,trans*-dienes were eluted singly, but the *cis,cis*-diene isomer remained on the column.

— 10 —

PREPARATION OF 9,15-OCTADECADIENOATE ISOMERS

R. O. Butterfield, C. R. Scholfield, and H. J. Dutton

Linolenic acid was reduced with hydrazine to produce a mixture containing a maximum of dienoic acids. After methylation this mixture was separated into trienoic, dienoic, monoenoic, and saturated esters by countercurrent distribution (CCD) with acetonitrile and hexane. The dienoic ester fraction was further fractionated by CCD with methanolic silver nitrate and hexane to separate pure *cis,cis*-9,15-octadecadienoate and the equimixture of *cis,cis*-9,12- and 12,15-octadecadienoates.

Following isomerization of the *cis,cis*-9,15-octadecadienoate with selenium, the geometric isomers were fractionated by CCD with methanolic silver nitrate and hexane. Pure *trans,trans* and pure *cis,cis* isomers were isolated, as well as an unresolved mixture of *cis, trans* and *trans,cis* isomers. The characteristics of these isomers and related compounds are compared as determined by CCD, infrared absorption, and capillary gas-liquid chromatography.

— 11 —

BIOASSAY OF LIPIDS

A Motion Picture produced by O. S. Privett and M. L. Blank, The Hormel Institute. Presentation and comments by O. S. Privett.

— 12 —

DETERMINATION OF THE GLYCERIDE STRUCTURE OF FATS: GAS LIQUID CHROMATOGRAPHY (GLC) OF OXIDIZED GLYCERIDES

C. G. Youngs and M. R. Subbaram

A method has been developed for the determination of glyceride composition of natural fats, which involves oxidation of the fat by permanganate-periodate, esterification of the oxidized glycerides, and subsequent GLC using a flame ionization detector. Quantitative analyses, requiring about 4 hr and 20 mg of sample are reported. The method gives the distribution of individual saturated acids within the glycerides. Glyceride composition of four vegetable oils has been determined using the above procedure.

— 13 —

THE TRIGLYCERIDE COMPOSITION OF CUPHEA MINIATA SEED FAT

C. Litchfield, Margaret Farquhar, and Raymond Reiser

The triglycerides of *Cuphea miniata* (var. Firefly) seed fat were separated according to the number of double bonds per molecule using preparative thin layer chromatography on $AgNO_3$ impregnated silicic acid. The recovered fractions were quantitated using the chromotropic acid procedure. Each fraction was also analyzed by gas-liquid chromatography for molecular weights of the triglycerides present and for fatty acid composition. The triglyceride composition of the total fat was calculated from these results.

Since *Cuphea miniata* seed fat contains over 84% decanoic acid, it was expected that each triglyceride molecule would contain at least two molecules of decanoic acid. Results showed this to be generally true, but several minor component triglycerides not conforming to this pattern were also found.

— 14 —

PANCREATIC LIPASE HYDROLYSIS OF TRIGLYCERIDES BY A SEMIMICRO TECHNIQUE

F. E. Luddy, R. A. Barford, S. F. Herb, and R. W. Riemenschneider

The positional specificity of pancreatic lipase has been utilized by some investigators in studies on glyceride composition and structure of fats. The published methods for lipase hydrolysis are designed for 500 mg or more of triglycerides, a severe restriction for its use with small fractions obtained in chromatographic separations of fats. The present paper describes an adaptation suitable for 25–50 mg of sample. The reaction products, i.e., fatty acids, diglycerides, monoglycerides, and unreacted triglycerides were separated and recovered by thin-layer chromatography. The fatty acid composition of each was determined by GLC. Evidence obtained indicates that different acyl groups vary somewhat in rate of hydrolysis and that reaction conditions affect not only the over-all rate but also the extent of position shifting of the fatty acids. The data confirm that to a large extent the fatty acids of the monoglycerides are representative of those in the 2-position of the triglyceride. Precise quantitative interpretations of glyceride structures based on these acids, however, are open to question.

— 15 —

THE USE OF MICROCHROMATOPLATES FOR THE QUANTITATIVE TLC ANALYSES OF TISSUE PHOSPHOLIPIDS AND NEUTRAL LIPIDS

J. J. Peifer, R. Muesing and F. Janssen

Microchromatoplates, prepared by "dipping" lantern or microscope slides into dispersion mixtures of silicic acid, have proven to be most useful for the rapid qualitative thin-layer chromatographic (TLC) analyses of lipids. During the last two years, this method has been extended to the quantitative TLC analyses of the major phospholipid and neutral lipid components isolated from the cardiovascular tissues. The microchromatoplates are prepared, the samples applied and fractionated and the major phospholipid fractions are chemically analysed within the time that is usually required for the preparation of the larger coated chromatoplates. By the combination of chemical analyses, the use of internal standards and gas liquid chromatographic (GLC) techniques, the molecular distribution of fatty acids in specific lipid esters are conveniently determined following the isolation of components from the microplates.

This modified TLC technique is particularly suited to the routine and multiple analyses of specific activities of tissue lipids labeled with carbon-14 and phosphorus-32.

The application of these techniques to the quantitative TLC analyses of tissue lipids from experimental animals will be illustrated.

— 16 —

QUANTITATIVE ANALYSIS OF LIPIDS BY THIN-LAYER CHROMATOGRAPHY

M. L. Blank, J. A. Schmit and O. S. Privett

A procedure is described for the quantitative analysis of neutral and phospholipids by thin-layer chromatography (TLC) employing densitometry. The chromatoplates are prepared with the usual solvent systems. The spots are charred under standard conditions and analyzed with a Photovolt Corp. densitometer equipped with a special stage designed for holding 20 × 20 cm chromatoplates. Each spot on the chromatoplate gives a peak of density values which is used for quantitative analysis.

Radioactive lipids are analyzed by autoradiography by the densitometry of radiograms of chromoplates developed from X-ray films.

The precision of the method is demonstrated on model mixtures of mono-, di- and triglycerides, neutral and phospholipids and C^{14} labeled lipids.

Results of the analysis of several samples of rat liver lipids compared closely to those obtained by silicic acid column chromatography.

— 17 —

BISGLYCERIDE-ETHERS. THE INTERMEDIATES OF ACYL MIGRATION

Dmytro Buchnea

The investigation of acyl migration in partially acylated glycerides has revealed the formation of bisglyceride-ethers prior to the acyl migration. Thus, the acyl migration is seen as a consequence of the hydrolysis or cleavage of the ether linkage, which connects two glycerol moieties.

In an attempt to demonstrate this observation more decisively, an optically active (D-1,2-distearoylglyceryl)-(D-1′,2′-isopropylideneglyceryl)-3,3′-ether was synthesised, and submitted to acid hydrolysis. The quantitative yield of the hydrolysis product was identified as L-1-monostearin. On recrystallization this gave 85% yield of theory, of pure L-1-monostearin, $[\alpha]_D$—3.6°, and on titration with periodic acid assayed 100% for the existence of vicinal hydroxyl groups. The inversion of the dextrorotatory (D-1,2-distearoylglyceryl)-(D-1′,2′-isopropylideneglyceryl)-3,3′-ether to levorotatory L-1-monostearin is due solely to the acyl migration, which is directed by the position of the ether linkage which connects two glycerol moieties.

Moreover, L-1 monostearin was converted to L-1,2-distearin and (L-1,2-distearoylglyceryl)-(L-1′,2′-isopropylideneglyceryl)-3,3′ ether. This ether has been reverted by acyl migration to the original D-form of 1-monostearin.

Furthermore, by synthesis of (L-1-benzyl-2-stearoylglyceryl)-(D-1′,2′-isopropylideneglyceryl)-3,3′-ether, and then hydrolysis of the ether linkage L-1-monostearin was obtained. The hydrolysis of of the ether linkage with the simultaneous acyl shift from 2- to 3′-position, converting the D-1,2-isopropylideneglyceryl-moiety to L-1-monostearin, offers the unequivocal experimental evidence that acyl migration in partially acylated glycerides is an intramolecular acyl exchange between two glycerol moieties within the bisglyceride-ether molecule. This is in contrast to the concept proposed by Emil Fischer, and generally accepted, in which a rearrangement within a single glyceride molecule proceeding via a cyclic orthoester intermediate, was postulated.

— 18 —

SOME LINSEED ESTERS OF METHYL α-D-GLUCOPYRANOSIDE PREPARED BY USING THE METHOXYCARBONYL BLOCKING GROUP

E. J. Dufek and W. J. DeJarlais

Three possible methoxycarbonyl esters of methyl 4,6-*O*-benzylidene-

(Continued on page 18)

Abstracts of Papers . .

(Continued from page 14)

α-D-glucopyranoside have been synthesized. The 2-*O*-methoxycarbonyl derivative (I) was more reactive than the 3-*O*-methoxycarbonyl derivative (II). Treatment of the 2,3-di-*O*-methoxycarbonyl compound with weak bases in anhydrous media gave nearly quantitative yields of II.

I was converted to the various possible 2-*O*-methoxycarbonyl-3-, 4-, and 6-substituted linseed esters. Removal of the blocking group yielded the corresponding 3-, 4-, and 6-linseed esters of methyl α-D-glucopyranoside. Thus, the use of the methoxycarbonyl to block the 2-*O*-position provides the first route to some unsaturated fatty esters of methyl α-D-glucopyranoside.

— 19 —

REACTION OF THE CYCLOPROPENE MOIETY OF VEGETABLE OILS WITH ACIDS

R. O. Feuge and Zigrida Zarins

The degree to which various acids react with the cyclopropene moiety in cottonseed and *Sterculia foetida* oils was established under a fixed set of conditions. From the standpoint of ease of reaction, acount of reactants required, and physical properties of the treated oil, three acids (citric, oxalic, and formic) were deemed to be the most desirable. The effect of temperature, time, and concentration on the reactions of citric and oxalic acids was established. On a molar basis citric acid was approximately half again as effective as oxalic acid in destroying the cyclopropene moiety, but the necessary reaction temperature was 40 degrees higher.

Citric acid reduced the iodine value of the treated oil and primarily produced polymers. Oxalic acid on the other hand produced only a very small amount of polymers, decreased the iodine value only slightly, and formed a large percentage of double bonds. No stoichiometric relationship existed between the amounts of oxalic acid used and the cyclopropene destroyed. During the reaction the oxalic acid decomposed into carbon dioxide and formic acid, which formed relatively unstable esters. Direct treatment of the oil with formic acid resulted in products similar to those formed with oxalic acid, except that the formic acid produced relatively large proportions of *unsym*-disubstituted olefinic compounds.

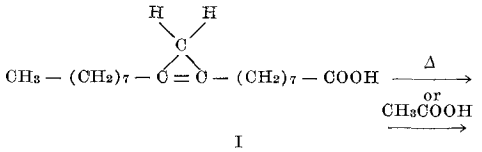
The reaction of the cyclopropene moiety with citric, oxalic, and formic acids differed in important respects from the previously accepted reaction of this moiety with acids. Probable mechanisms are discussed.

— 20 —

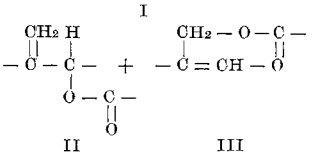
A RE-EXAMINATION OF THE POLYMERIZATION OF STERCULIC ACID

H. W. Kircher

Rinehart and co-workers have shown that sterculic acid (I) polymerizes or reacts with acetic acid with ring opening and formation of the allyl ester structures II and III. The structures were inferred from the products obtained from permanganate-periodate oxidations.

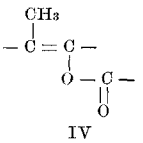


I



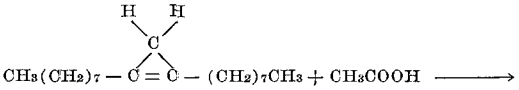
II III

Although Faure and Smith had suggested structure IV as part of the sterculic acid polymer on the basis of its infrared absorption, Rinehart was unable to find any chemical evidence for this structure.

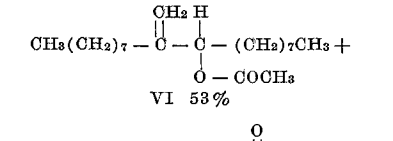


IV

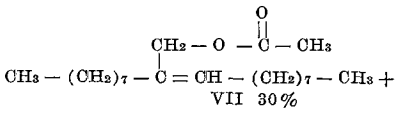
When sterculene (V) was used rather than sterculic acid, the products of the reaction with acetic acid were simpler and amenable to gas and thin-layer chromatography. Permanganate-periodate oxidations of the reaction products confirmed structures II and III and yielded no compounds expected from IV. The presence of IV was indicated, however, by alkaline hydrolysis of the products, reacetylation, and comparison of the infrared spectra and gas chromatograms of the hydrolytic and reacetylated materials with the original reaction product. A ketone band in the infrared and a new peak in the GLC separation diagram which did not disappear upon reacetylation indicated that sterculene reacted with acetic acid in the following manner:



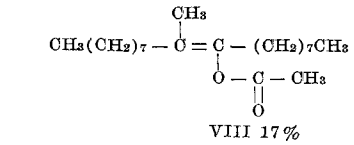
V



VI 53%



VII 30%



VIII 17%

The sterculene-acetic acid reaction products were ozonized. 2-Decanone was obtained, which proved that structure VIII was part of the reaction mixture. Here is an instance where a double bond not cleaved by permanganate-periodate was susceptible to ozonolysis. Comparable studies with the sterculic acid polymer will be discussed.

— 21 —

POLYUNSATURATED FATTY ACIDS WITH A "LONELY" DOUBLE BOND IN THE 5-POSITION

J. L. Gellerman and H. Schlenk

Lipids from leaves and nuts of the tree *Ginkgo biloba* contain several C_{20} and C_{18} acids with double bonds at the 5 and 11 positions. Any additional double bonds occur at positions higher than 11 and are in the common methylene interrupted pattern, in contrast to the "tetramethylene interruption" between 5 and 11. The group of isomers represents about 10% of the fatty acids in Ginkgo and includes nearly all the C_{20} acids. Linoleic and linolenic represent the greater part of the C_{18} acids; however, the isomers are present in the C_{18} series too, while they have not been found with C_{18} chain length.

The methyl esters were fractionated by liquid-liquid and gas-liquid chromatography. The individual esters or mixtures of isomers were identified by ozonization procedures before and after alkaline isomerization. Ozonization-hydrogenation established the positions of the extreme double bonds, ozonization-oxidation revealed the length of the internal fragments, and ozonization-hydrogenation after alkaline isomerization demonstrated the position of the "lonely" double bond.

Elucidation of the biosynthetic pathways to the unsaturation in position 5 and the possible correlation of the unusual lipids in Ginkgo with its early and unique place in evolution have been undertaken.

— 22 —

HYDROGENATION OF LINOLENATE. XI. NUCLEAR MAGNETIC RESONANCE INVESTIGATIONS

A. E. Johnston, C. A. Glass, and H. J. Dutton

Nuclear magnetic resonance (NMR) spectra have been obtained during the hydrogenation of methyl linolenate with platinum, nickel, and sulfur-poisoned-nickel catalysts and during the reduction of linolenic acid with hydrazine. Structural changes have been studied by "proton counting" techniques and include those for the 15,16-double bond (β-oleofinic methyl protons), allylic methylene (α-olefinic methylene protons), 1,4-pentadienes (α,α'-olefinic methylene protons), and total unsaturation (olefinic protons).

Amounts of double bonds in the 15,16 position and the 1,4-pentadiene structures decrease linearly with increasing saturation, but the slopes of lines differ for specific catalysts. Sulfur-poisoned nickel has the most negative slope, followed by electrolytic nickel, and then platinum and hydrazine. Amounts of α-olefinic structures with increasing saturation are roughly constant for hydrazine and platinum during reduction with the first mole of hydrogen; they fall to zero at complete saturation. For the two nickel catalysts, the α-olefinic structures increase during absorption of the first mole and a half of hydrogen before dropping to zero. The number of olefinic protons is inversely related to the degree of saturation as determined by iodine value, gas-liquid chromatography, and hydrogen absorption. The possibility of substituting NMR measurements for iodine value, alkali-isomerization spectrophotometric determination, and other structural analyses is discussed.

— 23 —

ELAIDINIZATION WITH MERCAPTANS

H. W. Kircher

Oleic acid or oleates are usually elaidinized with oxides of nitrogen at low temperatures or with selenium or sulfur at high temperatures. During rate studies on the addition of mercaptans to methyl sterculate and sterculene in dilute solutions, methyl oleate was used as a blank to check decay of the sulfhydryl reagents and the activity of "normal" double bonds to sulfhydryl addition. Very little addition of sulfhydryl to methyl oleate was observed, but the compound was appreciably isomerized to methyl elaidate even in the dark and in absence of catalysts. The reaction was studied with respect to the effect of solvents, temperature, light, peroxides and concentration of reactants and a mechanism is proposed.

— 24 —

THE SYNTHESIS AND EVALUATION OF NEW ACTIVE CHLORINE COMPOUNDS

I. R. Schmolka, M. Cenker, and M. Kokorudz

Twenty-seven organic compounds containing active chlorine were synthesized. The chlorine carriers included alkyl, aryl and acyl derivatives of urea, aliphatic and aromatic acyl derivatives of diamines, amides and imides of mono- and dibasic acids, sulfonamides and condensation products of urea with mono- and dicarbonyl compounds. These products were evaluated for bleaching and eighteen were found to be effective bleaches. Some of the better bleaches were evaluated for their ability to remove blueberry, tea and coffee stains. No one bleach was superior to the others for all three stains. All the bleaches were evaluated as germicidal agents. Four showed excellent germicidal activity against E. coli. Thermal stability measurements showed some of the new bleaches to be unstable.

— 25 —

APPLICATIONS OF SILICIC ACID-SILVER NITRATE CHROMATOGRAPHY

M. K. Bhatty and B. M. Craig

The use of silicic acid-silver nitrate chromatography as developed by de Vries is a very valuable technique. Enrichment of methyl ester fractions on the basis of unsaturation followed by GLC analysis has shown the presence of odd numbered fatty acids in all vegetable oils examined. Total saturated fatty acids may be quantitatively determined and the results show excellent agreement with GLC analysis. The technique coupled to permanganate-periodate oxidation is useful for the determination of isomeric unsaturated fatty acids. Samples of polyunsaturated fatty acids are readily obtained in high purity by using the technique in a form of displacement chromatography.

— 26 —

FRACTIONATION OF COMPLEX LIPID MIXTURES INTO GROUPS OF UNIFORM DEGREE OF UNSATURATION

H. K. Mangold and C. R. Houle

Vinylogous group separations of complex lipid mixtures by chromatography of acetoxymercuri-methoxy compounds on silicic acid constitutes a new type of fractionation: Mixtures of different classes of lipids, each differing in chain length and degree of unsaturation, can be segregated on the basis of the degree of unsaturation of their constituents. For example, saturated long-chain hydrocarbons, alcohols, aldehydes, and acids are separated, as a group, from the adducts of the corresponding monounsaturated lipids. The adducts of mono-, di- and triunsaturated lipids each migrate as a group.

This method is complementary to adsorption techniques, which essentially effect separation of lipids according to chemical classes; and it also supplements partition methods for fractionating classes into individual compounds.

The criterion of "chromatographic purity" of a lipid is not established unequivocally by the use of any single chromatographic technique. Instead, several different principles of fractionation are required to verify homogeneity of a sample.

— 27 —

EXPERIMENTS IN THE FORMULATION OF LIQUID DETERGENTS FROM TALLOW

R. G. Bistline, Jr., and A. J. Stirton

Many of the soaps and surface active agents which derive from tallow are quite water soluble and may be considered in the formulation of liquid detergents. Examples are: sodium oleate; tallow alcohol sulfates in the form of sodium oleyl sulfate, sodium 9, 10-dichlorooctadecyl sulfate. or sulfated nonionics $R(OC_2H_4)_2OSO_3Na$; the *N*-methyl tauride or isethionate ester of oleic acid; triethanolammonium α-sulfostearic acid; and salts of a variety of esters of α-sulfo fatty acids, such as sodium isopropyl α-sulfostearate, $C_{16}H_{33}CH(SO_3Na)CO_2CH(CH_3)_2$, and disodium 2-sulfoethyl α-sulfostearate, $C_{16}H_{33}CH(SO_3Na)CO_2CH_2CH_2SO_3Na$.

In simplified formulations using builder, water, and isopropanol, with compatibility as the first consideration, it was found that esters of α-sulfo fatty acids could be used as the only active ingredient, or in blends with soap or tallow alcohol sulfates. The liquid detergent formulations are described in terms of their appearance and stability, pH, viscosity, and foaming and detergent properties.

— 28 —

A METHOD FOR THE DETERMINATION OF SURFACE TENSION OF NONIONIC SURFACTANT SOLUTIONS AT ELEVATED TEMPERATURES

E. D. Berglund and S. B. Crecelius

The surface tension measurement of nonionic surfactant solutions at room temperature is not always a true indication of their value as a surfactant.

A method has been developed for the measurement of surface tension at elevated temperatures based on the capillary rise principle.

A number of different types of nonionic surfactant solutions at various temperatures and their surface tension versus temperature relationship were plotted. The results of these relationships were discussed. It was concluded that there is a wide variation in the behavior of nonionic surfactants at elevated temperatures depending on their structure, and this behavior cannot be predicted by measurement of surface tension at room temperature.

— 29 —

TEMPERATURE STUDIES OF COACERVATION IN AQUEOUS CATIONIC SOAP SOLUTIONS

Irving Cohen and Peter Economou

The coacervating quaternary ammonium salt, Hyamine 1622, forms a complex with molecular iodine. The micellar molecular weights and the charge properties of the homogeneous phase of this cationic soap system, as a function of NaCl concentration, I_2 concentration, and temperature, were determined from light scattering measurements. At constant temperature, an overall two stage growth process is indicated in these systems. At low NaCl concentration, the micelle grows to a limiting isotropic structure. At higher NaCl concentrations, the micellar growth is an exponential function function of the NaCl concentration. The Hyamine-I_2 complex systems show the typical micellar ionization suppression with increasing NaCl concentration encountered in a number of coacervating cationic soap systems. The infusion of small quantities of I_2 in an aqueous Hyamine-1622 solution produces changes in all of the characteristic properties of the system. Experimentally, detectable changes in the critical electrolyte concentration (CEC) necessary for two solution phase formation may be observed for I_2-Hyamine 1622 molecular ratios as low as 10^{-8}. Temperature studies of the aggregation numbers, micellar charges and C.M.C.'s, provide the basis for calculating the true values of the heats of micellization. The temperature dependence of the micellar molecular weights, (MMW), in the range of 17–47°C, is an exponential function, and plots of log (MMW) vs. 1/T give straight lines with positive slopes. At low NaCl concentrations (0–0.8 *M*), where there is an effective charge on the micelles, the slopes, d log (MMW)/d($\frac{1}{T}$), are very small At higher NaCl concentrations, where the micelles approach zero effective charge, (theta conditions), the slopes increase appreciably and are approximately constant over a wide range of NaCl concentration (0.09–0.24 *M*).

— 30 —

DYNAMIC FOAM TEST

W. G. Spangler

A test has been developed for measuring the amount of foam generated by a detergent composition under dynamic conditions and in the presence of soil.

Good correlation with actual practice results because the conditions of the test closely parallel practical laundry conditions.

The test can also be modified so that it can be used as a control for dishwashing.

— 31 —

CAKING TEST FOR DRIED DETERGENTS

Henry Watanabe and W. L. Groves

A test to measure the caking tendency of dried detergents is described. This test has good reproducibility and should be useful in screening new products, studying formulation changes and examining anticaking agents.

The test measures the force necessary to break cylinders formed from test detergent. Forming and breaking forces are extremely critical and are provided by an Instron tensile tester. The sensitivity of the test demands careful control of the environment; a number of critical factors are discussed. These include test procedure variables such as cylinder length, forming force and forming rate and time and product variables such as moisture content and particle size. An increase in the forming force and time, particle size within certain limits and moisture, increase the breaking force.

Results on drum dried products were found to correlate with spray dried products. Heavy duty products show a lower caking tendency than do light duty products. Anti-caking additives cause very marked differences in breaking force.

— 32 —

A NEUTRON ACTIVATION METHOD FOR SOIL REMOVAL MEASUREMENT

D. A. Netzel, C. W. Stanley, and D. W. Rathburn

A neutron activation analysis technique has been developed to determine the amount of particulate kaolinite soil removed from cotton fibers during a wash cycle.

When the aluminum, a constituent of the kaolinite lattice structure, is activated by neutron bombardment, short-lived aluminum-28 is produced. The amount of particulate soil present on a piece of cotton cloth before and after the wash cycle is determined by gamma scintillation counting of the aluminum-28, thus providing an absolute method for the determination of the per cent soil removed. The experimental technique and a comparison between a reflectance method and the neutron activation method for the determination of the per cent soil removed by a given surfactant will be presented.

— 33 —

PREPARATION AND PROPERTIES OF SEVERAL AMINE OXIDES

R. A. Reck

Much has been written in recent years concerning the use of amine oxides in detergent formulations. This paper will be concerned with the preparation and properties of six commercially available compounds. These compounds are varied according to long chain constituent and the other two substitutions on the nitrogen atom.

Data will be presented on detergent formulations, foaming characteristics, and wetting characteristics. Other miscellaneous applications also will be discussed.

— 34 —

TWO NEW STABLE POLYBROMINATED SALICYLANILIDES FOR ANTIBACTERIAL USE IN SOAP AND DETERGENT PRODUCTS

N. M. Molnar and S. Baron

The versatility of two selected hydrogenated salicylanilides as antiseptic agents and germicides in soap and detergent products for use on skin, hair, fabric and hard surfaces is discussed.

The germicidal preparations are:

A. A 1:1 mixture of 4',5-dibromo- and 3,4',5-tribromosalicylanilide (TEMASEPT), and

B. An essentially pure 3,4',5-tribromosalicylanilide (TEMASEPT II).

Both preparations show stability at elevated temperatures and compatibility with many surfactant vehicles. They are free of primary irritation and sensitization, including sunlight-induced irritation.

Temasept is currently being used in toilet bars, shampoos, laundry products, disinfectant sprays and multi-purpose disinfectant products.

The substantivity to fabrics is *not* characterized by an instability to heat or hydrolysis. No toxic aniline compounds are formed.

Temasept II is resistant to discoloration, making it particularly suitable for use in white and pastel colored toilet bars. It has excellent substantivity to skin and provides long lasting action against odor and infection-causing bacteria.

— 35 —

GAS CHROMATOGRAPHY IN LIPID INVESTIGATIONS

E. C. Horning

— 36 —

THIN-LAYER CHROMATOGRAPHY IN STUDIES OF LIPID METABOLISM

H. K. Mangold

Innovations of the thin-layer chromatographic technique will be summarized briefly.

Applications of TLC in work on the biosynthesis of fatty acids, triglycerides and phospholipids will be cited from the recent literature. Publications on the digestion, absorption and transport of fat will also be reviewed. Special attention will be given to studies concerned with the effect of dietary fats and of drugs on lipid metabolism.

— 37 —

DRUGS IN LIPID SYNTHESIS

W. L. Holmes

The implication of blood lipids in the pathogenesis of atherosclerosis and cardiovascular disease has stimulated a continuous search for hypolipemic agents. One aspect of these investigations, namely the attempts to lower blood and tissue cholesterol levels, has resulted in the development of many compounds which interfere with the synthesis of this sterol. This discussion will be concerned primarily with a review of several aspects of the investigations with a number of these inhibitors, with a major emphasis being placed on the more recently developed compounds. In addition to their effect on cholesterol synthesis, some of the inhibitors have been shown to affect other classes of lipids; brief mention will be made of this aspect of the problem.

— 38 —

THE EFFECT OF NEOMYCIN ON CHOLESTEROL METABOLISM

J. G. Hamilton, J. E. Muldrey, Grace A. Goldsmith, B. E. McCracken, and O. N. Miller

Samuel and Steiner found that neomycin caused a lowering of serum cholesterol in man. They suggested that its effect on serum cholesterol might be related to a change in the intestinal bacterial flora. This observation has been confirmed in a number of laboratories.

Large doses of neomycin (4-12 gms/day) are known to cause a malabsorption syndrome similar to idiopathic steatorrhea. The degree of malabsorption correlates with the size of the dose. Serum cholesterol is lowered by 2 g per day and the decrease in cholesterol is not correlated with size of the dose.

The effect appears to be more specific than a generalized malabsorption syndrome. There is an increased excretion of bile acids. A variation in the numbers of coliform bacteria (confirmed in this laboratory by variable excretion of 7-ketodeoxycholic acid) has been cited as evidence that the reduction in cholesterol level is not due to a disturbance of the intestinal flora, however, an invariable finding in this laboratory has been the complete hydrolysis of the conjugates and a failure of the conversion of cholic acid to deoxycholic acid. Both the hydrolysis of the conjugated bile acids and the concersion of cholic acid to deoxycholic are known to be bacterial in origin (probably obligate anaerobes).

Although the exact mechanism by which neomycin lowers serum cholesterol is unknown it appears to affect the absorption of bile acids and/or sterols. This effect on absorption is probably separate from a generalized malabsorption which is often caused by large doses of neomycin.

— 39 —

THERMAL DECOMPOSITION OF DIMETHYLLAURYLAMINE OXIDE

G. P. Shulman and W. E. Link

The principal reaction during thermal decomposition of dimethyllaurylamine oxide is deoxygenation to dimethyllaurylamine; 1-dode-

(Continued on page 23)

Abstracts of Papers . . .

(Continued from page 20)

cene is also formed. The rates of amine oxide decomposition have been determined in the range of 80–100C. Interpretation of the kinetic data shows that deoxygenation is a primary decomposition product. Comparison of the energy and entropy of activation for olefin formation (36.3 kcal, 12.4 e.u.) and amine formation (20.6 kcal, —30.8 e.u.) shows that these are competing processes with different transition states.

— 40 —

ANALYSIS OF DETERGENT MIXTURES CONTAINING AMINE OXIDES

H. Y. Lew

Long chain alkyldimethylamine oxides in detergent formulations have not only been difficult to analyze but also interfere with anionic active determination by the usual cationic titration method using methylene blue indicator. A new method has been devised for quantitative analysis of both amine oxides and anionic actives. Anionic actives are determined by cationic titration without interference from amine oxides by using bromocresol green indicator. Amine oxides are then determined by a relatively simple extraction followed by a normal methylene blue titration procedure. Low molecular weight aromatic sulfonates which would interfere are removed during the extraction step.

Analytical data are presented for a series of alkyldimethylamine oxides of different molecular weights in several experimental detergent formulations. The method is rapid and is amenable to both solid and liquid detergent formulations.

— 41 —

IDENTIFICATION OF SURFACE ACTIVE AGENTS IN ADMIXTURE BY THIN-LAYER CHROMATOGRAPHY

C. T. Desmond and W. T. Borden

The qualitative identification of the surface active agents commonly used in household detergent formulations is possible using thin layer chromatography. Successful separations have been done when the samples are spotted on Alumina G and developed with isopropanol. A variety of specific reagents may be used to color the spots and thereby supplement the Rf values as a means to identify the surfactant.

— 42 —

GAS LIQUID CHROMATOGRAPHY OF ACETATE ESTERS OF NONIONIC SURFACTANTS

L. Gildenberg and J. R. Trowbridge

A substantial improvement in the chromatographic stability of nonionic surfactants derived from ethylene oxide addition to fatty alcohol has been achieved through acetylation of the terminal hydroxyl function. For dodecyl alcohol adducts well separated peaks for species containing from 0–14 polyoxyethylene units have been obtained on a silicone column.

The assignment of peaks was verified by the addition of several single component species containing up to ten polyoxyethylene units. Polyglycols with up to six ethenoxy units were obtained by fractionation in a spinning band column of commercially available mixtures. Decaoxyethylene glycol was prepared from tetraoxyethylene glycol and 2-chloroethyl ether (Hibbert and coworkers, JACS *61*, 1905, (1939)). The desired monoalkyl ethers were obtained by a Williamson ether synthesis (Gingras and Bagley, Canadian J. Chem., *35*, 599–604 (1957)). Good resolution of the mixed fatty alcohol adducts has been obtained so far only for lower molecular weight adducts.

— 43 —

AN AUTOMATED VERSION OF THE METHYLENE BLUE METHOD FOR ANIONIC SURFACTANTS IN WATER

D. P. Lundgren and H. J. Keily

A standard methylene blue (MB) method for determining alkylbenzenesulfonate (ABS) and other anionic surfactants in water has been automated using modular units of the Technicon *Auto*-Analyzer. Samples containing up to 5.0 parts-per-million (ppm) ABS can be analyzed at a rate of 17 per hr. The method is particularly applicable for performing the large number of routine tests required for biodegradability studies of anionic surfactants.

Uniform molar responses are obtained for both linear and branched ABS due to the extremely high stage (and over-all) efficiency of the extraction step. This is accomplished by means of a large-volume sampler which permits the use of favorable chloroform-water ratios. The sampler was developed in this laboratory and can be used with any conventional fraction collector.

The accuracy and precision of the automated procedure has been checked against results obtained for the analysis of ABS in water using the American Public Health Association (APHA) method. Comparable data were found by both methods with a precision of ±0.2 ppm at the 95%-confidence level.

— 44 —

ALPHA SULFO FATTY ESTERS IN BIOLOGICALLY SOFT DETERGENT FORMULATIONS

E. A. Knaggs, L. Varenyi, J. A. Yeager, and E. Fischer

Salts of alpha sulfo tallow and coconut esters were subjected to river die-away, activated sludge and Warburg tests and results show these derivatives to be biologically soft detergents with disappearance curves approximately those of the fatty alcohol sulfates.

Selection of the proper fatty acid starting material, alcohol of esterification, and alkali for neutralization provides for a high degree of flexibility in tailor-making biologically soft surface active agents for a wide range of applications. Selected compounds have been found which exhibit remarkable foaming, lime soap dispersing, fabric and hard surface detergency, wetting and related surface active properties. These properties allow the formulation of a variety of synthetic and soap-synthetic combinations.

Primary emphasis is given to the presentation of data on surfactant formulation application studies. Salts of short chain alkyl esters of alpha sulfo tallow acid are ideally suited for "combo" soap bar and built heavy duty detergent applications. Salts of short chain alkyl esters of selected coconut fatty acids are uniquely suitable for light duty liquids, cosmetic and related surfactant applications.

An improved process for the manufacture of these sulfo esters has been developed which produces high yield, high purity and light colored products, and which should provide for their acceptance on a large scale in the detergent field.

— 45 —

BIODEGRADATION OF SOME TALLOW-BASED SURFACE ACTIVE AGENTS IN RIVER WATER

J. K. Weil and A. J. Stirton

The biodegradation of 33 anionic and 6 nonionic detergents has been studied in Schuylkill River water using the "Dye Away" procedure. Degens' methylene blue method was used to follow the disappearance of anionic detergents. Although not completely satisfactory, measurement of surface tension was the preferred method for following the disappearance of nonionic detergents. Water taken from the Schuylkill, Susquehanna, and Delaware rivers gave very nearly the same degradation rates and seasonable variations also showed little differences in degradation rates.

The results have been arbitrarily divided into 4 classes: 1) very soft detergents of the ester type, whose surface activity is readily destroyed, apparently by a hydrolytic process (1–3 days); 2) soft detergents which included nearly all of the simple alkanesulfonates and derivatives of the α-sulfo acids (3–7 days); 3) moderately hard detergents with some unusual structural features which effect a slight retardation of biodegradation (1–2 weeks); and 4) hard detergents characterized by branched alkyl substitution on a benzene nucleus (greater than 2 weeks).

At the end of one and a half days oxyethylated hexadecanol with an average of ten oxyethyl groups showed degradation by the surface tension method and also by disappearance of foam. Oxyethylated hexadecanol with an average of twenty oxyethyl groups showed degradation by the surface tension method in 43 hr but foam persisted after 25 days.

— 46 —

SOFT ALKYL BENZENES AS DETERGENT RAW MATERIALS

J. Morrisroe, R. W. Atwood, and R. E. Temple

Soft alkyl benzenes from foud or five different manufacturers of basic hydrocarbons are examined from the standpoint of how they relate to each other and to the hard alkyl benzenes on such properties as boiling range, completeness of sulfonation, bromine number, and aniline point. Data are presented on the surface tension, solubility, the emulsification properties, color, odor, and consistency of the sulfonates made from these soft alkyl benzenes.

— 47 —

ANALYSIS OF FATTY ALCOHOLS AS NITRATES BY THIN-LAYER CHROMATOGRAPHY AND INFRARED SPECTROSCOPY

D. C. Malins, J. C. Wekell, and C. R. Houle

Alcohol derivatives of fatty acids were fractionated and analysed by virtue of the unique spectral and chromatographic properties of the corresponding nitrates ($-ONO_2$).

Acetyl nitrate, which is formed *in situ* from nitric acid and acetic anhydride, reacts under mild conditions with olefinic double bonds and hydroxyl and amine groups. In the present work, fatty nitrates were obtained by reaction of this reagent with saturated alcohols, diols, hydroxy-esters, acyloins, glyceryl ethers, and mono- and diglycerides. The nitrate derivatives were characterized by a number of well defined bands in the infrared ($6.1 \pm 0.1\mu$, $7.9 \pm 0.1\mu$, $11.7 \pm 0.1\mu$, $13.2 \pm 0.1\mu$, and $14.4 \pm 0.1\mu$). They were readily and unambiguously identified by examination of their spectra and, in some cases, structures could be predicted.

In addition, fatty nitrates were fractionated and analysed by the complementary technique of thin-layer chromatography (TLC). Small quantities of hydroxy- compounds were conveniently nitrated in test tubes. The crude reaction mixtures, dissolved in diethyl ether, were then applied directly to the plate. Derivatives separated by TLC were eluted from the absorbent and analysed further by infrared spectroscopy. Mono- and dinitrates, having little affinity for polar absorbents, were separated from contaminants on thin layers of silicic acid by elution with pure petroleum hydrocarbons. Even weakly polar derivatives, such as esters and aldehydes, did not migrate in this system. Nitrate derivatives of hydroxy-esters, ketones, and ethers—which had greater absorbent affinity—were eluted with a slightly more polar solvent. Reaction products of acetyl nitrate with unsaturated compounds were generally too polar to interfere with the TLC of saturated nitrate esters. After separation by TLC, fatty nitrates were reduced back to alcohols by catalytic hydrogenation.

— 48 —

GAS-LIQUID CHROMATOGRAPHY OF POLAR FATTY DERIVATIVES

R. A. Morrissette and W. E. Link

The direct separation of fatty amides is achieved using a polyamide, Versamid 900, as the partitioning agent on a support which need not be previously impregnated with strong alkali or acid, procedures usually followed in the GLC separation of highly polar materials. The combination of a neutral support and polar substrate permits the separation of unsubstituted and substituted long chain fatty amides with as many as 24 carbon atoms with good resolution, in a reasonable time, and with good peak symmetry. The observed area responses agree with the weight percentages of standard amide mixtures, indicating that no loss of amides occurs on the column under the conditions employed.

The Versamid column has proved to be useful in the analysis of other polar fatty derivatives. Conjugated dienoic and trienoic acids run as their methyl esters are retarded sufficiently on Versamid 900 so that they may be estimated in the presence of other fatty acids. Mixtures of compounds with varying polarity, such as mono-, di-, and triacetin, and glycerol, may be separated easily. Hydroxyl and normal fatty acid methyl esters give equally symmetrical peaks. The versatility and stability of the polyamide when used as a column material at high temperatures will undoubtedly lead to other applications in the gas-liquid chromatographic analysis of fatty derivatives.

— 49 —

INFRARED ANALYSIS OF METHYL STEARATE FOR DEUTERIUM

Helen Ven Horst, W. K. Rohwedder, E. Selke, C. R. Scholfield, and H. J. Dutton

A comparison of the carbon-deuterium absorption band at 2140 cm for hydrazine-deuterated and catalytically deuterated methyl oleate shows that the hydrazine-deuterated material has one sharp peak, whereas the catalytically deuterated material has two shoulders on the main peak. While deuterium contents of methyl oleate, linoleate, and linolenate reduced with tetradeuterohydrazine to the corresponding di-, tetra-, and hexadeuterostearates agree with the independent and absolute determination of deuterium by mass spectrometry, the same unsaturated esters reduced with deuterium gas over platinum give low results as determined by infrared. The influence of stereoisomerism of the deuterium on the infrared absorption coefficients is discussed in relation to the development of a rapid analytical method.

— 50 —

THE GAS LIQUID CHROMATOGRAPHY OF HYDROXYSTEARIC ACID ESTERS

A. P. Tulloch

Hydroxy fatty acids now appear to occur in nature quite frequently. Therefore an investigation has been made of the extent to which gas liquid chromatography can be used to separate and determine the structure of the 17 isomeric hydroxystearic acids. The acids have been examined as their esters or acylated esters using several non-polar and polar liquid phases. A number of the isomeric acids have been separated and identified in this way.

— 51 —

CHARACTERIZATION AND IDENTIFICATION OF LIPIDS BY THEIR CRITICAL SOLUTION TEMPERATURES

H. H. O. Schmid, H. K. Mangold, and W. O. Lundberg

The mutual solubility of two liquids which are not miscible in all proportions is a function of the temperature. Rising temperature usually increases the solubility, possibly reaching a point, i.e. the upper critical solution temperature (CST), at which the two components become miscible in all proportions. The CST is characteristic for the two liquids involved. Determinations of CST values are especially valuable for substances which cannot be adequately characterized by their melting points. Minute amounts of sample (2–3 μl.) are required if the determination is carried out under the microscope.

CST values of homologous-vinylogous series of long chain hydrocarbons, alcohols, aldehydes, acids, methyl and ethyl esters, mono-, di-, and triglycerides have been determined by using several test substances.

The CST values depend on chain length, number of double bonds and functional groups of the compounds tested. Unlike the melting points, no alternating behavior between the members of homologous series is observed. Lipids occurring in various polymorphic forms can be characterized in the liquid state by their CST values. *Cis* and *trans* isomers yield different CST values.

It is possible that a mixture of lipids will yield the same CST with a certain test substance as one of the pure components, but identity as well as purity of a sample is ascertained if complementary purification techniques have not yielded fractionation and if the compound exhibits the accurate critical solution temperature.

— 52 —

METHODS FOR THE DETERMINATION OF CYCLOPROPENOID FATTY ACIDS. IV. APPLICATION OF THE STEPWISE HBr TITRATION METHOD TO THE ANALYSIS OF REFINED AND CRUDE COTTONSEED OILS

J. A. Harris, F. C. Magne, and E. L. Skau

A method is described for the determination of cyclopropenoid fatty acids in refined and crude cottonseed oils to within 0.01%. It is based upon a stepwise hydrogen bromide titration at 3C and

55C after removal of interfering substances by adsorption on activated alumina. Highly oxidized cottonseed oils must first be converted to methyl esters.

— 53 —

THE DETERMINATION OF DOWTHERM A IN FATTY ACIDS

J. E. Mehrens

In many cases, fatty acids are processed in vessels heated with Dowtherm. Occasionally, leaks develop which result in the contamination of the fatty acids with Dowtherm. Since Dowtherm has a very sharp and objectionable odor, it is important to be able to rapidly check the contents of the vessel routinely for the presence of Dowtherm.

The following method describes a GLC technique which will readily determine Dowtherm in fatty acids of C_{10}–C_{22} chain length down to levels of parts per million.

The equipment used was an Aerograph 90C with a 3/16" x 10' DEGS column operated at 180C and 50 ml/min helium flow rate. Standard mixtures of Dowtherm and fatty acids were prepared which contained a known weight percent of Dowtherm. These samples were injected and the resultant peak heights measured and plotted against concentrations. Unknowns were then injected and Dowtherm determined from the graph. Analysis time from start to finish was fifteen minutes.

While more efficient columns could be used such as silicones, etc., most commercial laboratories doing methyl ester analyses do not have the time or equipment versatility to continually change columns. For this reason, a DEGS column was chosen. It was noted that using this technique rendered detrimental effects upon the life of 1/4" columns but did not materially affect the life of 3/16" columns. Column life was three months under conditions of ten to twelve injections per day, five of which were fatty acids, the balance—methyl esters. This paper will be accompanied with slides illustrating calibration graphs and typical chromatograms.

— 54 —

DETERMINATION OF POLYOXYETHYLENE (20) SORBITAN MONOSTEARATE IN CAKE, CAKE MIXES, ICING AND CREAM FILLINGS

C. F. Smullin and A. D. Cooper

A procedure was developed for the determination of polyoxyethylene (20) sorbitan monostearate (TWEEN® 60) in cake, cake mixes, icing and cream fillings. The emulsifier is recovered from the sample by solvent extraction. After removal of the solvent, the extract is saponified with alkali and the fatty acids removed. The aqueous polyol solution is treated with barium phosphomolybdate to form a highly insoluble heteropoly acid complex with the polyoxyethylated moiety. The polyoxyethylene (20) sorbitan monostearate is determined by the use of a gravimetric factor which correlates the weight of complex obtained from known weights of emulsifier carried through the analytical procedure. The paper presents recovery data on typical cakes, cake mixes, icing and cream fillings.

— 55 —

THE USE OF THYROID ANALOGS IN THE TREATMENT OF PATIENTS WITH HYPERCHOLESTEROLEMIA AND CORONARY ATHEROSCLEROSIS

N. Tuna

Thyroid hormones and some of their analogs are known to affect the serum cholesterol. Analogs of the thyroid hormones having minimal metabolic effects but retaining the cholesterol lowering activity have been synthesized.

Sodium dextrothyroxine (sodium salt of 3,5,3',5'-tetraiodo-D-thyronine) is one of the most promising analogs.

Our experiences with sodium dextrothyroxine in patients with hypercholesterolemia and coronary artery disease will be reported. About 20 per cent decrease in serum cholesterol was observed in patients receiving 4–8 milligrams of sodium dextrothyroxine daily. Patients were followed from three months to three years. Side effects were minimal.

Various aspects of the metabolism of thyroid analogs and the mechanisms of their cholesterol lowering action will also be discussed.

— 56 —

THE ACTION OF ADRENAL HORMONES ON HEPATIC TRANSPORT OF TRIGLYCERIDES AND FATTY ACIDS

M. Heimberg, N. B. Fizette, and H. Klausner

Both adrenal cortical and medullary hormones appear to have a profound effect on hepatic transport and metabolism of lipids. In order to evaluate these hormonal actions on the liver without the multiple variables inherent in experiments on intact animals, we studied the transport of triglycerides and fatty acids in isolated perfused rat livers (Am. J. Physiology 202,353,1962) obtained from adrenalectomized and normal male animals. Epinephrine and norepinephrine have effects on net outward hepatic transport of triglycerides in opposition to that of cortisone. Both epinephrine and norepinephrine inhibit fatty acid uptake and outward triglyceride transport, when added to the medium perfusing livers obtained from normal animals. Triglyceride output and fatty acid uptake is depressed in livers from adrenalectomized rats. In fact, a net decrease in perfusate triglyceride concentration was observed in experiments using livers from adrenalectomized animals. The net triglyceride release by livers obtained from adrenalectomized, cortisone-treated rats, approached that observed with livers from normal control animals. The inhibition of triglyceride release in livers from adrenalectomized animals would thus appear to be primarily a result of cortical hormone deprivation.

Epinephrine not only reduced triglyceride release by the liver, but appeared as well to inhibit the uptake of a neutral fat emulsion labeled with tripalmitin-1-C^{14}. In experiments using a perfusion medium devoid of blood, it was observed that the rate of uptake of a neutral fat emulsion was stimulated in livers obtained from adrenalectomized rats. This increased rate of uptake was returned to the slower normal rate by addition of hydrocortisone to the medium *in vitro*. It appears from these experiments that hepatic lipid transport is sensitive to adrenal hormone action. The further relationship of these hormones to hepatic lipid synthesis and oxidation, and to the syndrome of fatty liver, is currently under investigation.

— 57 —

DRUGS AND LIPID TRANSPORT

M. Horning

— 58 —

POTENTIATION AND BLOCKADE OF SOME DRUG EFFECTS ON LIPID METABOLISM

S. E. Mayer

— 59 —

RESEARCH INTO FAT RANCIDITY BY GAS-LIQUID CHROMATOGRAPHY

P. A. T. Swoboda and C. H. Lea

Techniques will be described which are being used at the Low Temperature Research Station, Cambridge, England to analyse both qualitatively and quantitatively the volatile flavour constituents from autoxidised fats.

Initially a simple vacuum distillation technique using a short path cold finger type of pot still was developed for chemical assay of the total volatile carbonyls present, as an aid to organoleptic evaluation. Subsequently, following the development of the necessary sampling apparatus, gas chromatography is being applied to the separation and analysis of individual constituents.

Identification from retention measurements is confirmed by spectrophotometric and microchemical data on microgram fractions collected from the analytical gas chromatograph. Uultraviolet spectra and the spectra of thioarbituric acid and dinitrophenylhydrazine derivatives are used to characterise individual saturated and unsaturated carbonyl compounds. Chain length is established by micro-vapour phase hydrogenation, the hydrocarbon products from which are identified by gas chromatography.

Quantitative analysis requires rigorous checking of vacuum distillation recoveries, chromatographic artifacts and detector calibration. A simple and efficient total trapping technique has been developed for the quantitative collection of components eluted from the chromatograph, thus allowing independent chemical assay and flavour evaluation of individual components.

— 60 —

EFFECTS OF IONIZING RADIATIONS ON FATS. II. ACCUMULATION OF PEROXIDES AND OTHER CHEMICAL CHANGES

J. R. Chipault and G. R. Mizuno

The accumulation of peroxides, of carbonyl compounds and of reducing substances during irradiation and post-irradiation storage of pure fatty acid methyl esters have been studied.

The irradiation and storage of irradiated methyl myristate under vacuum results in formation of small quantities of these compounds. Irradiation under oxygen gives peroxides and carbonyl compounds in yields indicating that every ionization results in the formation of one mole of each group. The concentration of these compounds does not change during subsequent storage. The nature of the carbonyl and peroxidic groups is unknown. Antioxidants have no effect on the formation of these compounds during irradiation.

Irradiation of methyl linoleate under vacuum results in destruction of pre-formed hydroperoxides at a rate indicating that 1 mole of peroxide is destroyed for each three ionizations. Under oxygen, peroxides are formed with a G value of 21, indicating a chain reaction. Comparison of the formation of peroxides in methyl linoleate with that in methyl myristate indicate that approximately 1/8 of the peroxides formed during irradiation of methyl linoleate arise from the direct reaction of irradiation-induced free radicals with oxygen, while the rest is formed through a chain mechanism with an average chain length of 7.

Peroxides continue to increase in the irradiated methyl linoleate stored under oxygen and the rate of formation of these peroxides during storage increases with initial irradiation dose.

Antioxidants have some effect in retarding the formation of peroxides during irradiation of methyl linoleate and during post-irradiation storage, but the effect is small compared to their antioxigenic activity toward simple autoxidation. The effect varies with the nature of the antioxidant and with irradiation dose. Propyl gallate is much less effective than butylated hydroxyanisole and appears to be easily destroyed during irradiation.

— 61 —

PROOXIDANT EFFECT OF SOME CARBONYL COMPOUNDS IN VEGETABLE OILS

R. H. Anderson and T. E. Huntley

Certain carbonyl compounds which might be produced in browning degradations of sugars were found to act as prooxidants in

vegetable oils. Action of these compounds as prooxidants was apparent in oils held at 57° in open beakers but not in oils at 99° in the AOM Test.

— 62 —

A LIGHT TEST TO MEASURE STABILITY OF EDIBLE OILS

Helen A. Moser, C. D. Evans, and W. F. Kwolek

The effect of light on the flavor of edible oils and various food products is reviewed to show the importance of this factor in food studies and the need for a method of evaluation. Such a test, using fluorescent light in an easily assembled unit, has been developed and the parameters for its use were determined. Identical samples of soybean oil exposed on 10 different days and organoleptically evaluated show the method to be reproducible with a standard deviation of 0.79 using a scoring system of 1–10. This method was then applied to soybean, cottonseed, safflower, and hydrogenated-winterized soybean oils, and a light-exposure value was determined for each oil based on a comparison with accelerated storage procedures ordinarily used. Advantages of this light test are the short time required to complete the test, the reduction of variation by a controlled light source, the reproducibility of results, and the adaptability to related food products.

— 63 —

THE FLAVOR OF AUTOXIDIZED SOYBEAN OIL

F. D. Hill and E. G. Hammond

Commercial soybean oil was allowed to oxidize at room temperature in open flasks until "reverted" flavor developed. The flavor was concentrated by passing the oil through a Rota-a-film molecular still, the flavor being trapped in a condensor cooled with liquid nitrogen. It was not possible to remove all the flavor from the oil by this process. Since all of the flavors appear to be rather volatile compounds, this may be due to continuous generation of the flavor compounds during the distillation. Heating the oil as it passed through the still did not eliminate this problem. If enough heat was used to remove the autoxidation flavor, a heated flavor was generated. The contents of the liquid nitrogen trap consisted of a water layer and a small amount of "oil." The oily layer was diluted with petroleum ether and separated by gas chromatography on butanediol succinate columns. The flavor components were trapped in freshly deodorized soybean oil or in milk and tasted. On the basis of their retention times and flavors, the only significant flavor compounds in the oily layer were hexanal, vinylamyl ketone, and 2,6-nonadienal. The oily layer would not reproduce the original flavor of the autoxidized soybean oil. The aqueous layer, however, when added to freshly deodorized soybean oil did give the origianl flavor of the soybean oil. This layer was fractionated by gas chromatography and the fraction responsible for the autoxidized soybean oil flavor was found to travel at about the same rate as pentanal and hexanal on butanediol succinate columns. This important flavor compound is probably vinylethyl ketone.

— 64 —

RELATIONSHIP OF PEROXIDE VALUE AND THIOBARBITURIC ACID VALUE TO DEVELOPMENT OF UNDESIRABLE FLAVOR CHARACTERISTICS IN FATS

W. D. Pohle, R. L. Gregory, and B. Van Giessen

The peroxide value and thiobarbituric acid value were compared with the flavor score for a series of different types of fats, with and without added monoglyceride and with and without different stabilizers. The data indicated that the flavor score cannot be estimated from either the peroxide value or the thiobarbituric acid value. Either, can be used to follow the development of off flavors in a given product but the relative level may vary from product to product.

— 65 —

A STUDY OF METHODS FOR EVALUATION OF THE STABILITY OF FATS AND SHORTENINGS

W. D. Pohle, R. L. Gregory, T. J. Weiss, B. Van Giessen, J. R. Taylor, and J. J. Ahern

The data from several laboratory tests; AOM bomb, bomb with catalyst, Eckey and Schall Oven tests were compared with data from a storage test at 85F and organoleptic evaluation at selected intervals. Tests were carried out on lard, hydrogenated vegetable oil, and tallow, with and without added monoglyceride, and with and without selected antioxidants. A comparison of the data from the laboratory tests with that from the storage tests indicated; 1) that the different types of fats behave differently, 2) the laboratory tests cannot be used as an index of shelf stability except for a given type or formulation of fat when the relationship is known, 3) the bomb method is the most reliable for estimating shelf life, and 4) the bomb method with catalyst is less accurate than the bomb method, but may be used for control purposes where some accuracy may be sacrificed in the interest of shorter time.

— 66 —

SURFACE MIGRATION OF COBALT DRIERS IN PROTECTIVE COATINGS

J. H. Rolker, Jr.

An investigation was made of the vertical distribution of cobalt naphthenate present as a drier in films of vegetable oils and related polymers. The thickness of the films ranged from about 0.5–100 mil. The oils and polymers investigated included soybean, castor, linseed, dehydrated castor, blown linseed, heat bodied soybean and linseed, mineral oil, chemically modified soybean and linseed, urethane oils, vinyl toluene-oil and dicyclopentadiene-oil copolymers, and a water dispersible linseed oil based polymer. The analyses were made by determining the activity of Co^{60} Naphthenate tagged drier with a gas-flow proportional counter. Three separate types of experiments were performed. The first measured change in activity on drying of 4 mil, 100% solids polymers containing 0.06% Co. This study showed that there was, at most a 10% shift from a homogeneous distribution of cobalt. The second study compared the difference in activity between the top and bottom of cured polymer films of 0.8 mil and 8 mil thickness. These polymers contained 0.01% Co., 0.06% Co. or a mixture of 0.06% Co., 0.06% Pb and 0.03% Mn. Significantly more activity was found toward the top of the films. The presence of other driers made no significant difference. The third study examined the relationship of activity to sample thickness with a range of thicknesses for each polymer. An 0.06% Co. level was used. A substantial orientation of cobalt toward the surface was observed.

— 67 —

NOVEL URETHANE OILS

A. E. Rheineck and Sol Shulman

The formation of film forming polymers utilizing the reaction of the isocyanate group with compounds containing active hydrogen. especially hydroxyl, is one of the fastest growing developments in coatings.

Usually urethane coatings are prepared by the reaction of an aromatic diisocyanate (or polyisocyanate) and an aliphatic polyol.

In this study unsaturated fatty isocyanates were used with polyols, such as glycerol, pentaerythritol, etc. and the properties of the urethanes were compared with the corresponding esters.

Unsaturated isocyanates were prepared by two routes. One route involved the preparation of nitriles from unsaturated fatty acids and ammonia, followed by catalylic reduction of the nitrile to the unsaturated amine with subsequent conversion of the amine to the isocyanate using the well known phosgene method.

The other method consisted of conversion of the fatty acid to the acid chloride followed by reaction with sodium azide to produce the isocyanate where, in contrast to the isocyanate produced by the phosgene method, the olefinic chain possessed one carbon atom less than the fatty acid. Isocyanates based on linseed, safflower, and tall oil were thus obtained.

— 68 —

EVALUATION OF FATTY VINYL ETHER POLYMERS AND STYRENATED POLYMERS FOR METAL COATINGS

B. G. Brand, H. O. Schoen, L. E. Gast, and J. C. Cowan

A further evaluation of our fatty vinyl ether polymers as potential coatings for metals (particularly cans) includes studies on 12 linseed and soybean polymers including terpolymers with cyclopentadiene and styrenated derivatives. Films of these polymers were baked with and without driers and examined for flexibility, adhesion, chemical resistance, and hardness. Film properties were related to chemical composition of the polymers including the effect of styrenation, linseed versus soybean polymers, and content of cyclopentadiene. Films possessed excellent flexibility, adhesion, and hardness. Surprisingly, for all the polymers, the hardest films were obtained without driers.

— 69 —

THE RELATIONSHIP BETWEEN "GAS-PROOFNESS" AND STRUCTURE IN TUNG OIL

A. E. Rheineck and S. C. S. Peng

When tung oil is heat processed to make it "gas-proof," it was observed that the conjugated triene structure diminished as measured by the ultra-violet absorption characteristics. It appeared that at least less than 15% residual triene is necessary to have only the oil completely gas-proof. This low triene content is difficult to achieve without gelation if the oil is processed without resins.

Whether tung oil is gas-proof or not is related to film thickness, solvents and driers, as well as the method used to determine gas-proofness, e.g. turpentine with .01% Co and .1% Pb produced smooth films in air, whereas cellosolve produced wrinkled films. The method for determining gas-proofness is important.

During the heat processing, the presence of a conjugated diene, as an intermediate, was difficult to detect. If present, its life was short. The double bonds in the product exist as a nonconjugated diene. They are sufficiently far apart to make the shift to the conjugated structure impossible.

A series of structures for cyclic dimers which might be formed were postulated. Higher polymers are possible also. Ozonolysis degradation products indicated that a number of different structures are possibly formed in the gas-proofing reaction.

— 70 —

PREPARATION AND PROPERTIES OF SOME WATER-DISPERSIBLE INDUSTRIAL COATINGS FROM TUNG OIL

D. L. Wood, L. L. Hopper, Jr., and F. G. Dollear

Tung oil and tung oil methyl esters can be utilized in a water-dispersible resin by first adducting with acrylic acid and then cooking into an alkyd with polyethylene glycol, a triol and phthalic anhydride. These resins are readily dispersible in water without the use of special blenders or mixers and without the need of solubilizing agents, buffers or emulsifiers. They can be diluted easily without

separation. The tung dispersions are quite stable on aging, even during freeze-thaw cycles.

With the addition of a melamine-formaldehyde resin, usually about 30% based on resin solids, the tung oil or tung methyl ester coatings can be baked for industrial uses, such as automotive and appliance coatings.

As metal primers, the water-dispersible tung coatings exhibit good hardness, adhesion and flexibility. They are resistant to water, salt water, detergent, mineral spirits and gasoline. They give better adhesion and flexibility to commercial topcoats.

As a topcoat, the tung methyl ester enamel exhibits good gloss, hardness, adhesion, flexibility and chemical resistance. However, discoloring appears in baking and this problem must first be solved in order for use in white enamels.

— 71 —

SOME PROPERTIES OF THE GLYCEROL ESTERS OF THE ISOLATED AND CONJUGATED CIS, TRANS ISOMERS OF LINOLEIC ACID

A. E. Rheineck and D. D. Zimmerman

A series of glycerol triesters of the various geometric isomers of fatty acids high in linoleic acid were prepared and studied for rate of drying, after-tack, rate of oxidation and polymerization characteristics.

The acids derived from safflower oil have the isolated *cis, cis* structure. These were isomerized to the conjugated *cis, trans* by the alkali method. Re-esterification with glycerol showed no appreciable change in isomeric structure of the esters. The conjugated *trans, trans* oils were prepared by iodine isomerization of the *cis, trans* compound. The conjugated *trans, trans* compounds polymerize more readily than the conjugated *cis, trans*. Little or no difference was found in the drying rate of these two conjugated structures.

The isolated *trans, trans* oil was prepared by treatment of safflower oil with selenium or nitrous acid.

Drying time studies showed that oils with a high content of conjugated or isolated double bonds dried at a slower rate than blends of these two types. Thus, a minimum dry time occurred with mixtures containing 35% ± 5% of either conjugated *trans, trans* or *cis, trans* oils with the remainder being isolated *cis* oils. This is related to the faster oxygen uptake and peroxide formation of the *cis* structure which then is a peroxide initiator for vinyl type polymerization.

The drying rates of *trans* oils are directly proportional to the *trans* content.

— 72 —

PHOSPHATIDYLSERINE-BOVINE SERUM ALBUMIN INTERACTIONS

D. G. Therriault and J. F. Taylor

Interaction of bovine serum albumin with phosphatidylserine was studied by turbidimetric methods. It was found that bovine serum albumin will bind phosphatidylserine. Analysis by the law of mass action for multiple equilibrium has led to the conclusion that bovine serum albumin possesses a heterogeneity of binding sites for phosphatidylserine. The maximum number of sites and the respective affinity constants were calculated to be: $k_1 = 1.96 \times 10^4$, $n_1 = 2$; $k_2 = 3.9 \times 10^2$, $n_2 = 31$.

It was proposed that the different binding sites in bovine serum albumin may be attributed to changes in the tertiary or secondary structures of albumin initiated by the binding of phosphatidylserine.

The observation was made that removal of fatty acids from crystalline bovine serum albumin resulted in the appearance of a substance with sedimentation rate similar to that of the albumin dimer, $S_{20\,w} = 6.41S$. Even though extraction of the lipid did not affect the —SH content of bovine serum albumin, the dimerization could be reversed by treatment with cysteine. This treatment resulted in a product which was free of lipid, with the same centrifugal and electrophoretic characteristics as native crystalline bovine serum albumin.

— 73 —

BINDING OF STEROIDS TO PROTEINS

U. Westphal

Steroid compounds form dissociable complexes of low binding energy with numerous proteins of different origin as can be demonstrated by various physiochemical procedures. This interaction has definite physiological consequences in case of the steroid hormones. The sites of attachment between Δ^4-3-ketosteroids and human serum albumin appear to be located at the alpha side of the steroid molecule. The affinity of interaction with serum albumins is increased by entrance of electron-repelling groups (alkyl) into the steroid, and decreased by electron-attracting groups (—OH; =O; halogen) ("polarity rule"). This rule is reversed in interactions with certain proteins which have a higher content of aliphatic hydroxyl groups. It was concluded from competition studies with higher fatty acids that the attachment of Δ_4-3-ketosteroids to serum albumin does not take place at the anion-binding sites. The SH-group of serum albumin is not involved in the interaction with testosterone.

The α_1-acid glycoprotein (orosomucoid) from human serum was found to have a particularly high binding affinity for progesterone. Removal of sialic acid results in a decrease of this binding affinity. Complex formation with the orosomucoid leads to physiological inactivation of progesterone.

A highly specific interaction occurs between the adrenocorticoid hormones and the corticosteroid-binding globulin (transcortin) in serum of human and other species. For a quantitative test, the endogenous corticosteroids have to be removed by dialysis at 37C. Cortisol, corticosterone and related hormones are bound by transcortin; aldosterone interacts with serum and transcortin-containing fractions more strongly than with albumin. The "transcortin" activity of rat serum increases after adrenalectomy and hypophysectomy; injection of corticosterone into adrenalectomized rats reverses this effect. The general increase of the total α-globulin fraction in adrenalectomized rats (15–16%) is smaller than the increase in "transcortin" activity (100%). The corticosteroid-binding serum proteins of different mammalian species (rat, rabbit, steer, horse) were found to be α-globulins. Their binding affinities towards different corticosteroids will be discussed.

— 74 —

COMPOSITION AND FUNCTIONAL ROLE OF SOME LIPOPROTEINS OF INDUSTRIAL SIGNIFICANCE

J. D. Mullen and D. E. Smith

Eggs, milk and wheat, which constitutes a major portion of our food supply, contain lipoproteins which determine many characteristic properties of these foods. Current knowledge of the physical-chemical structures of these lipoproteins of egg yolk, milk fat membranes and wheat gluten are summarized. The relations between lipoprotein structure and industrial function are considered. This includes a discussion of structure modification by physical and chemical treatments.

Chemical additives are being used in this laboratory to study the mechanisms involved in determining rheological properties of wheat flour dough. Examples are described whereby flour dough with very poor rheological properties (*i.e.*, unsuitable for commercial bread manufacture) is markedly improved by addition of low levels of certain sulfated polysaccharides or surface active agents. (These agents have found considerable use in the study and isolation of blood and mitochondrial lipoprotein systems). The complexes between these improving reagents and the gluten lipoproteins of the flour dough are readily studied by moving boundary electrophoresis. These procedures and results are described.

— 75 —

EVALUATION OF BUTYL STEARATE, BUTYL OLEATE, BUTYL RICINOLEATE AND METHYL LINOLEATE AS POLY (VINYL CHLORIDE) PLASTICIZERS

G. R. Riser, F. W. Bloom and L. P. Witnauer

An investigation of butyl stearate, butyl oleate, butyl ricinoleate and methyl linoleate was made to determine the extent of their compatibility with poly(vinyl chloride) and their plasticizing effects on the physical properties of the resulting molded sheets. These monoesters were evaluated as the sole plasticizer and in combination with DOP. The results showed the materials to have limited compatibility in general, being less than 10% of the total mix. In combination with DOP these compounds were found to improve the color, low temperature flexibility and heat stability of the molded poly(vinyl chloride) sheets over those containing DOP as the sole plasticizer. Tensile strength, percent elongation and light stability properties were essentially comparable to those found for pure DOP.

— 76 —

THE PREPARATION AND PLASTICIZING CHARACTERISTICS, THE PIPERIDIDES OF LONG-CHAIN FATTY ACIDS AND THE FATTY AMIDES OF OTHER CYCLIC IMINES

R. R. Mod, F. C. Magne, and E. L. Skau

Forty-eight N-acyl derivatives of cyclic imines have been prepared, characterized and screened as plasticizers for poly(vinyl chloride-vinyl acetate) copolymer. Among these were the piperidides of decanoic, palmitic, stearic, oleic, erucic, ricinoleic, epoxystearic, epoxyoleic, diepoxystearic, sebacic, pinic, cottonseed, hydrogenated cottonseed, rapeseed, *Limnanthes douglasii*, animal, 2-ethylhexanoic, naphthenic and dimer acids, as well as the N-oleoyl derivatives of a number of substituted piperidides and other cyclic imines including pyrrolidine, piperazine, cyclohexylenimine, tetrahydroquinoline, 3-azabicyclo[3.2.2]nonane, dipyridylamine, and carbazole.

In general these amides of the cyclic imines exhibited exceptionally high plasticizing efficiencies. The compatibilities observed were the best to date for fatty acid derivatives on the basis of both individual and ternary fatty acid composition-compatibility data. Several of these amides exhibited low-temperature characteristics in the adipate plasticizer range without the adverse volatility characteristics of the adipates. There are indications that some of these amides have appreciable antifungal activity and also show some promise as nitrile rubber softeners. It has been concluded that cyclic imines will in general produce fatty acid derivatives of better than average compatibility as vinyl plasticizers.

— 77 —

PREPARATION OF ALCOHOLS FROM FATTY CYCLIC ACIDS

E. W. Bell, J. P. Friedrich, L. E. Gast, and J. C. Cowan

Saturated C_{18} and C_{20} cyclic alcohols have been prepared by catalytic hydrogenation of methyl esters of the following: linseed cyclic monomeric acids, pure C_{18} saturated cyclic acids, ethylene adduct of conjugated soybean fatty acids, and ethylene adduct of conjugated octadecadienoic acids. The conversion of ester to alcohol was 84–97%, based on hydroxyl determination. Gas-

(Continued on page 30)

Abstracts of Papers . .

(Continued from page 28)

liquid chromatography showed the conversion of ester to alcohol to be almost quantitative. The hydrogenations were carried out by using 10%, by weight, copper chromite catalyst, initial hydrogen pressure of 2,100 psi, and 3 hr at 280C.

Preliminary evaluations indicate that C_{18} and C_{20} saturated cyclic alcohols have potential in cosmetic formulations.

— 78 —

ACID-CATALYZED CONVERSION OF EPOXYESTERS TO HYDROXYESTERS

G. Maerker, E. T. Haeberer, and W. C. Ault

Esters of 9,10-epoxystearic acid (epoxidized oleic acid), dissolved in 1,4-dioxane, were treated at room temperature or below, first with aqueous acid and then with water to convert them to 9,10-dihydroxystearates in high yields. Ester functions remained intact. Glycidyl 9,10-epoxystearate, ethylene glycol bis-(9,10-epoxystearate) and catechol bis-(9,10-epoxystearate) were converted to the corresponding tetrahydroxy esters by this method.

Hydration of methyl epoxystearate with concentrated sulfuric acid at room temperature led to the formation of considerable amounts of by-products, principally methyl 9(10)-ketostearate. Side reactions were inhibited by diluting the acid catalyst with water.

Treatment of methyl 9,10-epoxystearate wtih diluted (24%) fluoboric acid gave methyl 9,10-dihydroxystearate in 88% yield. Under similar conditions glycidyl stearate did not react, and only one oxirane function of glycidyl epoxystearate was converted to the glycol. An explanation is offered, based on different hydration paths available, to account for the difference in reactivity of glycidyl esters and internal epoxides toward dilute acids.

— 79 —

REACTION OF DICHLOROCARBENE WITH FATS AND OILS

H. E. Kenney, Daria Komanowsky, and A. N. Wrigley

Dichlorocarbene was generated in the presence of lard, olive, safflower, tung, and menhaden oil. When solutions of fats or oils in ethyl trichloroacetate were mixed with sodium methoxide in n-heptane at 2C, unsaturated components were transformed in high conversion to dichlorocyclopropane derivatives; fats were converted to methyl-and-ethyl esters (90%) or remained as glycerides (10%). The proportion of dichlorocyclopropane rings was the same in the glycerides as in the ester products. The reactivity of safflower oil required metered addition of reactants at 25–55C.

Properties were measured on dichlorocyclopropane fatty esters freed from glycerides but containing saturated esters. Chlorine content ranged from 12–33%. Compatibility with silicone oils was substantially improved. Viscosities and densities increased with chlorine content. Viscosity indices were 135, 150, 49, 67, and 79 for products from lard, olive, safflower, tung, and menhaden oil.

Dichlorocarbene could be generated without alcoholysis of glycerides by decomposition of sodium trichloroacetate. This gave dichlorocyclopropanes from safflower oil unsaturates in 50% conversion, but failed with lard.

Analyses were performed by thin-layer, argentation, and gas-liquid chromatography.

— 80 —

METHANESULFONIC ACID-CATALYZED ADDITIONS. IV. ADDITIONS TO METHYL LINOLEATE

Abner Eisner, Theodore Perlstein, and W. C. Ault

The study of the methanesulfonic acid-catalyzed addition of nucleophiles to olefinic bonds has been extended to include linoleic acid. A number of the substances (phenols, phenyl ethers, and arylthiols) that have been successfully added to oleic acid and cyclohexane were tried similarly in an investigation with methyl linoleate. These include: phenol, o-chlorophenol, 2-naphthol, resorcinol, methyl salicylate, anisole, phenetole, p-toluenethiol, p-chlorobenzenethiol, and 2-naphthalenethiol. The additions to methyl linoleate in the methanesulfonic acid catalyst-solvent medium take place as readily as they did with oleic acid and good yields of products were obtained. The recovery of the products however, is complicated by the presence of poly-addition products and polymeric material along with the monoaddition products.

As observed previously in the oleic acid studies, evidence for the formation of ether intermediates was obtained. Infrared study of chromatographically separable fractions of the products of the reaction resulting from the addition of 2-naphthol to methyl linoleate revealed the presence of a naphthoxy addition product as well as the expected hydroxy-naphthyl derivatives. As before, the only identified products from the arylthiol additions were thioethers.

(Continued on page 33)

Abstracts of Papers . . .

(Continued from page 30)

— 81 —

PURIFICATION OF ALDEHYDES FROM SOYBEAN OIL BY BASE EXCHANGE RESINS

R. E. Beal

Self-polymerization of aldehydes, prepared on a pilot-plant scale in stainless-steel equipment from soybean oil or its methyl esters by ozonolysis and chemical or catalytic reduction, is substantially reduced when the mixed aldehydes are treated with a hydrogen-form cation resin. Such polymerization, which darkens the aldehydes and increases their viscosity and molecular weight, occurs slowly at room temperature and rapidly at high temperatures.

Triglyceride aldehydes prepared from soybean oil are steam stripped under vacuum at temperatures up to 100C to remove volatile byproducts. Treatment with cation resin before stripping reduced the Gardner color of the stripped aldehyde product from 18 to 10 with an accompanying decrease in viscosity.

Methyl azelaaldehydate (MAZ) made from soybean methyl esters was distilled under vacuum to effect purification. Resin treatment increased distillation yields. Addition of inorganic salts to MAZ followed by distillation indicated that iron, zinc, and other cations catalyze polymer formation to varying degrees.

— 82 —

PREPARATION OF MALONDIALDEHYDE TETRAMETHYL ACETAL BY OZONOLYSIS OF POLYUNSATURATED FATTY ACIDS

P. Fitton, E. H. Pryde, and J. C. Cowan

Malondialdehyde has been prepared, as its tetramethyl acetal, as one of the major products from the ozonolysis of fatty acids that contain a 1,4-diene group. Esters of linseed fatty acids that contained 13.3% methyl linoleate and 51.1% methyl linolenate were ozonized at 0° in methanol, and the products were reduced with Lindlar catalyst and hydrogen. A catalytic amount of acid was added to facilitate acetal formation, and malondialdehyde tetramethyl acetal was isolated in 70% yield. It was contaminated with a small amount of caproaldehyde dimethyl acetal. Methyl azelaaldehydate dimethyl acetal was obtained as the major product in over 90% yield.

— 83 —

RECENT TRENDS IN HYDROGEN PLANT TECHNOLOGY

B. J. Mayland, R. L. Harvin, and C. R. Trimarke

Catalytic processes for producing hydrogen for the vegetable oil industry and other industries have been in commercial use since the early thirties. They supply the major portion of the hydrogen by the hydrocarbon-steam reforming route for the generation with associated steps for purification. This paper outlines the present status of the hydrocarbon-steam reforming process as practiced in the United States. Recent trends and developments in design, engineering and operations are reviewed with emphasis on reliability and safety of the operation.

— 84 —

HOMOGENEOUS CATALYTIC HYDROGENATION OF UNSATURATED FATS: COBALT CARBONYL

E. N. Frankel, V. L. Davison, E. Emken, and H. J. Dutton

Homogeneous hydrogenation of unsaturated fats by cobalt carbonyl has been compared with the previously reported catalyses by iron and manganese carbonyls. Soybean methyl esters, methyl linoleate, and linolenate have been treated at 75–150C, hydrogen pressures of 500 to 3,000 psi, and 0.02 molar concentration of catalyst. Little hydrogenation occurred at hydrogen pressures below 1,000 psi. Some decomposition of the catalyst was observed at temperatures above 100C as evidenced by the formation of insoluble metallic cobalt. However, the catalytic hydrogenation is still considered homogeneous since any heterogeneous catalysis from finely divided cobalt would be inactivated by carbon monoxide evolved from the decomposition.

The partially hydrogenated products were analyzed by gas-liquid chromatography (packed and capillary columns), infrared and ultraviolet spectroscopy, countercurrent distribution, thin-layer and silver-nitrate ion-exchange chromatography, and oxidative cleavage. The products of hydrogenation are similar to those observed with iron and manganese carbonyls, but the reaction differs in showing much less accumulation of conjugated dienes, less selectivity toward linolenate, complete absence of monoene hydrogenation to saturates, less double bond migration, and more *trans* isomerization. No evidence was found for a stable complex between cobalt carbonyl and unsaturated fats as previously found with iron carbonyl.

The greater activity of the cobalt carbonyl catalyst at lower temperatures could be attributed to the lower stability of the active intermediate cobalt hydrocarbonyl and its complexes with unsaturated fats.

— 85 —

DEUTERIUM TRACER STUDIES OF THE MECHANISM OF HOMOGENEOUS CATALYTIC HYDROGENATION OF SORBIC ACID WITH PENTACYANOCOBALTATE II

A. F. Mabrouk, E. Selke, W. K. Rohwedder, and H. J. Dutton

Exchange of deuterium and hydrogen during homogeneous catalytic reduction of sorbic acid with pentacyanocobaltate II has

been investigated in three types of experiments: 1) Isotopic exchange between D_2 and H_2O, 2) H_2-D_2O exchange, and 3) D_2-anhydrous methanol exchange. In contrast to experiments with heterogeneous catalysts, where complete exchange and equilibration occur readily (W. K. Rohwedder, et al., JOACS, in press), mass spectrometric analysis of the gas phase above the pentacyanocobaltate II shows slow, incomplete exchange during the course of reduction of either catalyst alone or catalyst and substrate.

Mass spectra of methyl hexenoates from the deuterium exchange experiments have been examined. The cracking patterns of these esters were compared with those reduced with hydrogen and with authentic 2-, 3- and 4-hexenoates. During catalytic reduction of sorbic acid in either aqueous or methanolic solutions, no exchange of deuterium in gas or solvent for carbon-bonded hydrogen was evident. Little or no exchange occurred with the hydrogen of valeric or sorbic acids in the presence of pentacyanocobaltate deuteride ion $[Co(CN)_5D]^{3-}$ and deuterium oxide.

Results are in accordance with the sequence of reactions reported earlier (A. F. Mabrouk, et al., submitted to JAOCS) for the homogeneous catalytic hydrogenation of sorbic acid with $[Co(CN)_5]^{3-}$ and with the theory that H_2 or D_2 saturating the double bond is that present in either $[Co(CN)_5H]^{3-}$ or $[Co(CN)_5D]^{3-}$ formed during preparation of catalyst and in its "aging" reaction.

— 86 —

THE EFFECT ON MARGARINE TEXTURE OF CRYSTALLIZING THE FAT IN THE DISPERSE PHASE

H. Lavery and H. B. Oakley

In general the rheological behaviour of butter and margarine is different and there are a number of reasons why this difference exists.

One possible reason is the fact that butterfat is crystallized in the disperse phase whereas margarine is usually crystallized in the bulk phase. The influence which fat crystallization in the disperse phase has on the texture of margarine forms the subject matter of this paper.

Thre separate methods of crystallizing margarine fat in the disperse phase are described: 1) Spraying fat on to cold water, 2) Spraying fat in a cold atmosphere, and 3) Chilling a concentrated oil-in-water emulsion followed by phase inversion.

The rheological behaviour of margarines prepared by these processes is shown to be very similar to that of butter.

— 87 —

SEEDING OF SOME MELTED FATS BY MECHANICAL WORKING

N. V. Lovegren, B. Bradshaw, and R. O. Feuge

The tempering of fat products to convert their components to stable polymorphs is an important and sometimes troublesome problem of practical importance in the manufacture of fat and fat-containing products, particularly chocolate and chocolate-type confections. It has been found that tempering may be accomplished by intense mechanical working of the rapidly-chilled, supercooled liquid fat, either alone or in combination with other materials.

Mechanical working can be accomplished in a low pressure homogenizer. Under proper conditions sufficient amounts of the melted fat are converted by the mechanical working to numerous, minute crystals of the highest melting polymorph. These act as seed crystals for the rapid conversion of the remainder of the fat to the highest melting form on solidification. Best results are obtained when the temperature of the supercooled liquid fat is near the melting point of the alpha-polymorph. With cocoa butter near this temperature, usually two passes through a low pressure homogenizer are sufficient to produce the required seed crystals. The maximum permissible temperature approaches the melting point of the highest-melting polymorph. Very little seed (1% or less by weight) is necessary for cocoa butter to solidify rapidly to the stable form as shown by X-ray diffraction patterns.

The process for developing seed crystals by mechanical working of melted fats may be used with milk chocolate, chocolate liquor, borneo tallow, and even a high-melting fat such as hydrogenated pecan oil, which is essentially tristearin.

— 88 —

VERNONIA ANTHELMINTICA (L.) WILLD. THE EFFECT OF STORAGE ON THE EPOXY LIPID COMPONENTS OF THE SEED OIL

W. E. Scott and C. F. Krewson

Accessions of *Vernonia anthelmintica* (L.) Willd. seed from India and Pakistan varied somewhat in the amount of oil that they contained, but the oils did not vary significantly in their epoxy content. Storage of the whole seed for periods up to 3 years did not affect the quality of the oil or the activity of the seed enzyme system. The epoxy lipid components of low FFA *Vernonia* oil and trivernolin were not affected by storage at 100C for 6 months when the products were sealed under nitrogen. Addition of a stabilizer also prevented loss of oxirane oxygen from trivernolin but not from the oil. When stored at room temperature, exposure to light lowered slightly the epoxy content of both the oil and the trivernolin samples that were not sealed under nitrogen.

— 89 —

SOYBEAN UNSAPONIFIABLES: HYDROCARBONS FROM DEODORIZER CONDENSATES

C. D. Evans, J. Oswald, and J. C. Cowan

Molecular distillation of deodorizer condensates, followed by chromatography on alumina, gave substantial quantities of hydrocarbons free of other unsaponifiable constituents. Squalene comprised 50% of the hydrocarbon fraction and contained practically all the unsaturation. A crystalline, saturated hydrocarbon fraction of 4.2% was composed primarily of C_{29} and C_{31} paraffins. An unresolved fraction was composed of two major components, each estimated to contain about 30 or 35 carbon atoms. Minor amounts of many hydrocarbons with chain lengths of 15–35 carbon atoms were present but not completely identified.

— 90 —

CONTINUOUS REFINING OF CRUDE COTTONSEED MISCELLA

L. S. Crauer and H. Pennington

A system for continuous refining of crude cottonseed miscella in a two stage process is described. The effects of crude oil quality, oil:hexane ratios, temperature, mixing conditions, and chemical treatment on process control are noted. The chemical reactions in the process are followed microscopically. Comparative yields and finished oil quality data are given.

— 91 —

THE VARIABILITY OF LYSINE AND AVAILABLE LYSINE IN AND NUTRITIVE QUALITY OF COMMERCIAL COTTONSEED MEALS

Fairie Lyn Carter and V. L. Frampton

Total and available lysine in commercial cottonseed meals both vary widely, as does the protein quality. Total lysine (expressed as grams of lysine per 16 grams of nitrogen) in commercial meals varies from 3.5–4.2, while the available lysine varies from 2.2–3.75. The variance between meals produced by screw press, prepress solvent extraction and solvent extraction is no greater than the variance among meals produced by the same methods. That is, the differences in these two variables between meals produced by the three methods is no greater than the differences among meals produced by the methods. Available lysine is important because nonruminant animals can only use available lysine—lysine that is bound is not used by the animal. By the way of contrast, meals produced from raw cottonseed by solvent extraction in the absence of heat have total and available lysine levels of about 4.2 grams per 16 grams of nitrogen. Protein efficiency ratios determined for commercially produced cottonseed meals are invariably less than 2,0, while the ratios determined for meals produced on the extraction of raw cottonseed with a solvent mixture composed of acetone, hexane, and water invariably have been greater than 2.0.

— 92 —

MUSTARD SEED PROCESSING: SIMPLE METHODS FOR FOLLOWING HEAT DAMAGE TO PROTEIN MEALS

J. E. McGhee, L. D. Kirk, and G. C. Mustakas

Processed mustard seed contains a considerably higher content of reducing sugar than other oilseeds. During processing, this natural reducing sugar is supplemented with glucose released by enzyme hydrolysis of the thioglucoside, and the total content reaches a value of 3% of the defatted mustard meal. This quantity of reducing sugar in mustard seed is three times more than that in soybean meal. Consequently the browning reaction, which degrades protein, presents a greater problem in processing oilseeds containing thioglucosides than oilseeds free of thioglucosides. In developing the processing of mustard seed, the degree of heat treatment given the protein meals had to be determined. Several indirect methods were developed for following the effects of heat treatment on protein quality. Nitrogen solubility index, optical density of aqueous extracts, and reducing sugar content correlated well with degradation of heat-labile amino acids, such as lysine, arginine, and histidine, and gave an index of protein quality.

— 93 —

VERNONIA ANTHELMINTICA (L.) WILLD. EXTRACTION OF OIL OR TRIVERNOLIN FROM THE SEED

C. F. Krewson and W. E. Scott

Agricultural scientists are searching for new cash crops for the American farmer to grow mainly in relief or replacement of those now in surplus. This search is beginning to yield real, although modest, hopes and results. Developmental research on *V. anthelmintica* (ironweed), an import from Pakistan and India, is a part of this program. The seed of this species bears a unique oil, 22–28%, rich in a single component, trivernolin, the triglyceride of 12,13-epoxyoleic acid, 55–60% of the weight of the oil. Optimum conditions for the production of this oil or of trivernolin were developed which consisted of controlling the high lipolytic enzyme activity which followed grinding or flaking the seed. This control was achieved either by a rapid extraction technique in which whole seed was flaked as a slurry with the seed and extracting solvent, or by autoclaving the whole seed prior to flaking. Trivernolin was obtained as a major end-product

(Continued on page 40)

Abstracts of Papers . . .

(Continued from page 36)

without isolation of the oil. Cold solvent procedures appeared to offer advantages over hot extraction techniques. In the temperature range of 4 to 8C the yield of oil was as high as with boiling (39–59C) solvent (petroleum naphtha) and lighter colored oils were produced. The lower temperatures also discouraged lipolytic activity and made it unnecessary to resort to extremely rapid extraction procedures. An improved quality *Vernonia* oil was produced by removal of the unsaponifiable material (about 10%); this upgraded the epoxy lipid content and reduced color and odor. *Vernonia* oil and trivernolin produced as will be described has been distributed to a number of industrial and educational agencies for evaluation in a variety of fields. In the 1963 growing season a total of about 50 acres were planted to *Vernonia* seed in this country in 24 locations to learn more about how to grow this seed domestically for oil production.

— 94 —

PRODUCTION AND NUTRITIONAL EVALUATION OF EXTRUSION-COOKED FULL-FAT SOYBEAN FLOUR

G. C. Mustakas, E. L. Griffin, Jr., L. E. Allen, and O. B. Smith

A process to prepare full-fat soybean flours for human consumption on a commercial scale by high-temperature, short-time extrusion cooking was studied. Biological evaluations were made of samples produced experimentally by this process to determine the best conditions for preparing a flour of maximum nutritive value. Twelve full-fat soybean flours, processed under different combinations of time, temperature, and moisture conditions, were evaluated by chemical analyses, biological assays, available lysine content, organoleptic and bacteriological tests, and oxidative stability storage tests. Flour of high nutritive value and good stability can be prepared by preheating unextracted soybean flakes or grits to 200–210F, premixing and adding sparge steam at 212F to adjust moisture to 16–21%, and using an extruder holding time of 1 to 1½ min with final extrusion temperature reaching 242–290F, followed by cooling, drying, and grinding. Clinical testing of the product with infants 4–12 months of age on a fairly extensive scale has begun in two Far Eastern countries under sponsorship of UNICEF, and the completed results of the clinical studies should be available by late 1964.

— 95 —

GENETIC CONTROL OF FATTY ACID BIOSYNTHESIS IN RAPESEED (BRASSICA NAPUS)

R. K. Downey and B. M. Craig

Selection and isolation of rapeseed plants, the oil of which contained no erucic acid also resulted in a simultaneous selection for low to zero eicosenoic acid. Genetic analysis of the F_2 and F_3 populations indicated that the production of these fatty acids was controlled by two gene pairs. The reduction of erucic and eicosenoic acids is compensated primarily by an increase in oleic acid without a corresponding decrease in oil content. A logical explanation for this phenomenon is a genetically controlled chain lengthening mechanism for the production of eicosenoic and erucic acids with oleic acid as the immediate precursor.

— 96 —

GAS CHROMATOGRAPHIC DETERMINATION OF RESIDUAL HEXANE IN HEXANE-EXTRACTED OILSEED FLAKES

L. T. Black and G. C. Mustakas

A method was developed to determine residual hexane in solvent-extracted oilseed flakes by gas liquid chromatography (GLC). After residual hexane was extracted with pure iso-octane, the quantity extracted was determined by GLC. Analyses were run on three different columns. Each of these contained a stationary phase of a different polarity. Column efficiency was also varied to obtain maximum speed and accuracy. The attenuator was used to amplify the low signal output of the extracted residual hexane.

Detector response was checked against samples containing iso-octane and commercial normal hexane of known proportions. The results showed detector response for hexane and iso-octane nearly equal.

Accuracy of the method was established by analyzing samples of soybean flakes containing 0.01–7.5% hexane. The lower limit of accuracy of this method is approximately 0.02%, and the time required for analysis is relatively short.

Other extraction solvents that are infinitely soluble in iso-octane can be analyzed by this procedure.

— 97 —

DETERMINATION OF FOREIGN MATERIALS OF PLANT ORIGIN IN COTTON LINTERS

K. A. Jurbergs and D. J. Dowling

The present article describes a test procedure which can be used for determination of foreign materials mainly of plant origin in raw cotton linters. Laboratories that perform the cellulose yield test require very little additional equipment to carry out this test. A portion of the dry cellulose sample, remaining after the completion of the standard AOCS cellulose yield test, is bleached, formed into a hand sheet, and the total projected area of the visible dirts is determined on both sides of the hand sheet. The determination of the dirts is patterned according to principles used in Technical Association of Pulp and Paper Industries Standard Procedures T213 M-43 and T437 M-43.

There are three main groups of foreign materials encountered: stalks, cockle burs, and cotton seed hulls. The numbers and distribution of these particles vary with the general geographic locality and individual shipments of cotton linters. The test procedure described considers only such dirt particles which survive the major purification steps in the manufacturing of pulp, and are undesirable from the quality point of view of the finished product.

— 98 —

HIDDEN OIL LOSS IN THE COTTONSEED CRUSHING INDUSTRY

Walter Szutowicz

Hidden oil losses are known to exist in the cottonseed crushing industry. The condition is revealed by discrepancies between high laboratory determinations for oil in the seed and the corresponding lower production figures in the oil mill itself. Using two different experimental approaches it was found that the answer to the problem lies in fixation or binding of the oil in the meal. The experiments are described and a preliminary evaluation of the results is given.

— 99 —

STUDIES OF THE PHOSPHOLIPID-GLYCOLIPID COMPLEX OF WHEAT ENDOSPERM

Mary E. McKillican

The phospholipid-glycolipid complex was isolated chromatographically from the "free" (hexane soluble) and "bound" (hexane resistant, water saturated n-butanol extractable) lipids of wheat endosperm. The individual phopholipid and glycolipid components of the complex were separated by column and thin-layer silicic acid chromatography. Further identification was made by hydrolysis followed by thin-layer or paper chromatography. The fatty acid and sterol composition of the components was studied by means of gas-liquid chromatography.

A hitherto unreported sterol-containing glycolipid and a comparison of fatty acid composition and sterol composition of the phospholipid and glycolipid components are reported.

— 100 —

COMPETITIVE INHIBITION IN THE METABOLISM OF POLYUNSATURATED ACIDS

R. T. Holman

In fat deficiency the synthesis of 5,8,11-eicosatrienoic acid is increased, and this acid is a predominant polyunsaturated acid in tissue. When linoleate, linolenate, arachidonate, eicosapentaenoate or hexaenoate is fed, the synthesis of 5,8,11-eicosatrienoate is suppressed, and other polyunsaturated acids may be synthesized from these. When linolenate is fed, the synthesis of arachidonate from linoleate is suppressed and when linoleate is fed, the synthesis of polyunsaturates from linolenate is suppressed. These several observations indicate that the synthesis of polyunsaturated acids of one family is inhibited competitively by the presence of members of other families in the dietary fat. Thus, the pattern of polyunsaturated acids in tissues is regulated by the composition of the dietary fat and competitive inhibition in the metabolism of polyunsaturated acids.

— 101 —

INFLUENCE OF EFA-DEFICIENCY, EFA AND MARINE OIL SUPPLEMENTS ON THE HYPERCHOLESTEREMIA OF RATS. THE EFFECTS OF TOTAL UNSATURATION

J. J. Peifer

Adult male rats were preconditioned with an atherogenic diet containing tallow as the major source of fat calories and sufficient linoleate to prevent the onset of the EFA-deficiency syndrome. One group of rats were fed a similar atherogenic diet in which hydrogenated coconut oil was the sole source of fat calories. The preconditioned rats had part of their dietary tallow subtituted by the following supplements: palmitate, oleate, cottonseed oil, linseed oil, corn oil, or the complex mixtures found in menhaden, tuna and herring oils. The preconditioned rats were fed these supplements for a minimum of one month and blood lipid changes were measured at periodic intervals.

The EFA-deficiency did not appear to aggravate the hypercholesteremia resulting from the ingestion of the atherogenic diets. Furthermore, partial substitution of tallow by palmitate or oleate did not aggravate the hypercholesteremic condition of the rats. The marine oil supplements had significantly greater hypocholesteremic activities than the plant oils; for this reason, small caloric intakes of the marine oils led to a significant lowering of the plasma lipids. Some of the results suggest that the effects of the marine oils must be related to factors other than their total unsaturation, their total polyunsaturation or their EFA-activities.

— 102 —

STUDIES ON THE METABOLISM OF LINOELAIDIC ACID IN THE ESSENTIAL FATTY ACID-DEFICIENT RAT

O. S. Privett and M. L. Blank

Male weanling rats of the Sprague Dawley strain were made essential fatty acid-deficient by maintaining them on a fat-free

diet for four months. The animals were then fed a supplement of 5% methyl linoelaidate for 19 days and killed by exsanguination. The livers, kidneys and epididymal tissues were taken for studies on the composition of the lipids.

These studies showed that linoelaidic acid was deposited in the liver and kidney lipids, mainly in the form of phospholipids. Some linoelaidic acid was also deposited in the triglycerides of epididymal fat. Isolation analysis of the fatty acids showed no evidence that linoelaidic acid was converted to higher polyunsaturated fatty acids in the EFA-deficient rat.

— 103 —

RELATIONS BETWEEN AMOUNTS OF DIETARY AND BODY FATTY ACIDS IN MICE

B. M. Craig and J. M. Bell

Weanling mice were fed on a basal grain diet to which was added 2, 4 and 6% of safflower and palm oils and a vegetable shortening. After 14 days the animals were sacrificed, and total body fat was extracted. Fatty acid compositions of dietary and body fat were measured by GLC. The relations between dietary and body fat were studied with respect to amount of each fatty acid. Linear relations were found between dietary and body levels for linoleic and oleic acids with values of 0.28 and 0.20 g per gram respectively. Levels of myristic, palmitic, palmitoleic and stearic acid in the diet had very small effects on levels in the body fat.

— 104 —

THE RELATIONSHIP OF SINGLE DIETARY POLYUNSATURATED ACIDS TO THE FATTY ACID COMPOSITION OF LIPIDS FROM SUBCELLULAR PARTICLES AND UNFRACTIONATED TISSUES

J. J. Rohm and R. T. Holman

Supplements of purified fatty acid methyl or ethyl esters were fed at levels of 0.8% of dietary calories to each of seven groups of weanling rats for a period of sixty days. The esters were 9,12-octadecadienoate (18:2); 9,12,15-octadecatrienoate (18:3); 10,13-nonadecadienoate (19:2); 5,8,11,14-eicosatetraenoate (20:4); 5,8,11,14,17-eicosapentaenoate (20:5); 4,7,10,13,16,19-docosahexaenoate (22:6); and 12:3-epoxyoctadeca-9-enoate (epoxyoleate). The effects of these diet supplements upon the fatty acid compositions of the non-phospholipids and phospholipids from liver microsomal and mitachondrial cellular particles, and unfractionated lipids from livers, testes, epididymal and heart tissues were determined by gas-liquid chromatography.

Epoxyoleate and 19:2, which are structurally related to linoleic acid, did not function as essential fatty acids when judged by the progress of chemical and biological symptoms of fat deficiency. Dietary 20.5 and 22:6, both related to linolenate, were less active in preventing the occurrence of biological symptoms of fat deficiency than linoleate was, but more active in depressing the content of eicosatrienoate. The *in vivo* conversion of 20.5–22.5 of the linolenate family of fatty acids was shown to be reversible. Docosapentaenoic acid of the linoleate family was identified by reductive ozonolysis and shown to increase only with an increase in dietary 18:2 or 20:4.

— 105 —

STRUCTURAL ANALYSES OF THE DEPOT AND DIETARY FATS OF RATS FED FRESH AND OXIDIZED COTTONSEED AND OLIVE OILS

Hans Kaunitz, Ruth Ellen Johnson, Merle Blank, and O. S. Privett

Four matching groups of weanling male rats were placed on purified diets containing fresh cottonseed or olive oil or the same oils after 40 hr of aeration at 60C. They were maintained on these diets for sixteen months, after which, six representative members of each group were sacrificed. Triglycerides from dietary and pooled epididymal fat pads were prepared by florisil and silicic acid chromatography. The fatty acid compositions of the original dietary oils and depot fat extracts and of the purified triglyceride fractions were found to be in good agreement. Structural analyses of the triglycerides were carried out by reductive ozonolysis according to the method of Privett and Blank (JAOCS *40*: 70, 1963). The results showed that, although the structure of the depot fat was strongly influenced by the dietary fat, the animal produced molecular types not present in the dietary fat. Oxidation of the dietary fats exerted only a mild effect on the structure of the depot fat triglycerides.

— 106 —

PLATELET PHOSPHATIDES, THEIR SEPARATION, FATTY ACID COMPOSITION AND CLOTTING ACTIVITY

V. Mahadevan, E. Cubero and W. O. Lundberg

Platelet concentrates were prepared from pig blood by differential centrifugation, and homogenized in an omni-mixer with chloroform-methanol (2:1). The lipids obtained on evaporation of the extract were fractionated by silicic acid gradient-elution column chromatography using increasing concentrations of methanol in chloroform. Twenty ml fractions were collected and monitored by thin-layer chromatography (TLC). The following compounds were identified in the order in which they were eluted: phosphatidic acid (2:7%); mixture of phosphatidyl ethanolanime (PE) and phosphatidyl serine (PS) (26:9%); inositol phosphatide (4:8%); unidentified component (3:0%); lecithin (29:0%); and spingomyelin (13:1%). The PE and PS were further separated by TLC. The plasmologen contents of PE, PS, and lecithin were determined by iodine absorption. The distribution of the fatty acids and aldehydes in the various phospholipids was determined by gas-liquid chromatography of the liberated methyl esters of the fatty acids and dimethyl acetals. The activity of these phosphatides in blood clotting tests was determined and compared with total platelet lipid.

— 107 —

INTERCONVERSIONS OF LONG-CHAIN FATTY ACIDS IN THE RAT

D. Sand, N. Sen and H. Schlenk

10,13-Nonadecadienoic acid was fed to fat-deficient rats. The acid is metabolized since, in the organ fat of the rats, an appreciable amount of heptadecadienoic acid was found. However, odd-numbered higher unsaturated acids could not be detected. The biological inefficiency of 10,13-nonadecadienoic acid which has been established by other investigators is most likely connected with the failure to convert into longer-chain odd-numbered polyenoic acids according to the usual pattern.

This raises the question, which mono- and dienoic acids are suitable substrates for elongation and desaturation in the animal. Isomerism of mono-, di- and trienoic acids in the rat was investigated from birth over a period of more than 6 months of fat-deficient diet. Isomeric acids identified were Δ^{6}-, Δ^{7}- and Δ^{9}-C_{18}; Δ^{8}-, Δ^{9}- and Δ^{11}-C_{18}; $\Delta^{5,8}$-, $\Delta^{6,9}$- and $\Delta^{8,11}$-C_{18}; $\Delta^{5,8,11}$-C_{18} and -C_{20}. They can be correlated by 2-carbon addition or degradation and the pattern of methylene-interrupted desaturation. Some of the acids are, of course, well known and some of the conversions had been established by other investigators. By means of randomly labeled acids the conversions Δ^{7}-$C_{18} \longleftrightarrow \Delta^{9}$-$C_{18}$ and Δ^{9}-$C_{18} \longrightarrow \Delta^{11}$-$C_{18}$ have been established additionally.

Δ^{6}-, Δ^{9}- and Δ^{7}-C_{18} or the corresponding C_{18} acids are precursors for polyenoic acids in the rat with efficiencies increasing in this order. However, none of them matches the efficiency of linoleic acid as precursor where the second double bond obviously promotes the rate of conversion.

This is not the case with 10,13-nonadecadienoic acid although it has the linoleic type terminal structure A Δ^{10}-monoene has not been found in the rat. Apparently, a fatty acid with a double bond in position 10 is not produced by the rat and, when such compound is administered, its further conversion is blocked due to an unfavorable distance between carboxyl group and double bond.

— 108 —

LENTICULAR PHOSPHOLIPIDS OF EXPERIMENTAL ANIMALS

G. L. Feldman

Bovine and rabbit lenses are commonly used in ophthalmic research because of their large size and ease of procurement. However, great caution should be exercised when attempting to utilize animal data to explain human phenomena. To emphasize this point, the phospholipid composition of bovine and rabbit lenses is compared to previously reported human data (Feldman *et al.*, JAOCS, in press). Lecithin, cephalin and sphingomyelin are the major phospholipids, but their distribution differs widely between the species. Similarly, the fatty acid content of each phospholipid reflects marked species differences, particularly in the relative amounts of palmitate and oleate. In addition to the fatty acids, unidentified components exist that are probably dimethylacetals of the aldehydogenic group of plasmologen. These are particularly abundant in the bovine cephalin fraction.

NOTICE

A joint AOCS-ASTM Committee on Polymerized Fatty Acids has been formed to establish official AOCS and ASTM methods for these products. Those wishing to participate in the committee's work should write its chairman, G. G. Wilson, Chemical Div., General Mills, Inc., Kankakee, Ill.

• *Local Section News*

Northeast Section

The first regular meeting of the Northeast Section will be held on Tuesday, October 29, in the Della Robbia Room of the Sheraton Motor Inn, 39th and Chestnut Streets, Philadelphia, Pa. A get-together is scheduled for 6 p.m., with dinner following at 7:00 p.m.

Speaker at the after dinner meeting will be John Showell, U.S.D.A., Eastern Regional Laboratory, Wyndmoor, Pa. He has chosen the subject, "Novel Reaction for Production of 4-Hydroxy Stearic Acids."

With such a central location in the Delaware Valley area, convenient to good transportation, a record attendance is expected.