CHAPTER II

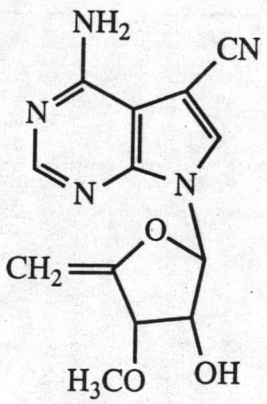
HISTORICAL

Chemical Constituents of The Genus *Mycale*

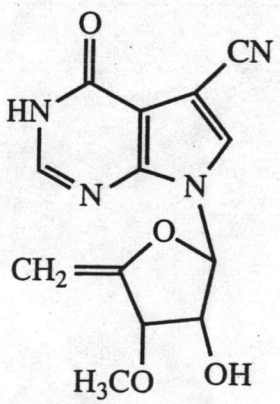
Many groups of compounds have been isolated from the Genus *Mycale* such as nucleosides, terpenoids, amides, macrolides, halogenated acetogenins and fatty acids.

1. Nucleosides

Two cyano containing purine nucleosides, mycalisine A [1] and mycalisine B [2] were isolated from *Mycale* sp. (Kato *et al.*, 1985). Mycalisine A inhibited cell division of fertilized starfish (*Asterina pectinifera*) eggs at 0.5 μg/ml, while mycalisine B showed activity at 200 μg/ml.



Mycalisine A [1]



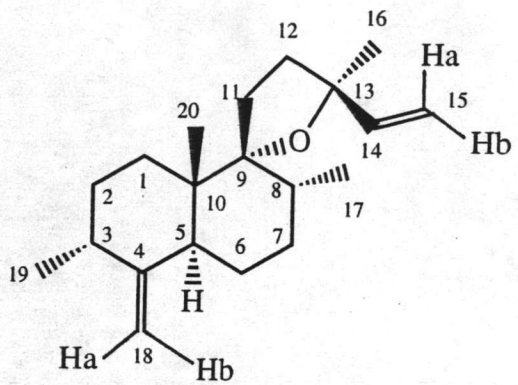
Mycalisine B [2]

2. Terpenoids

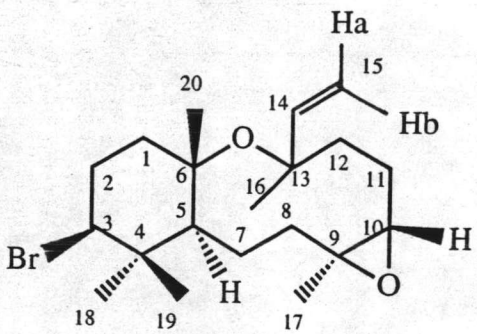
Terpenoids consist of isoprene units (C-5 unit) in their carbon skeletons. The number of these isoprene units in the compound that has given rise to simple primary classification system. Terpenes which isolated from *Mycale* spp. are diterpenes and norsesterterpene cyclic peroxides.

2.1 Diterpenes

Diterpenes consist of four isoprene units (20-carbons). Two diterpenes were isolated from *Mycale rotalis* (Bowerbank), Rotalin A [3], and Rotalin B [4] (Corriero *et al.*, 1989).



Rotalin A [3]

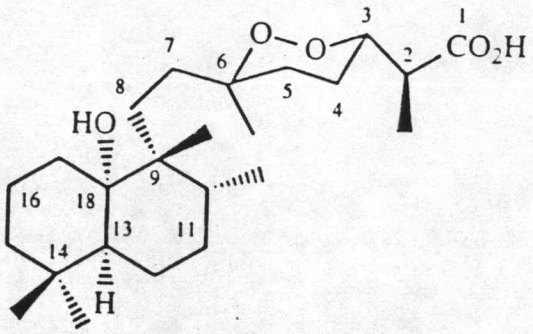


Rotalin B [4]

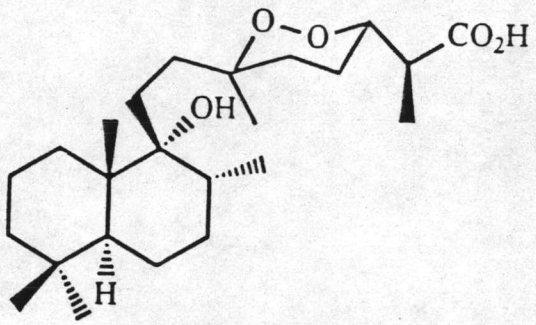
2.2 Norsesterterpene cyclic peroxides

Norsesterterpene cyclic peroxides consist of 24-carbons in their main skeletal. Seven norsesterterpene cyclic peroxide were isolated from *Mycale* spp.

Mycaperoxide A [5] and Mycaperoxide B [6] were isolated from a Thai sponge, *Mycale* sp. (Tanaka *et al.*, 1993). Mycaperoxides A and B showed significant cytotoxicity (IC-50 = 0.5-1.0 μg/ml) against the cell lines of P-388, A-549, and HT-29 and displayed antiviral activity (IC_{50} 0.25-1.0 μg/ml) against vesicular stomatitis virus and *Herpes simplex* virus type-1. Both compounds also inhibited the growth of the gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*.

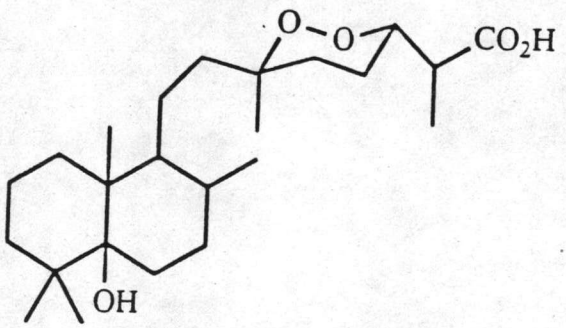


Mycaperoxide A [5]

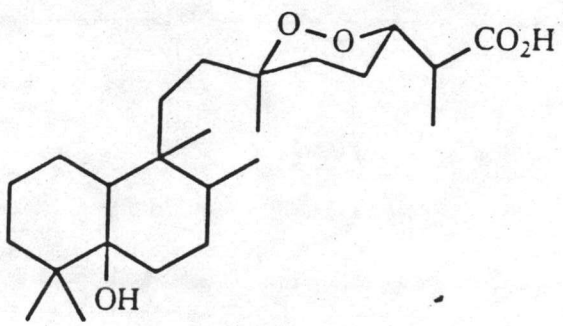


Mycaperoxide B [6]

Two norsesterterpene cyclic peroxides [7, 8] were isolated from dichloromethane part of *Mycale* (*Carnia*) cf *spongiosa* (Capon, 1991).

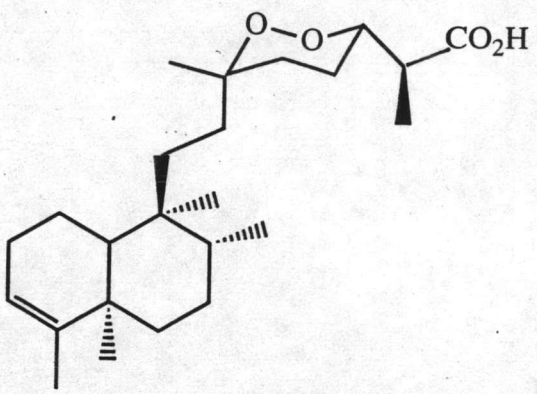


[7]

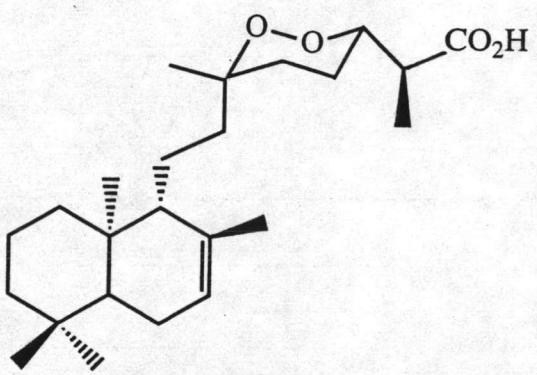


[8]

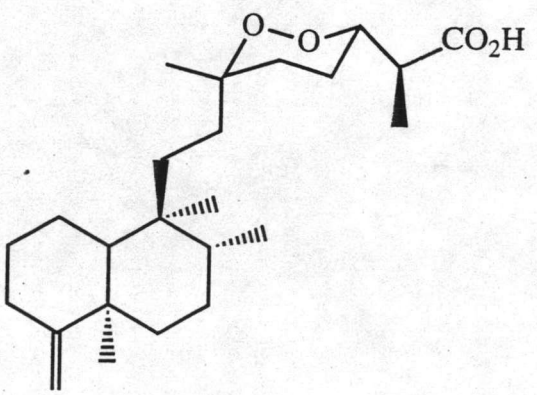
And two of these compounds [9, 10] were isolated from *Mycale ancorina* Whitelegge. Both are isomeric of *enantio*-sigmosceptrellin A [11] (Capon and Macleod, 1987).



[9]



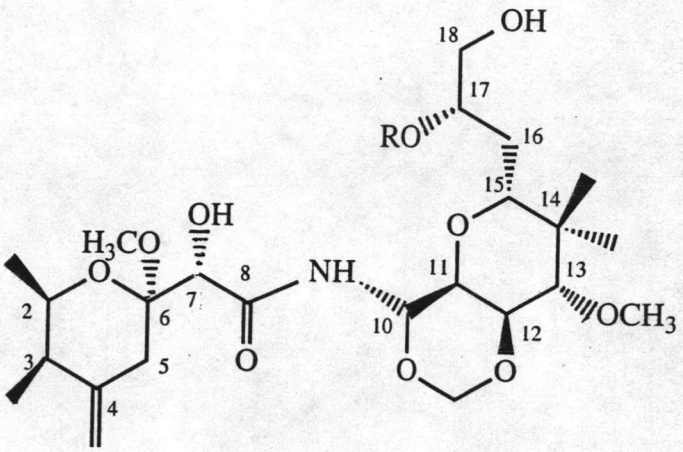
[10]



[11]

3. Amides

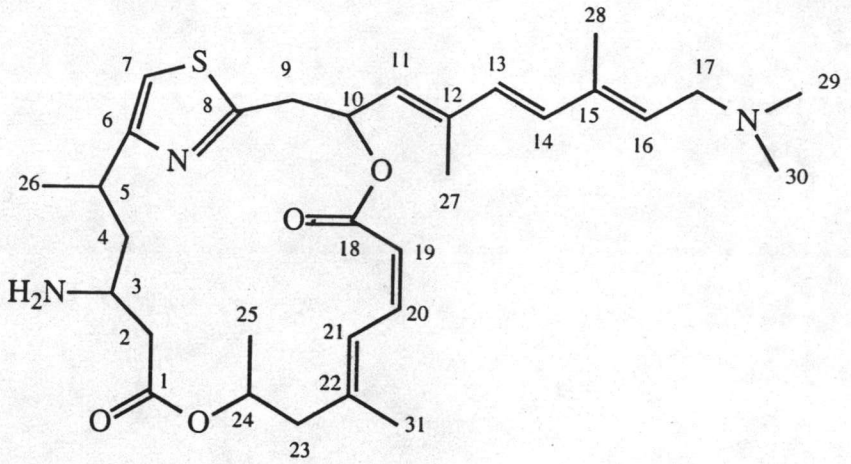
Two oxygenated cyclic amides were isolated from *Mycale* sp. (Perry *et al.*, 1990). Mycalamides A and B [12,13] showed cytotoxicity (IC_{50} 3.0 ng/ml, 0.7 ng/ml, respectively) against cell lines of P-388 and displayed antiviral activity (minimum active dose 3.5-5.0 ng/disc, 1.0-2.0 ng/disc, respectively) against Polio virus type I and *Herpes simplex* virus type I.



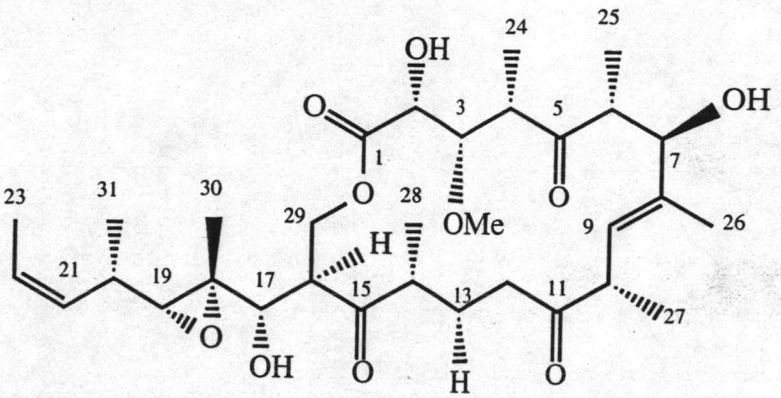
Mycalamide A [12], R=H
Mycalamide B [13], R=CH_3

4. Macrolides

Five macrolides were isolated from *Mycale* spp. Pateamine [14], a thiazole containing macrolide with potent in *vitro* antifungal and cytotoxic to cell lines of P-388 at IC_{50} = 0.15 ng/ml (Northcote, Blunt, and Munro, 1991) and 13-deoxy tedanolide [15], a peroxide containing macrolide were isolated from *Mycale adhaerens* Lambe (Fusetani, Sugawara, and Mutsunaga, 1991). 13-deoxy-tedanolide showed remarkable cytotoxicity against cell lines of P-388 at IC_{50} = 94 pg/ml.

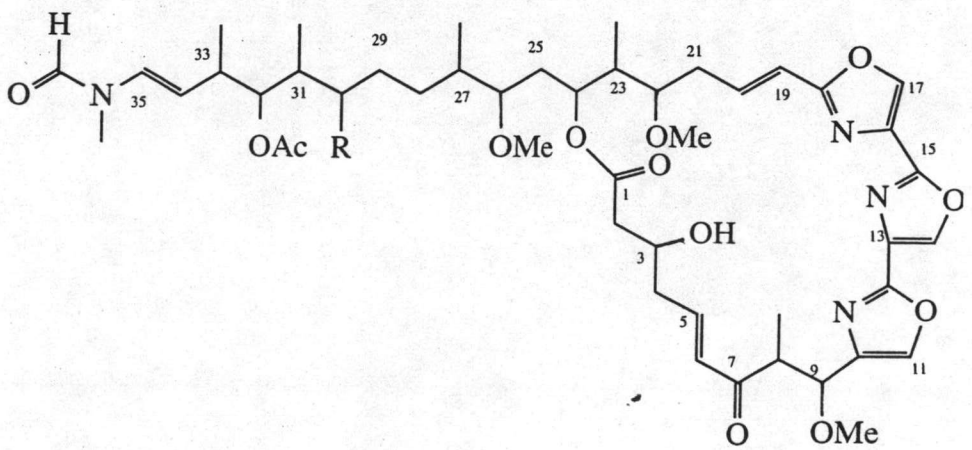


[14]



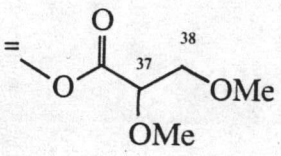
[15]

Mycalolides A-C [16-18], oxazole containing macrolides were isolated from *Mycale* sp. Mycalolides A-C showed antifungal activity against many pathogenic fungi and cytotoxic activity against cell lines of B-6 melanoma at IC_{50} = 0.5-1.0 ng/ml (Fusetani *et al.*, 1989).

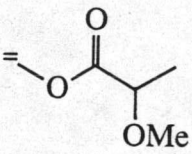


Mycalolide A [16], R = O

Mycalolide B [17], R =

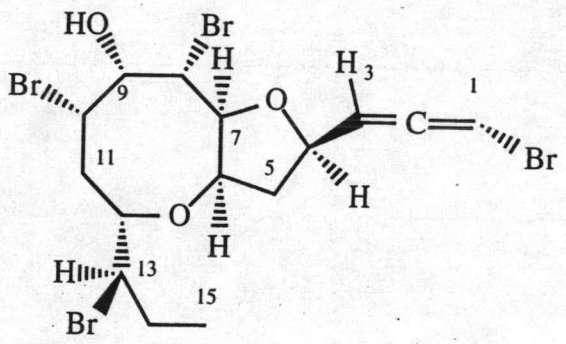


Mycalolide C [18], R =

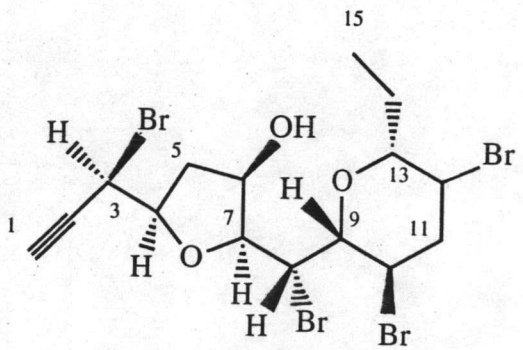


5. Halogenated acetogenins

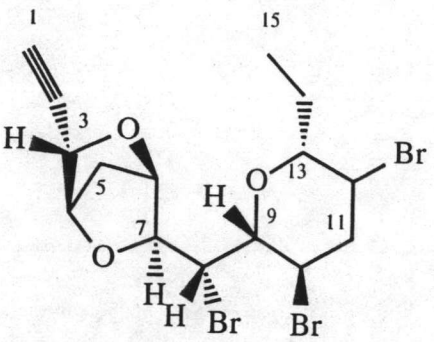
Three polybrominated C-15 acetogenins [19-21] were isolated from *Mycale rotalis* (Giordano *et al.*, 1990, Notaro *et al.*, 1992). Characteristics of cyclic member in this group were present of oxane ring of various sizes an enyne or allenic side chain and at least one halogen atom.



[19]



[20]



[21]

6. Fatty acids and fatty aldehydes

Fatty acids exist in nature, but few cases are known of very long chain monounsaturated acids longer than twenty two carbons however, sponges have provided the most intensity examples of long chain phospholipid fatty acid. Many of fatty acids and one of fatty aldehyde were found in *Mycale laevis* are shown in Table 1

Table 1. The phospholipid fatty acids and aldehydes.

Compounds	Equivalent chain length
[22] 17-Tetracosenal (24:1)	24.32
[23] Tetradecanoic (14:0)	14.00
[24] 13-Methyltetradecanoic (15:0)	14.59
[25] 12-Methytetradecanoic (15:0)	14.66
[26] Pentadecanoic (15:0)	15.00
[27] 9-Hexadecenoic (16:1)	15.71
[28] 11-Hexadecanoic (16:1)	15.75
[29] Hexadecenoic (16:0)	16.00
[30] 2-Methyl-5-hexadecenoic (16:1)	16.80
[31] Heptadecanoic (17:0)	17.00
[32] 11-Octadecenoic (18:1)	17.71
[33] Octadecanoic (18:0)	18.00
[34] Nonadecanoic (19:0)	19.00
[35] Eicosatetraenoic (20:4)	19.13
[36] 13-Eicosenoic (20:1)	19.71
[37] Eicosanoic (20:0)	20.00
[38] Heneicosanoic (21:0)	21.00
[39] 13-Docosenoic (22:1)	21.70

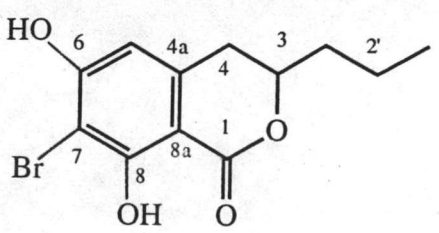
Table 1. (continue)

Compounds	Equivalent chain length
[40] 15-Docosenoic (22:1)	21.77
[41] Docosanoic (22:0)	22.00
[42] 16-Tricosenoic (23:1)	22.78
[43] Tricosanoic (23:0)	23.00
[44] 5,9-Tetracosenoic (24:2)	23.52
[45] 15-Tetracosenoic (24:1)	23.76
[46] 17-Tetracosenoic (24:1)	23.86
[47] 19-Tetracosenoic(24:1)	23.90
[48] Tetracosenoic (24:0)	24.00
[49] 5,9-Pentacosadienoic (25:2)	25.00
[50] 16-Pentacosenoic (25:1)	24.78
[51] 18-Pentacosenoic (25:1)	24.87
[52] Pentacosanoic (25:0)	25.00
[53] 24-Methyl-5,9-pentacosadienoic (26:2)	25.25
[54] 5,9-Hexacosadienoic (26:2)	25.59
[55] 17-Hexacosenoic (26:1)	25.78
[56] 19-Hexacosenoic (26:1)	25.87
[57] Hexacosanoic (26:0)	26.00

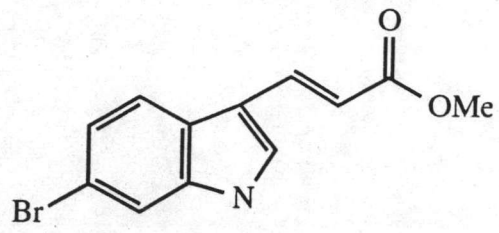
Reference: Carbelleira, Negron, and Reyes, 1992.

7. Miscellaneous compounds

Hiburipyranone [58] and Bromoindole [59] were isolated from *Mycale adhaerens* Lambe (Fusetani, Sugawara, and Matsunaga, 1991). Hiburipyranone showed cytotoxicity against cell lines of P-388 at IC_{50} = 0.19 ng/ml.



[58]



[59]

Chemistry of Nucleosides

Nucleosides consist of two parts, pyrimidine bases or purine bases and sugars. Nucleosides is found in the nucleic acid in the form of nucleoside phosphate esters (nucleotides). In nature sugar which substitutes on the bases is D-ribose or 2'-deoxy-D-ribose. The main sugar component of RNA is D-ribose. In DNA this sugar is replaced by 2-deoxy-D-ribose.

1. Pyrimidine bases (Figure 2)

The pyrimidine bases are derivartives of the parent compound pyrimidine, and the bases found in the nucleic acids are cytosine found in both DNA and RNA, uracil found in RNA and thymine and 5-methyl cytosine found in DNA. In certain colifages cytosine is replaced by 5-hydroxymethylcytosine or glucosylated.

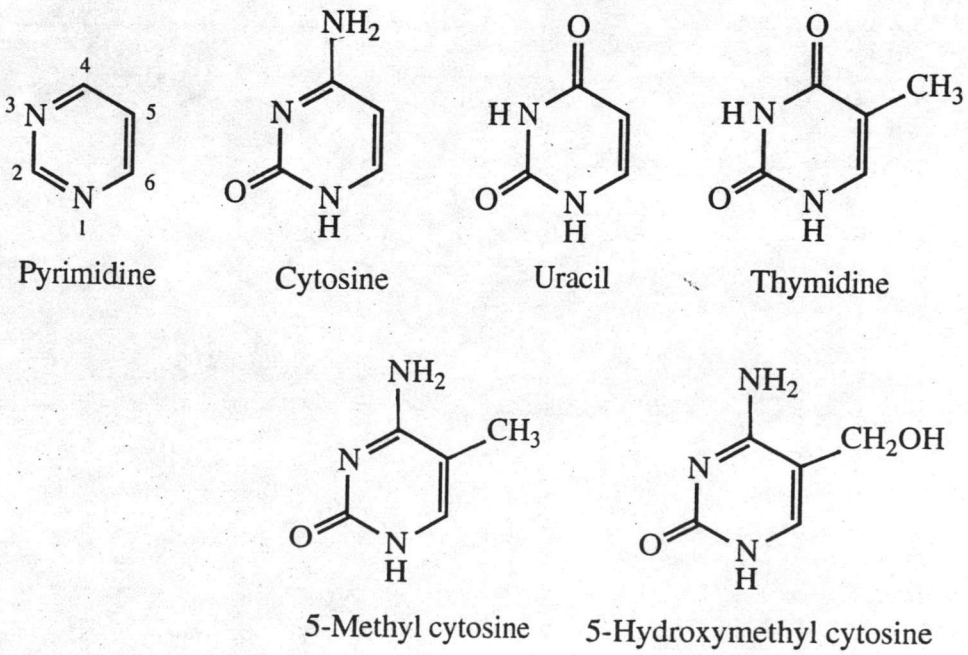


Figure 2. Pyrimidine bases

2. Purine bases (Figure 3)

They are derivative of purines which is form by the fusion of a pyrimidine ring and imidazole ring. The bases found in the nucleic acids are adenine and guanine.

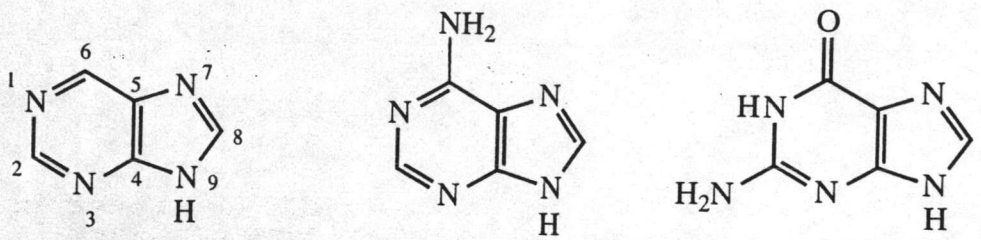


Figure 3. Purine bases

Generally, sugars substitute on position N-9 for purine nucleosides and N-1 for pyrimidine nucleosides. Sugars in nucleosides occur in the β–D-furanose form. In cell, free nucleosides are found in very low abundant. Biosynthesis are not known, but nucleoside may be hydrolyzed product of nucleotides and nucleic acids. Nucleic acids and nucleotides are constantly being degraded to nucleosides and free bases, the bases are to some extend degraded further to carbon dioxide and ammonia. Plants and other organisms can reconvert the nucleosides, deoxy nucleosides, and bases to nucleotides and deoxynucleotides by so called salvage reaction.

The ribonucleosides have three position (2', 3', 4' hydroxyl) that may be substituted with phosphate. In other way, the deoxyribonucleosides have only two position (3', 5' hydroxyl) that may be substituted with phosphate. The ribo-nucleoside 5'-phosphate may be further phosphorylated at position 5' to yield 5'-di and tri phosphates. Example,the adenosine 5' phosphate (AMP) may be phosphorylated to adenosine 5' diphosphate (ADP) and adenosine 5' triphosphate (ATP). The phosphate ester of nucleosides is nucleotides. Nucleotide is the component of nucleic acid. Nucleic acids act as storage and translation genetic information.

3. Biosynthesis of pyrimidine nucleotides (Ross, 1981)

3.1 Formation of UTP and CTP (Figure 4)

In all organism, the main route for the *de novo* synthesis of pyrimidines involves formation of orotic acid, using carbon dioxide, aspartate, and amide group of

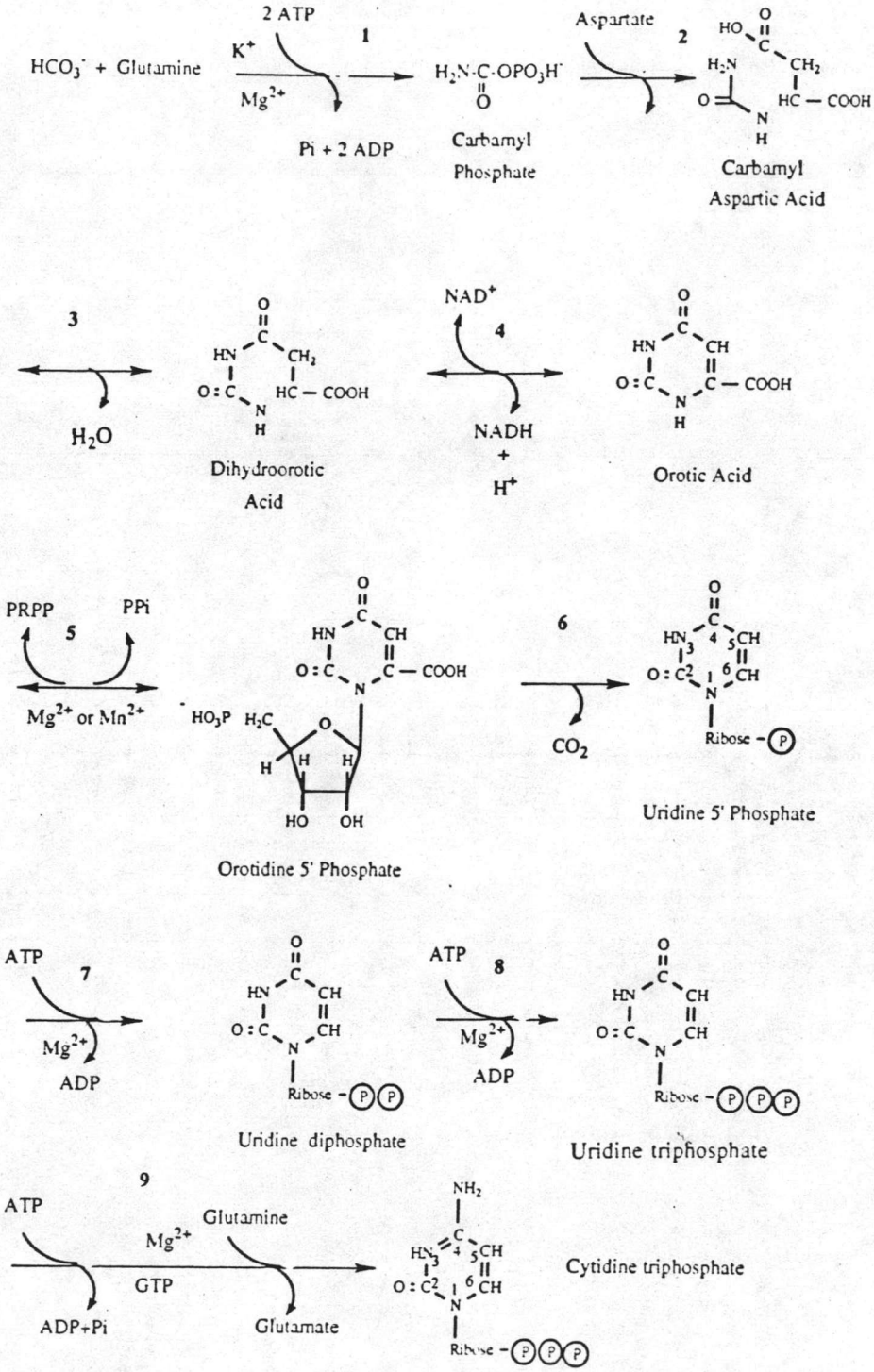


Figure 4. The *de novo* pathway of UTP and CTP formation

glutamine, with ATP as an energy sources (reactions 1-4 Figure 4). The phosphoribosyl moiety of 5 phosphoribosyl 1-pyrophosphate (PRPP) is added to orotic acid, yielding orotidine 5' phosphate (OMP), (reaction 5). Subsequently, the carboxyl group of OMP is removed, forming UMP and UMP is succesively phosphorylated to UDP and UTP (reactions 6-8). The amino group form another glutamine is added to UTP to form CTP.

3.2 Formation of thymidine nucleotides *de novo*

Formation of thymidine nucleotides are found in two mechanism. The first mechanism deoxyuridine nucleotides are substrate. The second mechanism deoxycytidine nucleotides are substrate

3.2.1 From deoxyuridine nucleotides

In animals deoxy-UDP is converted reversible to deoxy-UMP by a kinase in the present of ADP. In *E. coli*, deoxy-UDP is converted to deoxy-UTP by ATP-dependent kinase, and PPi is removed by a specific pyrophosphatase, forming deoxy-UMP. Deoxy UMP is transported to cell. Subsequent methylation of deoxy-UMP to yield TMP is catalized by thymidylate synthetase (TMP synthetase). The source of the methyl group transferred to the 5-position of the pyrimidine ring in deoxy UMP is N^5,N^{10}-methylenetetrahydrofolic acid (N^5, N^{10}-methylene THFA).

3.2.2 From deoxycytidine nucleotides

In animals but not in several bacterial species, thymidine nucleotides also arise from deoxycytidine nucleotides. The key enzyme is a deaminase that removes ammonia from deoxy-CMP, forming deoxy UMP. The latter is then converted TMP by TMP synthetase.

4. Biosynthesis of purine nucleotides

4.1 Formation of IMP

The first step in purine nucleotides biosynthesis is involves addition of the amide group of glutamine to the phosphoribosyl moiety of PRPP, displacing pyrophosphate and glutamate. The subsequent steps are formation of inosine 5'-monophosphate (IMP), a central intermediate in production of both adenine and guanine nucleotides.

4.2 Conversion of IMP to AMP and GMP

When C-2 or C-6 of the IMP is amminated, GMP or AMP is produced. GMP and AMP are further phosphorylated to GDP, ADP and GTP, ATP.

5. Formation of deoxyribonucleotides

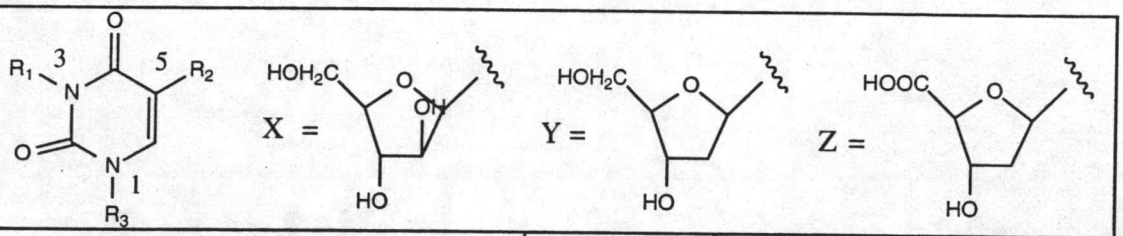
The ribonucleotides are reduced to deoxyribonucleotides by two distinct reduction mechanism exist in nature. In most *E. coli* strains and in animals, nucleoside diphosphates (UDP, CDP, ADP) are substrate, and NADPH is an indirect reductant. Thioredoxin-S-S is reduced by NADPH to thioredoxin-(SH2). Thioredoxin-(SH2) provides a hydride in that displace the 2'-hydroxyl group from ribose moiety of the nucleoside diphosphate. A second mechanism occures in several bacteria species, euglenoid flagellate and some fresh water blue green algae. Nucleoside triphosphate are reduced to deoxynucleoside triphosphate, and a coenzyme deoxyadenosyl cobalamin donate the hydride ion that replaces the 2'-hydroxyl group of deoxyribose. NADPH and thioredoxin are still necessary, and electron transport occures form NADPH to thioredoxin to 5'-deoxyadenosylcobalamin to the nucleoside triphosphate.

Distribution of Pyrimidine and Purine nucleosides

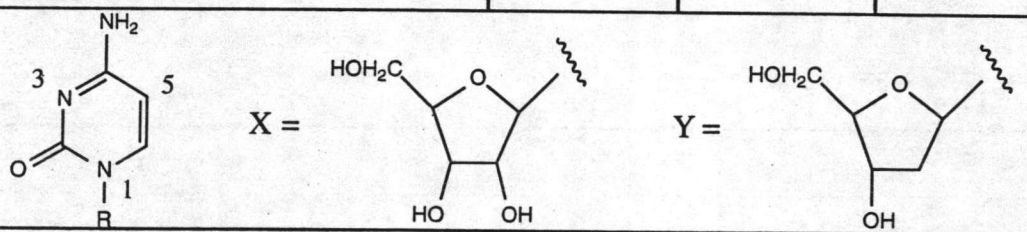
Table 2. Sources of pyrimidine nucleosides and derivatives

Compound	Sources	References
[60] Spongothymidine	*Cryptotethia crypta*	Bergman and Burke (1955)
[61] Spongouridine	*Cryptotethia crypta*	Bergman and Burke (1955)
[62] Thymidine	*Tridid*emnum *cereum*	Dematte *et al.*, (1985)
[63] 2-Deoxyuridine	*Tridid*emnum *cereum*	Dematte *et al.*, (1985)
[64] Uracil	*Tridid*emnum *cereum*	Dematte *et al.*, (1985)
[65] Thymidine-5'-carboxylic acid	*Aplidium fuscum*	Dematte *et al.*, (1986)
[66] 2'-Deoxyuridine-5'-carboxylic acid	*Aplidum fuscum*	Dematte *et al.*, (1986)
[67] Cytidine	*Cryptotethia crypta*	Cohen (1963)
[68] 2-Deoxycytidine	*Tridid*emnum *cereum*	Dematte *et al.*, (1985)

Table 3. Chemical structures of pyrimidine nucleosides and derivatives



Compounds	R_1	R_2	R_3
[60] Spongothymidine	H	CH_3	X
[61] Spongouridine	H	H	X
[62] Thymidine	H	CH_3	Y
[63] 2'-Deoxyuridine	H	CH_3	Y
[64] Uracil	H	H	H
[65] Thymidine-5'-carboxylic-acid	H	CH_3	Z
[66] 2'-Deoxyuridine-5'-carboxylic-acid	H	H	Z

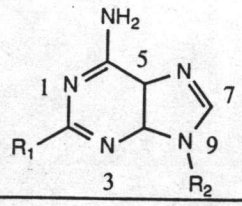


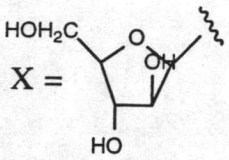
Compounds	R
[67] Cytidine	X
[68] 2'-Deoxycytidine	Y

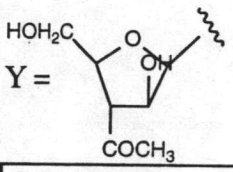
Table 4. Sources of purine nucleosides and derivatives.

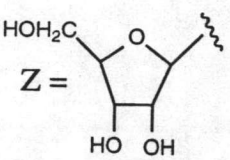
Compound	Sources	References
[69] Spongosine	*Cryptotethia crypta*	Bergmann and Burke (1956)
[70] 9-β-D-Arabinofurano-syl adenine	*Eunicella cavolini*	Cimino, Rosa, and Stefano (1984)
[71] 3'-Acetate-9-β–D-ara-binofuranosyl adenine	*Eunicella cavolini*	
[72] Adenosine	*Cryptotethia crypta*	Cohen (1963)
[73] 3-Methyladenine	*Tapsentia genitris*	Stoller, Braekman, and Daloze (1988)
[74] 4-Amino-5-bromo-pyrrolo [2,3-d] pyridine	*Echinodictyum* sp.	Kazlaukas *et al.*, (1983)
[75] Tubercidin	*Streptomyces* .sp.	Ramasamy, Robins, and Revankar (1986)
[76] 5-Iodo-5-deoxy-tubercidin	*Hypnea valendiae*	Kazlaukas *et al.*, (1983)
[77] Toyocamycin	*Streptomyces* sp.	Ramasamy *et al.*, (1986)
[78] Sangivamycin	*Streptomyces* sp.	Ramasamy *et al.*, (1986)
[79] Inosine	*Tapes japonica*	Baker and Murphy (1981)
[80] 2-Deoxyinosine	*Tridídemnum cereum*	Dematte *et al.*, (1985)
[81] Hypoxanthine	*Tridídemnum cereum.*	Dematte *et al.*, (1985)
[82] 1-Methylisoguanosine	*Tedania digitata*	Quinn *et al.*, (1980)
[83] 1,3,7-Trimethylguanine	*Latrunculia brevis*	Perry, Blunt, and Munro (1987)

Table 5. Chemical structures of purine nucleosides and derivatives.

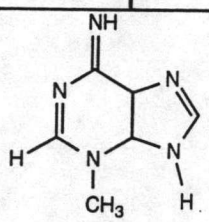




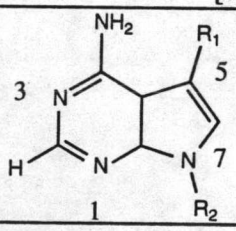


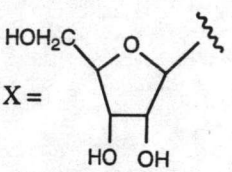


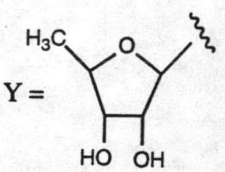
Compound	R_1	R_2
[69] Spongosine	OCH_3	Z
[70] 9-β-D-Arabinofuranosyl adenine	H	X
[71] 3'-Acetate-9-β–D-arabinofuranosyl adenine	H	Y
[72] Adenosine	H	Z



[73] 3-Methyladenine

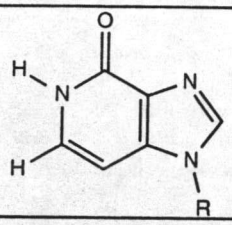


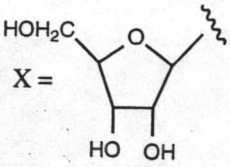


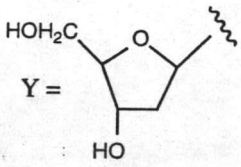


Compounds	R_1	R_2
[74] 4-Amino-5- bromo-pyrrolo [2,3-d] pyridine	Br	H
[75] Tubercidin	H	X
[76] 5-Iodo-5'-deoxy tubercidin	I	Y
[77] Toyocamycin	CN	X
[78] Sangivamycin	$CONH_2$	X

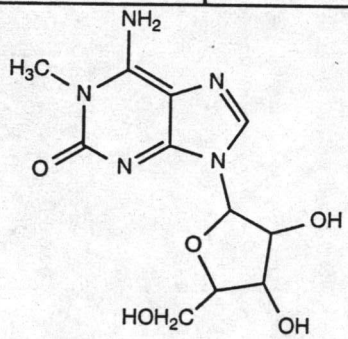
Table 5. continue



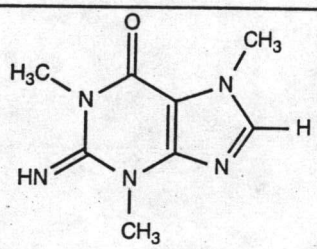
X = 

Y = 

Compounds	R
[79] Inosine	X
[80] 2'-Deoxyinosine	Y
[81] Hypoxanthine	H



[82] 1-Methyl isoguanosine



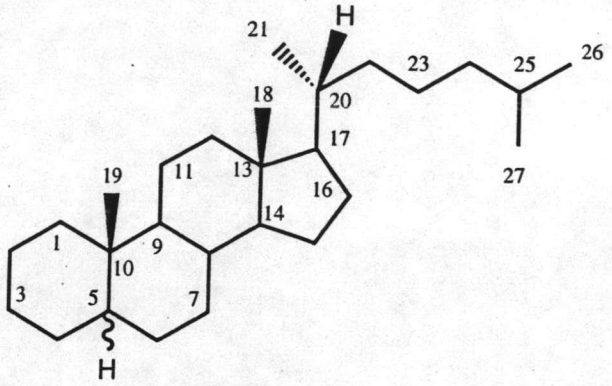
[83] 1,3,7 -Trimethylguanine

Chemistry of Steroids

Steroids are widely distributed in the animal and plant kingdom. The basic skeletons consist of 17 carbon atoms arranged in the form of a perhydrocyclo pentenophenanthrene. Steroids may be sterols; such as cholesterol, stigmasterol, and gorgostanol or steroidal ketones; such as ecdysone, testosterone, progesterone. Steroidal ketones are not found only in terrestrial animals but also found in marine organisms. They include wide variation in structure, and encompass compounds of vital importance to life, such as cholesterol, bile acids, vitamin D, sex hormones, corticoid hormones, cardiac aglycones, antibiotic, and insect moulting hormones.

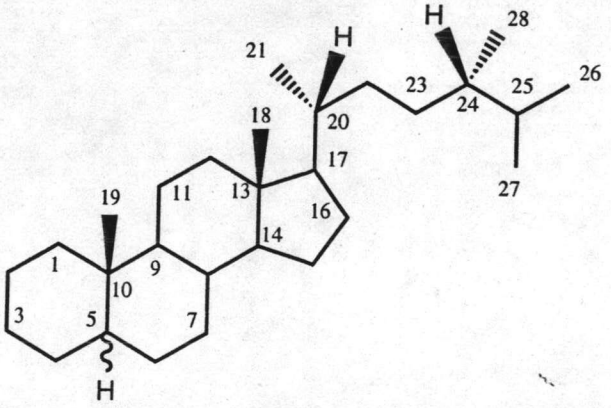
The numbers of skeletal carbon atoms used to classify the different type of steroids such as;

a) Cholestanes (C-27); the cholestane skeleton [84], which derives its name from cholesterol, can be regarded as the parent from which almost all other sterols are derived (Hill *et al.*, 1991).

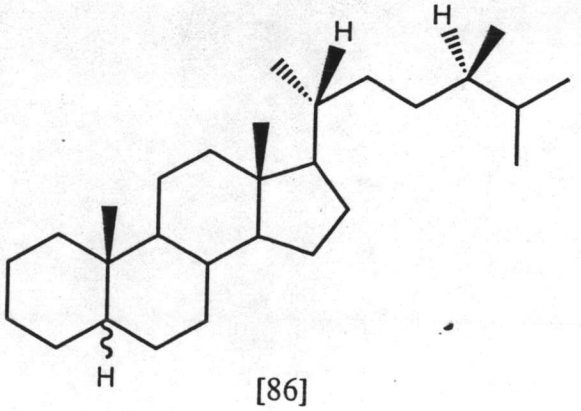


[84]

b) Ergostanes and campestanes (C-28); the 24-methyl-cholestane sructure is termed either ergostane or campestane, depending upon the configuration at C-24. Ergostanes [85] are 24 Hβ configuration (24-*S*) whereas campestanes [86] are 24 Hα configuration (24-*R*).

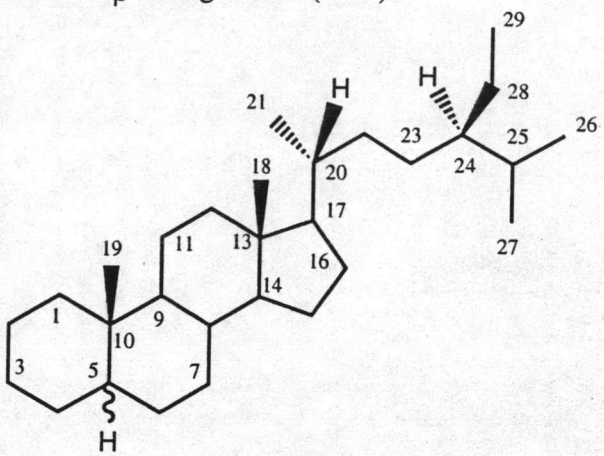


[85]

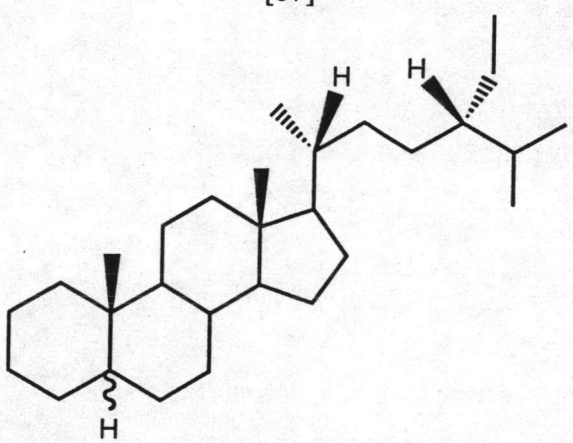


[86]

c) Stigmastanes and poriferastanes (C-29); these are the 24-ethylcholestanes, epimeric at C-24. Stigmastanes [87] have the 24 H_α configuration (24-*R*) and poriferastanes [88] are 24 H_β configuration (24-*S*).

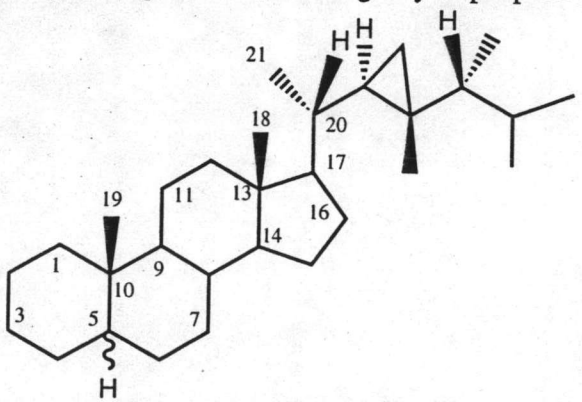


[87]



[88]

d) Gorgostanes (C-30); gorgostanes [89] are the parent hydrocarbon of widely occurring of group of sterols in marine organism. Their skeleton comprise ergostanes with an addition of methyl group at C-23, forming a cyclopropane ring.



[89]

Biosynthesis of steroids (Luckner, 1990)

Steroids originate from squalene. Squalene is firstly converted by squalene epoxidase, a mixed function oxygenase requiring O_2 and NADPH, in to squalene 2,3 oxide in which the configuration at C-3 is *S*. The next step is the cyclization of (3*S*)-squalene-2,3-oxide. In photosynthetic organisms, the cyclization product is cycloartenol while in non-photosynthetic organism it is lanosterol. The cyclization of squalene 2,3-oxide to cycloartenol is catalized by squalene 2,3-oxide:cycloartenol cyclase while cyclization to lanosterol is catalized by squlene 2,3-oxide:lanosterol cyclase. The cyclization is initiated by an electrophilic attack by H^+ on the epoxy oxygen while the squalene 2, 3-oxide is held in the chair-boat-chair-boat-unfold conformation.

Conversion of cycloartenol

Cycloartenol is primary cyclization product of plant sterol. The reaction in the formation of plant sterol that are unique are the opening of the cyclopropane ring of the sterol nucleus, alkylation of the side chain at C-24 and desaturation at C-22.

Conversion of lanosterol

In animals, lanosterol is conversed to cholesterol by oxidative removal of methyl groups in positions 14α, 4α, and 4β.

Side chain formation

Side chain formation, alkylation at C-24 involves either a single or double methylation with S-adenosylmethionine as the methyl donor. The orientation at C-24 may be a 24α- methyl or ethyl or a 24β-methyl or ethyl. Thus, the chirality at C-24 may be *R* or *S* configuration. For saturated side chain, a α methyl or α ethyl substituted on C-24 produce 24*S* configuration. Thus a β methyl or β ethyl produce 24 *R* configuration. For unsaturated side chain, a α methyl or α ethyl substituted on C-24 produce 24 *R* configuration. A β methyl or β ethyl produce 24 *S* configuration.

Distribution of Steroidal Ketones

Table 6. Sources of steroidal ketones

Compounds	Sources	References
[90] Cholest-4-en-3-one	*Stellata clarella*	Sheikh and Djerassi (1974)
	Muricea atlantica	Flores and Rosas (1966)
	Gracilaria textorii	Kanazawa and Yoshio (1972)
	Pyrocystis lunula	Kokke *et al.*, (1982)
[91] 24-Norcholesta-4, 22-dien-3-one	*Stellata clarella*	Sheikh and Djerassi (1974)
[92] Cholesta-4, 22-dien-3-one	*Stellata clarella*	
[93] Ergost-4, 22-dien-3-one	*Stellata clarella*	
[94] Ergost-4, 24-(28)-dien-3-one	*Stellata clarella*	
[95] (24 *E*)-Stigmast-4, 24(28)-dien-3-one	*Stellata clarella*	
[96] (24 *E*)-24-Ethylcholest-3, 5, 24(28)-trien-7-one	*Fucus evanescens*	Ikekawa *et al.*, (1972)
[97] 24 -Methylcholest-4-en-3-one	*Plexaura homomalla*	Popov *et al.*, (1976)
[98] Gorgost-4-en-3-one	*Plexaura homomalla*	
[99] Dinosterone	*Crypthecodinium cohnii*	Wither *et al.*, (1978)
[100] 23, 24*R*-Dimethylcholest-4,22-dien-3-one	*Pyrocystis lunula*	Kokke *et al.*, (1982)
[101] 24*S*-Methylcholest-4-en-3-one	*Pyrocystis lunula*	
[102] 3β-Hydroxyporiferast-5-en-7-one	*Gracilaria edulis*	Das and Srinivas (1992)
[103] Poriferast-3, 5-dien-7-one	*Gracilaria edulis*	
[104] Procesterol	*Calotropis procera*	Khan and Malik (1989)
[105] 6β-Hydroxy-stigmast-4-en-3-one	*Callicarpa formosana*	Chen, Lai, and Kuo (1990)
[106] Cholest-4-en-3, 6-dione	*Cinachyra terentina*	Aiello *et al.*, (1991)
[107] (24*R*)-24-Ethylcholest 4-en-3,6 dione	*Cinachyra terentina*	Aiello *et al.*, (1991)