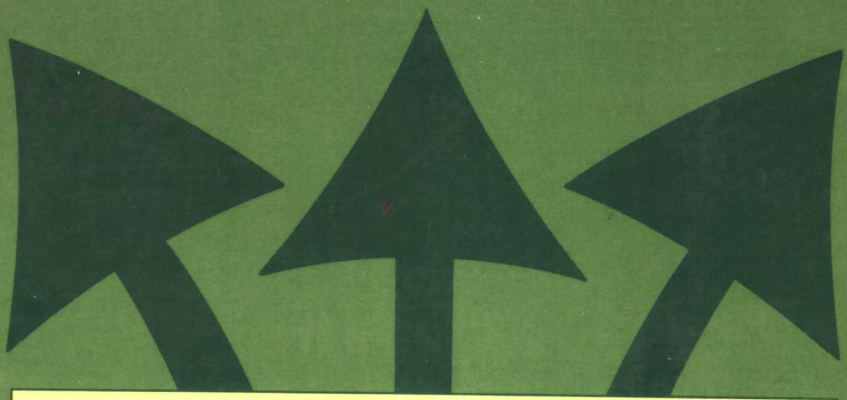

THE BIOSYNTHESIS OF SECONDARY METABOLITES

Second Edition

RICHARD B. HERBERT

CHAPMAN AND HALL

The Biosynthesis of Secondary Metabolites

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Preface

The chief purpose of such new editions is to bring the text up to date. This I have tried to do. The danger with such endeavours is that the book becomes corpulent. This I have tried to avoid. In so doing I am evermore conscious of how much material has had to be omitted. As before the references (ca. 770 of them) which are cited are intended in part to provide the reader with access to material that has been omitted.

I am deeply grateful to Mrs Marjorie Romanowicz for preparing the typed manuscript for this edition with customary accuracy and efficiency, to my wife Margaret for checking the references, and to research students Karl Cable, Lucy Hyatt, Mashupye Kgaphola and Andrew Knaggs for helping with the checking of the manuscript.

Richard Herbert
April 1988

Preface to the first edition

This is a book about experiments and results of experiments. The results described are the fruit of thirty years' labour in the field of secondary metabolism.

Secondary metabolism, more than any other part of the chemistry of life, has been the special preserve of organic chemists. Investigation of secondary metabolism began with curiosity about the structures of compounds isolated from natural sources, i.e. secondary metabolites. Coeval with structure determination there has been a curiosity about the origins and mechanism of formation of secondary metabolites (or natural products as they have been called). It is the experimental outcome of this curiosity that is described here.

This account is primarily intended to be an introduction to the biosynthesis of secondary metabolites. I have also endeavoured, however, to make the book as comprehensive as possible. This has meant that some of the material has had to be presented in abbreviated form. The abbreviated material is largely confined to particular sections of the book. The paragraphs marked with a vertical rule can be omitted by the reader wishing to acquire a general introduction to the subject.

A blend of the most significant and the most recent references is cited to provide the reader with ready access to the primary literature. This is clearly most necessary for the material presented in abbreviated form. Relevant reviews are also cited.

In compiling this account I am indebted to the following people: Mrs M. Romanowicz for typing the manuscript with accuracy and speed, to my wife, Margaret, for checking the references, to final year PhD students, Stuart H. Hedges and William J.W. Watson for critically reading and checking the typed manuscript, and John E. Cragg for proofreading.

Richard Herbert
June 1980

Contents

1 Introduction

1.1 PRIMARY AND SECONDARY METABOLISM

1.1.1 Introduction

Since prehistoric times man has used plant extracts to heal and to kill. Folklore abounds in references to the use of plant extracts in the healing of a variety of illnesses; examples of applications as agents of death range from that of calabar beans and hemlock as judicial poisons to that of the South American curare arrow poisons [1]. In modern times organic compounds isolated from cultures of micro-organisms, as well as from plants, have been used for the cure of disease (e.g. penicillin and tetracycline antibiotics). These organic compounds from natural sources form a large group known as natural products, or secondary metabolites.

Study of the metabolism, fundamental and vital to living things, has led to a detailed understanding of the processes involved. A complex web of enzyme-catalysed reactions is now apparent, which begins with carbon dioxide and photosynthesis and leads to, and beyond, diverse compounds called primary metabolites, e.g. amino acids, acetyl-coenzyme A, mevalonic acid, sugars, and nucleotides [2, 3]. Critical to the overall energetics involved in metabolism is the coenzyme, adenosine triphosphate (ATP), which serves as a common energy relay and co-operates, like other coenzymes, with particular enzymes in the reactions they catalyse.

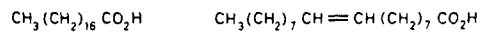
This intricate web of vital biochemical reactions is referred to as primary metabolism. It is often displayed usefully in chart form [4], and to the eye appears very much like an advanced model railway layout, not least because of the way primary metabolism proceeds in cycles (e.g. the citric acid cycle). The organic compounds of primary metabolism are the stations on the main lines of this railway, the compounds of secondary metabolism the termini of branch lines. Secondary metabolites are distinguished more precisely from primary metabolites by the following criteria: they have a restricted distribution being found mostly in plants and micro-organisms, and are often characteristic of individual genera, species, or strains; they are formed along

specialized pathways from primary metabolites. Primary metabolites, by contrast, have a broad distribution in all living things and are intimately involved in essential life processes (for further discussion of parts of primary metabolism see sections 1.1.2 and 5.1). It follows that secondary metabolites are non-essential to life although they are important to the organism that produces them. What this importance is, however, remains, very largely, obscure.

It is interesting to note that secondary metabolites are biosynthesized essentially from a handful of primary metabolites: α-amino acids, acetyl-coenzyme A, mevalonic acid, and intermediates of the shikimic acid pathway. It is these starting points for the elaboration of secondary metabolites which allow their classification, and also their discussion as discrete groups (Chapters 3 to 7). In the remainder of this chapter various aspects of biosynthesis of general importance to the discussion in Chapter 3 and succeeding chapters is reviewed. The first examples of primary and secondary metabolite biosynthesis will be found in sections 1.1.2 and 1.1.3. Chapter 2 is devoted to a brief discourse on the various techniques used in studying the biosynthesis of secondary metabolites.

1.1.2 Fatty acid biosynthesis [2, 3, 7]

Fatty acids, e.g. stearic acid (*1.1*) and oleic acid (*1.2*), are straight chain carboxylic acids found predominantly as lipid constituents. They are primary



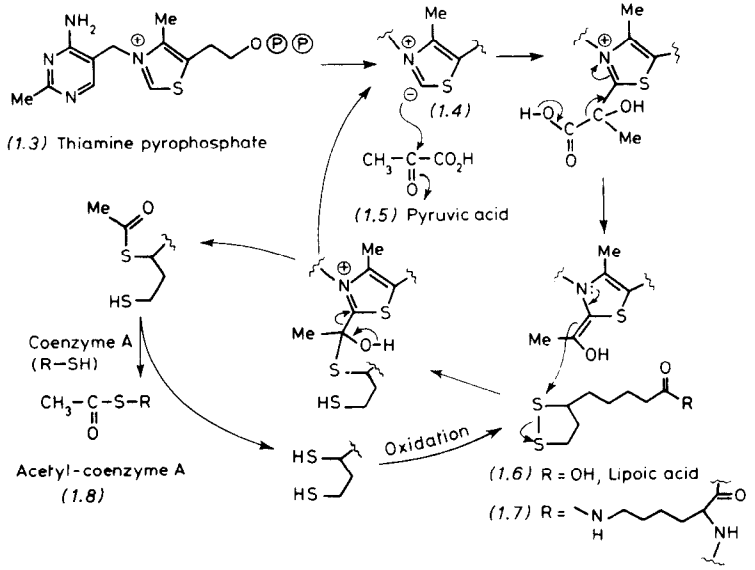
(1.1) Stearic acid

(1.2) Oleic acid

metabolites formed under enzyme catalysis by linear combination of acetate units. In this they are similar to the polyketides which are secondary metabolites (Chapter 3). Like many primary metabolites, their biosynthesis is understood in intricate detail. Much less detail is generally available on secondary metabolite biosynthesis.

Fatty acids are synthesized in a multienzyme complex from a crucially important primary metabolite, acetyl-coenzyme A (*1.8*). The principal source of acetyl-CoA (*1.8*) is pyruvic acid (*1.5*) and the conversion of (*1.5*) into (*1.8*) involves the coenzymes, thiamine pyrophosphate (*1.3*)* and lipoic acid (*1.6*) (Scheme 1.1). The key to the action of thiamine is the ready formation of the zwitterion (*1.4*) at the beginning and end of the reaction cycle. The lipoic acid (*1.6*) is enzyme linked via the side chain of a lysine residue (*1.7*). The disulphide functionality is thus at the end of a long (14 Å) arm. It has been suggested that this arm allows the lipoate to swing from one site to another within the multienzyme complex and transfer (and oxidize) the

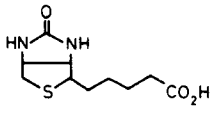
*Ⓟ = phosphate in (*1.3*) and subsequent structures, cf (*1.10*).



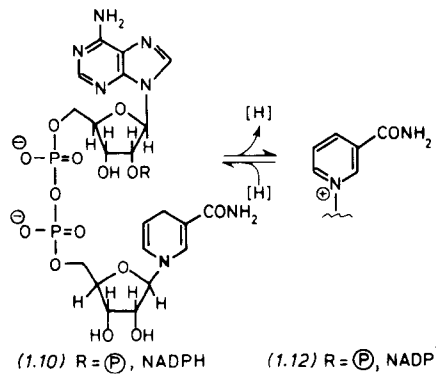
Scheme 1.1

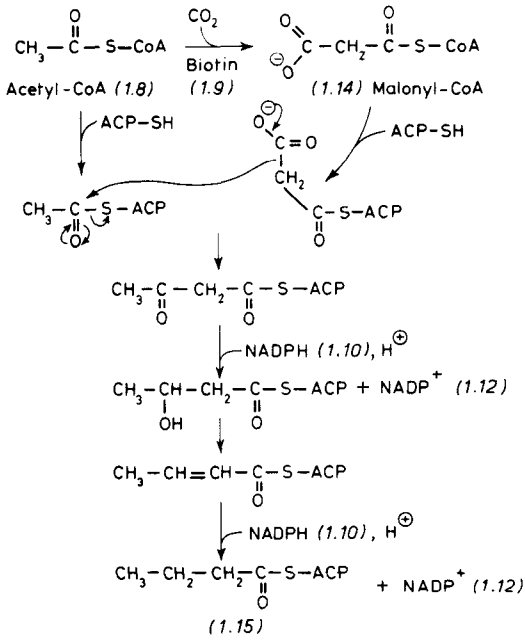
acetyl group [5]. In the sequence shown, pyruvic acid (*1.5*) loses carbon dioxide giving coenzyme-bound acetaldehyde, which is oxidized to the CoA ester of acetic acid.

A similar long arm is apparent in biotin (*1.9*), again enzyme bound through a lysine residue. The coenzyme (*1.9*) assists in the carboxylation of acetyl-CoA (*1.8*) with carbon dioxide yielding malonyl-CoA (*1.14*). Exchange of both acetyl-CoA and malonyl-CoA occurs with acyl carrier proteins (ACP) having free thiol groupings. Condensation then occurs between acetyl-S-ACP and malonyl-S-ACP with simultaneous decarboxylation; the



(1.9) Biotin





Scheme 1.2

carboxylate anion is transferred into the new bond (Scheme 1.2) [6]. Subsequent steps involve reduction, dehydration and double-bond saturation. They implicate in part the widely utilized reducing coenzyme, NADPH (*1.10*) (Scheme 1.2). The first sequence gives butyryl-S-ACP (*1.15*) and for the generation of longer chains, as (*1.1*), the sequence, of malonyl-CoA addition, reduction, dehydration and reduction, is repeated the requisite number of times.

It has been most elegantly demonstrated that the carboxylation of acetyl-CoA (*1.8*) to give malonyl-CoA (*1.14*) proceeds with retention of configuration; subsequent condensation of malonyl-CoA with acetyl-CoA proceeds with inversion of configuration at the malonyl methylene group. Further, elimination of water (Scheme 1.2) takes place (in yeast) in a *syn* stereochemical sense [8].

In examining the overall conversion of pyruvate into a fatty acid (Schemes 1.1. and 1.2) it is interesting to note the exploitation of particular chemical properties of sulphur:

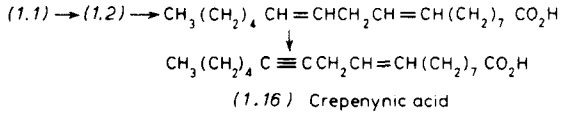
1. as an easily reduced disulphide (*1.6*)
2. as an easily oxidized dithiol
3. in reactive thioesters which aid the Claisen-type condensation reactions.

Also of crucial importance for the condensation is the use of a malonic acid

derivative (*1.14*) as a source of a stable anion. (Further discussion of fatty acid biosynthesis in relation to polyketide formation is taken up in Chapter 3.)

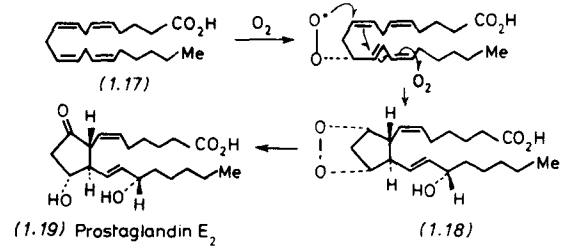
1.1.3 The biosynthesis of polyacetylenes and prostaglandins

The formation of unsaturated fatty acids, e.g. oleic acid (*1.2*), which are also primary metabolites, may occur by at least two routes, one aerobic the other anaerobic. Essentially though, both involve desaturation of a fully saturated fatty acid [2]. Polyacetylenes, e.g. crepenynic acid (*1.16*), which are secondary metabolites, also apparently derive by step-wise desaturation of a saturated fatty acid [9]. The path to crepenynic acid (*1.16*) is illustrated in Scheme 1.3.



Scheme 1.3

Prostaglandins, e.g. prostaglandin E_2 (*1.19*), are physiologically active secondary metabolites found in mammals. The biosynthesis of these compounds also involves an unsaturated fatty acid, e.g. arachidonic acid (*1.17*). Formation of the characteristic prostaglandin skeleton involves the formation of an intermediate endoperoxide (*1.18*). Some of the succeeding steps are indicated in Scheme 1.4 [10, 11].



Scheme 1.4

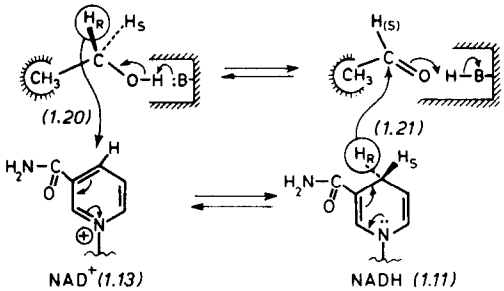
It is interesting to note the formation of polyacetylenes (and prostaglandins) from fatty acids, since it is unusual for secondary metabolites to be formed from a fatty acid. The route that dominates the formation of these metabolites is the polyketide one (Chapter 3).

1.2 STEREOCHEMISTRY AND BIOSYNTHESIS

1.2.1 Chirality and prochirality [12]

It is a common observation that enzymes deal stereospecifically with their substrates; the acceptable substrates must have a particular stereochemistry and the products in turn are formed with a particular stereochemistry (for an illustration of this see particularly section 5.1). This stereospecificity is associated in many instances with reactions involving chiral centres.

In addition to the stereospecificity of reactions associated with chiral centres there is further stereospecificity to be found in reactions at prochiral centres. The conversion of ethanol (*1.20*) into acetaldehyde (*1.21*) by the enzyme, alcohol dehydrogenase, with NAD^+ as co-enzyme provides a simple illustration. The methylene group in (*1.20*) is prochiral*. Oxidation of the ethanol proceeds stereospecifically with removal of the *pro-R* proton from

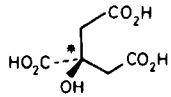


Scheme 1.5

*The methylene group of ethanol has two identical groups (H) on a tetrahedral carbon atom and two groups different from these and from each other (CH_3, OH), and so is *pro*-chiral: a single change in one of the identical groups (H) makes the centre **chiral**. The identical groups may be distinguished as *pro-R* and *pro-S*. In order to do this the priority of one of the identical groups (H) is raised over the other. If this is done for the hydrogen projecting above the plane of the paper in (*1.20*) then the configuration at the methylene carbon atom becomes *R*. So the hydrogen that is raised in priority is termed *pro-R*. [The reader can try labelling the carboxy-methyl groups in (*1.22*) similarly; answer: (*1.64*).] The acetaldehyde double bond is also prochiral: the two faces of the double bond are not identical (addition may give a chiral product). These faces may be labelled *re* and *si* by following the normal priority rules for chirality (*re* = *R*, *si* = *S*). The face of (*1.21*) viewed from above is *re* (for further discussion see [13]). An alternative way of defining prochirality is as follows. A prochiral molecule is one which has a plane of symmetry [for both (*1.20*) and (*1.21*) this is in the plane of the paper] but no axis of symmetry in the plane [rotation of e.g. (*1.21*) in the plane of the paper around an axis running through the carbonyl group does not give a molecule identical with (*1.21*), until, of course, a rotation of 360° has been carried out].

this group. The reverse reaction involves stereospecific addition of a proton to the *re*-face (in this case, the top face as seen by the reader) of the acetaldehyde carbonyl group (i.e. proton removal and addition to the same side of the two molecules). The reaction is, moreover, stereospecific with regard to co-enzyme. The interconversion of NADH and NAD^+ involves, respectively, removal of a proton from a prochiral centre and proton addition to form one. Removal and addition again involves the same face of the molecules concerned.

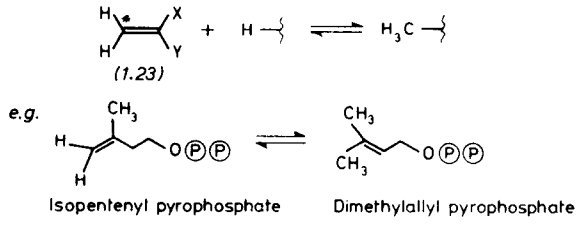
The stereospecific proton removal from (*1.20*) to give (*1.21*) can be understood simply as follows: imagine that at the active site of the enzyme there is one binding site specific for the ethanol OH and one specific for CH_3. This uniquely locates the molecules on the enzyme surface as shown in (*1.20*). Now imagine that the enzyme/co-enzyme proton removal can only occur physically from above the plane of the paper. This results in unique removal of the *pro-R* proton. There is no way on this model that the *pro-S* proton can be removed. A similar argument can be applied to proton addition to (*1.21*) and to proton removal from, and addition to, co-enzyme. [This argument was first applied some thirty years ago to citric acid (*1.22*) in relation to its place in the citric acid cycle. Citric acid has a prochiral centre (*) [14].]



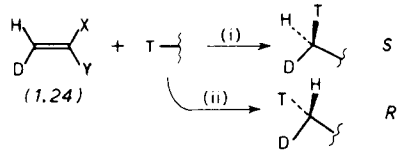
(*1.22*) Citric acid

1.2.2 Chiral methyl groups [15, 16]

Enzyme reactions involving methyl groups show none of the stereochemistry associated with prochiral centres as, e.g., the methylene group in ethanol (see above). The hydrogen atoms are indistinguishable (provided they are all one hydrogen isotope, see below) but enzyme-catalysed reactions do occur which involve methyl groups in various ways. Since enzymes are involved the reactions are expected to proceed with a particular stereochemistry. Three main classes of reaction can be identified [15]. By way of illustration we shall briefly examine one of these (Scheme 1.6) (for further discussion see



Scheme 1.6

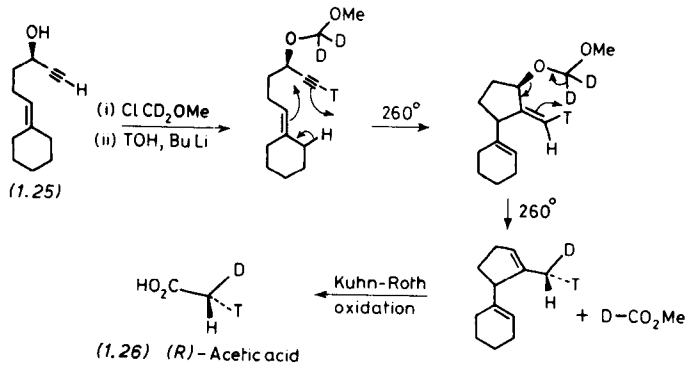


Scheme 1.7

section 4.4). Proton addition to C* of the double bond in (*1.23*), with formation of a methyl group, can, in principle, occur from above or below the plane of the double bond. If the three protons involved in the generation of the methyl group are labelled with the three isotopes of hydrogen [^{1}H, ^{2}H, and ^{3}H, indicated in Scheme 1.7 as, respectively, H, D, and T] then the methyl group generated will be chiral. The stereochemical course of the reaction can be deduced, provided that:

1. the chirality (*R* or *S*) of the asymmetric methyl group thus generated can be determined
2. the substitution of hydrogen isotope around the double bond in (*1.24*) is known, e.g. as shown.

The central analytical problem is to determine the chirality of methyl groups generated or modified in biochemical reactions. The solution is dazzlingly ingenious. First, samples of chiral acetic acid [as (*1.26*)] of known absolute configuration were synthesized. All molecules of acetic acid contained deuterium (and hydrogen) but, as is customary, only very few molecules were also labelled with tritium. Only very few molecules were therefore chiral. Since the analysis is for tritium, however, this does not matter. [Subsequently an economical and supremely elegant synthesis of chiral acetic acid has been developed (Scheme 1.8), which involves two stereospecific concerted reactions and transfer of the chirality in (*1.25*) into (*1.26*) [17].]



Scheme 1.8