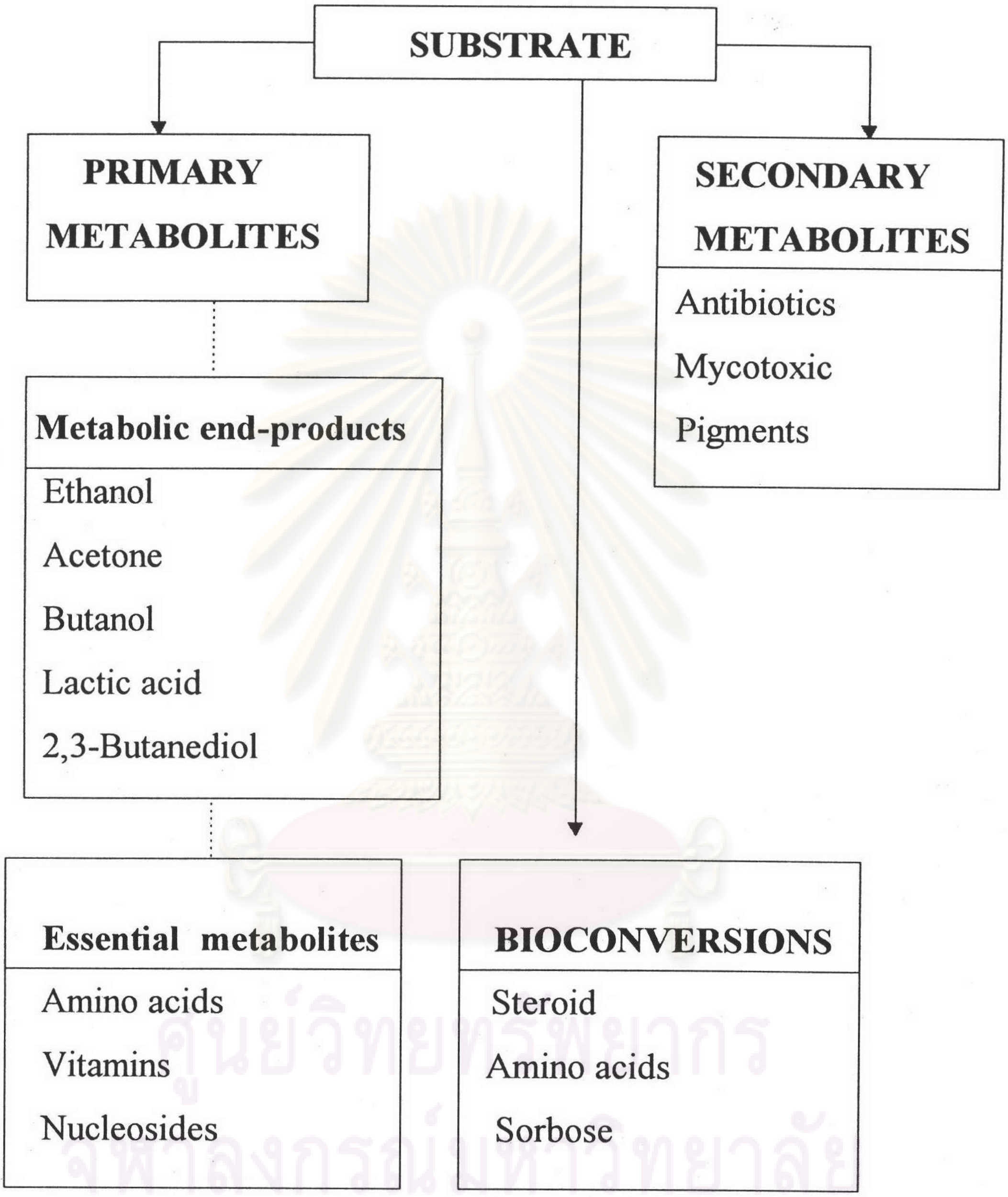
CHAPTER II

HISTORITICAL

Microorganisms had been subjugated by men in many different ways. These encompassed the production of industrially important materials including fine chemicals (e.g. pharmaceuticals) and bulk chemical, the manufacture of single-cell protein from diverse substrates, the processing or recycling of waste materials, and coping with what was loosely called the energy crisis (Primrose, 1987).

Microorganisms were used to produce a wide variety of low molecular weight compounds . These compounds could be subdivided into those whose production was associated with growth (primary metabolites) and those whose synthesis occured after growth ceased (secondary metabolites). Primary metabolites could be further subdivided into those such as vitamins and amino acids, which normally were produced in quantities only sufficient for cell growth, and those such as ethanol and lactic acid, which were produced in large quantities because they were normal metabolic end-products. Microbes were also used to effect chemical transformations; that was, the desired product was not a normal metabolic of the cell but was produced as a result of enzymatic conversion of an unusual substrate.



Schem 3. The different classes of low molecular weight compounds synthesized by microorganisms.

added to the culture medium. Often such substrates could not support growth, they simply underwent bioconversion (Schem 3.).

Secondary metabolites were molecules synthesized by microorganisms late in the growth cycle. They were not required for growth and their real function was not known. Since many of them, e.g. antibiotics, were inhibitory to other organisms they may impart an ecological advantage on the producing organism. The best known of the secondary metabolites were the antibiotics; others include mycotoxins and pigments. Over 2500 antibiotics had been described of which the majority were produced by actinomycetes. The diversity of molecular structures was impressive and many of them were produced as mixtures of related compounds.

Unlike primary metabolites such as amino acids, secondary metabolites as products were developed at a time when little was known about their pathways of biosynthesis. Even today details were unclear for many of them. Consequently two approaches had been used to obtain enhanced yield of product. One of these was the random mutation and selection proceduer. The other involved screening of hundreds of culture medium additives as possible precursors of the desired product.

MUTATION

A mutation was any change in the nucleotide sequence of DNA. Since genes consisted of specific nucleotide sequences, mutations effected gene function and were observed as inheritable changed in genes. Mutations resulted in (a) changed in the proteins coded for by genes or (b) the lack of synthesis of those proteins. When the gene for an enzyme mutated, the enzyme coded by the gene may become inactive or less active because its amino acids sequence had changed. Such a change in genotype may be disadvantageous or even lethal if the cell lose a phenotypic traid it needed. Yet, a mutation could be beneficial if, for instance, the altered enzyme coded by the mutated gene had a new activity that benefited the cell. Table 1 showed the activities changed of the mutants (Tortora, 1989).

Table 1. Kinds of mutants.

Description	Nature of change	Detection of mutant
Nonmotile	Loss of flagella; nonfunctional flagella	Compact colonies instead of flat,spreading colonies
Noncapsulated	Loss or modification of surface capsule	Small, rough colonies instead of larger, smooth colonies
Rough colony	Loss or change in lipopolysaccharide outer layer	Granular, irregular colonies instead of smooth, glistening colonies
Nutritional	Loss of enzyme in biosynthesis pathway	Inability to grow on medium lacking the nutrient
Sugar fermentation	Loss of enzyme in degadative pathway	Do not produce color change on agar containing sugar and a pH indicator
Drug resistant	Impermeability to drug target was altered or drug was detoxified	Growth on medium containing a growth inhibitory concentration of the drug

Table 1(cont.) . Kinds of mutants.

Description	Nature of change	Detection of mutant
Virus resistant	Loss of virus receptor	Growth in presence of large amounts of virus
Temperature sensitive	Alteration of any essential protein so that it was more heat	Inability to grow at a temperature normally supporting growth (e.g., 37°) but still growing at a lower temperature (e.g., 25°)
Cold sensitive	Alteration in an essential protein so that it was inactivated at low temperature	Inability to grow at a low temperature (e.g., 25°) that normally supported growth

The most common type of mutation involving single base pairs was base substitution or point mutation, in which a single base at one point in the DNA was replaced with a different one. Then, when the DNA replicated, the result was a substituted base pair. If a base substitution occured in a portion of the DNA molecule that coded for a protein, then the mRNA transcribed from the gene would carry an incorrect base at some position. when the mRNA was translated into protein, the incorrect base could cause the insertion of an incorrect amino acid in the protein. Thus, the base substitution in DNA could result in an amino acid substitution in the synthesized protein. This was known as a missense mutation .

By creating a stop (nonsense) codon in the middle of an mRNA molecule, some base substitutions effectively prevented the synthesis of a functional protein. only a fragment of protein was synthesized. A base substitution thus resulting in a nonsense codon was call a nonsense mutation.

Beside the base pair mutations, there were also changes in DNA called frameshift mutations. Here, one or a few nucleotide pairs were deleted or inserted in the DNA. This could shift the “translational reading frame”, that was, the three-by-three grouping of nucleotides recognized as codons by the tRNAs during translation. For example, inserting one nucleotide pair in the middle of a gene caused many amino acids downstream from the site of the original mutation to change. Frameshift mutations almost always resulted in a long stretch of missense and an inactive protein produced for the mutated gene. In most cases, a nonsense codon would eventually be generated and thereby terminated transition.

Base substitution and frameshift mutations may occure spontaneously because of occasional mistakes made during DNA replication. These spontaneous mutations were mutations that occured without known intervention of mutation-causing agents. Agents in the environment, such as certain chemicals and radiation, that directly brought about mutations called **mutagens**.

Many chemicals act as mutagens by interacting with DNA and its replication in a variety of ways (Table 2.). Certain mutagenic chemicals, called alkylating agents, change the biochemical structure of nucleotides by adding alkyl groups to them, e.g. N-methyl-N-nitro-N-nitrosoguanidine. Hydroxylamine and nitrous acid were the effective base pair mutagen (Figure 1.). Other chemical mutagents were base analogs, like 2-aminopurine and 5-bromouracil. These molecules were structural similar to normal to nitrogenous bases but they had slightly altered base pairing properties (Figure2.). Some antiviral and antitumor drugs were base analog.

Table 2. Some Mutagenic Chemicals.

Mutagens	Mode of Action
5-Bromouracil (5BU)	Incorporated like thymine: faulty pairing with guanine
2-Aminopurine	Incorporated like adenine: faulty pairing with cytosine
Hydroxylamine (NH_2OH)	Chemical reaction with cytosine
Nitrous Acid (HNO_2)	Deamination of adenine and cytosine
Ethylmethane sulfonate (EMS)	Ethylation of guanine
Nitrosoguanidine/Nitrogen Mustarts	Cross-link DNA strands
Ethidium Bromide/Acridine Orange	Insert between base pairs

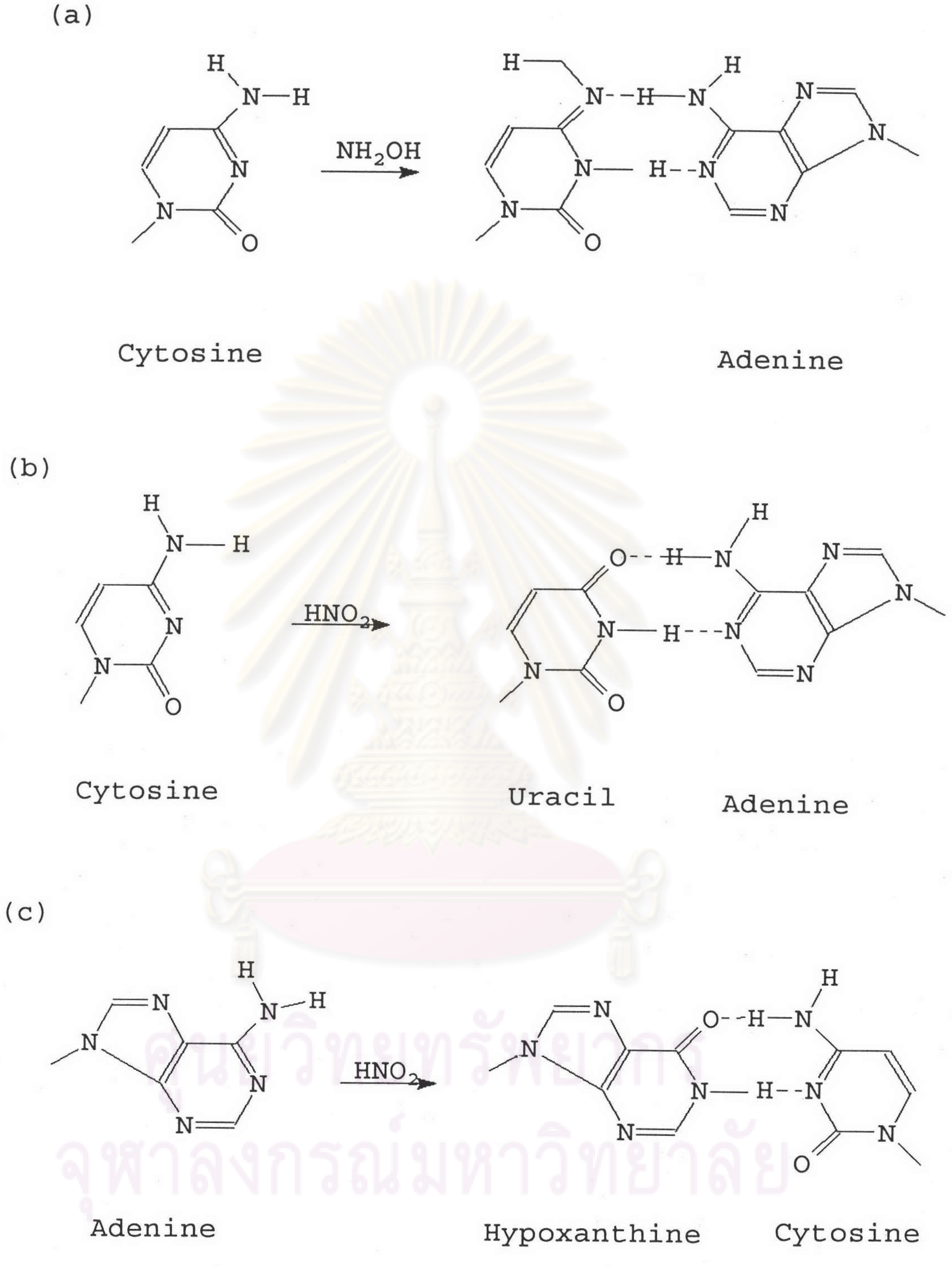


Figure 1. Mutagenesis by base pair mutagen.

(a) Hydroxylamine altered the cytosine.

(b) Nitrous acid altered the cytosine.

(c) Nitrous acid altered an adenine.

Normal nitrogenous base Analog

(a) Adenine 2-aminopurine

(b) Thymine 5-bromouracil

Figure 2. Base analogs and the nitrogenous bases they replace.

(a) 2-aminopurine and adenine.

(b) 5-bromouracil and thymine.

Mutations can also be induced by radiations. X-rays, gamma rays and other forms of ionizing radiation were potent mutagens. The penetrating rays of ionizing radiation caused electrons to pop out of their usual shells. The effected electrons bombarded other molecules and caused more damage, and many of the resulting ions and free radicals were very reactive. Some of these ions could combine with bases in the DNA. Such a combination resulted in mistakes in base pairing during replication and leaded to base substitutions. An even more serious outcome was the breaking of covalent bonds in the sugar-phosphate backbone of DNA. These caused physical breaked in chromosomes.

Another form of mutagenic radiation was ultraviolet light (UV), a nonionizing component of ordinary sunlight. The most important effect of UV light on DNA was the formation of harmful covalent bonds among the bases. Adjacent thymines in a DNA strand can crosslink to form thymine dimers. Such dimer, unless repaired, may cause serious damage or death to the cell because the cell cannot properly transcribe or replicate such DNA (Figure 3.).

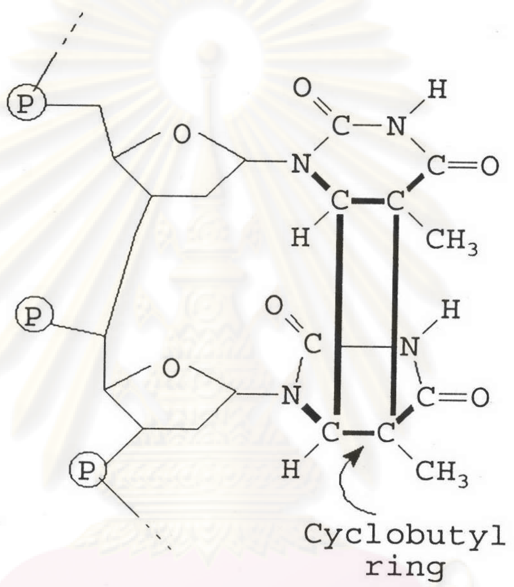


Figure 3. Mutagenesis by ultraviolet light.
Ultraviolet light crosslinked adjacent
thymines to form cyclobutylthymine dimer.

The mutation rate was the probability that a gene would mutate when a cell divided. The rate was usually stated as a power of 10, and because mutation were very rare, the exponent was always a negative number. Spontaneous mistakes in DNA replication occured at a very low rate because the replication machinery was remarkably faithful. Perhaps only once in 10^9 replicated base pairs does an error occured. Because the average gene had about 10^3 base pairs, the spontaneous rate of mutation was about once in 10^6 (a million) replication genes. Typically, a mutagen would increase the spontaneous rate from 10 to 1000 times; that was, a mutagen would produce a mutation rate of 10^{-5} to 10^{-3} per gene per cell generation (Tortora, 1989).

From the mutation experiments of *Zymomonas mobilis* CM141, a high ethanol tolerance strain by Supanwong and Chavapan (1986) at Chiang Mai University for a reduced levan formation strain by treatment with hydroxylamine. A mutant expected to produce no levan was found to produce red pigments under aerobic condition (1987). Physiological properties of the mutan differ from the parent strain in two respects. Firstly, growth was good under aerobic conditions and secondly pigments were produced as secondary metabolites. However the mutant still retained many important characteristics of the parent strain particularly the morphology and alcohol-fermentation properties (Table 3.). Large quantities of the pigment were secreted on to medium after the growth phase. This property was proved to be stable by performing ten successive transfers. It was stable when preserved frozen. The pigment production was faster when the medium contained 2 % glucose. Similar results were observed when 2 % sucrose was used.

Table 3. Properties of *Zymomonas mobilis* CM 141 and the pigment producing mutant.

Feature	*Zymomonas mobilis* CM 141	Mutant
Morphology	Rod	Rod
Gram stain	Negative	Negative
Flagella	+	+
Growth Condition	Anaerobic	Aerobic
Alcohol fermentation	15%(V/V) in 48 hours	12%(V/V) in 2 months
Pigment production	-	+

The pigments were extracted by methanol and chloroform (1:1) and were readily soluble in chloroform. Because of the lack of information in the literature on the nature of red pigments produced by *Zymomonas mobilis*, a study was performed.

NATURAL PIGMENTS

The pigments were the secondary metabolites of living cells. The chemical compounds of the natural pigments from plants, animals, and microorganisms were carotenoids, quinones, flavonoids, tetrapyrroles, andother non-polymeric N-heterocyclic pigments (Britton, 1983).

Carotenoids

Of all the various classes of natural pigments, carotenoids were problably the most widely distributed and were certainly among the most important. They were found throughout the plant kingdom in both photosynthetic and non-photosynthetic tissues, they were frequently encountered as microbial pigments.

Quinones

The quinones were a large and rather heterogenous collection of compounds. They ranged in color from pale yellow, through orange, red, purple and brown to almost black, and were important pigments in certain fungi and lichens.

Flavonoids

The flavonoids were almost exclusively products of higher plants. They included the anthocyanins which were responsible for perhaps the most striking of all plant colors, those of the brilliant red, purple and blue flowers and friuts.

Tetrapyrroles

The N-heterocyclic compound pyrroles was a very stable heteroaromatic system, but simple monopyrroles were seldom encountered in nature. Di- and tri- pyrroles were also rare, although the red bacteria pigment **prodigiosin** was now known to be a linear tripyrrole. In contrast, some of the most familiar of all natural pigments had cyclic tetrapyrrole structures, including such important substances as chlorophyll, the green light-harvesting pigment of plants, and haem, which forms the basis of the oxygen-transporting red blood pigments. Related tothese were the billins, which were linear tetrapyrroles. This group included the animal bile pigments, the phycobilin accessory photosynthetic pigments of some algae, and the chromophore of the plant photoregulatory pigment, phytochrome.

Other non-polymeric N-heterocyclic pigments

The basic skeletons of most of these pigments were condensed bi, tri- or oligocyclic heteroaromatic ring systems and their partially reduced derivatives. In these compounds electronic excitation was usually relatively easy, espectially in ring systems with extended conjugation or when several substituents were present, and yellow, red, purple or blue colors may be produced. Of the several groups of these pigments to be described, the purines and pteridines were extremely important substances, synthesised by all living organisms, but they were used as pigments only by a small number of animals. The closly related riboflavin was produced only by plants and microbes but was also extremely important in animals as a vitamin, though it rarely served as a pigment. The ommochromes were exclusively animal products (arthropots). The phenazine group was produced only by bacteria, which also elaborated a miscellany of other nitrogenous pigments. The betalains, which did not have condensed ring systems, were exclusively plant products, and were not present in or used by animals.

PHOTOSYNTHETIC PIGMENTS

The ultimate source of energy for life was solar or light energy. That process by which light energy was transformed to chemical energy and ultimately used in cell synthesis was called photosynthesis. The generalized equation for this process in plants, algae, and cyanobacteria was

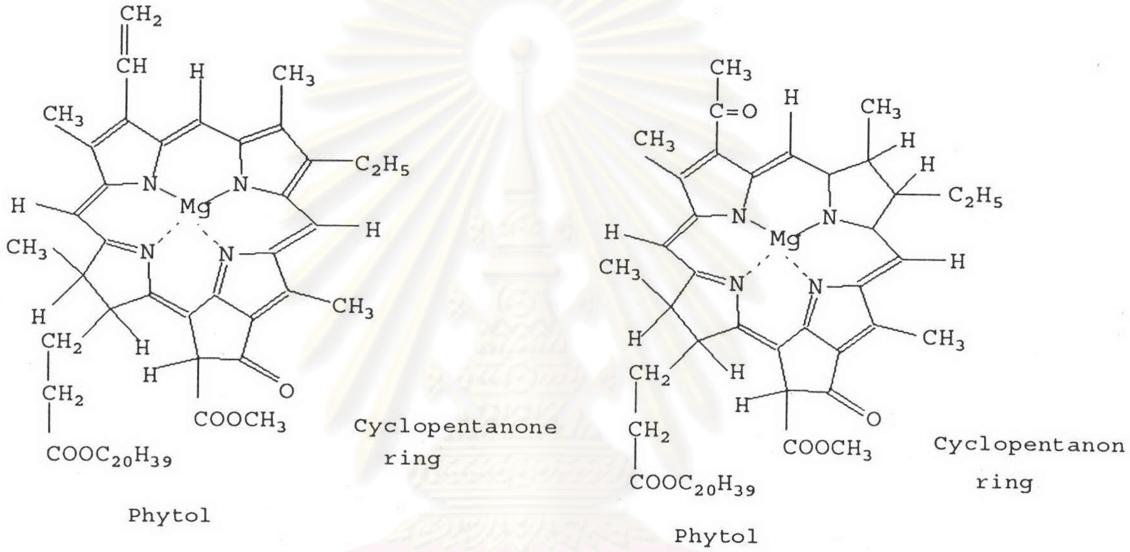
$$2CO_2 + 2H_2A \xrightarrow{\text{light}} 2(CH_2O) + 2A + O_2$$

where H_2A was the electron donor and A was the oxidized product.

The generalized reaction of photosynthesis encompasses two phases: photophosphorylation, in which light energy was converted to chemical energy, and the utilization of this energy for biosynthesis.

The chlorophylls were the primary pigments in the photosynthetic process. Like the heme component of the cytochromes and certain other respiratory enzymes, the chlorophylls were prophyrins containing a nucleus of four pyrrole rings, to which a metallic ion was chelated. However, this metallic ion in chlorophyll was magnesium, not iron. Chlorophyll molecules also contain a cyclopentanone ring fused to the tetrapyrrole nucleus, and one of the pyrrole rings was esterified with an alcohol such as phytol.

There were at least seven kinds of chlorophylls.Those present in the cyanobacteria and eucaryotic chloroplasts are designated as chlorophyll-*a* and -*b*, whereas those found in the other phototropic bacteria were designated as bacteriochlorophylls-*a*, -*b*, -*c*, and so on. The latter differ chemically from the other chlorophylls in the nature of the atoms and molecules attached to the tetrapyrrole nucleus. The structures of chlorophyll-*a* and bacteriochlorophyll-*a* were illustrated in Figure 4.



Chlorophyll-*a*

Bacteriochlorophyll-*a*

Figure 4. The chemical structures of chlorophyll-*a* and bacteriochlorophyll-*a*.

Major groups of Phototrophic bacterias and their pigments were shown in Table 4.

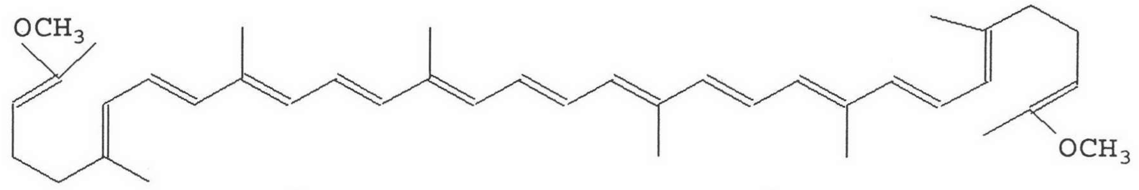
Table 4. Major groups of Phototrophic Eubacteria.

Taxonomic group	Metabolic Process	Pigments	Photosynthetic Electron Donors	Carbon Source(s)
Purple bacteria (Chromatiaceae and Rhodospirillaceae)	Anoxygenic	Bacterio-chlorophyll -*a* or -*b* and carotenoids	H_2S, S, H_2 and organic compounds	Organic and/or CO_2
Green bacteria (Chlorobiaceae and Chloroflexaceae)		Bacterio-chlorophyll -*c*, -*d*, or -*e* and low levels of -*a* and carotenoids	H_2S , S, H_2 and organic compoumds	Organic and/or CO_2
Cyanobacteria	Oxygenic	Chlorophyll -*a*, phycobili-proteins and carotenoids	H_2O (or H_2S)	CO_2
Prochlorophytes		Chlorophyll -*a* and -*b* and carotenoids	H_2O	CO_2

Associated with the chlorophylls as the light-havesting pigments were the carotenoids. These were long chain hydrocarbon molecules composed of a conjugated double-bond system.The chemical structures of carotenoids are shown in Figure 5.

The halobacteria were normally pigmented red because of the high carotenoid content of their membranes, which function in preventing photochemical damage. In the presence of atmospheric oxygen, the halobacteria were aerobic chemoorganotropbs oxidizing amino acids and other organic substrates for energy. Below the surface which was anaerobic conditions, energy may be obtained by photophosphorylation. The photoreactive pigment was bacteriorhodopsin; which was chemically similar to the rhodopsin pigment of the human eye.

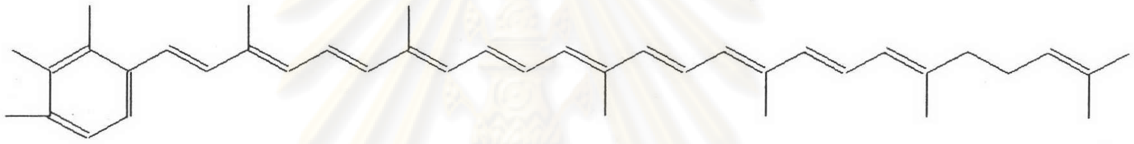
In the cyanobacteria and the red algae, the primary light-harvesting pigments were the phycobiliproteins. These were soluble, linear tetrapyrroles that were conjugated to proteins.



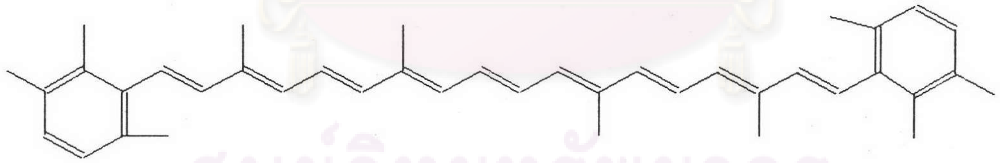
Spirillaxanthin

a. Aliphatic carotenoids

(Purple sulfur and nonsulfur bacteria)



Chlorobactene



Isorenieratene

b. Aryl carotenoids

(Green sulfur bacteria)

Figure 5. The chemical structures of some carotenoids of the purple and green phototrophic bacteria.

Prodigiosin was the characteristic red, water - insoluble pigment of the bacteria, first found from *Serratia marcescens* (*Bacillus prodigiosus*) in 1929 (Bentley). The structure was completely assignment in 1960 as the result of partial and total synthesis (Wasserman et al., 1960). The chemical formula of prodigiosin was $C_{20}H_{25}ON_3$ and was a linear tripyrrole pigment contained one methoxy group and two active hydrogen atoms. The main structure was 5-(2-pyrryl)-2,2'-dipyrrylmethene called prodigiosene (Hearn et al., 1970) (Figure 6.). Ten naturally occuring prodigiosin pigments were isolated.

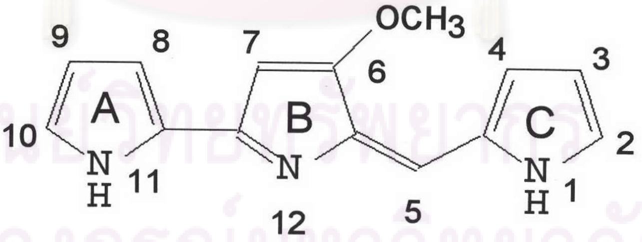
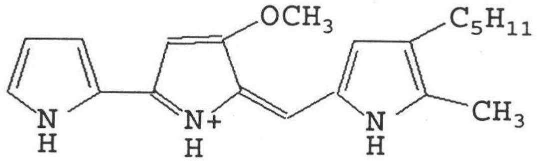


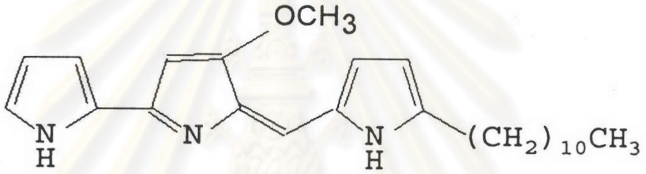
Figure 6. Prodigiosene.

The numbering system indicated on the formula was particularly convenient for naming prodigiosin and the prodigiosin-like compounds substituded on the pyrrole rings adjacent to the methene carbon. Three carbon atoms in the bipyrrole portion of the molecule and one in the monopyrrole were not numbered because substitutions on them would destroy the basic linear tripyrrole structure of prodigiosene (Hearn et al., 1970).

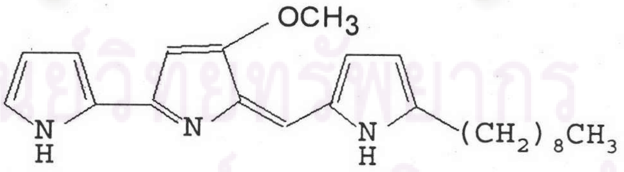
Prodigiosin (I) (Figure 7.) was first isolated from *Serratia marcescens* and latter conclusively identified from *Serratia marinorubra* and from two unamed aerobic, Gram-negative, rod shaped, mesophilic marine bacteria not members of the genus *Serratia* (Lewis and Corpe, 1964), from *Pseudomonas magnesiorubra* (Gandhi et al., 1973), and from *Vibrio psychroerythrus* (D'Aoust and Gerber, 1974).



(I) Prodigiosin

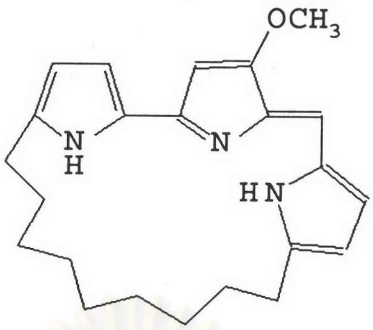


(II) undecylprodigiosin

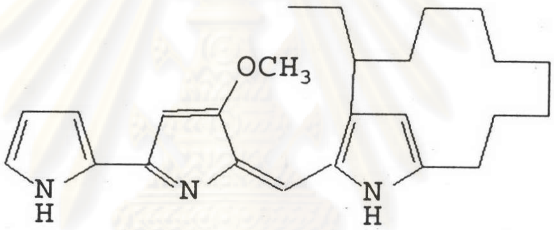


(III) Nonylprodigiosin

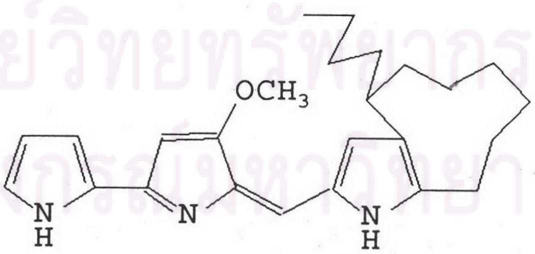
Figure 7. Prodigiosin and its derivatives.



(IV) Cyclononylprodigiosin

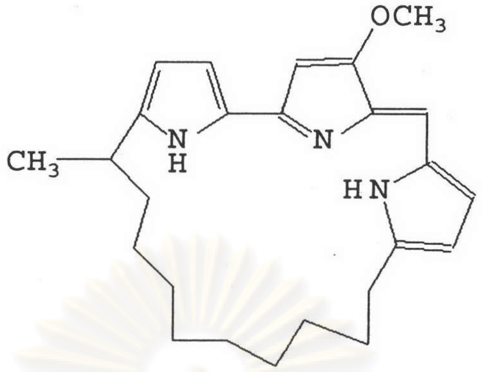


(V) Metacycloprodigiosin

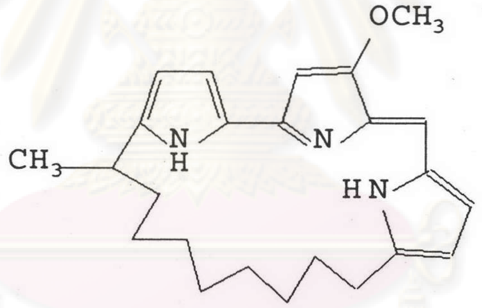


(VI) Butylcycloheptylprodigiosin

Figure 7 (cont.). Prodigiosin and its derivatives.

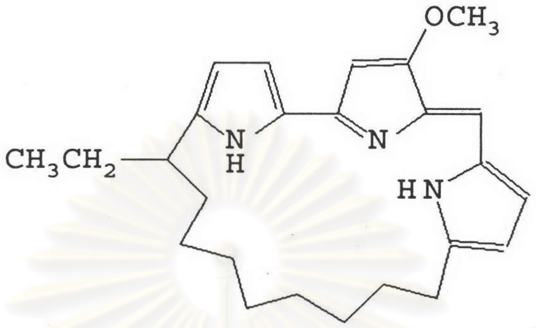


(VII) Cyclomethyldecylprodigiosin

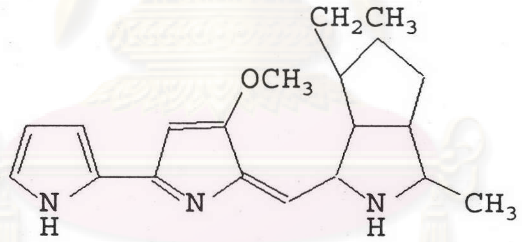


(VIII) Cyclomethylnonylprodigiosin

Figure 7 (cont.). Prodigiosin and its derivatives.



(IX) Cycloethylnonylprodigiosin



(X) ethylcyclopropylprodigiosin

Figure 7 (cont.). Prodigiosin and its derivatives

Undecylprodigiosin (II) (prodigiosin-25C) was reported from *Streptomyces longisporus* (Wasserman et al., 1966), *Actinomadura pelletieri* (Gerber, 1971), and *Streptoverticillin rubrireticuli* (Gerber and stahly, 1975).

Several red strains of *Actinomodura pelletieri* and *A. madurae* (Gerber, 1969) were investigated, and all were found to produce pigments with the characteristic of prodigiosin. The main component was nonylprodigiosin(III) and reinvestigated somewhat, a different pigment predominated, it was cyclononylprodigiosin (IV) (Gerber, 1971).

There were several prodigiosins present in the red strains of bacteria. In every case, thin-layer chromatography showed the presence of more than one component. The total amount of pigment and the relative amounts of each component variable. The macrocyclic prodigiosin was the miner component. These were metacycloprodigiosin(V) from *Streptomyces longiosporus ruber* (Wasserman et al., 1966), butylcycloheptylprodigiosin(VI) from *Streptomyces spp.* and *Streptoverticillium rubrireticuli*(Laatsoh et al., 1991 and Gerber and Stahly, 1971), cyclomethyldecylprodigiosin (VII), cyclomethylnonylprodigiosin (VIII) and cycloethylnonylprodigiosin (IX) from *Actinomadura pelletieri* and *Actinomodura madurae* (Gerber, 1973) ethylcyclopropylprodigiosin (X) from *Alteromonas rubra* (Gerber and Gauthier, 1979).

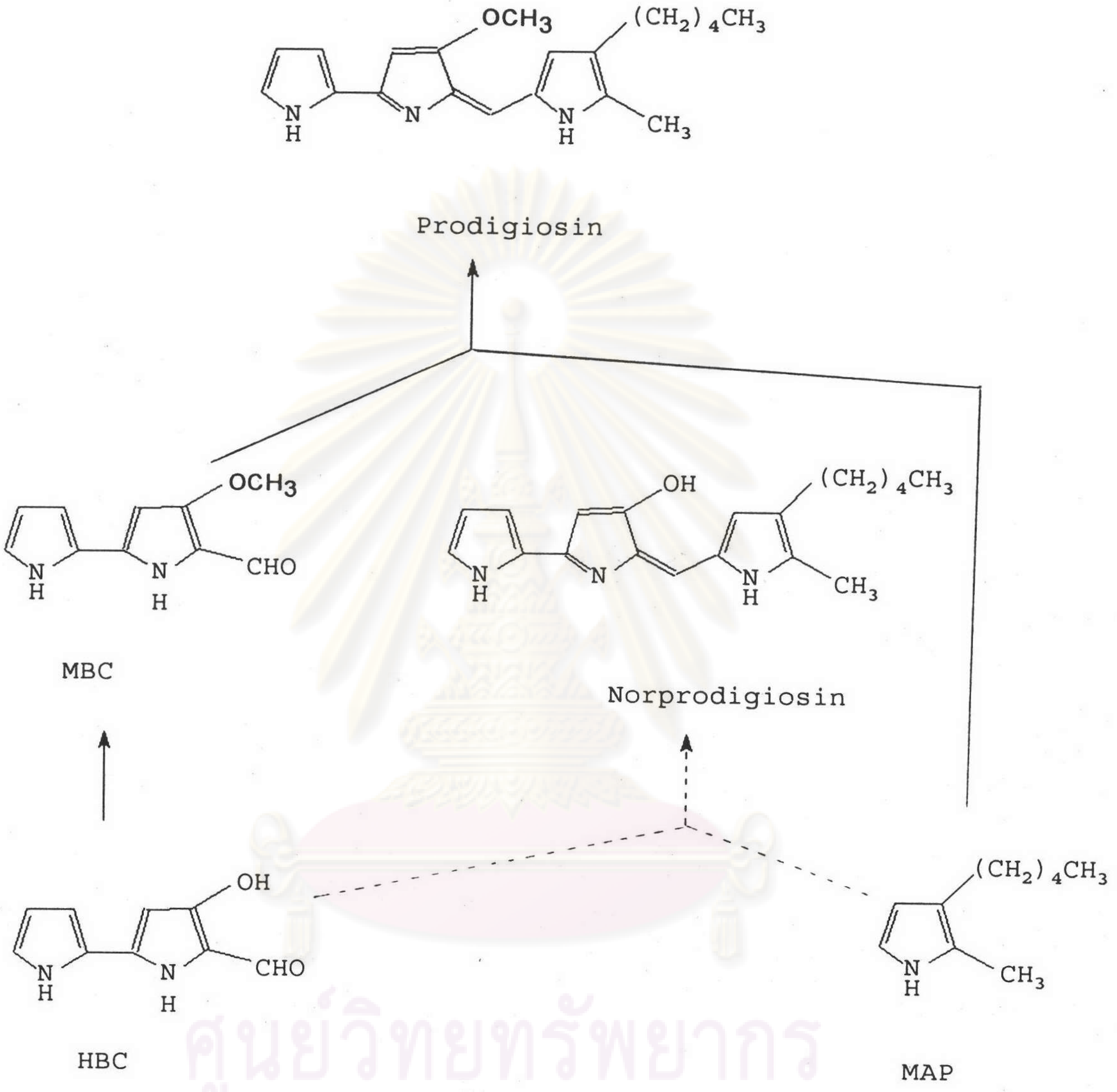
Table 5. Prodigiosin pigments and its sources.

Pigments	Sources
Prodigiosin	*Serratia marcescens* *Serratia marinorubra* *Pseudomonas magnesiorubra* *Vibrio psychroerythrus*
Undecylprodigiosin	*Streptomyces longisporus* *Actinomadura pelletieri* *Streptoverticillin rubrireticuli*
Nonylprodigiosin	*Actinomodura pelletieri* *Actinomodura madurae*
Cyclononylprodigiosin	*Actinomodura pelletieri* *Actinomodura madurae*
Metacycloprodigiosin	*Streptomyces longiosporus ruber*
Butylcycloheptyl prodigiosin	*Streptomyces spp.*
Cyclomethylundecyl prodigiosin	*Actinomadura pelletieri* *Actinomodura madurae*
Cyclomethylnonyl prodigiosin	*Actinomadura pelletieri* *Actinomodura madurae*
Cycloethylnonylprodigiosin	*Actinomadura pelletieri* *Actinomodura madurae*
Ethylcyclopropyl prodigiosin	*Alteromonas rubra*

BIOSYNTHESIS OF PRODIGIOSIN PIGMENTS

Considerable effort and experimentation had been applied to the problem of the biosynthesis of prodigiosin. Biosynthesis was complex and involved two different pathways to the intermediate precursors, 4-methoxy-2,2'-bipyrrole-5 carboxaldehyde (MBC) and 2-methyl-3-pentylpyrrole (MAP) (Shrimpton, 1963), plus enzymatic coupling to form prodigiosin (Gargallo, 1987) (Scheme 4.). It had also been shown that 5-aminolevulinic acid was not incorporated into prodigiosin, thus, the route was unrelated to that of porphyrin biosynthesis.

Isotope incorporation experiments using ^{14}C showed that proline (Shrimpton,1963), glycine (Hubbard and Rimington,1950), methionine (Qadri and Williams, 1973), and acetate (Hubbard and Rimington,1950), were important precursors of prodigiosin. It was concluded that proline was incorporated intact into ring A and one carbon of ring B; glycine and methionine were incorporated into ring B, and the labeling pattern in ring C was not clarified (Tanaka et al., 1972).



Scheme 4. Biosynthesis pathway for prodigiosin.

The use of ^{13}C as a precursor circumvents the need for chemical degadation. Using [1-^{13}C] and [2-^{13} C] acetate (Cushley et al., 1971), as well as [3-^{13}C]-L-alanine, [carbonyl-^{13}C]-D,L-proline, [2-^{13}C] glycine, [3-^{13}C]-D,L-serine (Cushley et al., 1971), and [CD_3)-DL-methionine, the labeling pattern shown in Figure 8. was established for prodigiosin. Thus, each of the three pyrrole rings was assembled in a different way : ring A from proline, ring B from acetate, glycine, and the carbonyl of proline, ring C from acetate and alanine. All were different from other naturally occurring pyrroles (Wasserman et al., 1973). When this approach was extented to the streptomyces pigments undecylprodigiosin and metacycloprodigiosin, similar results were obtained, except for ring C as summarized in Figure 8 (Wasserman et al., 1974). In these two pigments glycine was involved in ring C as well as ring B and the mode of incorporation of acetate in ring C was different.

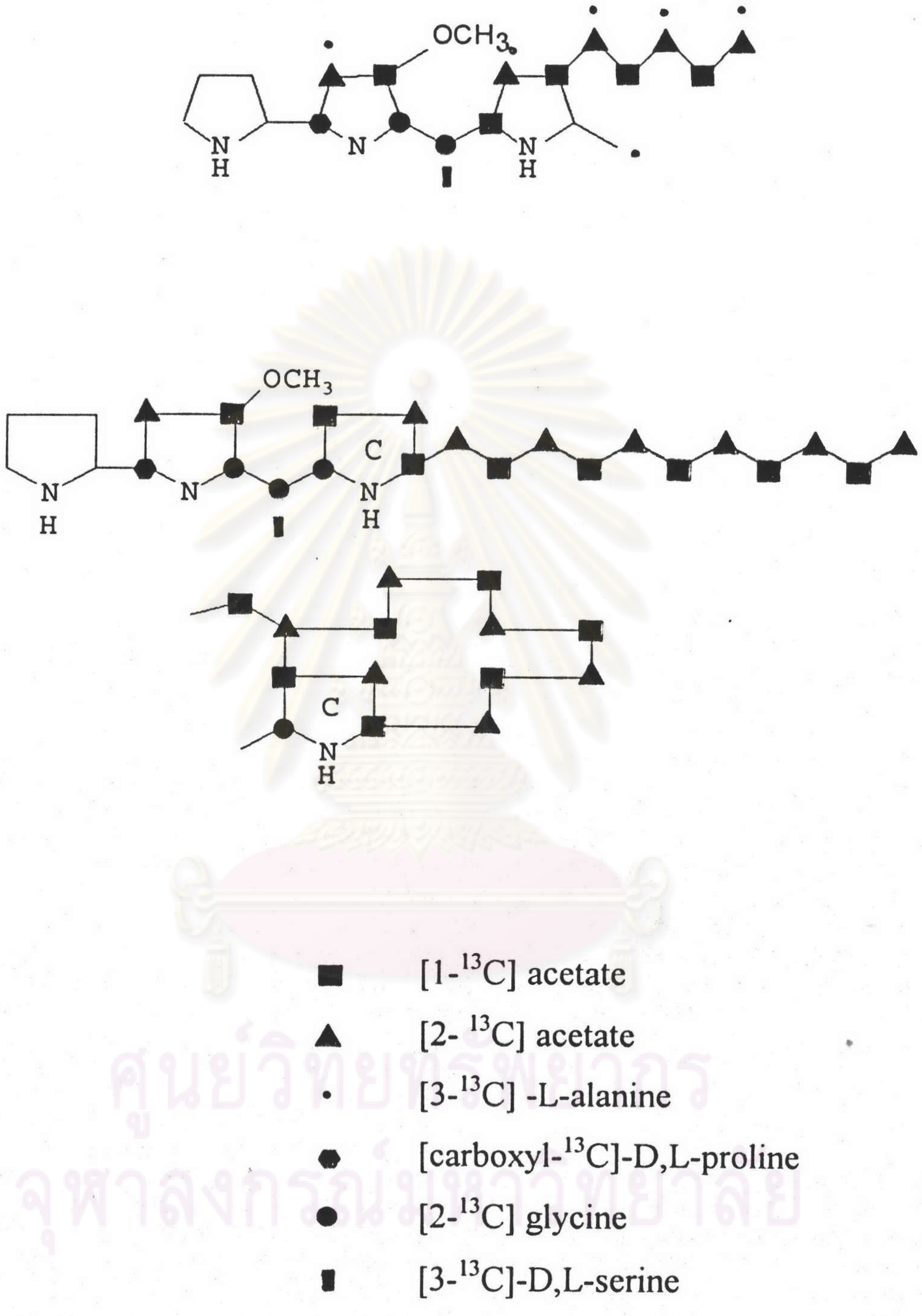


Figure 8. Incorporation patterns of acetate, glycine,alanine, proline, serine, and methionine in prodigiosin, undecylprodigiosin and metacycloprodigiosin.

BIOLOGICAL ACTIVITY OF PRODIGIOSIN PIGMENTS

Cytotoxic Activity

Prodigiosin , prodigiosene , and 2-methyl-3-pentylprodigiosene (desmethoxyprodigiosin) were subjected to comparative in vitro cytotoxic and antimicrobial evaluation in efforts to define the role the prodigiosin peripheral substituents play in contributing to potentiating the observed propertied of the prodigiosene (Table 6.).

Table 6. In Vitro Cytotoxic Activity of Prodigiosin.

Substance	IC_{50}, mcg/ml[a]			
	L1210[b]	B16[c]	9PS(P388)[d]	9KB[e]
Prodigiosin	0.02	0.03	0.00037	0.04
Prodigiosene	17	6	0.07	6.4
Desmethoxyprodigiosin	12	24	0.03	0.7

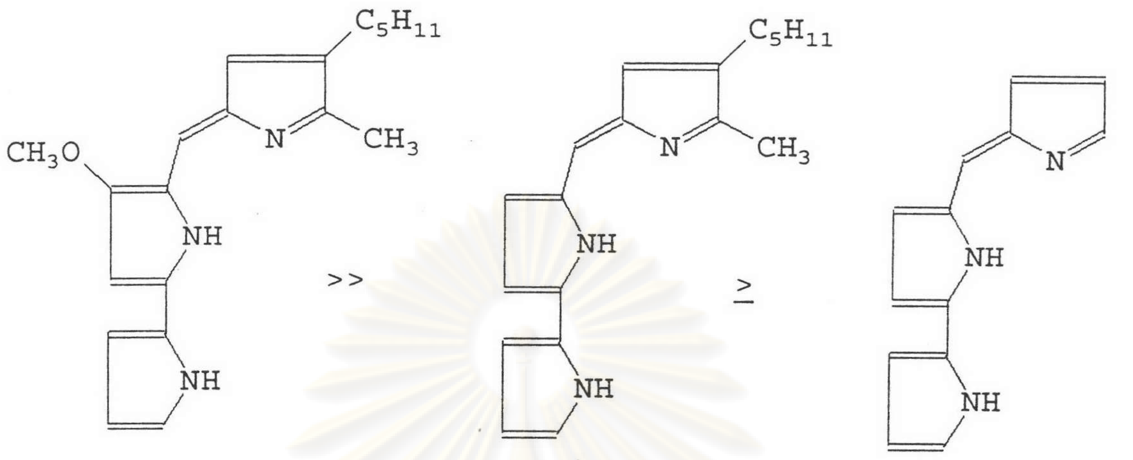
[a] Inhibitory concentration for 50% cell growth relative to untreated control (IC_{50})

[b] L-1210 mouse lymphocytic leukemia cell culture

[c] B16 mouse melanoma cell culture

[d] P388 mouse leukemia cell culture

[e] Human epidermoid carcinoma of the nasopharynx



Prodigiosin 2-methyl-3-pentyl prodigiosene Prodigiosene

Figure 9. The diminishing cytotoxic activity of the prodigiosin after the sequential removal of the prodigiosin peripheral substituents.

The sequential removal of the prodigiosin peripheral substituents resulted the diminishing observed cytotoxic potency. Prodigiosin showed exceptionally the *in vitro* activity against P388 leukemia (ID_{50}=$3.7x10^{-4}$mcg/ml =$3.7x10^{-10}$ mcg/ml) and displayed substantial *in vitro* cytotoxic activity against L1210,B16, and 9KB cell lines (ID_{50} =0.02,0.03, and 0.04 mcg/ml, respectively). The cytotoxic activity may be attributed to the presence of the peripheral prodigiosene C-6 methoxy substituent (Boger and Patel, 1988).

Antimalarial activity of prodigiosin

The prodigiosin had been considered too toxic for the therapeutic use, although it has evidently been used in the clinical treatment of some cases of disseminated coccidioidomycosis (Wier et al., 1952). Prodigiosin showed definite activity against the parasite, *Plasmodium berghei* (Castro, 1967).

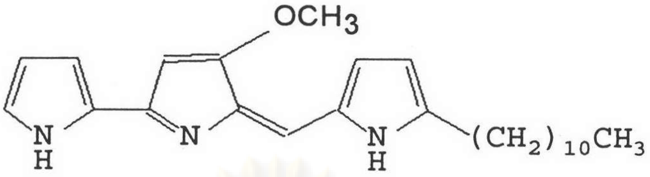
Toxigenic studies with the antibiotic pigments

Considering the antibiotic nature of prodigiosin and in spite of the importance of many other antibiotics served in the current therapy, it was interesting to note that only a few of properties were recommended to be used during pregnancy. This was due to the fact that many antibiotics as well as other drugs, which were comparatively well tolerated by the maternal organism, might exert adverse effects on the fetus at certain stages of the embryogenesis.The most fear outcome deterring the usage of these drugs was the ranges of the adverse and toxic effects which might result in fetal death or malformations. The experiment indicated that prodigiosin extracts had toxic effects on chicken embryos (Kalesperis et al., 1975).

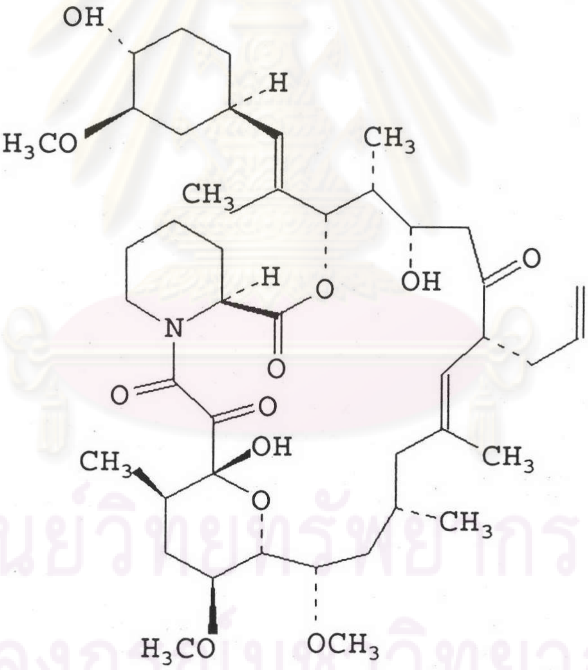
Selective immunosuppression

The immunomodulating substances, which attacked specific target sites of cells, were expected to be the tools for the study of cellular and biochemical events of the immune response, and also provided a useful prototype of drugs for the immunotherapy. Several groups had tried to search for immunomodulating substances among microbial metabolites by using the immunological assay system. Some new immunoactive low molecular weight substances had been discovered (Kahoma et al., 1992).

Prodigiosin 25-C inhibited the responses of murine splenocytes to T cell specific mitogens, concanavalin A (con A) and phytohemagglutinin (PHA), stronger than to a B cell specific mitogen lipopolysaccharide (LPS) (Nakamura et al., 1986). Prodigiosin 25-C inhibited both con A and interleukin-2(IL-2)-dependent proliferative response of con A-primed splenocytes (Nakamura et al., 1989). It was also shown that prodigiosin 25-C inhibited the induction of H-2 specific cytotoxic T lymphocyte (CTL) both *in vitro* and *in vivo*, but at the same dose as used *in vivo* in the supression of CTL induction, prodigiosin 25-C did not inhibit the anti-sheep red blood cell (SRBC) antibody production.



Prodigiosin 25-C



FK 506

Figure 10. Structure of prodigiosin 25-C and FK 506.

When comparing the immunosuppressive property of prodigiosin 25-C with FK 506 (Figure 10.), an antibiotic of the macrolide family, which was isolated from *Streptomyces tsukbaensis* (Tocci et al., 1989), *in vitro* and *in vivo*, the results demonstrated that only prodigiosin 25-C selectively inhibited CTL induction without affecting functions of the helper T cells and B cells (Tsuji et al., 1990).

Prodigiosin 25-C had the immunomodulating properties with preferentially supression of the induction of cytotoxic T cells (Tsuji et al, 1992).