FATTY ACID METABOLISM in MICROORGANISMS

By Klaus Hofmann

CHEMISTRY OF MICROBIAL PRODUCTS

Fatty Acid Metabolism in Microorganisms

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In recognition of the importance of cooperation between chemist and microbiologist the E. R. Squibb Lectures on Chemistry of Microbial Products were established with the support of The Squibb Institute for Medical Research in 1955. The lectures are presented annually in the fall at the Institute of Microbiology, Rutgers, the State University of New Jersey, New Brunswick, New Jersey.

PREFACE

The invitation to deliver the 1962 Squibb Lectures on Chemistry of Microbial Products was an honor which provided me with a welcome opportunity to summarize our studies dealing with some phases of fatty acid metabolism in microorganisms and to bring them into focus with recent developments. The experimental work has been carried out in the chemistry and biochemistry departments of the University of Pittsburgh since 1947. The book is divided into three chapters, which deal, respectively, with the discovery and chemistry of cyclopropane fatty acids, the chemical nature of monounsaturated fatty acids in bacteria, the quantitative estimation of fatty acids in bacterial lipids, the biosynthesis of the cyclopropane ring, and finally the anaerobic biosynthesis of monounsaturated fatty acids in microorganisms. The presentation is critical and influenced by the author's own point of view. No attempt is made to provide a comprehensive summary of the literature, and apologies

are offered to investigators whose contributions have been omitted.

The experimental studies could not have been carried out without the devoted help of a number of former students and colleagues, and I wish to express my very sincere appreciation to Drs. Henis, Jucker, Liu, Lucas, Marco, Miller, O'Leary, Panos, Sax, Tausig, Yoho, Young, and the late Dr. Orochena for their untiring efforts. I also wish to express my thanks to Professors Axelrod and Jeffrey of the University of Pittsburgh for many helpful discussions.

This little book will fulfill its mission if it stimulates further inquiry into the neglected but intriguing field of microbial lipid metabolism.

K. HOFMANN

Pittsburgh, Pennsylvania
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K. H.

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CHAPTER

1

LACTOBACILLIC ACID, A NOVEL MICROBIAL METABOLITE

1. DISCOVERY OF LACTOBACILLIC ACID

The observation (1–7) that unsaturated fatty acids exert a marked sparing action on the biotin requirements of certain lactic acid organisms prompted initiation of our systematic studies on the chemical nature of bacterial fatty acids. These studies, which led to the discovery of lactobacillic acid and to the recognition of *cis*-vaccenic acid as an important constituent of bacteria, provided the structural foundation for investigations of fatty acid metabolism in these lower forms of life.

The chemical nature of the fatty acids of *Lactobacillus arabinosus* (8, 9), *Lactobacillus casei* (10), *Agrobacterium* (*Phytomonas*) *tumefaciens* (11), and of a group C *Streptococcus* species (12) was determined in detail. The organisms were grown on essentially lipid-free media and fatty acids were isolated in the usual manner. Autoclaving with dilute acid must precede extraction, since some 80% of the fatty acids are present in the bacteria in a "bound" form not soluble in mixtures of acetone and ether. The lipids were

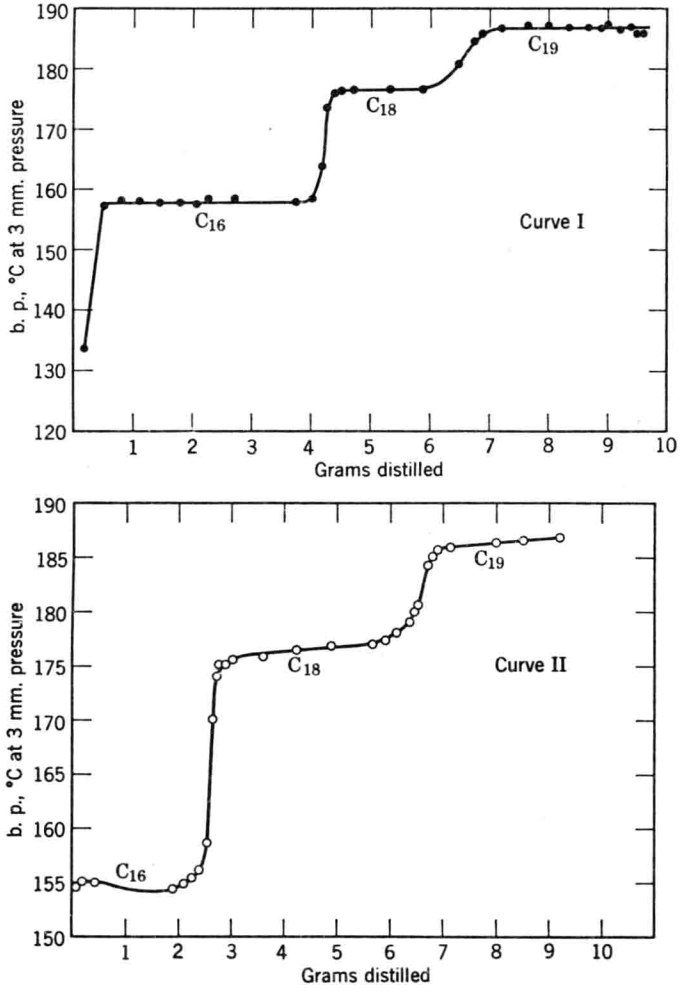

Fig. 1.1. Separation of methyl esters of fatty acids derived from *L. arabinosus* (curve I); *L. casei* (curve II); *Streptococcus hemolyticus*, group C (curve III); *Agrobacterium tumefaciens* (curve IV).

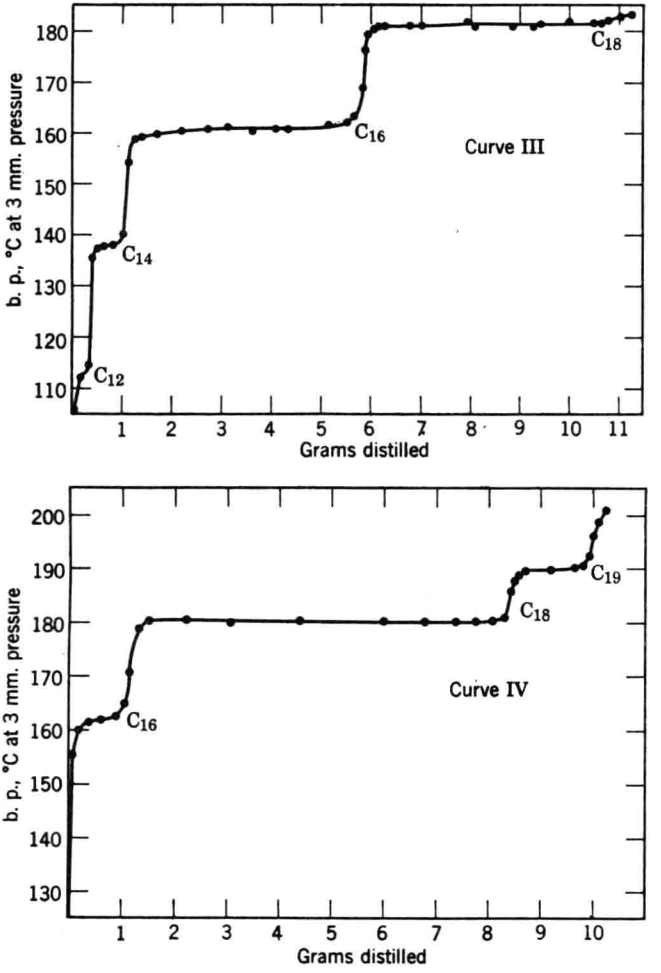

Fig. 1.1 *continued.*

saponified, the fatty acids converted into the methyl esters, and the ensuing ester mixture was separated into various components by fractional distillation. Inspection of typical distillation curves (Fig. 1.1), relating boiling point to amount distilled, shows the prominent presence of fatty acids containing 16 and 18 carbon atoms with lower fatty acids being present in small proportions. The presence of esters with boiling points above methyl stearate in the ester mixture derived from *L. arabinosus, L. casei,* and *A. tumefaciens* is of particular interest. Saponification of this highest boiling fraction gave a low melting (28–29°) crystalline acid of the composition $C_{19}H_{36}O_2$, which was given the name

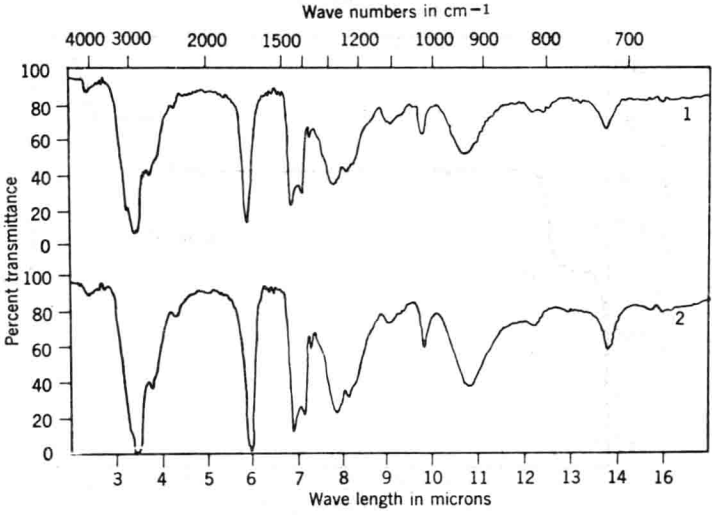

Fig. 1.2. Infrared absorption spectra of cyclopropane fatty acids. (1) "Phytomonic" acid from *A. tumefaciens.* (2) Lactobacillic acid from *L. casei.*

TABLE 1.1

Comparison of Main X-ray Spacings of Lactobacillic Acid from *L. arabinosus* and from *A. tumefaciens*

L. arabinosus	*A. tumefaciens*
Main Short Spacings	
4.65 M *	4.67 S †
4.35 M	4.38 M
4.07 M−	4.30 S
3.78 W ‡	4.07 W+
3.58 W+	3.81 W
3.42 W−	3.61 W
	3.43 W−
Long Spacing	
41.0	41.4

* M = medium
† S = strong
‡ W = weak

lactobacillic acid in view of its first isolation from a lactobacillus. Lactobacillic acid from *L. arabinosus* and *L. casei* is identical as concerns melting point, infrared absorption spectrum, and x-ray diffraction pattern. Lactobacillic acid is also identical with phytomonic acid (11), a compound previously isolated from *A. tumefaciens* whose true chemical nature had not been recognized by earlier investigators (13–17). The matching infrared absorption spectra (Fig. 1.2), x-ray diffraction patterns (Table 1.1), and melting points offer unequivocal evidence for identity. Lactobacillic acid appears to be a major constituent of the bacterial phospholipids (18).

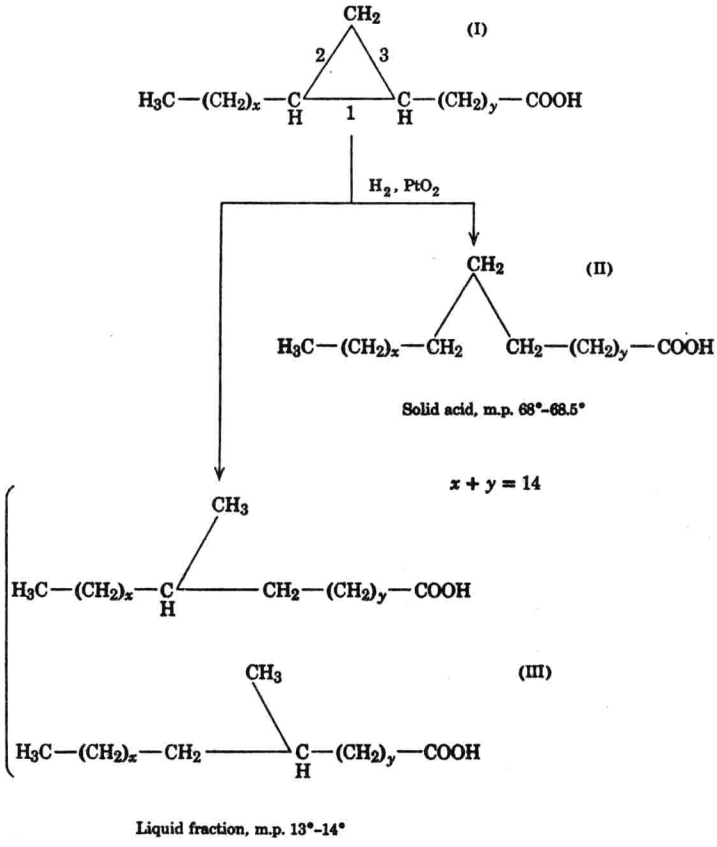

Fig. 1.3. Hydrogenolysis products of lactobacillic acid.