

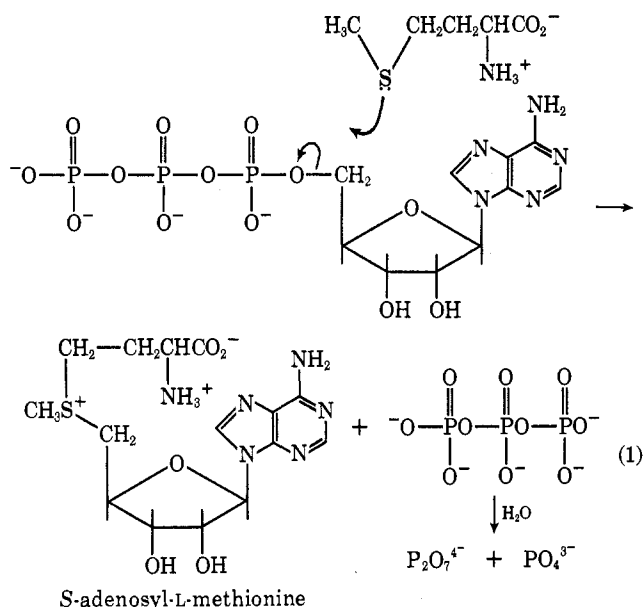
# Biosynthesis of Cyclopropane Rings

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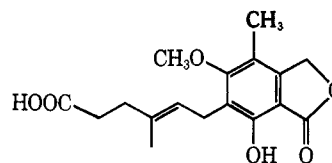
We were led to a study of cyclopropane ring formation in natural products through an interest in biological methylation reactions, the early history of which is contained in a lively account written by Challenger<sup>1</sup> in the 1940's. We now know that the nearly universal biological alkylating agent is an activated derivative of the amino acid methionine. Cantoni<sup>2</sup> showed that this agent, *S*-adenosyl-L-methionine, is formed by the reaction of adenosine triphosphate and methionine.



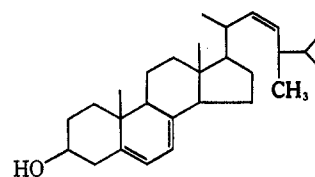
In a variety of enzymatic reactions, *S*-adenosylmethionine can serve as an alkylating reagent by donating a methyl group to oxygen, nitrogen, or sulfur atoms. Representatives of all classes of biological compounds can serve as acceptors. Transmethylating enzymes have been discovered which act upon DNA, RNA, proteins, carbohydrates, and lipids and are involved in the formation of such important compounds as antibiotics, hormones, lecithin, transfer RNA, and plant sterols.<sup>3</sup>

In 1954, Birch, Elliott, and Penfold<sup>4</sup> recognized the possibility of enzymatic carbon alkylation processes, and in 1957, Birch, *et al.*,<sup>5</sup> reported that a carbon-bound methyl group in mycophenolic acid arose by alkylation. This paper was followed by the experi-

ments of Alexander and Schwenk,<sup>6,7</sup> which demonstrated the origin of the "extra" methyl group of ergosterol from the methyl group of methionine.



mycophenolic acid (1)



ergosterol (2)

Hofmann and his colleagues<sup>8-11</sup> established the occurrence of cyclopropane fatty acids in bacteria and indicated that the methylene bridge in the ring arose from a one-carbon unit. O'Leary,<sup>12</sup> and later Chalk and Kodicek<sup>13</sup> and Liu and Hofmann,<sup>14</sup> established that the methylene bridge was derived from the methyl group of methionine.

Several naturally occurring fatty acids with cyclopropane and cyclopropene rings in the chain are now known, as shown in Table I. In most cases the cyclopropane rings have the *cis* configuration, but *trans* rings in the mycolic acids have been reported.<sup>15</sup> In no case is the absolute configuration known for the cyclopropane ring of a fatty acid. The simple cyclopropane acids of bacteria occur in the form of esters as a part of phospholipid molecules, while the cyclopropene acids of plants are present principally as components of triglycerides.

Hofmann's isolation of lactobacillic acid from *Lactobacilli* was based in part upon the ability of this and other fatty acids to serve as essential nutrients for this group of organisms under conditions of limited biotin.

(6) G. J. Alexander, A. N. Gold, and E. Schwenk, *J. Amer. Chem. Soc.*, **79**, 2967 (1957).

(7) G. J. Alexander and E. Schwenk, *J. Biol. Chem.*, **232**, 611 (1958).

(8) K. Hofmann, R. A. Lucas, and S. M. Sax, *ibid.*, **195**, 473 (1952).

(9) K. Hofmann and S. M. Sax, *ibid.*, **205**, 55 (1953).

(10) K. Hofmann, D. B. Henis, and C. Panos, *ibid.*, **228**, 349 (1957).

(11) K. Hofmann, W. M. O'Leary, C. W. Yoho, and T. Y. Liu, *ibid.*, **234**, 1672 (1959).

(12) W. M. O'Leary, *J. Bacteriol.*, **78**, 709 (1959).

(13) K. J. I. Chalk and E. Kodicek, *Biochim. Biophys. Acta*, **50**, 579 (1961).

(14) T. Y. Liu and K. Hofmann, *Biochemistry*, **1**, 189 (1962).

(15) D. E. Minnikin and N. Polgar, *Chem. Commun.*, 1172 (1967).

(1) F. Challenger, *Chem. Rev.*, **36**, 315 (1945).

(2) G. L. Cantoni, *J. Amer. Chem. Soc.*, **74**, 2942 (1952).

(3) S. H. Mudd and G. L. Cantoni, *Compr. Biochem.*, **15**, 1 (1964).

(4) A. J. Birch, P. Elliott, and A. R. Penfold, *Aust. J. Chem.*, **7**, 169 (1954).

(5) A. J. Birch, R. J. English, R. A. Massy-Westropp, M. Slaytor, and H. Smith, *Proc. Chem. Soc. London*, 204 (1957).



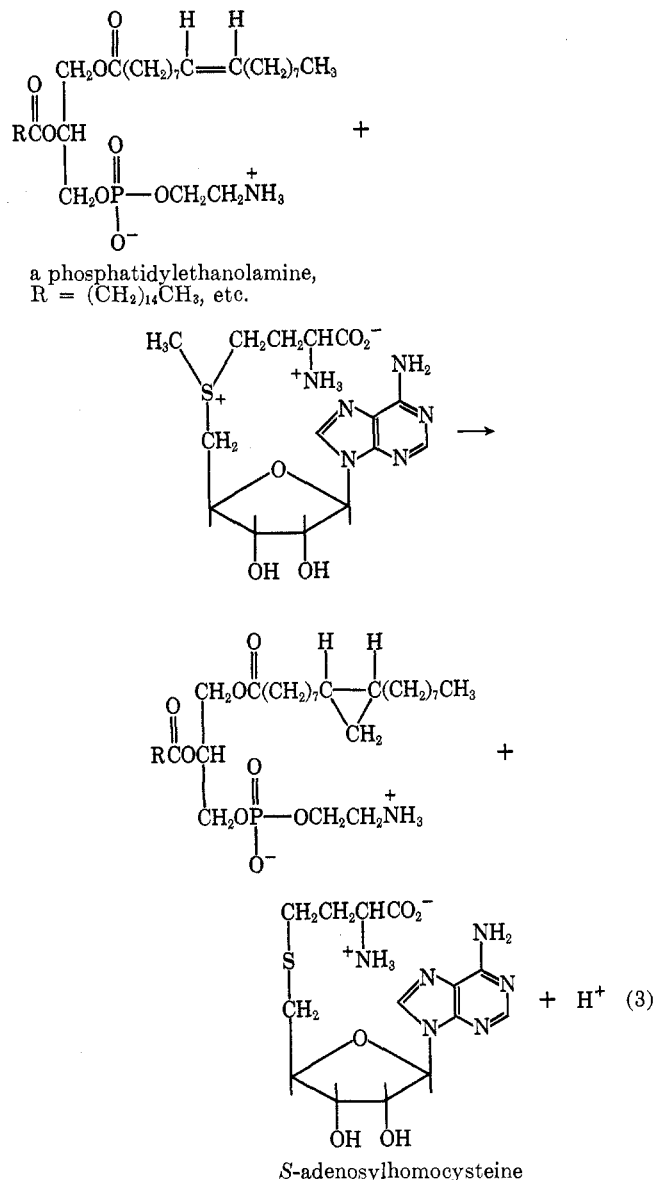
propane acids<sup>21,22</sup> provided evidence that most of these acids were present in phospholipid molecules and that they were not evenly distributed between the 1 and 2 positions of the glyceride molecules. In *Escherichia coli* 80% were found in the 2 position, while in *Clostridium butyricum* 65% were in the 1 position. This pattern was confused, however, by the fact that the ratio of cyclopropane acid to unsaturated acid precursor varies widely during the growth cycle in batch cultures.<sup>23</sup> Extensive investigations of this phenomenon by Knivett and Cullen<sup>24</sup> have shown that formation of cyclopropane fatty acids by *E. coli* cells is promoted by acid pH, low oxygen tension, high temperature, and Mg<sup>2+</sup> ions. The low pH and oxygen tension of batch cultures in the stationary phase of growth thus promote formation of these acids.

Another question which can be answered from outside the cell is that of positional isomers. Goldfine and Panos<sup>25</sup> have used Golay capillary gas chromatography to analyze positional isomers of olefinic and cyclopropane acids from *Clostridium butyricum*. Although this organism has a high proportion of hexadec-7-enoic acid compared to hexadec-9-enoic acid (ratio 2:1), the addition of the methylene bridge occurs nearly exclusively at the 9,10 position (ratio of 7,8 isomers to 9,10 isomer 1:19). In the case of the octadecenoic acids, the 11,12 isomer is preferred over the 9,10 isomer by a ratio of 3:1 when approximately equal amounts of olefinic acid are present. The preference would appear, therefore, to be for the double bond seven carbons from the methyl end of the chain.

Studies of biosynthesis from outside the organism, while informative, cannot supply all details about the chemical mechanisms involved in the process. It ultimately becomes necessary to move to the enzyme level where questions about cofactors and active sites can be answered.

We incubated bacterial cell extracts with *S*-adenosylmethionine labeled with <sup>14</sup>C in the methyl group and determined the level of radioactivity in fatty acids obtained by saponification of the incubation mixtures. In this way we could demonstrate the enzymatic synthesis of cyclopropane fatty acids.<sup>26</sup> The most promising enzymatic extract was obtained from the anaerobe *Clostridium butyricum*, and we were able to purify the enzyme, which we ultimately termed cyclopropane synthetase.<sup>27</sup> Most enzymatic reactions of fatty acids previously investigated involved the coenzyme A esters. We found no evidence for any reaction of the free fatty acids or their coenzyme A esters in the cyclopropane synthetase system. The only fatty acid derivatives which would serve as substrates in the reaction and the

only fatty acid containing products that could be found in the unsaponified incubation mixtures were phospholipids. One of the effective substrates was a phosphatidylethanolamine with a high content of olefinic fatty acids. A typical reaction catalyzed by the enzyme is given by eq 3.



It was possible to imagine that the enzyme might actually remove the acyl chains from the phospholipid molecules to form ester-bound groups at the active site where the alkylation reaction could take place. This idea was rejected when it was shown that the enzyme could operate on phospholipid derivatives in which the long alkyl chains were bound by ether instead of ester linkages.<sup>28,29</sup>

The nature of the substrates for the reaction imposes difficulties, for they are both complicated molecules; furthermore, the phospholipids are water insoluble, while the enzyme and *S*-adenosylmethionine are water soluble. We found that the rate of the reaction was

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(22) J. G. Hildebrand and J. H. Law, *Biochemistry*, **3**, 1304 (1964).

(23) J. A. Croom and J. J. McNeill, *Bacteriol. Proc.*, 170 (1961).

(24) V. A. Knivett and J. Cullen, *Biochem. J.*, **96**, 771 (1965).

(25) H. Goldfine and C. Panos, *J. Lipid Res.*, in press.

(26) H. Zalkin, J. H. Law, and H. Goldfine, *J. Biol. Chem.*, **238**, 1242 (1963).

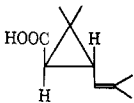
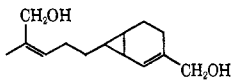
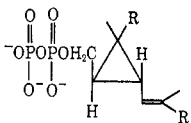
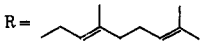
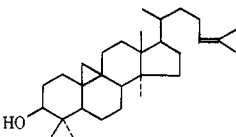
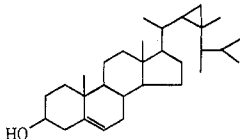
(27) A. E. Chung and J. H. Law, *Biochem.*, **3**, 967 (1964).

(28) A. E. Chung and H. Goldfine, *Nature*, **206**, 1253 (1965).

(29) P. J. Thomas and J. H. Law, *J. Biol. Chem.*, **241**, 5013 (1966).



Table II

Class of compd	Typical structure	Typical source <sup>b</sup>	Ref
Amino acids	$\text{CH}_2=\text{c-C}_3\text{H}_5\text{CH}_2\text{CH}(\text{NH}_3^+)\text{CO}_2^-$ Hypoglycin	<i>Blighia sapida</i>	Akee fruit c
	$\text{CH}_2=\text{c-C}_3\text{H}_5\text{CH}(\text{NH}_3^+)\text{CO}_2^-$	<i>Litchi chinensis</i>	Litchi d
	$\text{CH}_2=\text{c-C}_3\text{H}_5\text{CH}(\text{CH}_3)\text{CH}(\text{NH}_3^+)\text{CO}_2^-$	<i>Aesculus californica</i>	California buckeye e
Terpenes <sup>a</sup>		<i>Chrysanthemum cinerariaefolium</i>	f
	Chrysanthemum acids 	<i>Allomyces</i>	Water mold g
	Sirenin 	Probably ubiquitous in sterol-producing organisms	h
Sterols <sup>a</sup>	Presqualene pyrophosphate R = 	Many plants	i, j
			
Gorgosterol		Sea fans (horny corals)	k

<sup>a</sup> The list of cyclopropane terpenes and sterols is a very large one, and the examples given are not adequately representative. The reader is referred to a paper by R. Soman, *J. Sci. Ind. Res.*, **26**, 508 (1967). <sup>b</sup> *c-C*<sub>3</sub>*H*<sub>5</sub> = 1,2-disubstituted cyclopropyl. <sup>c</sup> E. V. Ellington, C. H. Hassall, J. R. Plimmer, and C. E. Seaforth, *J. Chem. Soc.*, 80 (1959). <sup>d</sup> D. O. Gray and L. Fowden, *Biochem. J.*, **82**, 385 (1962). <sup>e</sup> L. Fowden and A. Smith, *Phytochemistry*, **7**, 809 (1968). <sup>f</sup> L. Crombie and M. Elliott, *Fortsch. Chem. Org. Naturst.*, **19**, 120 (1961). <sup>g</sup> L. Machlis, W. H. Nutting, and H. Rapoport, *J. Amer. Chem. Soc.*, **90**, 1674 (1968). <sup>h</sup> H. Rilling and W. W. Epstein, *ibid.*, **91**, 1041 (1969); *J. Biol. Chem.*, **245**, 4597 (1970). <sup>i</sup> D. H. R. Barton, *J. Chem. Soc.*, 1444 (1951). <sup>j</sup> P. Benveniste, L. Hirth, and G. Ourisson, *Phytochemistry*, **5**, 31 (1966). <sup>k</sup> N. C. Ling, R. L. Hale, and C. Djerassi, *J. Amer. Chem. Soc.*, **92**, 5281 (1970).

in Table II. Among the illustrative examples chosen are compounds with unusual biological activity. The cyclopropane amino acids are hypoglycemic and toxic,<sup>40</sup> the chrysanthemum acids are constituents of the insecticidal pyrethrins,<sup>41</sup> sirenin is the sex pheromone of the water mole *Allomyces*,<sup>42</sup> presqualene pyrophosphate is an important intermediate in the biosynthesis of triterpenes and sterols,<sup>43</sup> and cycloartenol may be a precursor of the phytosterols.<sup>44,45</sup>

(40) R. Bressler, C. Corredor, and K. Brendel, *Pharm. Rev.*, **21**, 105 (1969).

(41) See Table II, footnote f.

(42) A. W. Barksdale, *Science*, **166**, 831 (1969).

(43) See Table II, footnote h.

(44) See Table II, footnote j.

For most of these compounds we have little information about the process of ring construction. In the case of presqualene pyrophosphate, the enzymatic synthesis of which has been well studied,<sup>43</sup> two molecules of farnesol pyrophosphate are joined during ring formation. This is most likely an alkylation reaction similar to those involving *S*-adenosylmethionine except that the leaving group is pyrophosphate. We must await further experimental evidence before we can attempt to formulate general principles of ring formation in the remaining natural products.

(45) L. J. Goad in "Natural Substances Formed Biologically from Mevalonic Acid," T. W. Goodwin, Ed., Academic Press, New York, N. Y., 1970, p 45.