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# Progress in the Chemistry of Lipides in South Africa in the Past Fifty Years

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WITH THE UNIFICATION of South Africa in 1910 the country passed from the heroic period to one of steady progress and economic growth. Government research laboratories, concerned primarily with agriculture and mining were first set up, and during the following 10 years many fully-fledged universities were established. These new institutions made possible a slow but steady increase in scientific research and provided the scientific personnel who have played so great a role in both the economic and scientific development which has taken place in recent years.

In 1945 the Council for Scientific and Industrial Research was set up, and since then rapid progress has been made in the establishment of a large number of scientific laboratories and research institutes to serve special industries and needs.

In 1909, where this survey begins, there was very little systematic research in South Africa. Investigations in fat chemistry were chiefly concerned with the casual investigation of indigenous plants as sources of vegetable oils suitable for burning and soap-making. Many seed fats and waxes were analyzed in a rudimentary way by analysts in the Cape Colony by the Welcome Chemical Research Laboratories, England, but chiefly at the Imperial Institute, London.

Lipide research really began in South Africa with the work initiated by W. S. Rapson, then at the University of Cape Town, now vice president of the Council for Scientific and Industrial Research, Pretoria. During a period of about 10 years W. S. Rapson and his associates carried out an extensive survey of the lipides, principally of fish caught off the South African coast. These investigations, reported in some 33 papers, covered the seasonal variation in yields of fish oils and the physical and chemical properties of these oils. Extensive use was made of the Hilditch ester fractionation technique in the determination of the composition of the fatty acid fractions, and computation forms relating to this method were devised to simplify the calculation of the results (1). The difficulty of getting accurate unsaponifiable determinations with a number of marine oils was traced to the presence of large proportions of glyceryl ethers, and the effect of the presence of these substances on the accuracy of the S.P.A. method was examined. A method of determining these substances by means of periodate oxidation was also developed.

Particular importance was placed on the occurrence, distribution, and determination of vitamin A, and these investigations led to the rapid commercial exploitation of fish liver oils for the manufacture of vitamin A concentrates on a large scale (2, 3). The work also led to the manufacture of a wide range of products, particularly drying oils for paints. Commercial production of fish oils began in 1946 and four years later had reached 11,000 tons a year. At one factory a propane segregation (Solexol) plant was set up (4) to produce high vitamin A concentrates and to upgrade marine oils by removing color bodies

and the more saturated glycerides so that they could be used as drying oils for paint manufacture.

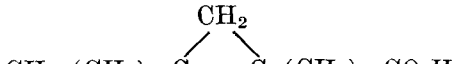
The development in recent years of powerful but mild techniques of separating unstable lipides has made possible a new approach to the problem of the composition of fish oils, particularly pilchard oil, by D. A. Sutton and co-workers. Thus it was found that the sequence of lithium-soap-acetone separation, urea complex fractionation, molecular distillation in a triple-effect still (5), and reversed-phase partition chromatography (6, 7, 8) permitted the separation of individual acids from the mixed pilchard acids in reasonable states of purity. The structures of the acids isolated were determined by means of oxidation, but the usual methods of locating the original carboxyl group among the oxidation products by esterifying and identifying the half ester formed was not found very satisfactory. Instead it was found better to lengthen the chain by one carbon, using the Arndt-Eistert procedure, then to compare the products of oxidation with those from the original acid.

Using the above procedures together with spectroscopic and other usual methods, the following acids, with all *cis* double bonds, were identified: all *cis-n*-hexadecatetra-6,9,12,15-enoic, all *cis-n*-eicosapenta-5,8,11,14,17-enoic, all *cis-n*-docosahexa-4,7,10,13,16,19-enoic. An octadecatetraenoic acid was also isolated, and the structure was determined on the basis of a previously prepared but less pure specimen. Evidence has thus accumulated that these structures are common in marine oils and the highly unsaturated acids of animal phosphatides (9) in spite of a voluminous literature in which doubly interrupted structures have been proposed.

## Vegetable Oils

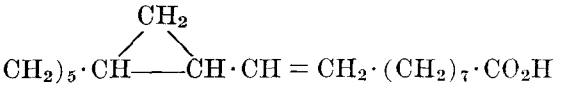
The early interest in vegetable oils, particularly seed oils, has continued through the years, more recently at the National Chemical Research Laboratory (10) and also by F. Hawke of the University of Witwatersrand. During these studies several unusual fatty acid components were identified. Hawke showed the presence of 9,12-hexadecadienoic acid in the acid mixture derived from the seed fat of *Acacia giraffae*. J. R. Nunn isolated the unstable sterculic acid ($C_{19}H_{34}O_2$) in a pure form by low temperature crystallization after the nonpolymerizing acids of the seed oil of *Sterculia foetida* had been removed as their urea complexes. The acid polymerized rapidly on standing with a progressive loss of free carboxyl and a gain of combined carboxyl function. On partial hydrogenation a dihydrosterculic acid was obtained, which gave no color with tetranitromethane but, on further hydrogenation, yielded a mixture of $C_{19}$ acids. Oxidation of sterculic acid with permanganate gave azelaic and pelargonic acids so that one carbon atom was lost in the process. Ozonolysis at low temperature, followed by hydrogenation of the product, yielded an acid ($C_{19}H_{34}O_4$) which was a 1:3-diketone. Further oxidation of the latter again gave pelargonic and azelaic acids. It was therefore 9:11-diketononadecanoic acid.

Dihydrosterculic acid had a band in the infrared in the same position (9.85 $\mu$) as that of the disubstituted saturated *cyclo*propane acid of *Lactobacillus arabinosis* (11). The latter acid cleaves in the three possible ways on hydrogenation, thus providing an explanation of the formation of a mixture on complete hydrogenation of dihydrosterculic acid. From these observations sterculic acid was formulated as shown.



Confirmatory evidence of the presence of the *cyclo*propane ring in dihydrosterculic acid has been obtained by Hofmann *et al.* (11).

Later Nunn's structure was rejected in favor of



However it was supported by infrared evidence, and a re-investigation of the structure in the National Chemistry Research Laboratory, in collaboration with J. C. Smith of the University of Oxford (12), confirmed the original structural allocations.

The seed oils of certain indigenous *Ximenia* species were found to show an ultraviolet absorption band at 229 $\mu$ which was traced to the presence of an unusual fatty acid, ximenynic acid ($C_{18}H_{30}O_2$). The position and the intensity of the absorption band indicated the presence of a conjugated ene-yne system. On hydrogenation three moles of hydrogen were absorbed to give stearic acid. Ozonolysis yielded *n*-heptaldehyde and azelaic acid. The structure was thus established as

$$CH_3\cdot(CH_2)_5\cdot CH{:}CH\cdot C{:}C\cdot(CH_2)_7\cdot CO_2H$$

and not

$$CH_3\cdot(CH_2)_5\cdot C{:}C\cdot CH{:}CH\cdot(CH_2)_7\cdot CO_2H$$

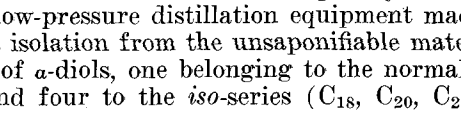
since the latter could not yield *n*-heptaldehyde because the twelfth carbon atom has no hydrogen atom.

Besides these structural investigations of vegetable oils, a method of preparing unsaturated, long-chain alcohols by means of lithium aluminum hydride reduction has been worked out (13). The infrared absorption spectrum of olive oils was examined (14).

With the production in South Africa in recent years of large amounts of a variety of seed oils, particularly peanut oil, a vigorous expansion of the fats industry has been possible. During the last World War many new industries sprang up to supply products then no longer available from overseas. Since the war these new industries have become firmly established and are able to compete on their own terms. Thus South Africa now produces, from oils and fats, a remarkably wide range of products, margarine (15), soaps, detergents, and paints, both for local and export markets.

## Sebaceous Secretions

One of the first research programs initiated by W. S. Rapson and his colleagues in 1949 was the study of the composition of wool wax, the sebaceous secretion of sheep (16). Earlier studies had shown this substance to be of unusual complexity. High-efficiency, low-pressure distillation equipment made possible the isolation from the unsaponifiable material of a series of $\alpha$-diols, one belonging to the normal series ($C_{16}$) and four to the *iso*-series ($C_{18}$, $C_{20}$, $C_{22}$, $C_{24}$)



Urea fractionation proved most valuable for the separation of the aliphatic alcohols from the unsaponifiables; with the aid of solvent distribution and distillation a series of α-hydroxy acids, four belonging to the normal series ($C_{12}$, $C_{14}$, $C_{16}$, $C_{18}$) and one *iso*-α-hydroxy acid ($C_{18}$), were isolated.

Besides these hydroxy acids a group of higher molecular weight acids were isolated by adsorption chromatography at elevated temperatures. As derivatives were too high boiling for satisfactory separation by fractional distillation, they were converted to large ring lactones. Distillation, followed by hydrolysis, yielded ω-hydroxy *n* acids ($C_{30}$ and $C_{32}$). They were identified by oxidation to the corresponding dicarboxylic acids, one of which was synthesized, and by removal of their hydroxy groups to yield the *n*-fatty acids (17).

These hydroxy acids of wool wax total 40–45% of the acid fraction and, as the pure wax does not have an appreciable hydroxyl value, it is clear that the wax is largely composed, as suggested on analytical grounds, of estolides or double esters. The fatty acid fraction, free from hydroxy acids, did not show an iodine value. Unsaturated acids must therefore be absent.

Besides these aliphatic acids a third group of resinous acids were isolated. The constitution of this group has not yet been elucidated, but recent investigations indicate that they are not true wool wax components but artifacts produced by autoxidation of the steroid and triterpene moieties of the wax while the sheep are exposed to the weather. In addition, some model wool wax components have been synthesized, and mohair and karakul waxes have been examined. Some technological work has been carried out to provide wool wax components of commercial value (18).

Human sebum (surface skin fat), like wool wax, is largely composed of waxes, the acid constituents of which had been extensively investigated. A study of the unsaponifiable constituents, using methods similar to those used with wool wax, led to the isolation of 12 new aliphatic alcohol constituents which were identified as normal alcohols ($C_{14}$, $C_{16}$, $C_{18}$, $C_{20}$, $C_{22}$, $C_{24}$), *iso*-alcohols ($C_{20}$, $C_{22}$, $C_{24}$), and unsaturated normal alcohols

$$CH_3 \cdot (CH_2)_8 \cdot CH{:}CH \cdot (CH_2)_{2n} \cdot CH_2OH$$

($C_{20}$, $C_{22}$, $C_{24}$). The position of the double bond was determined by hydroxylation of the double bond and oxidation with lead tetra-acetate to a mixture of decanoic acid and a α-hydroxy acid. The former was identified by means of reversed-phase chromatography and the latter by further oxidation to a dicarboxylic acid.

## Cutin

Earlier work in other laboratories had indicated that plant cuticles were made up of a thin sheet of cutin, a hydroxy-acid polymer, but little was known of the composition of the component acids. In an investigation by M. Matic of the cutin of *Agave americana* (19) the hydroxy acids were elegantly separated by means of countercurrent distribution and reversed-phase chromatography into five acids, 80% of the mixture, and identified as threo-9:10:18-trihydroxyoctadecanoic acid, 10:18-dihydroxyoctadecanoic, 10:16-dihydrohexadecanoic, 18-hydroxyoctadecanoic, and 18-hydroxyoctadec-*cis*-9-enoic acids, the latter isolated as its dihydroxy derivative. These structures were identified by reduction to the corresponding unhydroxylated fatty acids and by graded oxidation to known fragments.

## Autoxidation of Methyl Linoleate

American spectroscopic evidence that both *cis-trans* and *trans-trans* isomers are contained in the primary reaction product, methyl monohydroperoxido-linoleate, was confirmed (20). The peroxide was separated from unreacted methyl linoleate and other impurities by solvent partition methods, then converted to the corresponding methyl monohydroxylinoleate by sodium borohydride reduction. The *trans-trans* hydroxy compound was separated as its urea adduct and hydrogenated to give a mixture of methyl 9- and 13-hydroxystearates which were separated on neutral alumina (following Bergström) and shown to be present in about equal amounts. The *cis-trans* hydroxy compound, obtained less pure as the urea complex raffinate, was hydrogenated to give the same hydroxystearates in the same proportion. No trace of methyl 11-hydroxystearate was found by chromatography, neither was any methyl 11-hydroxylinoleate detected by attempted anionotropic rearrangement of the total methyl monohydroxylinoleate. It was concluded that autoxidation of linoleate at low temperatures gives mainly, or solely, the 9- and 13-peroxido *cis-trans* isomers, the *trans* double bonds of which may be nearest to the peroxide group (21).

## Polymerization of Oils

Dr. Sutton and co-workers published investigations in this field. Heat-polymerized pilchard oil was fractionated by molecular distillation and solvent extraction, and the number of average molecular weights were determined by isothermal distillation. It was clear that only inaccurate information about polymer sizes and distributions was obtainable by such separation methods, and an accurate method, depending on the application of Flory-Stockmayer mathematics, was worked out by Hoeve (22) and applied to sunflower and linseed stand oils.

The structures produced during thermal polymerization of conjugated and unconjugated lipides were also studied. Chemical dehydrogenation and then oxidation of the thermal dimers from methyl β-eleostearate and from methyl linoleate gave prehnitic acid in moderate and low yields, respectively. Thus the Diels-Alder type of polymerization occurs in both cases although it may not be the sole mechanism in nonconjugated olefines. The recovered monomer from heated methyl β-eleostearate was examined, and it was found that much of this had undergone intramolecular cyclization to six-membered ring structures which contained a variety of diene types.

## Medical Biochemistry

Lipides have been investigated in the course of several studies in this field.

The widely differing incidences of coronary artery disease among white, Negro, and mixed racial groups in South Africa have stimulated a great volume of work including lipide researches. The changes in the serum cholesterol and fatty acid levels of healthy white and Negro subjects and of coronary patients,

brought about by changes in the amount and composition of dietary fats, have been studied by the groups of J. F. Brock (23) and A. Antonis (24). Recent studies have been concerned with the effects of dietary fats upon serum cholesteryl esters, triglycerides, and phospholipides and their component fatty acids, also upon fecal lipides and on bile acid secretion. These studies suggest that the serum cholesterol lowering effect, brought about by unsaturated fatty acid ingestion, may be caused by increased catabolism of sterols. Work on experimental atherosclerosis in rabbits led to an *in vitro* study of the effects of heparin and calcium ions on lipemic sera.

Lung lipides have been isolated from healthy and silicotic guinea pigs since it has been claimed that the increased lung lipides, noted in this disease, are themselves a factor in promoting fibrosis of the lung. The carcinogenicity of the nonsaponifiables from human cancerous and noncancerous livers has been studied. Autoxidation of such extracts occurs rapidly, using the aerobic conditions of isolation applied by previous workers, and this factor may play a role in the contradictory results recorded in the literature.

The first paper of a series entitled "Studies on Fat Metabolism in Kwashiorkor" has recently appeared; it deals with total serum cholesterol. The enlarged fatty liver, which is a characteristic of this disease, had previously been extensively studied by medical research workers in this country. Some interesting work on the changes induced in pooled human sera by treatment with ether has been done by H. B. W. Grieg. Following such treatment, the bulk of the cholesterol is found in the globulins precipitated by 40% saturation with ammonium sulphate whereas, in untreated serum, the bulk of the cholesterol is found in the proteins not precipitated. It is thought that the protein affected by ether treatment is an $\alpha_1$ globulin. A number of related studies of general interest to biochemists are being currently pursued.

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# Advances in Fat Research in Spain During the Last Fifty Years

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IT IS AN HONOR to be invited to describe the results of fat research in Spain, and I am very pleased that my country should be represented in this symposium. I also want to congratulate my American colleagues on having founded, back at the beginning of this century, a specialized scientific society whose work has made valuable contributions to the progress of science and fat technology.

Some general preliminary remarks are in order to interpret correctly Spanish achievements in the field of fat research.

In the first place, the Spanish fat production and processing industry consists of a large number of small factories with small technical staffs and without research laboratories of their own. At present, most of our industrial laboratories limit their work to the analytical control of raw materials and finished products and do not undertake any work of research or development.

As a result, during the period 1908–1941 research on fats may be considered the work of a small number of individuals interested in these problems, working either in private laboratories or at university centers. Only from 1941 on, there began to appear some research centers independent of the university as a result of the foundation of the Superior Council of Scientific Research and other institutions, such as the Oceanographic Institute, the National Council of Agronomic Research, including its provincial experimental farms, and so on. Finally in 1947 the Applied Research Department of the above-mentioned Superior Council of Scientific Research created the Institute of Fats and Their Derivatives with the collaboration of the private fat industry. This center has given impetus to research in the field of oils and fats, and we shall discuss it later on.

The second factor to be considered is the particular nature of the Spanish fat industry, which is mainly directed to the processing of one staple product: olive oil. Spain's annual production of olive oil averages about 350,000 metric tons and represents more than one-third of the world production. Other vegetable oils, such as cottonseed, flaxseed, and castor oils, are produced only on a small scale, and the production