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Lipids as renewable resources: current state of chemical and biotechnological conversion and diversification

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Abstract Oils and fats are the most important renewable raw materials of the chemical industry. They make available fatty acids in such purity that they may be used for chemical conversions and for the synthesis of chemically pure compounds. Oleic acid (1) from “new sunflower,” linoleic acid (2) from soybean, linolenic acid (3) from linseed, erucic acid (4) from rape seed, and ricinoleic acid (5) from castor oil are most important for chemical transformations offering in addition to the carboxy group one or more C-C-double bonds. New plant oils containing fatty acids with new and interesting functionalities such as petroselinic acid (6) from *Coriandrum sativum*, calendic acid (7) from *Calendula officinalis*, α -eleostearic acid (8) from tung oil, santalbic acid (9) from *Santalum album* (Linn.), and vernolic acid (10) from *Vernonia galamensis* are becoming industrially available. The basic oleochemicals are free fatty acids, methyl esters, fatty alcohols, and fatty amines as well as glycerol as a by-product. Their interesting new industrial applications are the usage as environmentally friendly industrial fluids and lubricants, insulating fluid for electric utilities such as transformers and additive to asphalt. Modern methods of synthetic organic chemistry including enzymatic and microbial transformations were applied extensively to fatty compounds for the selective functionalization of the

alkyl chain. Syntheses of long-chain diacids, ω -hydroxy fatty acids, and ω -unsaturated fatty acids as base chemicals derived from vegetable oils were developed. Interesting applications were opened by the epoxidation of C-C-double bonds giving the possibility of photochemically initiated cationic curing and access to polyetherpolyols. Enantiomerically pure fatty acids as part of the chiral pool of nature can be used for the synthesis of nonracemic building blocks.

Introduction

Average annual world oil production in the years 1996 to 2000 amounted to 105.0×10^6 t and will increase in the years 2016 to 2020 to 184.7×10^6 t (ISTA Mielke GmbH Hamburg 2002). Eighty to eighty-one percent of the produced oils and fats are consumed as human food; 5–6% as feed. Approximately 14%, 15–17 million tonnes are used by industry (Gunstone and Hamilton 2001). In contrast, the world consumption of fossil mineral oil was approximately $4,000 \times 10^6$ t in the year 2002. The chemical share was about 11% in the European Union (EU).

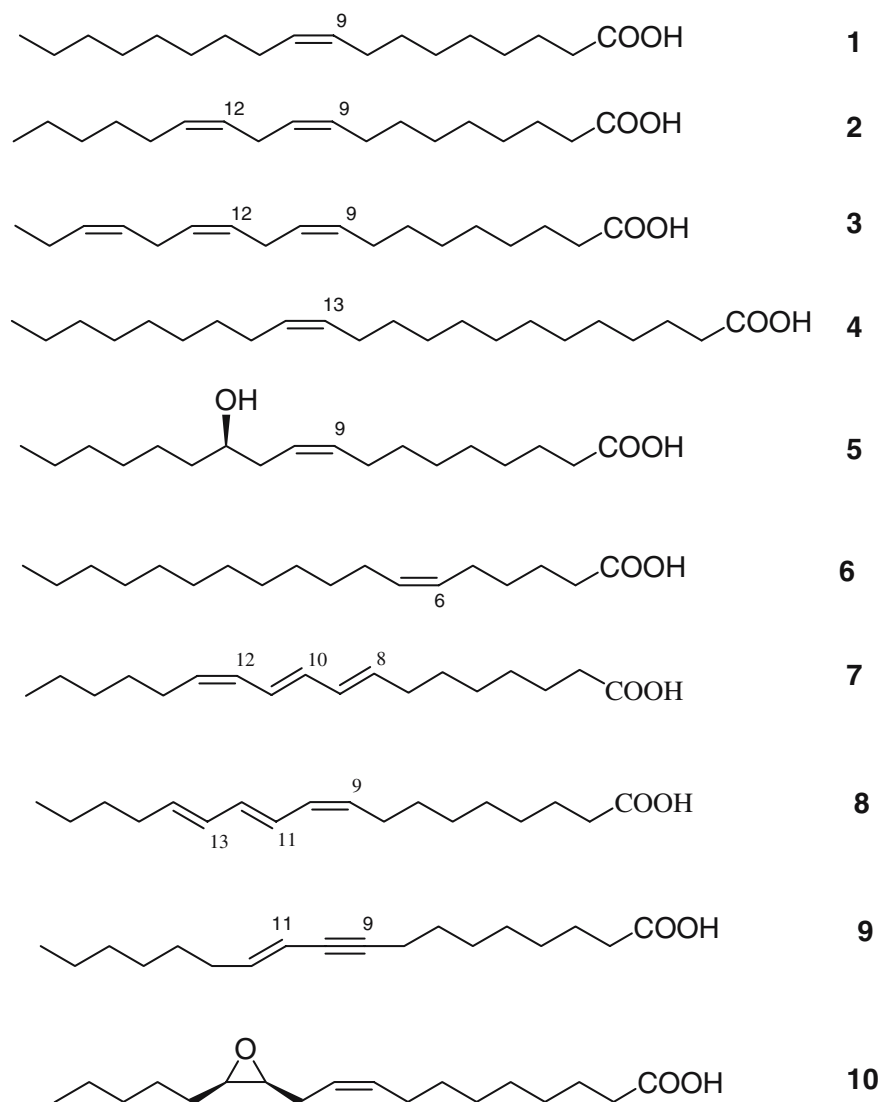
About 80% of the global oil and fat production were vegetable oils and only 20%, with declining tendency, were of animal origin. About one quarter of global production came from soybean, followed by palm oil, rapeseed, and sunflower. Coconut and palm kernel oil (laurics) contain a high percentage of saturated C12 and C14 fatty acids and are most important for the production of surfactants. These commodity oils make available fatty acids in such purity that they may be used for chemical conversions and for the synthesis of chemically pure compounds such as oleic acid (1) from “new sunflower,” linoleic acid (2) from soybean, linolenic acid (3) from linseed, erucic acid (4) from rapeseed, and ricinoleic acid (5) from castor oil (Fig. 1).

Where “nonfood” uses are concerned, genetic engineering approaches can make a special contribution to the expansion in the wealth of raw materials available to oleochemistry such as increasing the content of individual fatty acids or drastically changing the oil quality by the

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Fig. 1 Fatty acids as starting materials for the synthesis of novel fatty compounds:
1 Oleic acid, **2** linoleic acid,
3 linolenic acid, **4** erucic acid,
5 ricinoleic acid, **6** petroselinic acid,
7 calendic acid,
8 α -eleostearic acid, **9** santalbic acid,
and **10** vernolic acid



introduction of a new fatty acid, e.g., the development of high lauric rapeseed (Biermann et al. 2000a).

New plant oils

New plant oils containing new and interesting functionalities are becoming industrially available (Baumann et al. 1988; Gunstone 2001). Fatty acids containing the C-C-double bond in unusual positions of the alkyl chain and containing conjugated double bonds are most interesting from a chemical view point (Fig. 1). Moreover, fatty acids from the natural chiral pool are exciting substrates for stereoselective transformations to give enantiomerically pure products. We can hope that the increasing usage of renewable feedstocks will also eventually enlarge the agricultural biodiversity. It is to be expected that this is further boosted by the development of genetically engineered plants through metabolic engineering, an area (Biermann et al. 2000a) which is outside of the scope of this article. Significant advances were already made to produce

polyunsaturated fatty acids such as docosahexenoic acid, eicosapentenoic acid, and arachidonic acid in modified oil-seed crops (Singh et al. 2005; Huang et al. 2004).

Petroselinic acid (6) from the seed oil of *C. sativum* is an 18:1 acid with unsaturation at C6 (Meier zu Beerentrop and Röbbelen 1987). Meadowfoam (*Limnanthes alba*) oil contains approximately 65% 20:1 acid with double bond at C5. Both fatty acids show some novel reactivities based on the proximity of the double bond to the carboxyl group. Thus, cyclizations to cyclopentane (Metzger and Mahler 1993) and cyclohexanone (Metzger and Biermann 1993) derivatives were reported (Biermann et al. 2000a).

The seed oil of *C. officinalis* contains up to 60% of calendic acid [(8*E*,10*E*,12*Z*)-octadecatrienoic acid] (7) with a conjugated and stereochemically well-defined hexatriene system (Janssens and Vernooij 2001). α -Eleostearic acid [(9*Z*,11*E*,13*E*)-octadecatrienoic acid] (8) with a conjugated hexatriene as well is obtained from Chinese wood oil (tung oil). Both are drying oils and interesting applications in alkyd resins were reported. Both hexatriene fatty acids allow highly regioselective and stereoselective

Diels–Alder addition of maleic anhydride to the *trans, trans*-conjugated diene system (Metzger and Biermann 2006).

Santalbic acid (9) is the main fatty acid of the seed oil of sandalwood [*S. album* (Linn.)]. It contains a unique conjugated enyne system in the alkyl chain, which could be successfully exploited for highly regioselective additions (Biermann et al. 2000b) and for the selective synthesis of halogenated fatty compounds (Lie Ken Jie et al. 2003).

Vernolic acid (10) can be obtained from the seed oil of *V. galamensis* and of *Euphorbia lagascae*. Vernolic acid [(12*S*,13*R*,9*Z*)-12,13-epoxy-9-octadecenoic acid] is an enantiomerically pure unsaturated epoxy fatty acid with interesting applications as binder in coatings and preferentially in photocuring coatings (Crivello and Carlson 1996). Vernolic acid is an interesting substrate for the stereoselective synthesis of enantiomerically pure compounds.

Basic oleochemicals

With a production of 8.9×10^6 t in 1990, soaps still ranked first in worldwide statistics for industrial use of fats and oils and for surfactants (Schumann and Siekmann 2002). In 2005, the global production of oleochemicals—excluding soaps and biodiesel—was estimated to amount to 6.7×10^6 t/a. The basic oleochemicals—the production in 2000 is given in brackets—are free fatty acids (3.05×10^6 t/a), methyl esters (0.66×10^6 t/a), fatty alcohols (1.44×10^6 t/a), and amines (0.57×10^6 t/a) and glycerol (0.75×10^6 t/a) as a by-product (Gunstone 2001). The free fatty acids are obtained by hydrolysis of triglycerides with water in a continuous process at 20–60 bar and 250°C. The fatty esters are produced by transesterification of the triglycerides with the respective alcohol, mostly methanol. Hydrolysis and transesterification can be performed enzymatically at ambient temperature and normal pressure. However, economics restrict up to now the use of this technology (Bühler and Wandrey 1987). It was claimed that by applying modified technologies for fat splitting and direct transesterification of triglycerides, it would become possible to lower the enzyme concentration dramatically, resulting in an even more economic process compared to the classical methods. In addition, the quality of the products becomes better and even process units of around 2,000 t/a of feed become economic (Noweck et al. 2004).

The production of long-chain fatty alcohols is an important industrial process. Catalytic hydrogenation of fatty acid methyl esters gives long-chain fatty alcohols at approximately 200°C and 250–300 bar. Long-chain fatty alcohols are also produced from petrochemical feedstocks by the Ziegler Alfol process from ethylene and by hydroformylation of olefins (Noweck 2002). It is remarkable to note that the share of natural sources is rising. It is most important that fat alcohols derived from fats and oils as renewable feedstocks show a more favorable life cycle assessment (LCA) than petrochemical alcohols (Hirsinger 2001) and show that they are an important example that base chemicals derived from renewable feedstocks can be

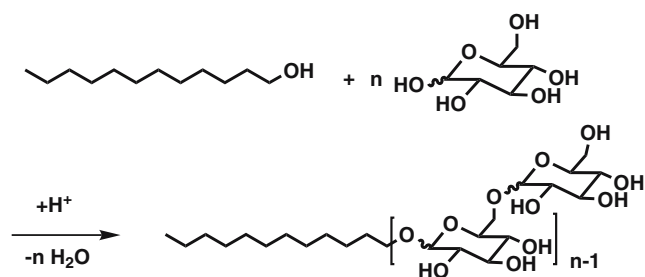


Fig. 2 Synthesis of alkylpolyglycosides by acid catalyzed reaction of lauric alcohol and glucose

commercially competitive. It is possible to perform the hydrogenation of the ester group with retention of the C–C double bond making oleyl alcohol easily available by hydrogenation of the methyl ester of high oleic sunflower or rapeseed oil.

Fatty amines are produced from fatty acids in a multistep process via nitriles followed by hydrogenation to give the primary amines, which are converted to tertiary amines and to quaternary ammonium compounds (quats) (Franklin et al. 2001).

The production of fatty acids and esters from triglycerides gives as a by-product about 10wt% of glycerol. New uses of glycerol and new chemical transformations to interesting products are of increasing importance. Fortunately, glycerol has a melting point of 20°C and could possibly be a suitable compound to be used as interior render with latent heat stores.

Fatty acid methyl esters have found an important new application as biofuel. The biodiesel production in the EU added up in 2003 to 1.4×10^6 t with steadily increasing tendency. Agenda 21 calls for “criteria and methodologies for the assessment of environmental impacts and resource requirements throughout the full life cycle of products and processes.” A simple metric for the production of biofuels is the overall energy efficiency that is the heating value of biofuel divided by the energy required to produce the biofuel. The biodiesel production in Germany from rapeseed has—without credits for the coproduct glycerol—an overall energy efficiency of 1.9 (Kraus et al. 1999) and the respective from soybean in the USA of about 3 (Sheehan et al. 1998).

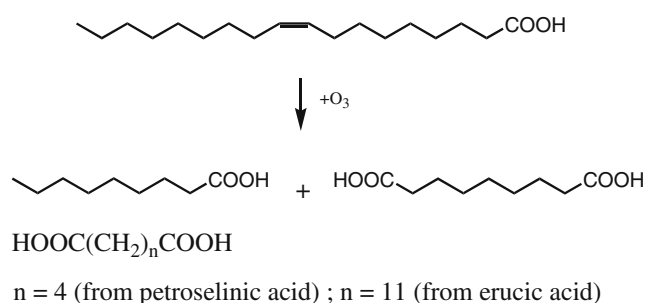
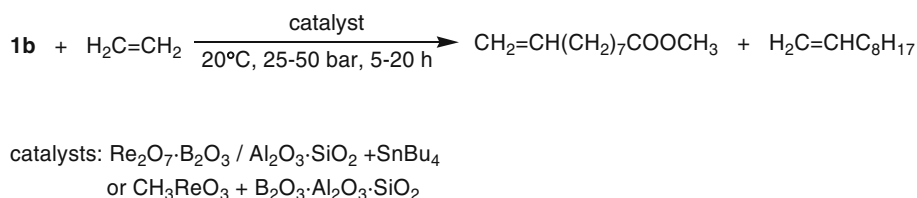


Fig. 3 Oxidative scission of oleic acid with ozone to give azelaic acid and pelargonic acid. Petroselinic acid 6 and erucic acid 4 give adipic acid (n=4) and brassylic acid (n=11), respectively

Fig. 4 Cometathesis of methyl oleate **1b** and ethylene to methyl 9-decenoate and 1-decene. The ester **1b** used (new sunflower) was 87% pure, the conversions and selectivities each >90%, and the yields were >80%



Vegetable oils are increasingly used as environmentally friendly industrial fluids and lubricants because of their biodegradability and their favorable water pollution class (Erhan and Perez 2002). The usage as insulating fluid for electric utilities such as transformers (Fields 2004; Lewand 2004) and as additive to asphalt to improve the surface properties (Carmen 2004) are interesting new industrial applications.

Current state of chemical conversion of oleochemicals

By means of simple industrial reactions, basic oleochemicals are available from vegetable oils in such purity that they may be used for further chemical conversions and for the synthesis of chemically pure compounds. The basic oleochemicals are chemically converted including enzymatic and microbial transformations (see section below for details) to a great variety of specialties, which are used for the production of cosmetics, lubricants, coatings, surfactants, and many other useful products. Here, chemists make profit from the synthetic input of nature. The chemical structure is essentially retained making the compounds biologically and easily degradable. At present, surfactants are the most important products of oleochemistry. About 430,000 t/a, which is one third of the industrially used fats and oils in Germany, are converted to surfactants. The most recent important innovation in oleochemistry is the production of alkyl polyglycosides by acid catalyzed reaction of lauryl alcohol and of glucose. Glucose is suspended in excess lauryl alcohol (2–6 mol) and the reaction is carried out at a temperature of 100–120°C in the presence of an acidic catalyst, typically a sulfonic acid. After removal of water as by-product (1 equiv relative to glucose) under vacuum, the product mixture comprises alkyl mono-, alkyl oligo-, and alkylpolyglycosides (Fig. 2). The average degree of polymerization for such an alkyl polyglycoside is largely dependent on the ratio of glucose to alcohol in the reaction mixture (von Rybinski and Hill 1998). It may be possible that an enzymatic production method could give well-defined products having improved properties. Alkyl polyglycosides are nonionic surfactants. They show a very good biodegradability and are used in detergents for home care applications and in cosmetics because of the good skin compatibility. They are produced on a scale of 70,000 t/a.

It was said that “more than 90% of oleochemical reactions were those occurring at the fatty acid carboxy group, while less than 10% have involved transformations of the alkyl chain. However, future progress will be along the lines of these latter types of reactions with their potential for considerably extending the range of com-

pounds obtainable from oils and fats” (Baumann et al. 1988). Modern methods of synthetic organic chemistry including enzymatic and microbial transformations were applied extensively to fatty compounds for the selective functionalization of the alkyl chain (Biermann et al. 2000a; Biermann and Metzger 2004b).

Diacids, ω -hydroxy fatty acids, and ω -unsaturated fatty acids

Diacids, ω -hydroxy fatty acids, and ω -unsaturated fatty acids derived from basic oleochemicals are of great interest. Diacids are important intermediates for the production of polyesters and polyamides. At present, the aliphatic dibasic acid with the highest production volume of 2.3×10^6 t/a is globally adipic acid, which is produced industrially by oxidation of a mixture of cyclohexanone and cyclohexanol (obtained by catalytic air oxidation of cyclohexane) with HNO_3 under formation of the coupled product N_2O and different nitrogen oxides (Musser 2000). Adipic acid is a basic chemical with a very high gross energy requirement of about 80 GJ/t, the production of which should be improved to be more sustainable (Eissen et al. 2002). An alternative access to various diacids from renewable feedstocks would be most interesting. Diacids such as azelaic acid (C_9) (Fig. 3) and brassylic acid (C_{13}) can be produced from oleochemical feedstocks by ozonolysis of oleic acid and erucic acid, respectively, giving as by-product nonanoic acid (Baumann et al. 1988). Adipic and lauric acid may be obtained from petroselinic acid. Because ozone is very expensive and the industrial ozonolysis presents some difficulties, an alternative process is required. A catalytic process using peracetic acid and ruthenium catalysts or H_2O_2 and Mo, W, or Re based catalysts was reported yielding only 50–60% diacids (Herrmann et al. 1989; Warwel and Rüschen gen. Klaas

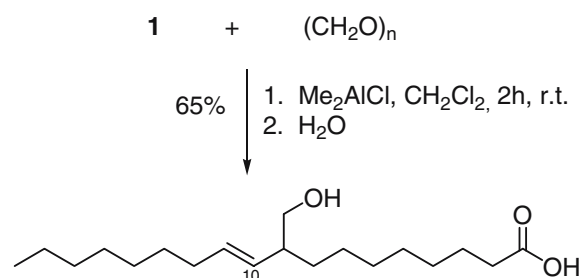


Fig. 5 Me_2AlCl -induced addition of paraformaldehyde to oleic acid (**1**) to give stereoselectively an unsaturated alkyl branched ω -hydroxy fatty acid

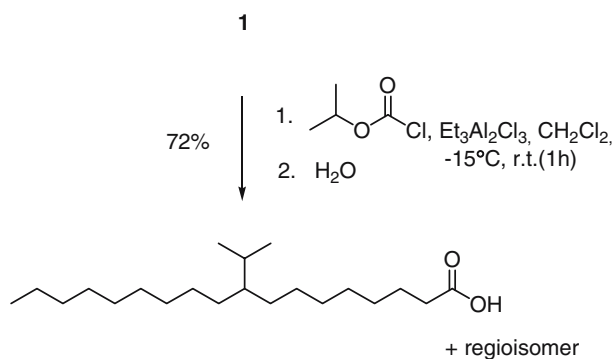


Fig. 6 Ethylaluminum sesquichloride induced reaction of oleic acid (1) and isopropyl chloroformate to give an alkyl branched fatty acid

1997). Obviously, a catalytic process using oxygen from the air has to be developed. Such a process would open the door for the production of a great variety of dibasic acids of different chain length from plant oils.

Long-chain diacids can also be obtained by metathesis of ω -unsaturated fatty acids. The 10-undecenoic acid is industrially produced by steam cracking of ricinoleic acid to give as a by-product heptanal, which is used in perfumes. While 9-decenoic and 13-tridecenoic acid can be obtained by comethathesis of ethene and oleic acid and erucic acid, respectively, giving 1-decene as a by-product (Fig. 4). It is interesting to note that an unsaturated C_{20} -diacid with the C-C double bond in position C 10 is obtained by metathesis of 10-undecenoic acid (Warwel et al. 1992, 2000).

Splitting ricinoleic acid with caustic soda in a ratio of 1:1 at 180–200°C gives as major products 2-octanone and 10-hydroxydecanoic acid. Using a ratio of 2:1 and 250–275°C, 2-octanol and sebacic acid (decane diacid) are obtained (Gunstone 2001).

Microbial ω -oxidation of fatty acids, which leads via ω -hydroxy fatty acids to diacids, is of great interest (Biermann et al. 2000b). Cognis developed a metabolically engineered strain of *Candida tropicalis* to oxidize a terminal methyl group of an alkyl chain. The reaction of oleic acid gives the respective unsaturated ω -hydroxy C_{18} -acid and finally the C_{18} -diacid (Craft et al. 2003).

Alkyl-branched ω -hydroxy fatty acids were synthesized by hydroformylation (e.g., linoleic acid) followed by hydrogenation of the carbonyl group (Fell and Meyers 1991; Kandamarachchi et al. 2002a,b); alkyl aluminum chloride mediated the addition of formaldehyde to the (*Z*)-configured C-C-double bond (e.g., oleic acid), which yielded stereoselectively (*E*)-configured alkyl-branched unsaturated ω -hydroxy fatty acids (Fig. 5) (Metzger and Biermann 1992).

Very special diacids produced on a commercial scale are dimer fatty acids obtained by heating of unsaturated fatty acids from tall oil at around 230°C with a montmorillonite. Distillation gives a monomer and a dimer fraction containing some trimers. These fractions are very complex mixtures, which are not fully identified (Brutting and Spittler 1994). Most important, the dimer acids are C_{36} -dibasic acids. They are used mainly for polyamides.

The monomer fraction is commercialized as “isostearic acid” being a highly complex mixture of linear, branched, cyclic, and aromatic C_{18} -fatty acids. Isostearic acid is used in cosmetics because of good spreadability, solubility in cosmetic formulations, and emolliency and also in lubricant areas because of low viscosity, low pour points, good oxidative and hydrolytic stability, and good solubility in various solvents. A method for the selective synthesis of alkyl-branched fatty acids by hydroalkylation of the C-C-double bond of unsaturated fatty acids using alkyl chloroformates in the presence of ethylaluminum sesquichloride was recently reported (Fig. 6) (Biermann and Metzger 1999, 2004a).

Epoxidation

Unsaturated fatty compounds are preferably epoxidized on an industrial scale by in situ performic acid procedure (Baumann et al. 1988). Numerous new epoxidation methods were applied to oleic acid (Biermann et al. 2000a). Chemoenzymatic epoxidation is of considerable interest because this method totally suppresses undesirable ring opening of the epoxide. Initially, the unsaturated fatty

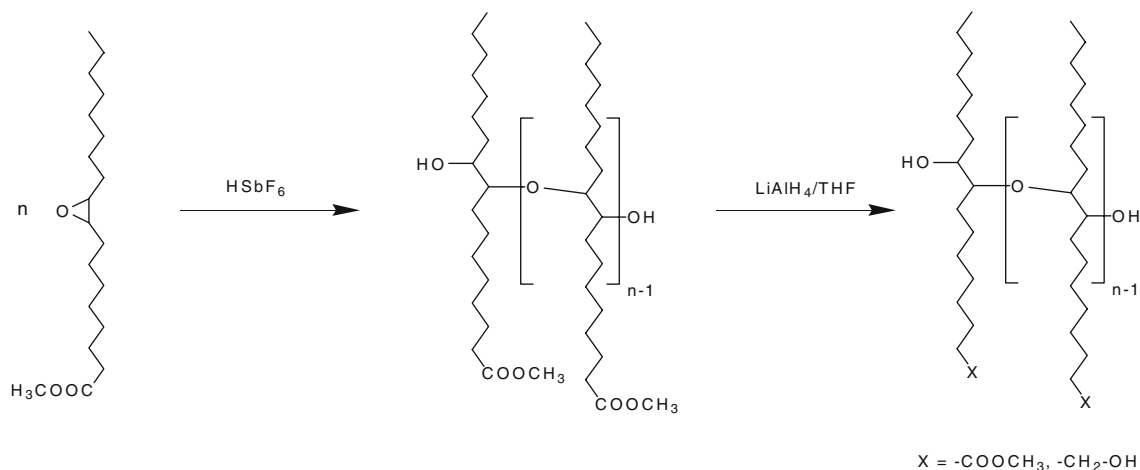


Fig. 7 Synthesis of polyetherpolyoles from methyl epoxystearate followed by partial reduction to give primary alcohols

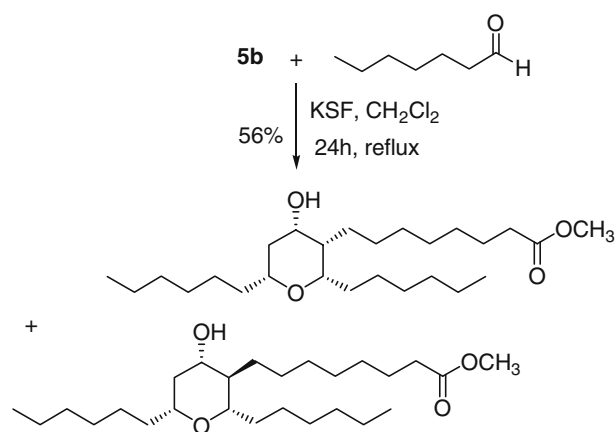


Fig. 8 Addition of heptanal to methyl ricinoleate *5b* in the presence of montmorillonite KSF to give the 4-hydroxytetrahydropyran as two diastereomers in a ratio of 2.7:1

acid or ester is converted into an unsaturated percarboxylic acid by a lipase-catalyzed reaction with H_2O_2 and is then self-epoxidized in an essentially intermolecular reaction (Rüsch gen. Klaas and Warwel 1997).

In the industry, vegetable oil epoxides are currently used mainly as PVC stabilizers. Interesting applications were opened by the possibility of photochemically initiated cationic curing (Crivello and Narayan 1992). The comparison of UV-curable coatings with linseed oil epoxide as binder to a binder produced on a petrochemical basis derived from propylene oxide by LCA showed clear advantages for the renewable raw material linseed oil (Eissen et al. 2002).

The preparation of polyols from epoxides of fatty acids and vegetable oils for polyurethane use was the subject of many studies (Guo et al. 2000, 2002; Petrovic et al. 2000). Recently, polyether polyols were obtained by acid-catalyzed ring-opening polymerization of epoxidized methyl oleate and subsequent partial reduction of ester groups gave primary alcohols (Fig. 7). Depending on the degree of reduction, polyols of different hydroxyl values were obtained, which were reacted with 4,4'-methylenebis (phenyl isocyanate) to yield polyurethanes (Lligadas et al. 2006).

Polyether polyols for polyurethanes are mostly produced from propylene oxide. Propylene oxide is one of the top 50 chemicals in terms of production: in 1997, 1.9×10^6 t were produced in the USA, about 1×10^6 t were produced in Germany, and approximately 4×10^6 t were produced worldwide. It is the top 50 chemical with the highest gross energy requirement of about 105 GJ/t (Eissen et al. 2002). Eventually, polyetherpolyols derived from epoxidized fatty compounds may substitute the petrochemical compounds in various applications.

It is also a challenge to synthesize suitable diisocyanates via diamino compounds derived from vegetable oils, making the production of polyurethanes possible completely from renewables.

Chiral and enantiomerically pure oleochemicals

It seems to be remarkable that enantiomerically pure fatty acids as part of the chiral pool of nature were not used very extensively for the synthesis of nonracemic compounds. In contrast, the stereochemistry is lost in most oleochemical transformations performed with, e.g., ricinoleic acid.

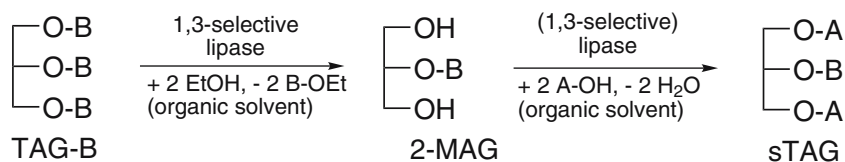
Ricinoleic acid [(12*R*,9*Z*)-12-hydroxyoctadecenoic acid] with a homoallylic alcohol functionality was reacted in a Prins-type reaction with aldehydes mediated by $AlCl_3$ and the environmentally more benign montmorillonite KSF (Fig. 8) to give with high stereoselectivity 2,3,6-trialkyl-4-chlorotetrahydropyrans, which are interesting building blocks (Biermann and Metzger 2006).

Chemical epoxidation of ricinoleic acid gives, with low diastereoselectivity, two diastereomers of epoxidized ricinoleic acid that were transformed in two steps in the enantiomerically pure hydroxy aziridines. The hydroxy-aziridines were tested for cytostatic/cytotoxic activity with regard to different tumor cell lines. It is interesting to note that the minor diastereomer in all cases showed stronger activity than the major diastereomer. Similar results were obtained for antimicrobial activity tested on different microorganisms. Vernolic acid was reacted quite analogously to give the respective aziridine (Fürmeier and Metzger 2003).

Table 1 Enzymes useful for lipid modification

| Enzyme | Applications | Examples |
|---------------|---|---|
| Lipase | Synthesis of structured triglycerides Enrichment of specific fatty acids Incorporation of specific fatty acids Synthesis of fatty acid derived products | Cocoa-butter equivalent, Betapol PUFA from fish oils PUFA into plant oils Emollient esters |
| Phospholipase | Removal of fatty acids in <i>sn</i> 1- or <i>sn</i> 2-position (PLA ₁ or PLA ₂) Removal of phosphate group (PLC) Head group exchange (PLD) | Degumming of oils Lyso-phospholipids Chiral diglycerides Phosphatidylserine |
| Monooxygenase | Hydroxylation of fatty acids | Precursor for polyesters/lactones |
| Epoxidase | Epoxidation of double bonds | – |
| Lipoxygenase | Synthesis of FA-hydroperoxides | – |

Fig. 9 Principle of the lipase-catalyzed two-step synthesis to obtain sTAG in high purity. *A* and *B* denote different fatty acids



Current state of enzymatic conversion of fats and oils

A broad range of enzymes can in principle be used for the conversion of fats and oils and their application in lipid modification is well documented in literature (Bornscheuer 2000, 2003; Bornscheuer et al. 2002). Table 1 provides a survey about useful enzymes, their application areas, and selected examples.

By far the most often used biocatalysts are lipases (EC 3.1.1.3, triacylglycerol hydrolases) for which fats and oils are their natural substrates. These enzymes do not require cofactors, many of them are available from commercial suppliers and they exhibit high activity and stability, even in nonaqueous systems. A plethora of publications on the use of lipases has appeared in the last two decades and only the most important and recent examples are highlighted here. Because lipases show chemo-, regio-, and stereo-selectivity, they can be used for the tailoring of natural lipids to meet nutritional properties, especially for humans. The most prominent example is the synthesis of cocoa butter equivalents (Quinlan and Moore 1993). Cocoa butter is predominantly a 1,3-disaturated-2-oleyl-glyceride where palmitic, stearic, and oleic acids account for more than 95% of the total fatty acids. Cocoa butter is crystalline and melts between 25 and 35°C providing the desirable “mouth feel.” Unilever (Coleman and Macrae 1977) and Fuji Oil (Matsuo et al. 1981) filed the first patents for the lipase-catalyzed synthesis of cocoa butter equivalent from palm oil and stearic acid. Both companies currently manufacture it using 1,3-selective lipases to replace palmitic acid with stearic acid at the *sn*1- and *sn*3-positions. Reactions are usually performed as transesterification or acidolysis of cheap oils using tristearin or stearic acid as acyl donors and a 1,3-specific lipase.

Structured triglycerides (sTAG) with a defined distribution of different fatty acids along the glycerol backbone are important compounds for a range of applications in human nutrition. sTAG containing medium-chain fatty acids at the *sn*1- and *sn*3-position and a long preferentially polyunsaturated fatty acid are used to treat patients with pancreatic insufficiency and to give rapid energy supply (i.e., for sports). Another important example is Betapol, which is used for infant nutrition. This sTAG [1,3-dioleoyl-2-palmitoyl glycerol (OPO)] contains oleic acid at the *sn*1- and *sn*3-position and palmitic acid at the *sn*2-position. Currently, Betapol is manufactured by interesterification of tripalmitin with oleic acid using a lipase from *Rhizomucor miehei* (Novozyme RM IM). However, the product contains only 65% palmitic acid in the *sn*2-position. To obtain higher purities and yields, a two-step lipase-catalyzed process was developed (Fig. 9). First, tripalmitin is subjected to alcoholysis with ethanol with a lipase from

Rhizopus delemar immobilized on a polypropylene carrier (EP-100) yielding 95% monopalmitin with a purity >90% after crystallization. Subsequent enzymatic esterification with oleic acid in *n*-hexane proceeded quantitatively within a few hours and the final OPO (yield 70%) contained up to 96% palmitic acid in the *sn*2-position (Schmid et al. 1999).

Another possibility is starting from 1,3-diglycerides (1,3-DAG), which are available on large-scale as cooking and frying oil (see below), 1,3-DAG can be obtained from glycerol and fatty acid vinyl esters (Berger et al. 1992) or via controlled acyl migration from 1,2-diglycerides. These 1,3-DAGs can then be esterified with a lipase exhibiting distinct fatty acid selectivity, i.e., the lipase must not act on the fatty acids present in the *sn*1 and *sn*3-position and solely catalyzes the introduction of the second type of fatty acid (B-OH in Fig. 10) into the *sn*2-position. It could be shown, that commercial lipase from *Pseudomonas cepacia* (Amano PS) and *Candida antarctica* (CAL-B) allow for the synthesis of sTAG. These biocatalysts are not 1,3-regiospecific, but rather show distinct fatty acid specificity (Bornscheuer et al. 2005; Wongsakul et al. 2004). Recently, the first lipase with distinct *sn*2-specificity was created by directed evolution of an esterase (Reyes-Duarte et al. 2005). This enzyme should be very useful for the synthesis of sTAG based on this methodology.

Recent examples for successfully industrialized processes include lipase-catalyzed production of zero-*trans* margarines (ADM and Novozymes) and diglyceride-based cooking and frying oils (Kao Corp. and ADM) (Watanabe et al. 2004). The zero-*trans* and reduced *trans* oils and fats are produced on industrial scale by transesterification using lipase from *Thermomyces lanuginosa* (TL IM) in combination with a cost-effective immobilization technology (Anonymous 2005). Thus, the use of isolated technical enzymes, in contrast to conventional chemical means, provided cost-effective, simple, and straightforward methods to obtain the desired products. In addition, these biocatalytic processes are environmentally friendly and can thus be seen as a result of sustainable developments. The technology developed by ADM and Novozymes received the Presidential Green Chemistry Challenge Award in 2005.

Lipases were also used on industrial scale to produce simple esters, e.g., for cosmetic applications (Fig. 11).

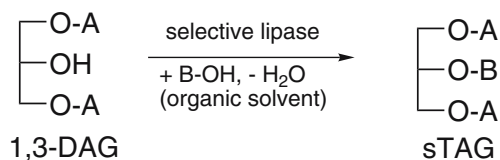
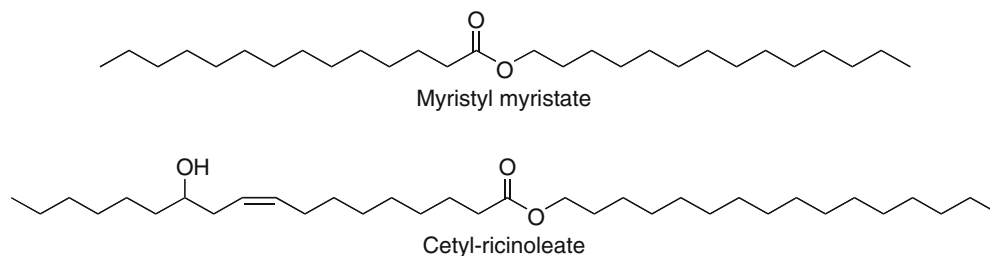


Fig. 10 Principle of the lipase-catalyzed two-step synthesis to obtain sTAG in high purity. *A* and *B* denote different fatty acids

Fig. 11 Myristyl myristate and cetyl ricinoleate are produced by lipase-catalyzed esterification



Prominent examples are cetyl ricinoleate and myristyl myristate (Heinrichs and Thum 2005; Hills 2003). Although both esters were chemically synthesized for a long time, enzyme technology allows higher yields and substantially purer products. The higher costs for the biocatalyst are compensated by savings in energy (ambient temperature instead of 160–180°C) and product purification (i.e., a bleaching and deodorization step can be omitted).

Phospholipase (PLA₁ and PLA₂) are used on large-scale for degumming—the removal of phospholipids—of natural fats and oils. Whereas the earlier process used a mammalian phospholipase from porcine pancreas specific for the *sn*2-position (PLA₂), this method was recently changed to the application of a microbial biocatalyst obtained from *Fusarium oxysporum*, which exhibits *sn*1-selectivity (PLA₁) (Clausen 2001). In both cases, lysophospholipids are formed, which are easily hydratable and therefore allow for the reduction of the phospholipid content below 10 ppm. The microbial enzyme is safer (with respect to enzyme supply and a lowered risk of contamination) and shows better performance in the process, and the products obtained from them are considered kosher and halal.

Whereas phospholipase C has no application in biocatalysis yet, phospholipase D can be used in the head group exchange. This allows for the synthesis of nonnatural phospholipids and the synthesis of compounds bearing natural head groups, i.e., phosphatidyl serine (Skolaut et al. 2005).

To our knowledge, the other enzymes listed in Table 1 do not have large-scale applications yet. One exception is the use of whole-cell systems, i.e., *Candida* yeast for the biohydroxylation to yield dicarboxylic acids as mentioned above. However, the progress made in gene technology, metabolic engineering, and biocatalysis should make the application of these oxidative enzymes feasible in the near future.

Future perspectives of chemical and biotechnological conversion

Most products obtainable from renewable raw materials may at present not be able to compete with the products of the petrochemical industry, but this will change as oil becomes scarcer and oil prices rise. It is very important to note that fat alcohols derived from fats and oils are an example that shows that base chemicals derived from renewable feedstocks can be commercially competitive

with petrochemical products. At present, it can be observed that petrochemical fat alcohols will increasingly be substituted. More examples will come up in the next few years. Middle- and long-chain diacids, ω -hydroxy fatty acids, ω -unsaturated fatty acids, and epoxidized vegetable oils may be the next candidates. The use of modern synthetic methods together with enzymatic and microbiological methods has led to an extraordinary expansion in the potential for the synthesis of novel fatty compounds. With the breeding of new oil plants, numerous fatty compounds of adequate purity are now available, which makes them attractive for chemical synthesis and industrial use. However, numerous synthetic problems have to be solved and solutions have to be found in the coming years.

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