Practical Implications of Fatty Acid Topography Modification by Unsaturation

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ABSTRACT

Unsaturated fatty acids are markedly different from their saturated counterparts in a number of areas that relate only to the physical properties given them by their topography. In systems where free single bond rotation is restricted, such as monolayers or biomembranes, the effectiveness of chain packing and the fluidity of the fatty chain region is critical and here may best be studied. Topography plays a role in many chemical reactions in terms of reaction rate and product stereochemisty, but is less critical here. Topography hindrances to reaction rate often may be overcome by raising the reaction temperature. When stereochemistry is important, however, the topography of the reactants must be considered. In tightly packed crystalline bulk or condensed monolayer states, the conformation assumed by the chain is its most stable one, excluding polymorphism. This conformation usually will approximate the one of greatest stability for the free molecule in a nonrestricting environment. However, because differences in energy between rotational conformers is generally small, departures from the preferred conformation will be abundant in the absence of constraints imposed by neighboring molecules. Therefore, in the absence of a restricting environment, topographical differences will be minimized. The counterpoint to this is that a large percentage of the fatty acids in use are in such restricting environments in biomembranes, soaps, wetting and foaming agents, fabric softeners, etc., or are purified through a process making use of differences in chain packing efficiency, crystallization, where their topography is the primary source of their utility.

INTRODUCTION

Fatty acids commonly are used in applications taking advantage of their interfacial activity. Commercial surfactant derivatives such as soaps, ethoxylates, alpha-sulfonates, quats, amines and amides for use in cleaning, emulsifying or fabric softening; corrosion inhibitors and other coating applications where the fatty acids often are polymeric; and biological compounds such as fats or phospholipid derivatives in cell membranes all are possible because of the polarity differences between the hydrophilic carboxyl group and the hydrophobic fatty chain. In such applications, the shape, or the space occupied by, or the *topography* of the fatty chain dramatically affects the way the chains interact and pack together. The nature of the hydrocarbon chain packing strongly influences melting point, foaming, micelle stability and structure, surface coverage, and cell membrane permeability and fluidity as well as most enzymatic reactions in living organisms. Other areas such as coatings and lacquers, polymer plasticizers/stabilizers and fat hardening often involve interfacial interactions with the carbon-carbon double bonds in the fatty chain. The steric environment or topography about this bond influences the rate of air-induced polymerization (gas/liquid interface), epoxidation (liquid/liquid interaction) and hydrogenation (gas/solid/liquid interfaces). The topographical differences between saturated, monounsaturated and polyunsaturated acids influence a number of physical properties, and given a degree of unsaturation, the geometrical or *cis-trans* isomerism naturally affects fatty acid packing and double bond reactivity.

All of this is well known; however, the implications of fatty acid unsaturation, regardless of the well developed double bond chemistry, on things like micellar structure, surface tension, density, cell membrane permeability and melting point, a diverse group of properties, are not immediately clear. The enforced "kink" in the hydrocarbon chain caused by unsaturation substantially changes the density to which these chains may be packed under similar thermodynamic conditions. That chain packing efficiency directly influences melting points is obvious. To those of us not trained in the surface sciences, it is less clear, but by no means less true, that chain packing plays an equally important role in the surfactant, micellar and biological systems mentioned earlier.

DEFINITIONS

I define topography from Webster's New Collegiate Dictionary of the American Language as "the configuration of a surface including its relief and the position of its natural and man-made features." In a chemical sense, for fatty acids, topography is a dynamic concept, highly dependent on temperature and environment. Also, given a set of physical conditions, the time-averaged shape of the molecules may be quite different from the instantaneous shape. Therefore, the topographies of fatty acids of a given chain length are not fixed in the sense that DNA or proteins are. They may vary over a wide range from rod-like to semi-spheroid, depending on external parameters, especially temperature and the local population density. Just as dense population centers such as Tokyo, Shanghai, New York or London give each individual less room to himself than one finds in parts of Texas, Siberia or Australia, so the shape and space occupied by the fatty acid molecule is restricted by encroachments of neighboring molecules into its rotational space. These restrictions affect the time-averaged topography and, together with the temperature, can cause roughly spherical shapes in the gas phase or relatively immobile rods in the crystalline phase.

In general, the fatty acid molecular motion of interest is rotational with respect to a fixed point rather than purely random, as gas molecules in an enclosed space. Most fatty acid applications are surfactant-related. Soaps, cleaning agents, wetting agents, biological membranes—in all of these a polar head group is immersed in one phase, usually aqueous, and the hydrocarbon chain in another phase, often its own phase. This has the effect of fixing the head group, to a first approximation, with respect to the tail. (In fact, the nature of the head group does affect packing of the chains. In a discussion of free fatty acids, however, this is a fixed variable.) Even in some derivatization reactions such as fatty acid hydrogenation or epoxidation, the double bond is the point of interest, coordinated with a catalyst particle or an oxygen source, and may be considered to be at the origin of a tricoordinate system, fixed with respect to its side chains. The energy required for some foreign body, such as another fatty chain, a catalyst particle or an oxygen bubble, to approach a kinetically active fatty acid is, among other things, a function of the most thermodynamically stable instantaneous shape of the chain being approached. This is a statement of steric hindrance. The greater the energy required for approach, the less likely the interaction is to occur. This phenomenon is seen readily in the liquid-solid transitions of the high melting, more readily packed *trans*-unsaturated acids vs the low melting, kinked, *cis*-unsaturated acids.

The liquid crystal-gel transition of phospholipids in bio-

membranes, an analogous transition to melting, is a practical application of this fact. Phospholipids, containing two fatty acid groups with a single polar head group, are the major structural feature of biomembranes. For these membranes to function and enable life, they must be in a fluid state, having flexibility and permeability. It is interesting to note that organisms functioning at different temperatures have fluid membranes derived from fatty acids with different melting points. Certain bacteria which prosper in relatively high temperature environments have phospholipids containing two high melting stearic acid groups. Human life, operating at a lower temperature, uses phospholipids containing one saturated acid such as palmitic or stearic, and one unsaturated, often oleic. This combination is less readily packed together than two saturated chains, and consequently is fluid at the lower temperature. Life at still lower temperatures, such as the US southern pine tree, needs a monounsaturated acid, oleic, and a diolefinic acid, linoleic, to maintain fluid membranes. These are the major constituents of US tall oil fatty acids and soybean oil. The degree to which membrane fluidity can be fine-tuned is represented in the tall oil fatty acid composition of northern trees from colder climates. Scandinavian tall oil, for example, contains a larger percentage of polyunsaturated acids, such as linolenic, which give fluid membranes at still lower temperatures.

EXAMPLES

Physical/Chemical Data

As mentioned earlier, the symptoms of these topography differences crop up in a number of different areas. The most obvious difference is the macroscopic, physical observation of the melting point. Table I shows the melting points of straight chain acids with identical carbon numbers but different topographies. Differences of 30-40 C in melting points occur between acids with various types of monounsaturation at a given position. A maximum difference of 80 C in the melting points of these C_{18} straight chain acids is found. A third observation is that unsaturation near the center of the chain is much more effective at altering packing efficiency, and thereby melting point, than unsaturation near either end. Introduction of a *trans* double bond in the middle of the chain (elaidic acid) lowers the melting point only 24 C vs stearic acid, whereas similar *cis* unsaturation (oleic acid) lowers the melting point 58 C. Figure 1 shows why. The kink in the center of the chain minimizes the number of uninterrupted methylene carbons, which tend to be in the thermodynamically favored *trans* conformation, thereby maximizing the packing distortion.

In addition, large differences in the melting point of stereochemical isomers of regiochemically identical polyunsaturated compounds occur. For regiochemical isomers, as the 9,12 vs the 9,11 or 10,12 dienoic acids, it is clear that the methylene interruption of polyunsaturated fatty acid systems which characterizes naturally occurring polyunsaturated fats causes lower melting points than shown by conjugated dienoic acids as a result of the decreased packing efficiency of the naturally occurring acids.

Physical phenomena that do not involve chain packing to such a great degree distinguish much less among the various acids of a given carbon number. The boiling points of selected acids shown in Table II demonstrate this. Still, the elaidic *(trans)* is close to stearic acid in boiling point with the severely kinked oleic acid being the most different from stearic acid.

The density data at 20 C for the liquid acids show that with increasing *cis* unsaturation, the density increases. The heat of fusion is a function of the energy required to convert the acid from its solid state to a liquid state, and therefore is a measure of chain packing efficiency. Increasing unsaturation, especially *cis*, reduces the energy required for this phase transition. This is reflected in the melting point data in Table I. The viscosity data show that, at a constant number of degrees over the melting point, the kinked oleic acid has a substantially greater viscosity than the flexible stearic acid.

Table III shows some surfactant data for selected fatty acid salts (8). The critical micelle concentrations (CMC) of the unsaturated acids are almost an order of magnitude higher than for stearic acid, while the surface tensions at the CMC are markedly lower than for stearic acid. The unsaturated acids show similar foaming tendencies, but both were much greater than stearic. The greatest differences between the unsaturated acids are their surface tensions, γ, at the CMC (much lower for elaidic acid) and the CMC

TABLE I

Melting Points (C) of Straight Chain, C_{18} Acids (1-3) (Stearic Acid = 69 C)

Position of unsaturation	*cis*-Double bond(s)	*trans*-Double bond(s)	Triple bonds	*trans, cis* Bonds	*cis, trans* Bonds
2	50	58	57	–	–
3	50	65	74	–	–
4	46	59	75	–	–
5	13	47	52	–	–
6	29	54	51	–	–
7	13	45	49	–	–
8	24	52	47	–	–
9	11	45	46	–	–
10	23	53	46	–	–
11	13	44	47	–	–
12	28	53	47	–	–
13	27	44	49	–	–
14	42	53	64	–	–
15	41	59	65	–	–
16	54	66	–	–	–
17	56		67	–	–
6,9	−11	15	–	–	–
9,12	−8	26	–	–	–
10,12	21	55	–	3	–
9,11	19	33-43	–	–	22
9,12,15	−10	29	–	–	–
9,11,13	–	71	–	–	48 *(c,t,t)*

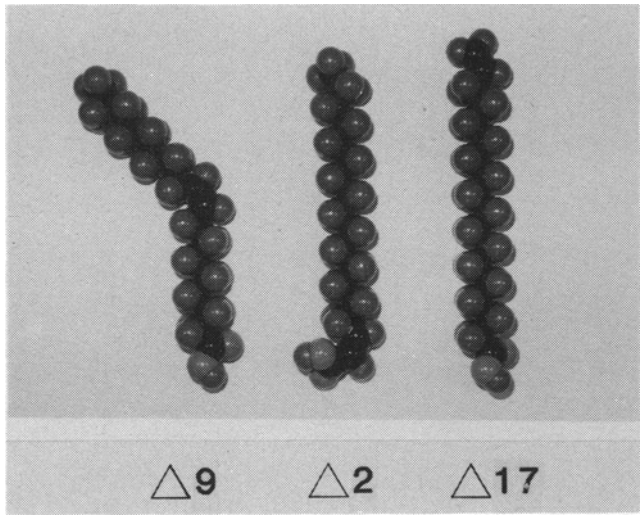

FIG. 1. Low energy conformations of *cis*-9-,2- and 17-octadecenoic acids.

themselves. These differences, together with the foaming data, prompted the authors to single out potassium elaidate as a surfactant showing "balanced properties."

Monolayer Studies

Marked differences also are seen with fatty acids having varying degrees of unsaturation in monolayer studies. Simplistically, fatty acids will spread on the surface of water to give monolayer systems where the hydrophilic acid group is immersed in the aqueous phase and the nonpolar hydrocarbon chain extends above the surface of the water. These experiments commonly are run in either a Langmuir film balance or by the Wilhelmy plate method. In the film balance, a film of amphiphilic molecules is allowed to form on a clean water surface. A movable float attached to a balance separates a clean water surface from the film-covered area. The molecular density, and therefore the degree of chain packing, may be increased or decreased by moving the float, and the surface pressure at a given surface area may be measured directly. A second point of interest is the equilibrium spreading pressure shown by all amphiphiles where, at a constant film surface area, there is a equilibrium between bulk fatty acid and acid in the film.

By varying the surface pressure over a wide range, a plot of surface pressure vs area per molecule is obtained, and a plot of stearic acid is shown in Figure 2 (9). Stearic acid forms a condensed film at room temperature, analogous to a two-dimensional solid. In the very steep section of the curve, above 25 dynes/cm and below ca. 20 $Å^2$/molecule, the observed molecular area most closely approximates the

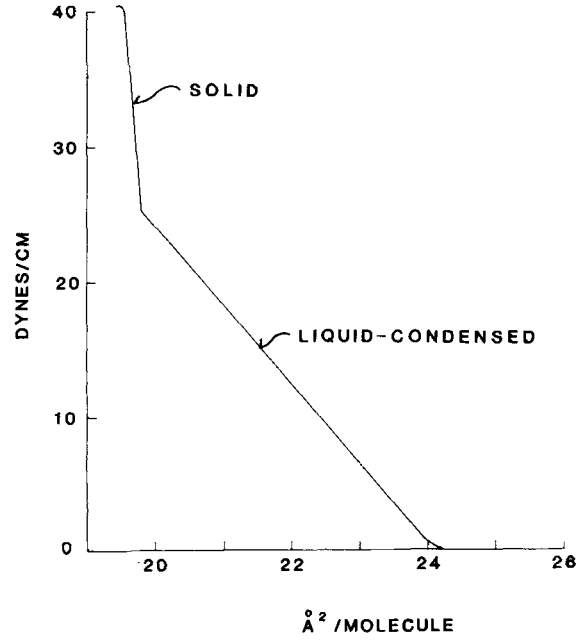

FIG. 2. Surface pressure vs area plot of stearic acid (9).

TABLE II

Physical Properties of Selected Fatty Acids

Acid	Boiling point (C @ 10 mm) (4)	Density d_4^{20} (mp) (5)	Viscosity, (Pa.s X 10^3), 30 C over the melting point (6)	Heat of fusion, ΔH_f(kJ/mol) (7)
Stearic, 18:0	227	–	5	63.2
Oleic, $18:1^{9c}$	223	.8905(11)	10.8	46.4-49.8
Elaidic, $18:1^{9t}$	226	–	–	59.9-62.3
Linoleic, $18:2^{9c,12c}$	224	.903(−8)	–	51.5
Linolenic, $18:3^{9c,12c,15c}$	224.5	.9164(−10)	–	39.3

TABLE III

Surfactant Data for C_{18} Fatty Acid Potassium Salts (8)

Acid	Temperature	CMC	γ at CMC	Foaming at CMC		Foaming at optimum concentration		
	(C)	(M X 10^3)	(dyne/cm)	Height (mm)	5-min stability (%)	Height (mm)	5-min stability (%)	Concentration (M X 10^3)
Stearic	40	–	–	0	0	25	70	6.5
	60	0.70	65.6	50	60	190	90	5
Oleic	40	5.31	45.0	208	90	210	90	6
	60	4.93	39.2	212	97	220	95	6-7
Elaidic	40	7.42	37.0	195	97	200	97	10
	60	6.50	32.8	230	95	230	95	8

true molecular cross-section. The further compressibility of the film at pressures above 25 dynes/cm is very small, comparable to that of solid bulk stearic acid. The lower part of the curve represents a state of greater compressibility with an extrapolated area of 24-25 $Å^2$ at surface pressure = 0. Debate continues about the nature of this compressible area, but the terms "solid" for the incompressible phase and "liquid-condensed" for the still dense but yet compressible phase below 25 dynes/cm commonly are used. The solid phase is thought to be densely packed chains vertical to the water surface. The liquid-condensed phase is considered to be a densely packed chain structure, but tilted to the interface as in Figure 3 (10). When a modification to the system is made that will affect the dense packing of the hydrocarbon chains, such as a temperature increase, chain branching or the introduction of unsaturation, other phases and discontinuities appear in the surface pressure vs area diagram. Figure 4 shows how the surface pressure vs area diagram of myristic acid changes with increasing temperature (12). At the lowest temperature, a condensed film is seen. As the temperature is raised, another discontinuity appears, followed by the gentle arcing curve characteristic of the liquid expanded phase. The effects of these data are seen in the temperature dependence of many surfactant properties such as wetting, foaming and detergency. Figure 5 shows how unsaturation at the center of a C_{18} chain affects chain packing thermodynamics (12). The stearic acid graph shows the solid and liquid-condensed phases, but the oleic and elaidic acids (Δ9-*cis*, Δ9-*trans*) both are shown to give expanded films by their plots. The effect, therefore, of unsaturation, even the relatively easily packed *trans* double bond, is to expand markedly the physical state of the monolayer, a system evidently very sensitive to chain packing efficiency. Interaction of the chain unsaturation with the aqueous subphase also has been suggested to contribute to monolayer expansion (12). However, lowering the temperature to near 0 C demonstrates the tendency of the elaidic film to condense, while the film of *cis*-unsaturated fatty acid remains expanded. Oleic acid is predicted to have a theoretical condensation temperature of −30 C (13,14). The surface pressure vs area diagrams of the Δ13 *cis* and *trans* C_{22} acids show the difference at room temperature between their packing efficiencies (Fig. 6). The *trans* acid remains a condensed film at room temperature while the *cis* acid is an expanded film (15). At a given temperature and surface pressure, molecular area increases in the order: stearic acid (22 $Å^2$), elaidic acid (35 $Å^2$), and oleic acid (37 $Å^2$) (Table IV) (12). Although these are not hard numbers or detailed topographical maps, they do represent the area each acid requires under identical thermodynamic conditions. Striking are (a) the similarity between the Δ9 *cis* and *trans* isomers, and (b) the large difference between the saturated acid, stearic, and the Δ9 *trans* acid, elaidic, especially considering the apparent topographical similarity of the Δ9 *trans* and saturated acids vs the Δ9 *cis* acid, oleic. Moving the double bond nearer either end of the chain results in monolayers showing more condensed character, i.e., more like a saturated acid. This is consistent with the melting point data discussed earlier (12).

The equilibrum spreading pressure of fatty acids is a quantity which reflects the surface pressure when bulk fatty acid and monolayer are in equilibrium. Alternatively, the equilibrium spreading pressure represents the surface pressure of a saturated solution of fatty acid (16). Therefore, the equilibrium spreading pressure is a measure of the tendency of fatty acid to remain in the bulk phase vs its tendency to interact with water and spread. In other words, the thermodynamic competition between the

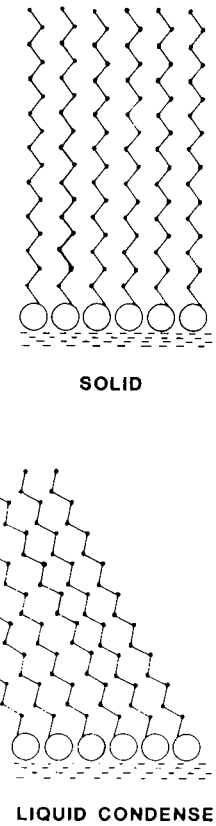

FIG. 3. Solids vs liquid-condensed monolayer structure (10).

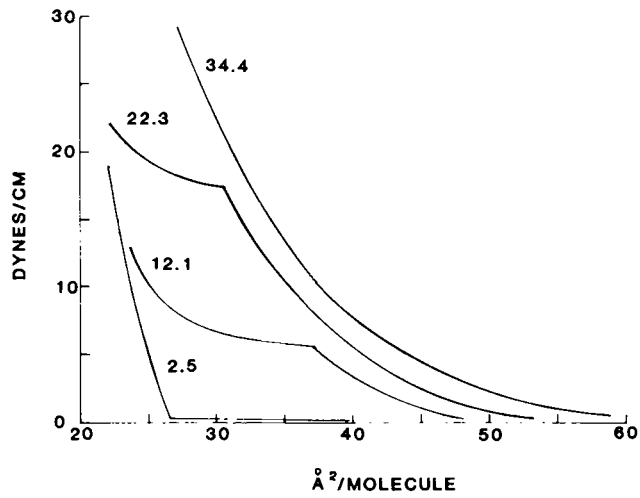

FIG. 4. Temperature dependence of surface pressure vs area plot for myristic acid (11).

desirable hydrophilic interaction of the polar head group with water vs the stability of the bulk phase energy sink is in balance. The more stable the acid is in bulk, or the more efficient its chain packing, the less likely the acid is to spread. Such acids tend to be solid. Conversely, things that increase the thermodynamic stability of the monolayer state such as soap formation or introduction of polar groups in the side chain which will interact with water favor the monolayer state. In addition to features which favor bulk or monolayer, traits such as chain branching or mid-chain *cis* unsaturation simply disfavor bulk by increasing its free energy through a decreased chain packing efficiency (such acids are liquid). This also shifts the spreading equilibrium toward monolayer, raising the equilibrium spreading

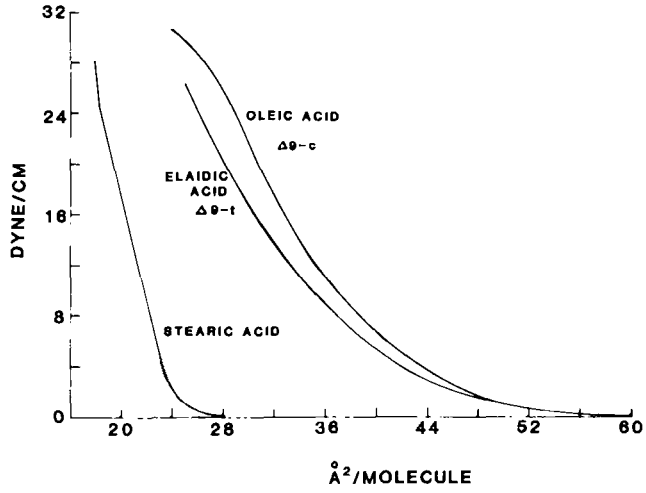

FIG. 5. Surface pressure vs area diagrams of midchain unsaturated C_{18} fatty acid vs stearic acid at 25 C (12).

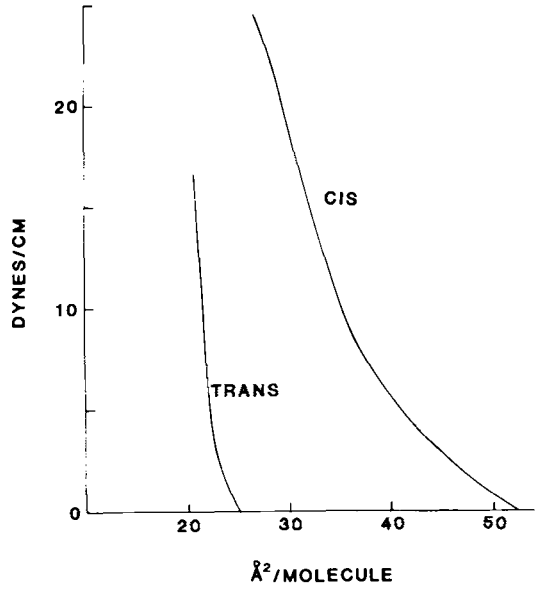

FIG. 6. Surface pressure vs area diagrams for *cis*- and *trans*-13-docosenoic acids (15).

TABLE IV

Molecular Area at 25 C and 10 dyne/cm (12)

Stearic acid	22 Å²
Elaidic acid (Δ9 *trans*)	35 Å²
Oleic acid (Δ9 *cis*)	37 Å²

TABLE V

Equilibrium Spreading Pressures (ESP) for Common Fatty Acids (17,18)

Acid	Melting point (C)	ESP 25 C (dyne/cm)
Stearic, 18:0	69	0.5
Palmitic, 16:0	63	9.5
Oleic, $18{:}1^{9c}$	11	30.3,28.9
Elaidic, $18{:}1^{9t}$	45	14.0
Linoleic, $18{:}2^{9c,12c}$	-8	28.5
Linoelaidic, $18{:}2^{9t,12t}$	26	26.7
Linolenic, $18{:}3^{9c,12c,15c}$	−10	27.2

pressure. Measurements of the equilibrium spreading pressure at different temperatures allow the calculation of several thermodynamic functions such as the free energy, enthalpy and entropy of spreading and the heat of fusion (16,17). These values are very useful data.

Some equilibrium spreading pressure data for common fatty acids are shown in Table V. The most stable acid in the bulk phase, stearic acid, shows the lowest equilibrium spreading pressure (18). Of all the unsaturated acids, oleic has the largest equilibrium spreading pressure, implying that its bulk state is more energetic with respect to its monolayer state than elaidic acid or even the polyunsaturated acids.

The spreading of monolayers has practical significance in a number of areas. The surface tension of water may be markedly reduced by monolayers. This results in many important commercial processes including foaming, wetting and detergency. The partial control of oil spills has been postulated through the use of surfactants of high spreading pressure. The surfactant, having a higher surface pressure than the oil, can surround the oil surface and cause it to contract to a thickness several orders of magnitude greater than the surfactant monolayer thickness (19).

Biological Systems

The impact of chain packing probably is most critical in biological systems, as evidenced by the almost exclusive use of *cis* vs *trans* unsaturated fatty acids by living organisms. In biological systems, fatty acids have several functions, among them energy storage, synthetic precursors for more complex molecules such as prostaglandins and providing form, shape and permeability to biological membranes as their lecithin or phospholipid derivatives. In the first examples, energy storage and synthesis, the fatty acid unsaturation plays a role in an enzymatic reaction; the packing together of fatty chains is not the goal, even though the chain topography is of paramount importance to the enzyme. In biological membranes, however, the density and effectiveness of fatty acid chain interaction is primarily responsible for most of the useful properties of these membranes. What, then, do *cis*-unsaturated acids offer over *trans* acids? What does unsaturation in the middle of the chain offer over unsaturation at either terminus? These questions are at the base of all fatty acid biochemical and biological research, and still cannot be completely answered.

Of all the possible fatty acids, some offering rich chemistry and lucrative derivatization reactions, we have stearic, palmitic, oleic and linoleic acids with some linolenic-type (trienoic) and some myristic acid. Although many successful, large-volume applications exist, these acids are chemically rather dull. Still, Nature has found them to be optimum for many life processes, so we should not complain too much.

In this area, I will not discuss enzymatic reactions such as fatty acid oxidation, chain elongation or phospholipid synthesis since the need for specific topographies in enzyme-catalyzed reactions is well known. Rather, I will address the applications of fatty acids and their derivatives where the physical chain-chain interaction is critical for the successful performance of a function.

Fatty acids in biological membranes generally are esterified and occur as phospholipids. Phospholipids are *bis*(fatty acid)glycerides with one of the primary hydroxyl groups of glycerol esterified to phosphoric acid, resulting in two fatty chains joined to one polar head group. Esterification of a second acid site on phosphorus with alcohols such as ethanolamine or choline give cephalins and lecithins, respectively, which are the major lipid components of most membranes in animal cells.

In addition to the variation in polar groups, each class of phospholipids shows a considerable variation in the fatty acids employed. The most common acids found are stearic acid, palmitic acid, oleic acid, linoleic acid, linolenic acid and arachidonic (C20:4) acid. The fatty acid content may be fine-tuned to give specific properties of fluidity, rigidity and permeabilities to membranes at different temperatures. Naturally, other components also occur in biological membranes, especially proteins and sterols such as cholesterol and sitosterol. Most commonly, unsaturated acids *(cis)* are located preferentially at the 2-position of the molecules, esterified to the glycerol secondary hydroxyl group. Saturated acids normally appear at the 1-position.

These phospholipids will form monolayers by spreading on water just as the free fatty acids described earlier do. Analogously to the fatty acids, saturated lecithins ([1,2-distearoyl]-3-lecithin) form condensed monolayers at room temperature with molecular areas of 37-50 $Å^2$, as might be expected for two closely packed saturated chains; replacement of one stearoyl chain with an oleoyl chain results in expanded monolayers at room temperature (20).

Table VI shows the molecular area occupied by synthetic lecithins with varying degrees of unsaturation at 10 dyne/cm and 22 C. A marked expansion of the monolayer occurs with increasing unsaturation. This expansion also is represented by monolayer permeability. Table VII shows that the permeability of pure lecithin liposomes towards glucose increases with increasing unsaturation of the fatty components. Incorporation of cholesterol into the film caused reduction in the area per molecule and in permeability.

Differential scanning calorimetry (DSC) analyses of (1,2-dioctadecenoyl) lecithins which were equilibrated with 25% water show a gel-to-liquid crystalline transition endotherm. The temperature of this transition, T_c, and the enthalpy, ΔH, are both lower for all *cis* unsaturated lecithins than for the saturated, (1,2-distearoyl) lecithin, as shown in Table VIII. The lecithins containing centrally located double bonds, $\Delta 7$-$\Delta 11$, undergo the transition at the lowest temperatures and have the lowest ΔH values (22). This is consistent with other data mentioned earlier showing that midchain, *cis* unsaturation causes a greater divergence of properties from saturated acids than does unsaturation near either end of the chain.

This low gel-liquid crystal transition temperature seen with unsaturated lecithins is critical in life processes. For instance, in the bacterium *Escherichia coli*, the proper functioning of the membrane system has been demonstrated to require that the membrane lipids be in a fluid or liquid crystalline state. Cooling the bacterium to temperatures where the lipids become rigid markedly slows the enzymatic activity of membrane-bound proteins (23).

In their native element, the membrane, lecithins and phospholipids in general occur in bilayers rather than monolayers. The bilayer structure, shown schematically in Figure 7, consists of two layers of phospholipid with their hydrophobic hydrocarbon chains in the middle and the polar head groups facing outward to an aqueous medium on either side of the membrane. Phospholipid bilayers also may be generated and studied in vitro, outside the living cell. Many workers have studied the structure-property relations of such bilayers with respect to the different fatty chains available. Nuclear magnetic resonance spectroscopy, NMR, has developed as one of the most useful tools in this work.

A large volume of work has been done by Seelig and coworkers in Basel on the molecular order, structural dynamics and conformation of the hydrocarbon chains of unsaturated lecithins using deuterium NMR of specifically labeled

TABLE VI

Molecular Area of Lecithin (at 22 C, 10 dyne/cm) (21)

Lecithin	Area ($Å^2$) ±2
1,2-Distearoyl	50
1-Stearoyl-2-oleoyl	82
1-Palmitoyl-2-linoleoyl	87
1-Palmitoyl-1-linolenoyl	90
1-Palmitoyl-2-arachidonoyl	92
1,2-Dilinoleoyl	102
1,2-Dilinolenoyl	107

TABLE VII

Glucose Permeability of Pure Lecithin Liposomes (21)

Lecithin	% Glucose released from liposome after 1 hr @ 40 C	Area, $Å^2$ (22 C, 12 dyne/cm)
1-Stearoyl-2-oleoyl	25	82
1-Palmitoyl-2-linoleoyl	50	87
1-Palmitoyl-2-linolenoyl	60	90
1-Palmitoyl-2-arachidonoyl	58	92
1,2-Dilinoleoyl	85	102
1,2-Dilinolenoyl	90	107

TABLE VIII

DSC Data for (1,2-Dioctadecenoyl) Lecithins (22)

cis-Double bond position	T_c (C)	ΔH (kJ/mol)
2	41	40.2
4	23	34.3
6	1	32.6
7	−8	31.8
8	−13	31.4
9	−21	32.2
10	−21	31.8
11	−19	32.6
12	−8	33.1
14	7	36.0
16	35	40.2
1,2-Distearoyl	58	44.8

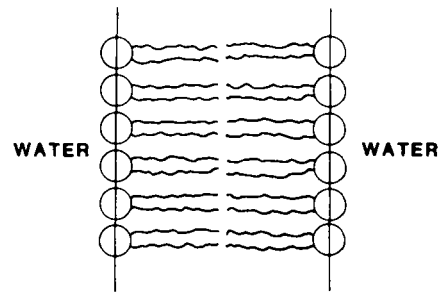

FIG. 7. Phospholipid bilayer structure.

lecithins (24-26). Many of the details glossed over by the "squiggly" lines of Figure 7 have been elucidated. In fact, distinct conformational constraints are imposed on phospholipids in biomembranes, both on the fatty hydrocarbon chains and the polar headgroups.

Regarding Seelig's technique of deuterium NMR, he has found specific deuterium labeling to be accomplished by chemical synthesis or biochemical incorporation. This substitution results in a nonperturbing probe which is readily

identified and is very sensitive to molecular order, electron density and anisotropic molecular structure. This technique was described as superior to the use of spin-labels and electron spin resonance (ESR) analysis because of the perturbation of chain packing introduced by the bulk of the midchain substituted spin-label. The deuterium NMR data, in the form of deuterium quadrupole splittings, may be used to calculate deuterium order parameters. Since these splittings are mainly determined by the average conformation of the phospholipid molecules, they provide structural information about the bilayer. Deuterium NMR quadrupole relaxation times give insight into the dynamics of the phospholipids.

Seelig also finds that neutron diffraction of the deuterium-labeled phospholipids provides unambiguous determinations of the average positions of each deuterium-labeled segment in the membrane. Together, these techniques have proven very powerful in determining details of biomembrane structure.

The first conclusion of this work, relative to the discussion of fatty chain topography, is that the conformations (in bilayers in vitro or in membranes in vivo) of the fatty acids at the 1- and 2-positions of the lecithin are not equivalent. The chain on the 1-position (*sn*-1) is extended perpendicularly to the bilayer surface at all methylene segments. The first CH_2 segment of the 2-position (*sn*-2) chain, on the other hand, is oriented parallel to the surface of the membrane and then bent sharply beyond this segment to assume an orientation perpendicular to the surface (see Fig. 8). This observation was found to hold true for several identically and nonidentically substituted (1,2-difatty) lecithins. As a result, the two chains remain out-of-step throughout the bilayer and the *sn*-1 fatty chain penetrates more deeply into the bilayer than the *sn*-2 chain, when both are of the same carbon number. This observation must be considered in the light of two others: (a) the aforementioned preference for unsaturated fatty acids on the 2-position, and (b) in naturally occurring phospholipids the *sn*-2 chain frequently has a larger carbon number than the *sn*-1 chain. This observation was corroborated by neutron diffraction and X-ray crystal structure analyses.

Conformational data for the hydrocarbon chain past C_2 are not as well defined. Rapid *trans-gauche* rotational isomerism occurs, giving a liquid-like character to bilayer interiors, but the hydrocarbon chains in the bilayer core are not as disordered as in a pure liquid hydrocarbon. The order in these chains may be represented by an order parameter, S, calculated from the deuterium quadrupole splitting. For chains in an all-*trans* configuration with rotation only about the long molecular axis, S = 1. For a completely statistical movement through all angles of space, S = 0. In general, phospholipids have relatively constant order parameters at 0.4-0.5, for saturated lipids, through C_9 with a subsequent decrease to 0.1-0.2 near the methyl terminus. Figure 9A shows the order profiles of the palmitic chain of (1,2-dipalmitoyl) lecithin and (1-palmitoyl-2-oleoyl) lecithin, each at 19 C above their gel-liquid crystal phase transition point. The increase in the order of the palmitic chain in the oleic-containing lecithin was attributed to a stiffening effect of the *cis* double bond. When compared at the same temperature, the oleic-containing lecithin is always more disordered than the saturated lecithin (Fig. 9B). In fact, at a constant temperature, incorporation of a *cis* double bond in the hydrocarbon chain reduces ordering in all parts of the hydrocarbon region.

Deuterium labeling on the double bond of the oleic chain of lecithin allowed order parameter studies which showed the nonequivalence of the C_9 and C_{10} positions as a distinct discontinuity of the order profile plot (Fig. 10). This nonequivalence rules out the possibility that the C=C bond is exactly parallel to the bilayer surface. The conclusion was that the most probable orientation of the *cis* double bond is tilted ca. 7-8° from parallel with the bilayer surface. Comparable work with *E. coli* cells shows the same results; therefore, average orientations and conformations are not strongly affected by the presence of membrane-bound proteins and other ingredients of biomembranes.

Analogous analyses also have been made on the *trans*-unsaturated isomer (1-palmitoyl-2-elaidoyl) lecithin. The order profile for the elaidic chain shown in Figure 10 does not possess the discontinuity in the C_9-C_{11} region seen with an oleic chain. In fact, the behavior of the elaidic chain through this region resembles a fully saturated chain (Fig. 9) more than that of a *cis*-unsaturated chain. From the equivalence of the C_9 and C_{10} order parameters it is possible to say that the C-D bond vectors are parallel to each other and make the same angle with the bilayer surface, but it was not possible to determine the orientation of the *trans* double bond.

The *cis* conformation of the Δ9 double bond in the *sn*-2 chain was found to disturb the parallel packing of the hydrocarbon chains more so than a *trans* conformation, thereby reducing the van der Waals interactions between adjacent chains. This effect is consistent with the earlier discussions on the effects of *cis* unsaturation and is reflected, in this case, in the lower gel-to-liquid crystal tran-

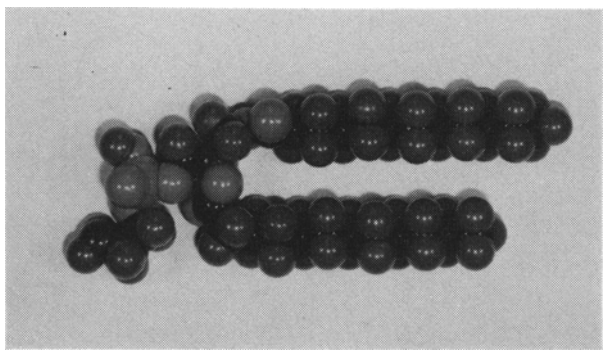

FIG. 8. Observed kink at 2-position of *sn*-2 chain in phospholipids (24-26).

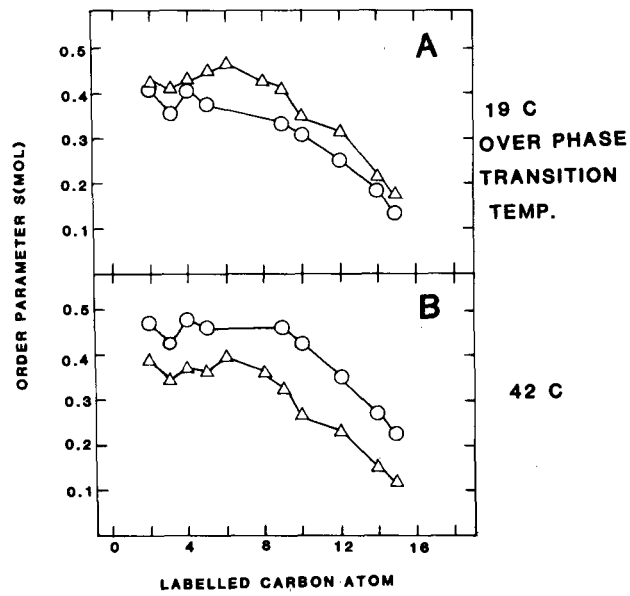

FIG. 9. Palmitic chain order profiles of lecithins (24-26). (△): (1-Palmitoyl-2-oleoyl) lecithin; (○): (1,2-dipalmitoyl) lecithin.

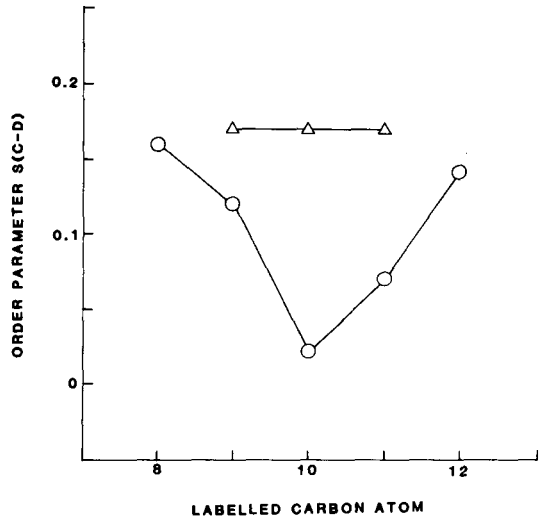

FIG. 10. Order profile for *cis* and *trans* chains of (1-palmitoyl-2-octadec-9-enoyl) lecithin (24-26). (△): Deuterated elaidic chain; (○) deuterated oleic chain.

sition temperatures for the (1-palmitoyl-2-oleoyl) lecithin, −5 C, vs the (1-palmitoyl-2-elaidoyl) lecithin, 26 C, vs the (1,2-dipalmitoyl) lecithin, 41 C.

Chemical Systems

The effects of *cis* vs *trans* unsaturation on the rates of hydrogenation, polymerization, oxidations and general additions are well known to organic chemists. Much of the same selectivity and rate differential that one sees in reactions of olefin stereoisomers in general may be applied to fatty acids. However, since these effects are well known and since, as mentioned earlier, the bulk of the fatty acids available to us as derivatizers are *cis*, midchain olefinic C_{18} acids, I will confine this discussion to three applications where topography effects may be readily observed or perhaps controlled.

Epoxidation of unsaturated fatty acid esters is a large application area for fatty acids. The products, long-chain fatty esters with a 3-membered epoxy ring near midchain, are mainly used as polymer stabilizers. Epoxidation generally is carried out by treatment of an unsaturated fatty ester with an epoxidizing agent containing a peroxide linkage, such as peracetic acid, perbenzoic acid or a hydrogen peroxide/carboxylic acid combination (Fig. 11).

In this reaction, stereochemistry is maintained. Thus, *cis* epoxides are generated from *cis* olefins and *trans* from *trans*. Epoxidation of linoleate yields two 9,10:12,13-diepoxystearate isomers. Both result from *cis* addition, with *syn* and *anti* positioning of the epoxide oxygens. Use of one equivalent of peracid enables preparation of monoepoxides from linoleate.

Hydrogenation of carbon-carbon double bonds is a process sensitive to topography. The proper thermodynamic conditions for interaction of three phases, the solid catalyst particle, the gaseous hydrogen bubble and the liquid olefin, must be attained to achieve an effective and efficient process. Fatty acid hydrogenation most often is of triglycerides, or fats, with the goal of either maximizing *cis, cis* linoleic content or maximizing monounsaturate content. This results in harder, more oxidatively stable fats. The relative oxidation rates for stearic, oleic, linoleic and linolenic acids are 1, 10, 100 and 150, respectively (27).

The selectivity required, namely, removal of only trienoic acids or removal of both tri- and dienoic acids, is possible due to rate differences. These differences are due more to chemical effects than to physical or topographical

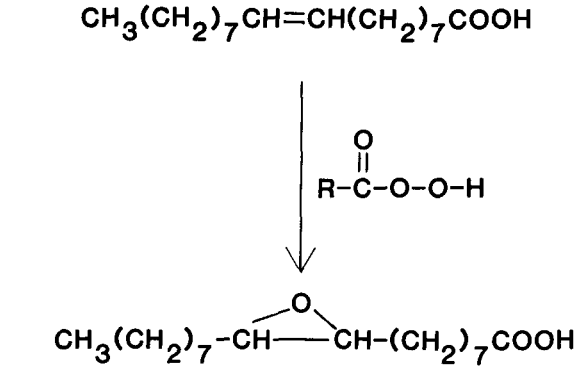

FIG. 11. Fatty acid epoxidation.

ones, but both are involved. Relative hydrogen reactivities are 100, 50 and 1 for linolenic, linoleic and oleic acids, respectively (28).

Since the hydrogenation catalysts can interact reversibly with double bonds, isomerization of *cis* double bonds to the thermodynamically more stable *trans* isomers is a common occurrence. Some catalysts are reported to give 90% isomerization to *trans* double bonds while others have been developed to minimize isomerization. The latter is a substantially more difficult task.

Often, polyunsaturated acids are asked to undergo cycloaddition reactions such as Diels-Alder reactions. These types of compounds may arise through cyclic intermediates with concerted shifting of electron density from the fatty acid unsaturation to form new bonds.

The conjugation of linoleic acid to a mixture of 9,11- and 10,12-octadecadienoic acids may be accomplished in a number of ways, and the reactions of such conjugated dienes with other olefins may result in substituted cyclohexene derivatives through a Diels-Alder reaction (Fig. 12). A wealth of such reactions may be run with conjugated isomers of linoleic acid, and one commercial product of growing importance is a C_{21} dibasic acid (trademark Westvaco DIACID® 1550) which results from the reaction of acrylic acid and tall oil fatty acids with a conjugation catalyst in a patented process (29,30).

The Diels-Alder reaction is very sensitive to topography

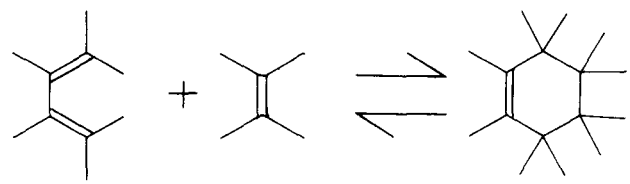

FIG. 12. Diels-Alder reaction.

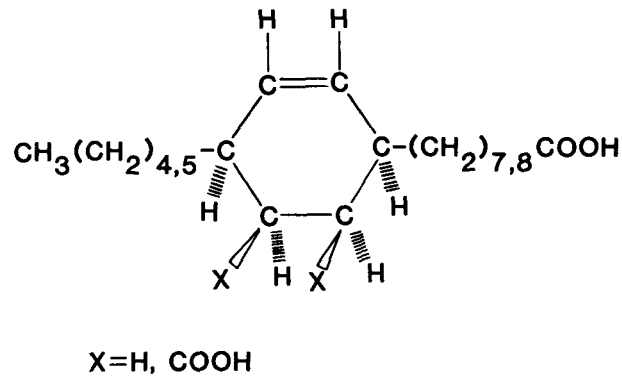

FIG. 13. Predicted stereochemistry of C_{21} dibasic acid.

as well as to differences in electron density. The distribution of *cis* and *trans* bonds in the conjugated system not only influences the isomers obtained in the Diels-Alder reaction but also the rate of reaction (Fig. 13).

Diels-Alder reactions of conjugated octadecadienoic acids from other sources have been described with ethylene, isoprene and other dienes, nitroethylene and β-nitrostyrene, α, β-unsaturated acids and maleic anhydride.

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The Chemistry of Dibasic and Polybasic Fatty Acids

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ABSTRACT

Current processes to produce dibasic and polybasic fatty acids including azelaic, sebacic, dodecanedioic, C_{21} diacid, fatty dimer and trimer acids are discussed. A number of alternative routes to produce azelaic, sebacic and fatty dimers are also discussed, including the preparation of azelaic and sebacic acids from butadiene and sebacic acid from adipic acid. Physical properties of the various dibasic acids are compared. Preparation of important derivatives and their applications is discussed, including: (a) esters and polyesters as vinyl plasticizers and as base stocks for synthetic lubricants; (b) polyamide resins in coatings, fibers, inks and adhesives; (c) salts as surfactants; and (d) amido amines and imidazolines as corrosion inhibitors. Dimer acids have found application in the petroleum industry as drilling mud thickeners in oil recovery.

INTRODUCTION

State-of-the art technology is presented on dibasic and polybasic fatty acids including azelaic acid, sebacic acid, dodecanedioic acid, C_{21} dicarboxylic acid and dimer acids. These higher molecular weight dibasic acids are well established and are important in many commercial applications today. Each of these five dibasic acids are produced in multimillion pound quantities. Only one of the five, dodecanedioic acid, is produced from nonrenewable resources.

Azelaic acid is produced commercially by Emery in Cincinnati, Ohio, and by Unichema in Gouda, The Netherlands. The process used is based on ozonolysis of oleic acid followed by the decomposition of the ozonide with oxygen. Oleic acid is mixed with pelargonic acid which functions as a solvent. This mixture is fed into an ozone absorber countercurrently in relation to a continuous flow of oxygen gas containing ca. 2% ozone. A reaction temperature of 25-45 C is maintained through external cooling. The ozonized oleic acid is then fed continuously into reactors at ca. 100 C where it is decomposed and oxidized as rapidly as possible.

The mixed oxidation products are then fed to a still where the pelargonic and other low boiling acids are removed as overhead while the heavy material is removed as residue. The sidestream contains azelaic acid and other acids which boil in the same temperature range. This sidestream product is treated with hot water to dissolve the azelaic acid and remove it from the water-insoluble acids such as palmitic and stearic acids. The azelaic acid is recovered by removing the water in an evaporator or through crystallization (1,2).

A generalized scheme showing reactions believed to occur during ozonolysis is shown in Figure 1. The first step in ozonolysis is thought to be the formation of a complex which rearranges to the 1,2,3-trioxolane or the initial ozonide. This ozonide is unstable and rearranges to a carbonyl fragment and a zwitterion. Recombination of these cleavage intermediates leads to the final ozonolysis product, 1,2,4-trioxolane. Oxidation of this final ozonide with molecular oxygen gives two carboxylic acids, azelaic and pelargonic acids (3-5).