TOPICS IN
LIPID CHEMISTRY

VOLUME I

Edited by

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PREFACE

The proliferation of primary journals is usually followed by the appearance of review volumes, which are now accepted as an important part of scientific literature and as a valuable source of information. It is therefore not surprising that widening interest in lipids by scientists of many disciplines should be reflected in the appearance of new primary journals and new review series.

The present series is launched with confidence that there is material to be reviewed, authors willing to write, and enough interested readers to make the whole project worthwhile. To avoid serious overlap with other excellent reviews in this field it will emphasise the chemistry, physics, and technology of this group of substances without wholly neglecting important biological aspects.

Each of the six reviews in this first volume is concerned with an important and developing area of lipid chemistry. All of them are written by busy men who are themselves involved in the work they describe. The Editor is grateful to them for agreeing to write and for responding to his subsequent pressures to write to a deadline. The manuscripts were received in the period September, 1968 to March, 1969 and the authors have been given the opportunity to insert additional relevant papers in the list of references, but not in the text.

It is hoped to offer a service to the readers of this series in the form of tabulated information with little or no comment. A start has been made in the present volume by listing books and reviews in this field which have been published from about 1962 onwards. It is hoped to continue this list in later volumes and to provide other useful information.

St. Andrews　　　　　　　　　　　　　　　　F. D. Gunstone.
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CONTENTS

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CYCLOPROPANE AND CYCLOPROPENE FATTY ACIDS

William W. Christie

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1

A. INTRODUCTION

In 1950, Hofmann and Lucas isolated from the lipids of the bacterium *Lactobacillus arabinosus* an unusual fatty acid, 'lactobacillic acid', which they suggested might contain a cyclopropane ring. In the series of investigations described in his monograph (1963), Hofmann and his colleagues demonstrated that the acid was 11,12-methyleneoctadecanoic acid (10-(2-hexylcyclopropyl)-decanoic acid (1)).

$$CH_3 \cdot (CH_2)_5 \cdot \overset{\displaystyle CH_2}{\overset{\displaystyle \triangle}{CH—CH}} \cdot (CH_2)_9 \cdot CO_2H \qquad (1)$$
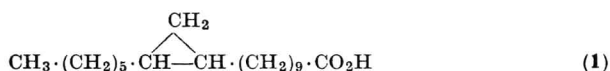
Soon afterwards, Nunn (1952) obtained an even more unusual fatty acid, 'sterculic acid', from the seed oil of *Sterculia foetida* and showed that it was 8-(2-octylcyclopropen-1-yl)-octanoic acid (2), i.e. an acid with a cyclopropene ring.

$$CH_3 \cdot (CH_2)_7 \cdot \overset{\displaystyle CH_2}{\overset{\displaystyle \triangle}{C=C}} \cdot (CH_2)_7 \cdot CO_2H \qquad (2)$$
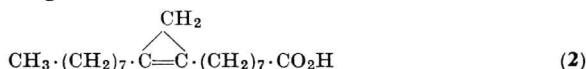
A large number of cyclopropane ring-containing natural products are known including mono-, sesqui-, di- and tri-terpenes, an amino acid, an alkaloid, a steroid, and steroidal alkaloids. The monoterpene, 3-carene (3), the triterpene, cycloartenol (4), and the amino acid, hypoglycine (5) are typical examples. The occurrence, chemistry, and biogenesis of such compounds have been recently reviewed (Soman, 1967).

(3)

HO

(4)

$$CH_2=\overset{\overset{\displaystyle CH_2}{\diagup\diagdown}}{C}-CH \cdot CH_2 \cdot CH(NH_2) \cdot CO_2H$$
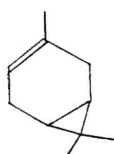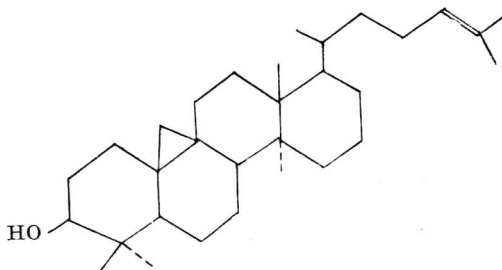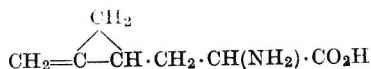
(5)

The structure and reactivity of cyclopropane derivatives have been comprehensively reviewed (Lukina, 1962). For a clearer understanding of the chemistry of cyclopropane fatty acids, it is instructive to consider briefly the molecular orbital representation of the cyclopropane ring. The carbon atoms are believed to be hybridised in such a way that their orbitals have greater p character than in a normal sp^3 bond. The two orbitals from any one carbon lie in the same plane at an angle of 104° to each other and, as a result, the C—C bonding orbitals are not directed towards each other so the bonds are described as 'bent' or 'banana' (Coulson and Moffit, 1949). Bonds in the cyclopropane ring, therefore, differ markedly from those in alkanes or higher alicyclic compounds with undistorted bond angles, and cyclopropane compounds have properties which are similar in many ways to those of alkenes. For example, they undergo addition reactions with electrophilic reagents but with simultaneous ring opening.

In cyclopropene derivatives, the double bond is much shorter than that found in normal olefins and a greater degree of 'bond-bending' must occur than in the corresponding cyclopropane compounds. As a result, the double bond has properties intermediate between those of unstrained olefins and acetylenes and the single bonds are the weakest points in the molecule because of the excessive 'bending'. The chemical and physical properties of cyclopropene compounds in general are fully discussed in these terms in the recent reviews by Carter and Frampton (1964) and Closs (1966). Cyclopropene fatty acids, therefore, undergo all the typical addition reactions of olefins, but also take part in a number of distinctive reactions in which the single bonds in the ring are cleaved.
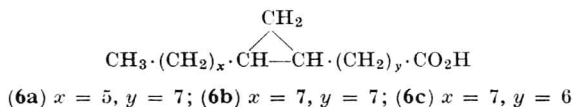
Although many aspects of the chemistry and metabolism of cyclopropane and cyclopropene fatty acids are very different, both types of fatty acid occur together in certain plants and the mechanisms of biosynthesis of both have many features in common. It is, therefore, logical and informative to review the chemistry, biosynthesis, and metabolism of both types of acid together.

B. CYCLOPROPANE FATTY ACIDS

1. *Occurrence*

(a) Naturally Occurring Cyclopropane Fatty Acids

Lactobacillic acid (**1**) was the first cyclopropane fatty acid to be found in nature but others have now been discovered. Evidence was obtained for the occurrence of a C_{17} cyclopropane fatty acid in the lipids of the bacterium *Escherichia coli* (O'Leary, 1959b; Dauchy and Asselineau, 1960) and this was proved to be *cis*-9,10-methylene-hexadecanoic acid (8-(2-hexylcyclopropyl)-octanoic acid, **6a**) by Kaneshiro and Marr (1961).

$$CH_2$$
$$CH_3 \cdot (CH_2)_x \cdot CH\!\!-\!\!CH \cdot (CH_2)_y \cdot CO_2H$$

(**6a**) $x = 5$, $y = 7$; (**6b**) $x = 7$, $y = 7$; (**6c**) $x = 7$, $y = 6$

This acid and lactobacillic acid with, on occasion, other isomers or homologues, have now been found as constituents of a large number of bacterial species. For example, *cis*-9,10-methyleneoctadecanoic acid (8-(2-octylcyclopropyl)-octanoic acid, **6b**) or 'dihydrosterculic acid' occurs with lactobacillic acid in at least one organism, *Salmonella tymphimurium* (Gray, 1962), but it may be more widespread and is probably the principal cyclopropane fatty acid of some species of protozoa (Meyer and Holz, 1966). C_{13} and C_{15} cyclopropane fatty acids (Goldfine and Bloch, 1961; O'Leary, 1962a) and a C_{21} cyclopropane fatty acid (Park and Berger, 1967) have been detected in bacterial lipids by gas chromatography, but their structures have not been further defined. Also, C_{17} and C_{19} cyclopropane aldehydes, linked to phosphoglycerides as vinyl ethers (plasmalogens), accompany the corresponding cyclopropane fatty acids in the lipids of *Clostridium butyricum* (Goldfine, 1964). Certain unique high molecular weight cyclopropane fatty acids have been found as constituents of the Mycobacteriaceae and these are discussed in Section B4.

Dihydrosterculic acid is a major constituent (17 per cent) of the seed oil of *Dimocarpus longans*, Sapindaceae (Kleiman, Earle, and Wolff, 1968), and it also accompanies the cyclopropene fatty acid, sterculic acid, in many species of the plant order Malvales (see Section C). However, dihydromalvalic acid (*cis*-8,9-methyleneheptadecanoic acid or 7-(2-octylcyclopropyl)-heptanoic acid, **6c**), the cyclopropane

analogue of the other major cyclopropene fatty acid, malvalic acid, has not yet been found in nature though it has been detected in *in vitro* experiments with seeds of the family, Malvaceae (Johnson, Pearson, Shenstone, Fogerty, and Giovanelli, 1967a).

(b) Cyclopropane Fatty Acid Content of Bacteria

The fatty acid spectrum of any bacterial species can vary considerably according to the conditions under which it is grown (Asselineau and Lederer, 1960; Kates, 1964). The concentration of certain nutrients, the presence or absence of oxygen, temperature, and the age of the culture can all affect the amount and type of lipid synthesised. In particular, increases in the proportions of cyclopropane fatty acids in bacterial cultures at later stages of growth have been observed in *E. coli* (Marr and Ingraham, 1962; Law, Zalkin, and Kaneshiro, 1963; Knivett and Cullen, 1965), *Serratia marcescens* (Law *et al.*, 1963; Kates, Adams, and Martin, 1964), *Agrobacterium tumefaciens* (Law *et al.*, 1963) and *L. arabinosus* (Croom and McNeill, 1961). It has been shown with *E. coli* that changes in the proportions of cyclopropane fatty acids can be induced by changes in pH, oxygen supply, temperature, or the concentration of certain inorganic ions (Knivett and Cullen, 1965, 1967).

As a result, the comparison of fatty acid composition of different bacteria has only limited use for taxonomic purposes unless the organisms are grown and harvested under strictly comparable conditions. Law *et al.* (1963), for example, suggested that the fatty acid composition of organisms in the late stationary phase of growth, when cyclopropane fatty acid formation has ceased, should be selected for comparison of species differences. Nevertheless, some useful correlations have been made, in particular by Kates (1964), on the basis of family affiliation. With few exceptions, cyclopropane fatty acids are found only as components of several gram-negative and a few gram-positive families of the order Eubacteriales, as listed in Table 1, although in certain other families of this order, they are conspicuously absent. It should be recognised, however, that much too often the identification of the cyclopropyl group in the fatty acids has been made solely by gas chromatographic retention times. Occasionally, this has been combined with limited chemical degradative procedures or structures have been deduced on biosynthetic

TABLE 1
Cyclopropane fatty acid content of bacteria

Bacteria	Cyclopropane acid (%)		Reference
	C_{17}	C_{19}	
Eubacteriales (gram negative)			
Enterobacteriaceae			
Escherichia coli	24	24	Law *et al.* (1963)
	6	< 1	Knox *et al.* (1967)
	9	3	Kanemasa *et al.* (1967)
Salmonella typhimurium	16	4[a]	Gray (1962)
Serratia marcescens	44	9	Law *et al.* (1963)
	32	3	Bishop & Still (1963)
	28	12	Kates *et al.* (1964)
	20	< 1	Cho & Salton (1966)
Aerobacter aerogenes	25	6	O'Leary (1962b)
Proteus P18 bacillary form	22	7	Nesbitt & Lennarz (1965)
L form	7	4	Nesbitt & Lennarz (1965)
Rhizobiaceae			
Agrobacterium tumefaciens	—	13	Hofmann & Tausig (1955)
	6	47	Kaneshiro & Marr (1962)
Eubacteriales (gram positive)			
Lactobacillaceae			
Lactobacillus arabinosus	—	30	Hofmann *et al.* (1952)
	—	47	Thorne & Kodicek (1962)
	—	15	Henderson *et al.* (1965)
Lactobacillus casei	—	16	Hofmann & Sax (1953)
	—	45	Thorne & Kodicek (1962)
	—	35	Henderson *et al.* (1965)
Lactobacillus acidophilus	—	30	Thorne & Kodicek (1962)
Lactobacillus delbrueckii	—	9	Hofmann *et al.* (1957a)
Streptococcus lactis	—	20	MacLeod *et al.* (1962)
Streptococcus lactis			
var. Multigenes	—	44	MacLeod & Brown (1963)
Streptococcus cremoris	—	18	MacLeod & Brown (1963)
Streptococcus agalactiae	—	2	MacLeod & Miller (1967a)
Streptococcus uberis	—	8	MacLeod & Miller (1967a)
Streptococcus dysgalactiae	—	30	MacLeod & Miller (1967b)
Bacillaceae			
Clostridium butyricum[b]	9	5	Goldfine & Bloch (1961)
Micrococcaceae			
Micrococcus cryophilus	—	1	Brown & Cosenga (1964)

TABLE 1—continued
Cyclopropane fatty acid content of bacteria

Bacteria	Cyclopropane acid (%)		Reference
	C_{17}	C_{19}	
Brucellaceae			
Pasteurella pestis	+ ve	—	Asselineau (1961)
Haemophilus parainfluenzae	< 1	< 1	White & Cox (1967)
Mycoplasmatales			
PPLO (strain 07)[c]	5	—	O'Leary (1962a)
Hyphomicrobiales			
Rhodomicrobium vanielii[d]	—	4	Park & Berger (1967)
Pseudomonadeles			
Pseudomonas fluorescens	3	8	Brian & Gardner (1968a)

[a] 80% lactobacillic acid, 20% dihydrosterculic acid.
[b] Also 0·4% C_{13} cyclopropane and 1·5% C_{15}.
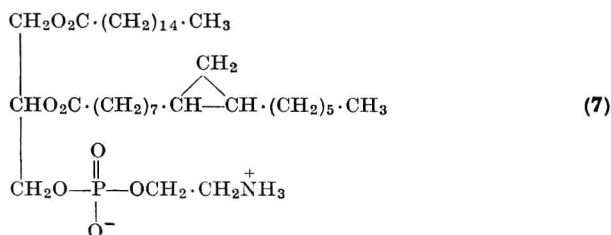[c] Also 2% C_{15} cyclopropane.
[d] Also 8% C_{21} cyclopropane.
See also Thiele, Busse and Hoffman (1968); Ballio, Barcellona and Salvatori (1968); and Brian and Gardner (1968b).

grounds, but only with a few bacteria has the position of the cyclopropane ring in the fatty acid been determined precisely.

(c) Cyclopropane Fatty Acids in Structural Lipids

In bacteria and protozoa, cyclopropane fatty acids have been found only as constituents of the phospholipids which may be in the solvent-extractable or bound form. There is considerable evidence (see Section D), in fact, that they are synthesised from the appropriate monoenoic acids while these are present in a phospholipid, specifically in the phosphatidyl ethanolamine, which is the principal phospholipid of most bacteria (Ikawa, 1967). In the protozoon Crithidia fasciculata (Meyer and Holz, 1966), cyclopropane fatty acids are found only in the phosphatidyl ethanolamine, but in the bacteria L. casei (Thorne, 1964), A. tumefaciens (Hildebrand and Law, 1964) and E. coli (Kanemasa, Akamatsu, and Nojima, 1967) all the polar lipid classes contain similar proportions of cyclopropane fatty acids.

B

In general, the positional distribution of fatty acids in bacterial phospholipids is in accord with that found elsewhere in nature with saturated fatty acids in position 1 and unsaturated in position 2. The phospholipids of regrettably few organisms that synthesise cyclopropane fatty acids have been examined in this detail but, in the phosphatidyl ethanolamine of *E. coli* and *S. marcescens* and the phosphatidyl ethanolamine and phosphatidyl choline of *A. tumefaciens* (Hildebrand and Law, 1964), the cyclopropane fatty acids are esterified predominantly in the 2-position, as determined by enzymatic hydrolysis with the specific phospholipase A of snake venom. More detailed examination of the extracellular phosphatidyl ethanolamine from *E. coli* (van Golde and van Deenen, 1967) has shown further that the major individual molecular species containing virtually all the cyclopropane fatty acid is (1-palmitoyl-2-*cis*-9,10-methylenehexadecanoyl)-phosphatidyl ethanolamine (**7**). One notable

$$CH_2O_2C \cdot (CH_2)_{14} \cdot CH_3$$

$$CHO_2C \cdot (CH_2)_7 \cdot \overset{\displaystyle CH_2}{\overset{\displaystyle \triangle}{CH\!\!-\!\!CH}} \cdot (CH_2)_5 \cdot CH_3 \qquad \textbf{(7)}$$

$$CH_2O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-OCH_2 \cdot CH_2\overset{+}{N}H_3$$

exception to this general distribution rule has been encountered, however, with the phosphatidyl ethanolamine of *C. butyricum* (Hildebrand and Law, 1964) in which unsaturated and cyclopropane fatty acids are found in greater abundance in position 1 (see also Thiele, Busse and Hoffman, 1968).

Bacterial phospholipids occur largely in the membranes where they form part of the structural units. Kodicek (1963) has pointed out that the necessary elasticity of a semi-permeable membrane will be obtained if the fatty acid chains resist being packed closely in a surface film. Branched-chain, polyunsaturated, and cyclopropane fatty acids might all be expected to have such properties, and it is conceivable that cyclopropane fatty acids, replacing monoenoic fatty acids, perform a function of this nature. The fact that cyclopropane fatty acids have the same positional distribution as unsaturated fatty acids in phospholipids lends credence to this opinion. X-ray studies of lactobacillic acid (Craven and Jeffrey, 1960) show,

in fact, that it has a similar shape and crystal structure to the corresponding monoenoic acid. Also, phospholipids containing either unsaturated or cyclopropane fatty acids are similar in their solubility characteristics in polar solvents and in their ability to form stable micellar dispersions (Law *et al.*, 1963; Rothfield and Pearlman, 1966; Law, 1967). Some of the earlier studies of the metabolic activity of lactobacillic acid showed that it had comparable biotin-sparing properties to those of *cis*-vaccenic acid in the Lactobacilli (Hofmann and Panos, 1954; Hofmann, O'Leary, Yoho, and Liu, 1959). Law *et al.* (1963) interpreted this phenomenon also by postulating that the cells require a structural phospholipid with certain physical properties which can be supplied by having either unsaturated or cyclopropane fatty acids in the molecule.

It is not known, however, why cyclopropane fatty acids should be preferred for this purpose to monoenoic acids or what advantages accrue to the bacteria in performing the energetically expensive reactions involved in the synthesis of cyclopropane fatty acids.

2. *Isolation and Structure Determination*

(a) Isolation and Preliminary Identification

In the earlier researches of Hofmann and his colleagues (Hofmann, 1963) lactobacillic acid was obtained by careful distillation of the methyl esters of the total fatty acids from bacteria. With the small quantities generally available from bacterial sources, however, this is seldom practical and preparative gas chromatography (GLC) is now commonly used for this purpose (Kaneshiro and Marr, 1961; Gray, 1962; Bishop and Still, 1963; Goldfine, 1964), occasionally in combination with silver-ion chromatography (reviewed by Morris, 1966) or chromatography of mercuric acetate adducts (Goldfine and Bloch, 1961; Meyer and Holz, 1966; Conacher and Gunstone, 1967) to separate saturated acids including cyclopropane fatty acids from unsaturated. Mercuric acetate is better avoided, however, as it can react irreversibly with cyclopropane compounds under such mild conditions that Lukina (1962) has suggested this reaction as a general test for the cyclopropyl group.

Analytical GLC is by far the most useful tool for estimating individual fatty acids. Methyl esters of cyclopropane fatty acids can often be tentatively identified by comparisons of relative retention times, carbon numbers (Woodford and van Gent, 1960),