# CHAPTER II

# HISTORICAL

# *BIEMNA FORTIS* (TOPSENT)

## TAXA AND DESCRIPTION

Sponges are complex, sedentary, filter - feeding metazoans that maintain an almost protozoan independence for each of their constituent cells. They are relatively unselective particle feeders and exist by pumping a very large volume of water through their tissue at low pressure. The body of sponge is organized around a system of pores, canals and chambers, which conduct the water current throughout the body (Apsimon, *et al.*, 1983).

The identification of spongs utilizes the characteristic of their skeletons, which are formed calcium carbonate spicules, siliceous spicules, and for spongin fibers. This characteristic leads to classification of sponges into 4 classes; Calcarea, Hexactinellida, Demospongeae and Sclerospongiae (Brusca and Brusca, 1990). Amonge these , the largest class is Demospongiae, which the genus *Biemna* belongs to (Wolfgang, 1986).

Phylum Porifera
Class Demospongiae
Subclass Ceractinomonpha
Order Poecilosclerida
Family Desmacellidae
Biemna fortis (Topsent)

The characteristic of *Biemna fortis* (Topsent) (Figure 1) is described as followed;

It is massive sponge with the growth form of burrowing base, erect digitate blind fistules (with epibiont cover) and burrowing in soft sediments. Its color is grey-brown or beige. It has ectosome skeleton, without special spicule, with protruding megascleres. The size is 1000 x 20-23 μm of style; 90-105 x 4-6 μm of L. Sigmas; 20 x 1 μm of S. Sigmas; 140 long of rephides. In choanosomal skeleton, they are choanosome with halechondroid reticulation, abundant dank collagen and abundant microscleres (Sollas, 1902; Hentschel, 1912; Desqueyroux - Faundez, 1981; Topsent, 1897).

**THE CHEMISTRY OF THE GENUS BIEMNA**

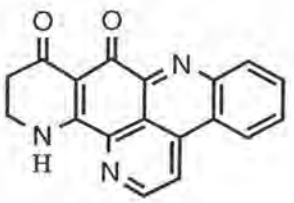
The sponge *Biemna* sp. was collected for studying its constituents in a few years ago. There are 2 main groups of chemical constituents isolated from the *Biemna* sp.

The first group of constituents found from the sponge *Biemna* sp. which was collected at Unten Harbor, Okinawa Island, Japan, is two new polycyclic aromatic alkaloids and two known alkaloids (Figure II.1) (Zeng *et al.*, 1993).

It is known that a series of polycyclic aromatic alkaloids possessing significant biological activities and unique structure features have been isolated from marine organisms in the past several years (Kobayashi et al., 1992). Biemnadin(**1**) and 8,9-dihydro -11-hydroxyascididemin (**2**) showed cytotoxicity against human epidermoid carcinoma KB cells($IC_{50}$ value : 1.73 and 0.209 mg/ml, respectively) and marine lymphoma L1210 cells ($IC_{50}$ values : 4.29 and 0.675 mg/ml, respectively). Whereas meridine (**3**) and 11-hydroxyascididemin (**4**) were previously obtained from extracts of tunicates. *Amphicarpa meridiaua*, *Leptoclinides* sp., and *Eudistoma* sp.

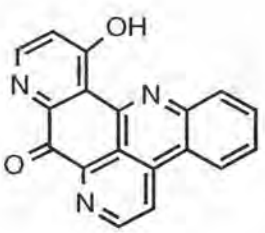

(1)

**Biemnadine**



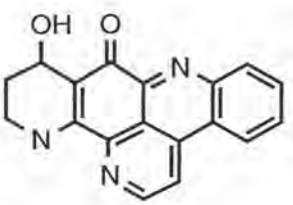

(2)

**8,9-dihydro-11-hydroxyascididemin**




(3)

**Meridine**




(4)

**11-hydroxyascididemin**

Figure II.1 Structure of alkaloids from *Biemna* sp.

The second group is steroid compounds. It is seemed to be major components group found in the *Biemna* sp. and steroid compounds are intensively studied for a long time. Its details would be discussed in the next section in this chapter.

**1. Introduction of Steroid**

The structure of steroid consists of cyclopentenoperhydrophenanthrene ring system, steroid nucleus which contains three fused cyclohexane ring (A, B, C) and a terminal cyclopenane ring (D), jointed with generally angular methyl groups at $C_{10}$ and $C_{13}$ and various carbons side chain at $C_{17}$. The conformation of steroid nucleus is that ring B and C are locked rigidly in the chair conformation by the *trans* fusions to rings A and D and the ring B and ring C union are a *trans* fusion indicated by X-ray crystallographic evidence.

Ring A is free to assume the boat form but the instability associated with the boat form of cyclohexane itself would be augmented by a strong interaction between methyl and hydroxyl groups at bow ($C_{10}$) and stern ($C_3$) position.

About the conformation, it is considered that the useful generalization which will be illustrated with numerous examples are as follows:

(i) A substituent is generally more stable in the equatorial than in the axial orientation.

(ii) An equatorial hydroxyl group is more easily acylated than an axial group at the same position. The rule applies as well to hydrolysis of the esters.

(iii) In chromatography on paper or on alumina, an equatorial alcohol is more strongly adsorbed than the axial epimer.

(iv) In an oxidation, since the point of attack is the C-H bond, a secondary alcohol is more vulnerable to attack if the hydroxyl group is axial rather than equatorial.

(v) Fission of a three-membered oxide ring gives a *trans*-diaxial product.

(vi) Eliminations occur more rapidly if the atoms or groups eliminated are *trans*-diaxial than if they are *trans*-diequatorial.

(vii) Conformational effects influence the course and rate of rearrangement reactions.

(viii) The equatorial or axial orientation of a substituent is often reflected in the infrared absorption spectrum.

A double bond (or an oxide ring) in one of the six - membered rings causes the ring to assume the half-chair conformation shown in perspective formula. The four carbon atoms associated with the olefinic system $C_6$-$C_1$-$C_2$-$C_3$ lie in a plane, and $C_4$ is above this plane and $C_5$ is below it. The bonds extending to hydrogen at $C_4$ and $C_5$ have the character of the normal axial or equatorial bonds of cyclohexane. But those at $C_3$ and $C_6$ are only approximately axial or equatorial and are described as quasi-axial(a') or quasi-equatorial(e').

## 2. Naturally Occurrung Steroids from Porifera

### 2.1 Steroids from Porifera

The isolation and structure elucidation during the period 1970-1972 have begun as an extremely productive period for discovery of new sterols. Whereas Scheuer's review of Marine sterol (Sheuer, 1973) listes forty different sterols, including well-known ones common to terrestial plants and animals, the present review covering the period 1972-1976 describes twice that many new ones. This explorasion in the discovery of new marine sterols resoundingly conferms the predictions made long ago (Bergmann, 1962) regarding the diversity of marine sterols. It also reveals more fully the member of candidates in the field from which cholesterol emerged as the dominant sterol in the evolution of animals.

Early work with marine animals resulted in the isolation of many sterols common to terrestrial plants plus a few unique to marine organisms. The carbon skeletons of the latter were the same as those already recognized in phytosterols, and their uniqueness frequently was derived from the position of unsaturation or extent of hydroxylation. These modifications, unusual in themselves, proved to be harbingers of many other unprecedented structural variations that emerged.

The work to date has clearly revealed that marine organisms contain a far more diverse array of sterols than terrestial plants and animals marine organisms produce sterols with a remarkable variety of side chains, unconventional nuclear structures, and assorted hydroxylation patterns (Sheuer, 1978; Braekman et al., 1978).

They are too various types of steroid of marine organism were found, in study, however only steroids of sponge were reviewed (Hill et al., 1986). Most publications have dealt with 3β-hydroxy-and 3β-(hydroxy-methyl)-A-nor-sterols containing unique or partially novel side chains (Bohlin et al., 1980; Li, and Djerassi, 1983). Presence of unusual sterols may be of phylogenetic significance and can also shed light on the complex structure-function role of the sterol in membranes. New sterols present in minor and trace amounts may offer important clues to biosynthetic or dietary pathways of the major compounds. Thus the discovery of nuclearly modified sterols, the 3β-(hydroxymethyl)-A-nor-sterans, has been of considerable value in studying the food chain, biosynthesis and chemotaxonomy of certain sponges. On the basis of direct incorporation experiments De Stefano and Sodano (De Stefano, and Sodano, 1980) suggested that cholest-4-en-3-one is an intermediate in the conversion of cholesterol to 3β-(hydroxymethyl)-A-nor-cholestane in the marine sponge *Axinella verrucosa*. Two sterols with a 5α-methoxy-$\Delta^{6,8(14)}$-nucleus **1** and **2** were isolated from *Axinella cannabina*, but they are presumed that these compounds may be artifacts, produced during the isolation procedure by reaction of methanol with the

corresponding 5α,8α-epidioxysterols (Itoh, Sica, and Djerassi, 1983). Several 5 α,8α-epidioxides (**3-12**) were isolated from a *Hyrtios* species (Koch et al., 1983), while *Thalysias junipertina* contained the same steroids (**3-12**) in addition to four related compounds (**13-16**) (Gunatilaka et al. 1981).

Furthermore., Koch *et al.* (Koch et al., 1983) described some sterols with functionalized side chains (**17-24**) from a *Hyrtios* species. Compound **20** as well as the C-24 aldehyde (**25**) and cholesta 5,25-diene-3β-24ξ-diol (**26**) were found in the extract of the Far Eastern sponge *Esperiopsis digitata* (Shubina et al., 1983). The biogenesis of the last named substances is not clear; they are possibly formed through oxidation, *in vivo*, of sterol precursors and as the result of oxidation during extraction and chromatography. Although there was no reference to biological activity of these sterols, there are reports of cytotoxic sulfated steroids from sponges. Halistanol sulfate (**27**), a trisodium sulfate of 24ξ,25-dimethylcholestane-2β,3α,6α-triol, has been isolated from the Okinawan species *Halichondria* cf. *moorei* as an antimicrobial constituent (Fusetani, Matsunaga, and Konosu, 1981). Makarieva et al.(1983) obtained a similar compound, sokotrasterol sulfate (**28**), differing only in the side chain, from *Halichondria* sp. They are presumed that biosynthesis of free sterols and trisulfated derivatives in these animals involves different precursors. Another group of sulfated sterols (**29-31**) having a wide variety of biological activities have been isolate form *Toxadocia zumi* (Nakatsu et al., 1983).

## 2.2 Steroids from *Biemna fortis* (Topsent)

Marine organisms such as sponges constitute a rich source of sterols possessing unusual is chains or unconventional ring system (Scheuer, 1973; Goad, 1978; Morris, 1977; Minale, 1977; Schmitz. 1978). Besides their chemical structure elucidation such as sterols raise interesting questions in term of biosynthesis and biological function (Djerassi, in press)

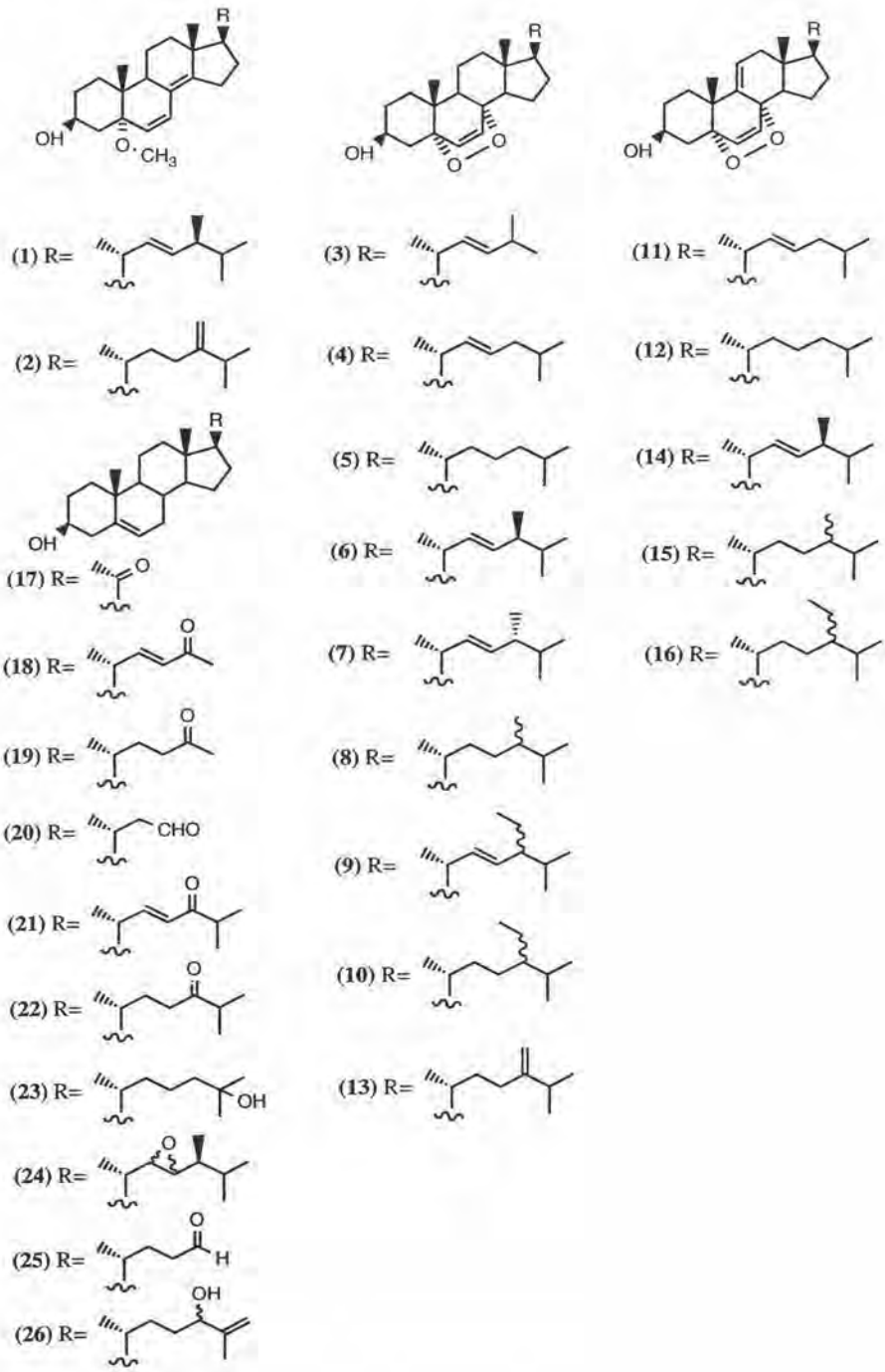



Figure II.2 Structure of the sterols from sponges

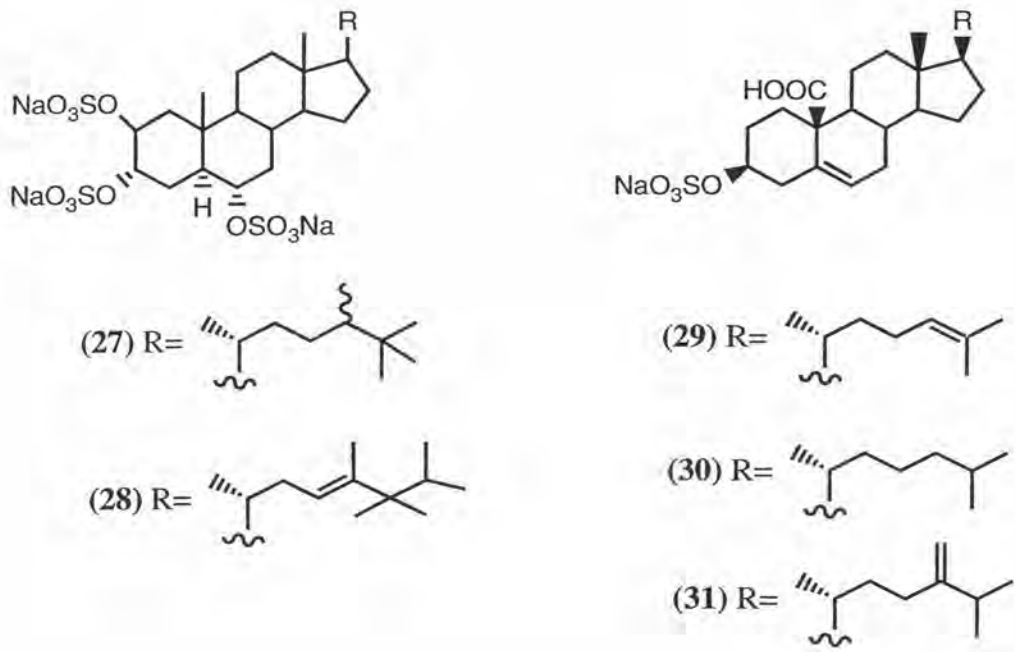



Figure II.3 Structure of the sterols from sponges (continued)

The steroidal components of *Biemna fortis* from the Red Sea; sponge was investigated and devided in 5 groups (Delsth, 1979; Kashman, 1982) as follow:

1). Mono - unsaturated sterols

1.1) Cholesterol (**1b**)

1.2) 24ξ-ethylcholest-5-en-3β-ol (**1g**)

1.3) (22*R*,23*R*,24*R*)-22,23-methylene-23,24-dimethylcholest-5-en-3β-ol (gorgosterol) (**1h**)

The two mono - unsaturated conventional sterols **1b** and **1g** were detected in small amount (less than 0.5% of the mixture) but are widely distributed among marine organisms (Goad, 1978).

The gorgosterol,**1h**, is more intriguing. The presence of this sterol is reported in soft corals (Goad, 1978; Minale, 1977) and in marine sediments (Wardroper, 1978) but it is first identification in sponge.

2). Di - unsaturated sterols

2.1) Cholesta-5,22-dien-3β-ol (**1a**)

2.2) (22*E*)-ergosta-5,22-dien-3β-ol (brassicasterol) (**1c**)

2.3) (22*E*,24*R*)-24-ethylcholesta-5,22-dien-3β-ol (Poriferasterol) (**1e**)

They are widely distributed in the marine environment (Goad, 1978) and amount to more than 25% of the mixture. The configuration assignments of **1c** and **1e** were base on $^{1}$H-NMR spectrum and compared with authentic samples (Rubinstein & Goad, 1974; Rubinstein, 1976; Nes & Krevitz, 1976).

3). Tri - unsaturated sterols

3.1) Cholesta-5,7,22-trien-3β-ol (**2a**)

3.2) (22*E*)-ergosta-5,7,22-trien-3β-ol (ergosterol) (**2c**)

3.3) (22*E*,24*R*)-24-ethylcholesta-5,7,22-trien-3β-ol (**2e**)

Ergosterol (**2c**) is one of the most widely distributed $\Delta^{5,7}$-sterols in marine organisms, notably the molluscs (Goad, 1978) and it is also being the major component (~37%) of *B. fortis*.

4). Tetra - unsaturated sterols

4.1) (22E)-ergosta-5,7,9(11),22-tetraen-3β-ol (**3c**)

4.2) 24ξ-ethylcholesta-5,7,9(11),22-tetraen-3β-ol (**3e** or **3f**)

They are displayed fragments characteristic of a sterols with a tri-unsaturated nucleus and an unsaturated side chain in high resolution mass spectrum. The C(24) stereochemistry assignment of compound **3c** employs NMR technique and compares with authentic sample (Windaus, 1932; Romo, 1951). Whereas compound **3e** or **3f** which was isolated by HPLC but decomposed prior to $^{1}$H-NMR analysis. Its C(24) stereochemistry could not be assigned. Between **3e** and **3f**, the former structure is more credible because all the other sterols with this type of side-chain (**1e**,**2e**) have a (24*R*) configuration in the mixture.

5). Penta - unsaturated

Two low molecular weight penta-unsaturated olefins with shorter GC retention time than cholesterol were also detected in the mixture. They are formed in yields of about 30% by action of a trace of hydrogen chloride on dehydroergosterol **3c** in chloroform solution at RT. (Nes, 1956; Nes & Steele, 1957). Solely based on their GC/MS, they could be such pentaenes : **4c** or **5c** or **6c** for the one with $M^{+}$ = 376 and for the other with $M^{+}$ = 390 which are essentially the same as those of **3c** and **3e** (or **3 f**) except that no fragment due to loss of water was observed. Further identification was not attempted because these components are probably artifacts arising from dehydration and (or) rearrangement of **3c** and **3e** during separation. This hypothesis is

supported by the fact that the amount of these derivatives in the sterol mixture was not only small but variable for three different collections of the sponge (total of 1.01, 0.05 and 0.2%) and increased slightly upon storage of the crystalline mixture during 2 months.

Recently, biemnasterol (7), the rare 22,25-diene side chain, and 24β-methylcholesta-5,7,22,25-tetraen-3β-ol (**8**) isolated from the marine sponge *Biemna* sp., collected at Okinawa Island (Zeng, Ishibashi, Kobayashi, 1993). Biemnasterol, 24 β-methylcholesta-7,22,25-trien-3β,5α,6β-triol, exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro, with $IC_{50}$ values of 3.0 and 1.3 μg/ml, respectively.

### 3. Biosynthesis of Steroid

Acetate - Mevalonate pathway is the biosynthesis pathway of steroid which is closely related to terpenoid. The sequence of reaction begins with the condensation of acetyl coenzyme A with acetoacetyl coenzyme A to form mevalonic acid. After that mevalonic acid is converted by way of the 5-phosphate into the 5-pyrophosphate by dehydration and decarboxylation give isopentenyl pyrophosphate (Harrison, 1990; Shoppee, 1964).

The connection in head-to-tail manner of two or more isoprene units gives geranyl pyrophosphate, farnesyl pyrophosphate geranyl geranyl pyrophosphate and the connection in tail-to-tail manner of two farnesyl pyrophosphate gives squalene which is important role in term of the intermediate of steroid biosynthesis. Finally, the different types of ring closures, degree of unsaturation, shift of double bonds, isomerism and wide variety of functional groups, give various types of steroids and triterpenoid compounds (Robinson, 1980; Nicholas, 1973).

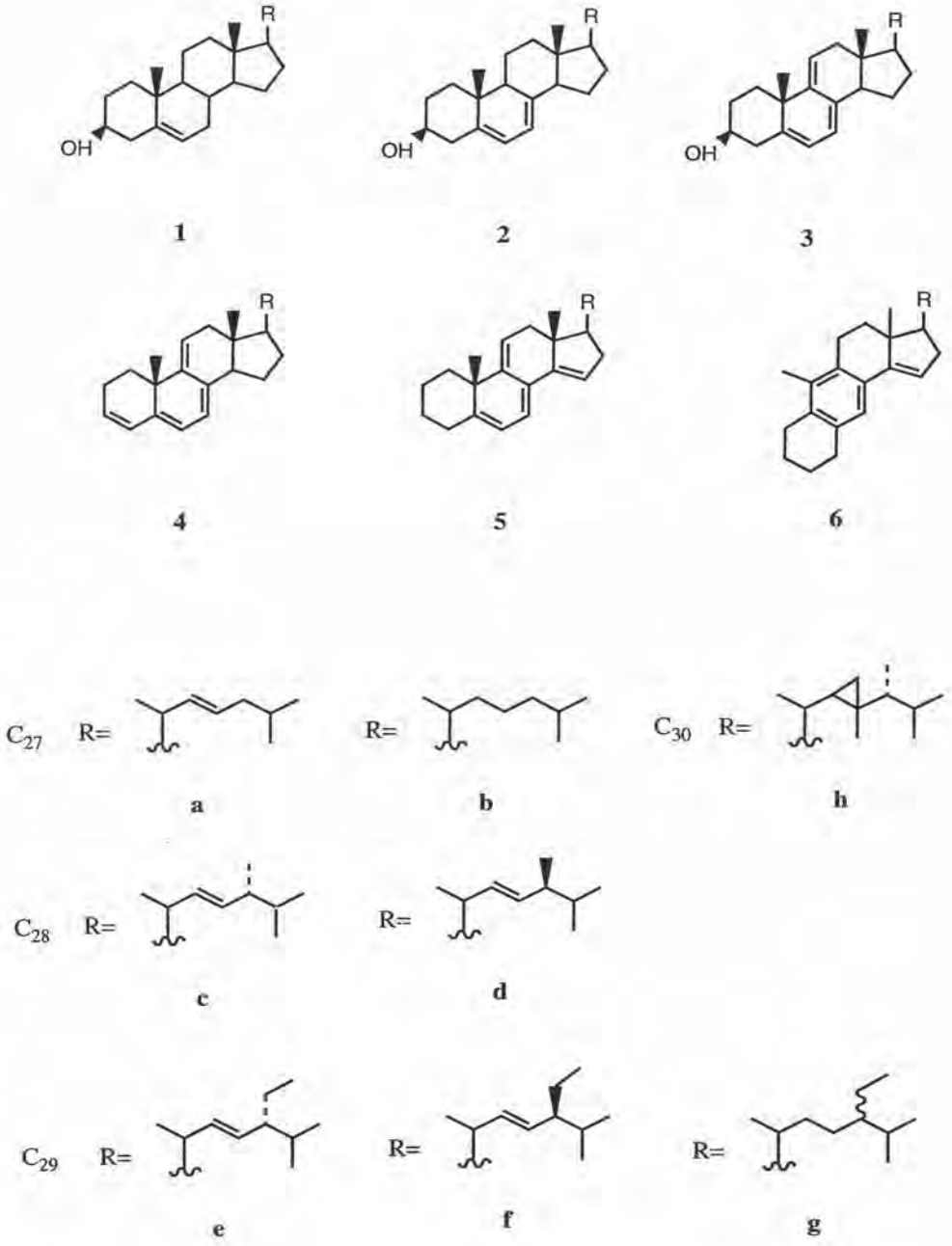
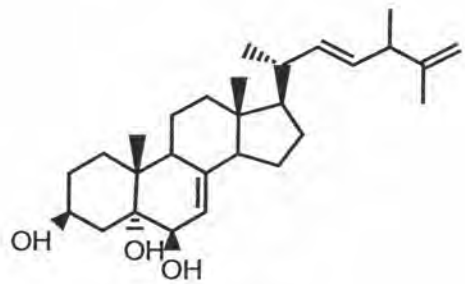
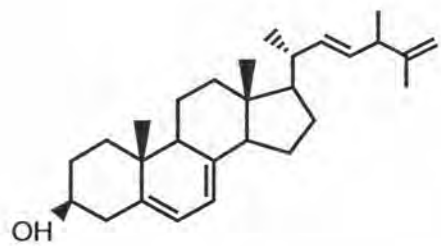



Figure II.4 Structure of the sterols of *Biemna fortis* (Topsent)




**7**

**Biemnasterol**




**8**

**24β-methylcholesta-5,7,22,25-tetraen-3β-ol**

Figure II.5 Structure of the sterols from *Biemna* sp.

A part from marine sterols which consists of various derivative or various types of substituents such as double bond, ketone group, polyhydroxy group, epoxide group, $C_{24}$-methyl substituent and heterocyclic rings in the side chain. The biosynthesis and metabolic transformations of marine sterols have been investigated by feeding radioactive precursors such as [1-$^{14}$C]acetate, [methyl-$^{14}$C]methionine, and [2-$^{14}$C] mevalonate in marine invertebrates (Stoilov, 1987; Goad, 1978; Voogt, 1976).