# CHAPTER II

# HISTORICAL

## THE GENUS *RENIERA*

### 1. Taxa and Description

The identification of sponge species has been the difficult works due to the variation of shape and color within species. Depending on its environment, a single sponge species may vary from an encrusting in an open area to an erect or branched one in sheltered area or deeper sea (Coleman, 1991). The shades of color of sponges are also able to vary although the specimens are in the same species and collected in the same location (Burton, 1926).

The identification of sponges utilizes the characteristic of their skeletons, which are formed calcium carbonate spicules, siliceous spicules, and/or spongin fibers. This characteristic leads to classification of sponges into 4 classes; Calcarea, Hexactinellida, Demospongiae, and Sclerospongiae (Brusca and Brusca, 1990). Among these, the largest and most difficult-to-identify is the class Demospongiae, which the genus *Reniera* belongs to (Rovirosa, De La Luz Vanquez, and San-Martin, 1990).

There were a lot of confusions and conflicts in classification and taxa of the genus *Reniera* (Pratt, 1923; Hyman, 1940; Rutzler, 1986; Brusca and Brusca, 1990; Rovirosa *et al.*, 1990). Here, the taxa of this genus is followed those given by Hyman (1940), Rutzler (1986), and Rovirosa *et al.* (1990) which provide the most complete information.

Phylum Porifera
Class Demospongiae
Subclass Monaxonida
Order Haplosclerina
Family Renieridae
Genus *Reniera*

Pratt (1923) described the characteristic of the sponges belonging to the genus *Reniera* as following;

*Reniera* Schmidt — form various, very fragile, easily pulverized, spongin very little developed, spicules straight needles joined at their tips, and arranged to form a net work numerous species.

The further details of the skeletons in the sponges of this genus were given by Burton (1926) as following.

...The spicules are oxea which may be straight or curved, stout or slender or bluntly pointed. Often they are replaced by styli, more rarely by strongyla, sometime isolated oxea, here and there, are centrotylote. The length of the spicules varies from about .080 to .150 mm, the thickness from .004 mm or less to .012 mm. Spicules from one part of the sponge may be of the larger size, while those from another part 2 cm away will be of the smaller size frequently in section it appears that the spicules fall into two distinct categories, large and small, but other sections from different parts of the same sponge show that is not true for the whole sponge...

Burton also reported that spongin of the sponge was only on the ends of the spicules and formed meshes, which were called isodictyal skeleton.

The sponges, *Reniera* sp., which were investigated in this work were found at the depth of 2-3 m in the vicinity of Si-Chang Island, Chonburi, Thailand. They are the hard, bright-blue sponges, and occur as the encrusting sheets with the thickness of 2-3 inches, approximately. The colonies cover on the rock or the coral reef and show a lot of oscula which look like the small vulcanoes. (Figure 1)

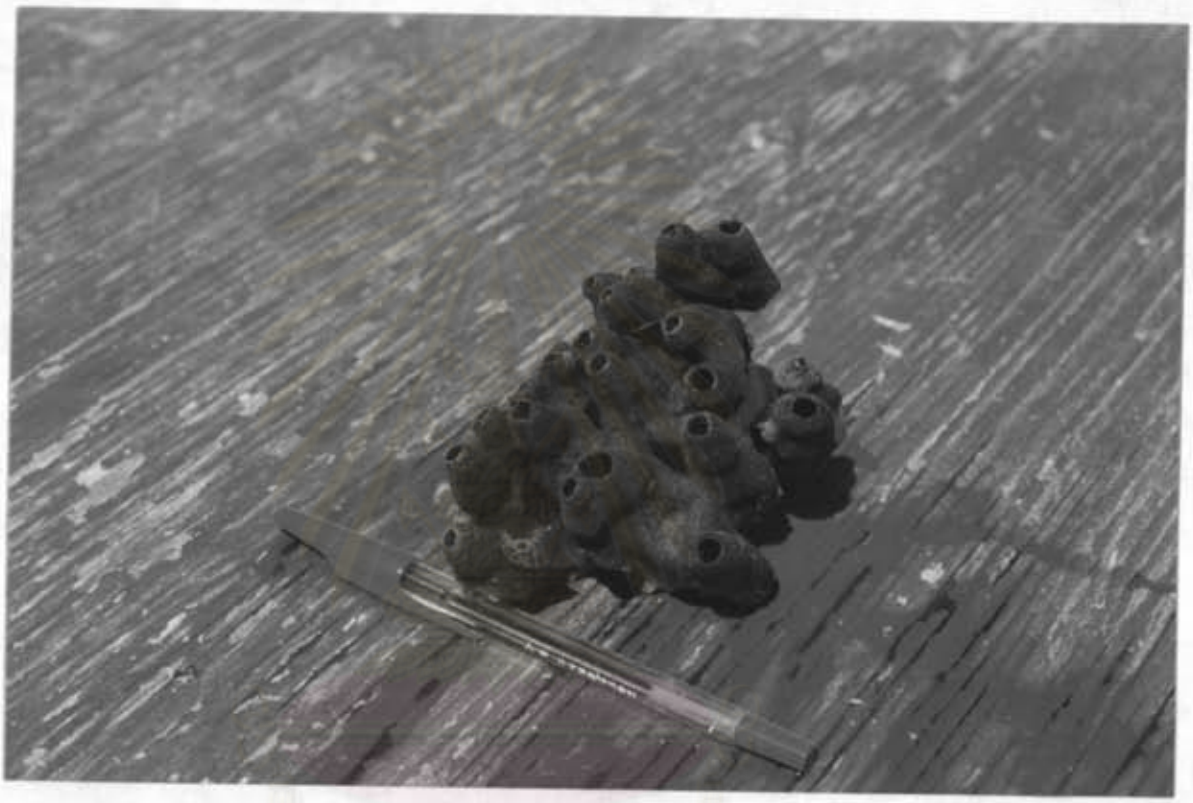
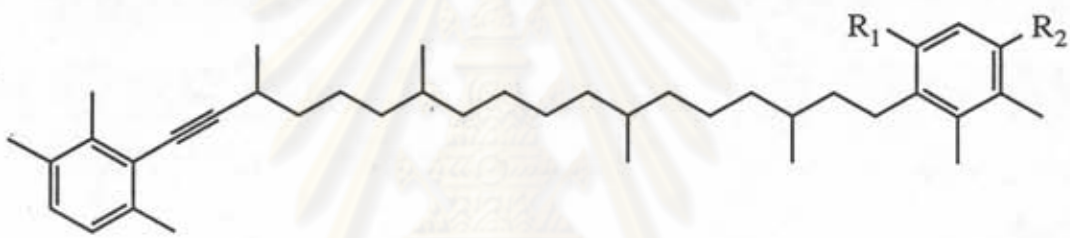


**Figure 1** The Thai sponge, *Reniera* sp.

## 2. The Chemistry of the Genus *Reniera*

Studies on the chemical constituents from the sponges of the genus *Reniera* began 20 years ago. There are 3 main groups of chemicals isolated from the *Reniera*, including acetylenic compounds, pentacyclic alkaloids, and isoquinoline quinones.

The first group of compounds found in the *Reniera* is the acetylenic compounds (Figure 2). Two acetylenic aromatic carotenoids [**1**,**2**] were isolated from *Reniera japonica* (Hamasaki, Okukado, and Yamaguchi, 1973). A few years later, 5 acetylenic compounds were reported. These compounds were renierin-1 [**3**], debromo-renierin-1 [**4**], 18-dihydrorenierin-1 [**5**], renierin-2 [**6**], and 18-hydroxyrenierin-2 [**7**]. All of these have long alkyl chain containing a double bond and 2 acetylenic bonds. In the case of compounds 3 and 5, the double bond is brominated (Cimino and De Stefano, 1977).




**1** ; $R_1$ = -H, $R_2$ = $-CH_3$
**2** ; $R_1$ = $-CH_3$ ; $R_2$ = -H


**3** ; $R_1$ = -Br, $R_2R_3$ = =O, $R_4$ = -H
**4** ; $R_1$ = -H, $R_2R_3$ = =O, $R_4$ = -H
**5** ; $R_1$ = -Br, $R_2$ = -H, $R_3$ = -OH, $R_4$ = -H
**6** ; $R_1$ = -H, $R_2$ = $R_3$ = -H, $R_4$ = $-CH_2OH$
**7** ; $R_1$ = -H, $R_2$ = -H, $R_3$ = -OH, $R_4$ = $-CH_2OH$

**Figure 2** Acetylenic compounds isolated from *Reniera* spp.

The pentacyclic alkaloids (Figure 3) are a group of compounds found in a Mediterranean sponge collected from the Bay of Naples, *Reniera sarai*. This group of alkaloids contains heterocyclic systems which link with macrocyclic alkyl groups. Sarain-1 and -2 [**8-9**] and isosarain-1 [**10**] have an unsaturated piperidine ring linked with a quinolizidine nucleus, and these two systems are joined together with 2 long alkyl chains to form 2 macrocyclic ring systems (Cimino, Spinella, and Trivellone, 1989). Sarain-A [**11**], another compound in this group, exhibited the central cage structure of heterocyclic rings in the middle of 2 cyclic alkyl chains (Cimino, Mattia, *et al.*, 1989; Cimino *et al.*, 1990).

The activities of sarains were studied, and they showed antiarrhythmic effect. There was no further study on their pharmacological activities because of their toxicity ($LD_{50}$ 200 mg/kg) (Cimino *et al.*, 1986).

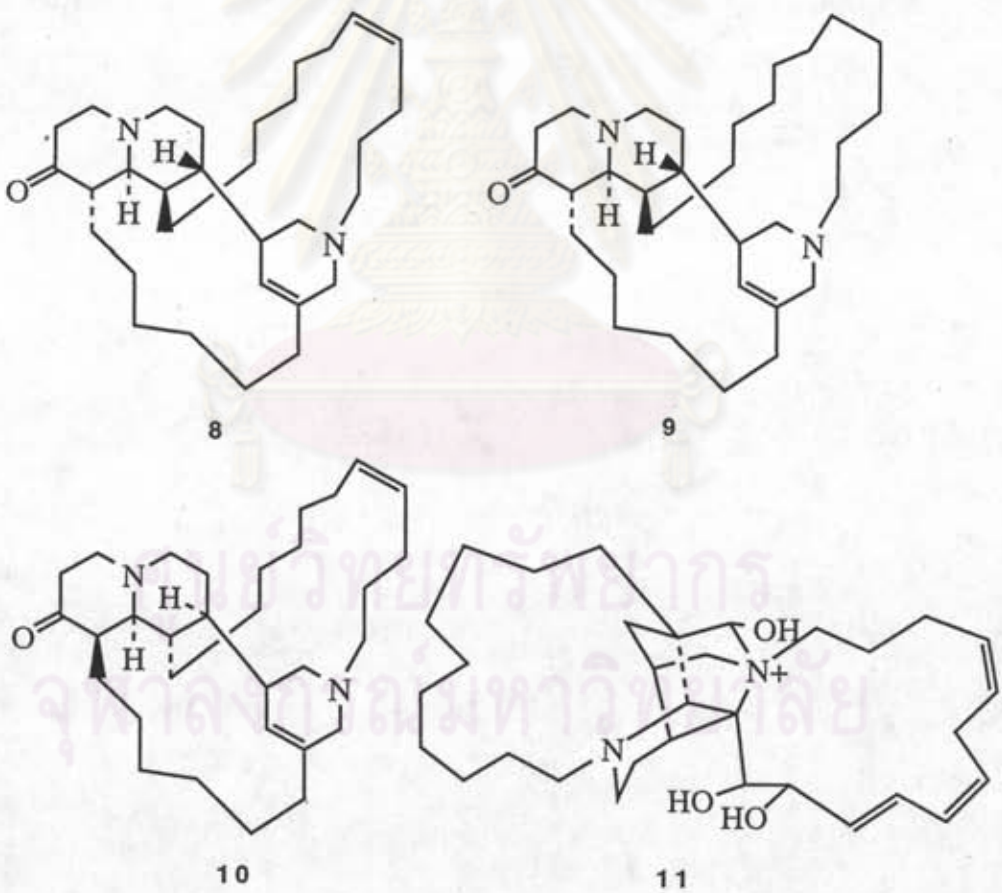



**Figure 3** Pentacyclic alkaloids from *Reniera* spp.

The last group, isoquinoline quinones, are the widely studied compounds. The details about their chemistry and bioactivities would be discussed in the next section in this chapter.

## THE ISOQUINOLINE QUINONES

### 1. Naturally Occuring Isoquinoline Quinones

Isoquinoline quinones is one group of compounds which was found in nature. The main biological sources of isoquinoline quinones are fungi in the genus *Streptomyces* and a marine sponge of the genus *Reniera*. Isoquinoline quinones were also found in other microorganisms such as *Pseudomonas*, and other marine species such as sponges of the *Xestospongia*, or a nudibranch of the *Jorunna*. These isoquinoline quinones have not been reported from plant sources (Arai and Kubo, 1983).

Naturally occuring isoquinoline quinones are classified into 3 types, as following;

1.1 naphthyridinomycin type
1.2 mimosamycin type
1.3 saframycin type

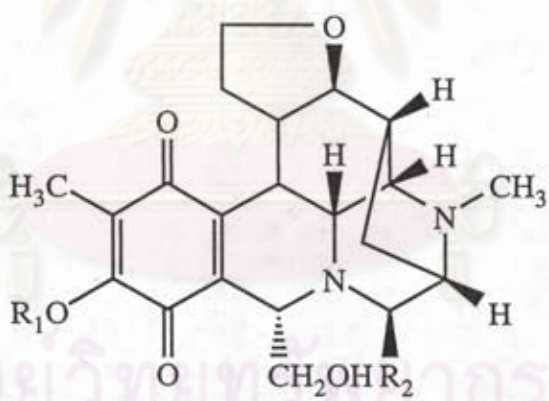
### 1.1 Naphthyridinomycin-type Isoquinoline Quinones

Naphthyridinomycin is the first antibiotic bearing isoquinoline quinone moiety which was isolated from *Streptomyces lusitanus* AYB-1026. The chemical structures of these antibiotics contain a quinone ring substituted with other 5 fused rings possessing 3 tertiary amines. Their sources and structures are shown in Table 1 and Figure 4, respectively.

**Table 1** Biological sources of naphthyridinomycin-type isoquinoline quinones

| compounds | sources | references |
|---|---|---|
| **12** naphthyridinomycin | *Streptomyces lavendulae* AYB-1026 | Sygusch *et al.*, 1974<br>Kluepfel *et al.*, 1975* |
| **13** SF-1739 HP | *S. griseoplanus* SF-1739 | Itoh *et al.*, 1982 |
| **14** naphthocyanidine | *S. griseoplanus* SF-1739 | Itoh *et al.*, 1982 |
| **15** cyanocycline A | *S. flavogriseus* no.40 | Hayashi *et al.*, 1982 |

note : * The structure was re-established from that of Sygusch *et al.* by Kluepfel *et al.*

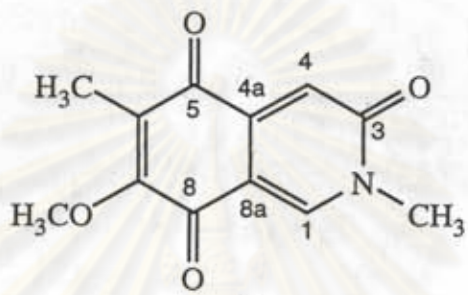


| | $R_1$ | $R_2$ |
|---|---|---|
| **12** naphthyridinomycin | $-CH_3$ | -OH |
| **13** SF-1739 HP | -H | -OH |
| **14** naphthocyanidine | -H | -CN |
| **15** Cyanocycline A | $-CH_3$ | -CN |

**Figure 4** Chemical structures of naphthyridinomycin-type isoquinoline quinones

### 1.2 Mimosamycin-type Isoquinoline Quinones

The compounds in this class are simple isoquinoline quinones. Their prototype is mimosamycin [**16**] (Figure 5) which was first isolated from the fungus *Streptomyces lavendulae* no.314 (Arai *et al.*, 1976). The main biological sources of these compounds are a fungus of the genus *Streptomyces* and 2 sponges of the genera *Reniera* and *Xestospongia*. Some compounds can be found in a nudibranch of the *Jorunna* which associates with the sponge containing these compounds.




**16**

**Figure 5** The chemical structure of mimosamycin [**16**]

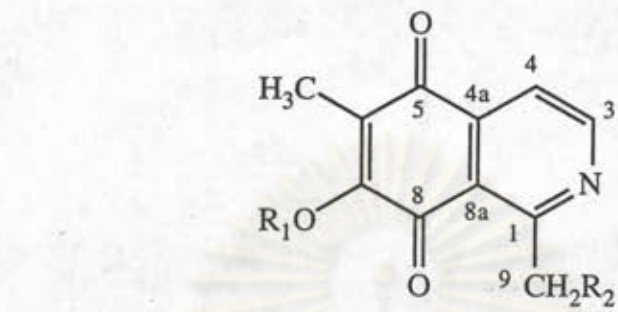
Substituted groups on the isoquinoline quinone nucleus are varied and depended on biological sources of each compounds. The pyruvamide, angelate ester, and other alkanoate ester groups are found as substituted groups at position 9 of isoquinoline quinones from the *Streptomyces*, the *Reniera*, and the *Xestospongia*, respectively.

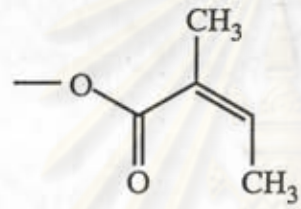
Lists of biological sources and chemical structures of compounds are shown in Tables 2 and 3, respectively.

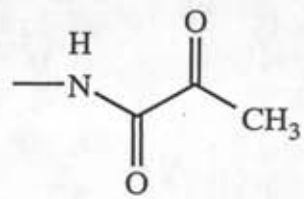
**Table 2** Biological sources of mimosamycin-type isoquinoline quinones

| compounds | sources | references |
|---|---|---|
| **16** mimosamycin | *Streptomyces lavendulae* no.314 | Arai *et al.*, 1976 |
| | *Reniera* sp. | Frincke and Faulkner, 1982 |
| | *Xestospongia caycedoi* | McKee and Ireland, 1987 |
| **17** renierone | *Reniera* sp. | McIntyre *et al.*, 1979 |
| **18** mimocin | *S. lavendulae* no.314 | Kubo *et al.*, 1980 |
| **19** *N*-formyl-1,2-dihydro-renierone | *Reniera* sp. | Frincke and Faulkner, 1982 |
| **20** *O*-demethylrenierone | *Reniera* sp. | Frincke and Faulkner, 1982 |
| **21** 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione | *Reniera* sp. | Frincke and Faulkner, 1982 |
| **22** renierol | *X. caycedoi* | McKee and Ireland, 1987 |
| **23** renierol acetate | *Xestospongia* sp. and *Jorunna funebris* | Kubo, Hitahara, and Nakahara, 1989 |
| **24** renierol propionate | *Xestospongia* sp. and *Jorunna funebris* | Kubo, Hitahara, and Nakahara, 1989 |
| **25** *N*-formyl-1,2-dihydro-renierol acetate | *Xestospongia* sp. and *Jorunna funebris* | Kubo, Hitahara, and Nakahara, 1989 |
| **26** *N*-formyl-1,2-dihydro-renierol propionate | *Xestospongia* sp. and *Jorunna funebris* | Kubo, Hitahara, and Nakahara, 1989 |

**Table 3** Chemical structures of mimosamycin-type isoquinoline quinones

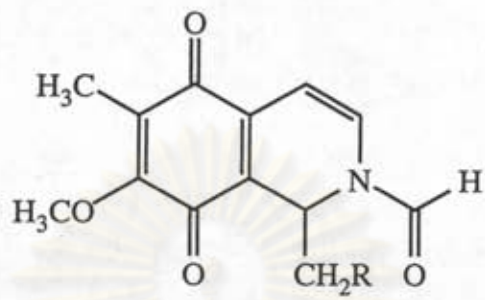



A = 


B = 


| compounds | $R_1$ | $R_2$ |
|---|---|---|
| **17** renierone | -$CH_3$ | A |
| **18** mimocin | -$CH_3$ | B |
| **20** *O*-demethylrenierone | -H | A |
| **21** 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione | -$CH_3$ | -H |
| **22** renierol | -$CH_3$ | -OH |
| **23** renierol acetate | -$CH_3$ | -$OCOCH_3$ |
| **24** renierol propionate | -$CH_3$ | -$OCOCH_2CH_3$ |

**Table 3 (cont.)**




| compounds | R |
|---|---|
| **19** *N*-formyl-1,2-dihydrorenierone | A |
| **25** *N*-formyl-1,2-dihydrorenierol acetate | -$OCOCH_3$ |
| **26** *N*-formyl-1,2-dihydrorenierol propionate | -$OCOCH_2CH_3$ |

### 1.3 Saframycin-type Isoquinoline Quinones

The skeleton of the compounds in this class is dimeric isoquinoline quinone (Figure 6). Two monomeric units of the compounds (ring A/B and ring D/E) are linked and established another fused ring called ring C. These isoquinoline quinones can be classified into 3 minor classes depended on their biological sources and substituted groups. These minor classes are saframycins, safracins, and renieramycins.

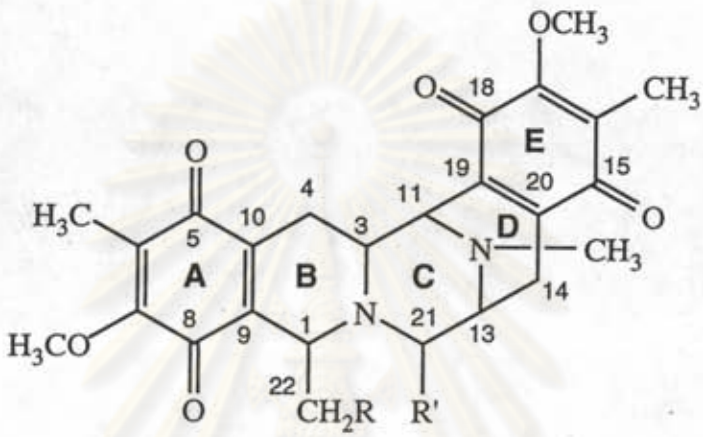
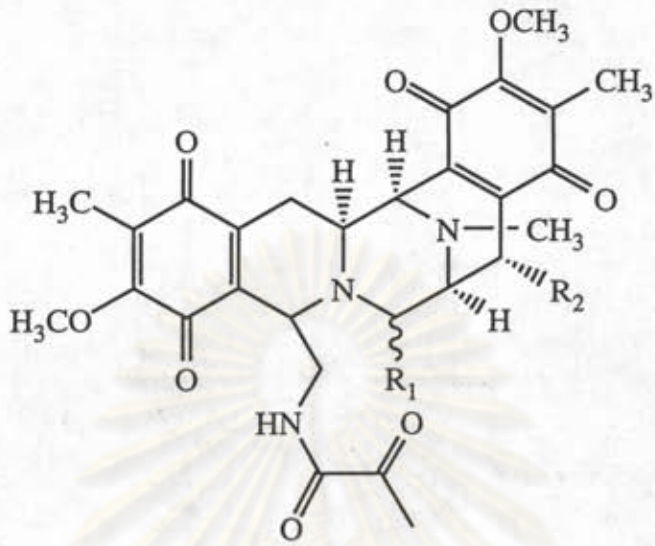



**Figure 6** The skeleton of saframycin-type isoquinoline quinones

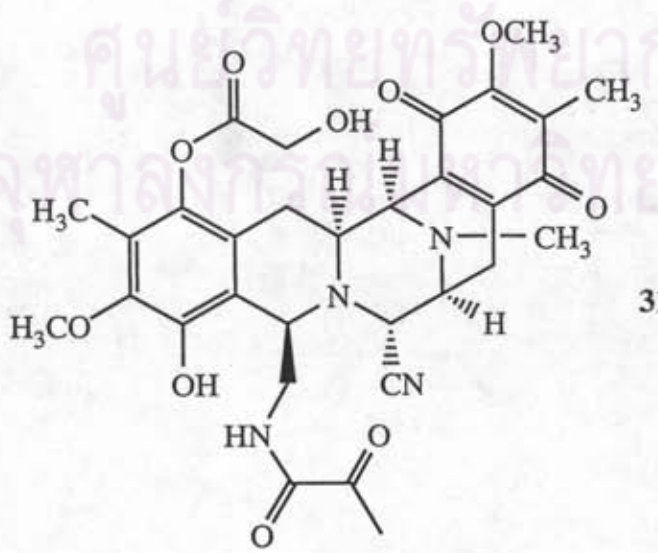
The saframycins [**27-33**] were isolated from *Streptomyces lavendulae* no.314, and contain pyruvamide substituted at position 22. The second group, safracins. [**34-35**], was isolated from *Pseudomonas fluorescens* A2-2, and contains an alanine amide substituted at position 22. The last group, renieramycins, was found in 2 marine sponges. Renieramycin A-F [**36-41**] were found in *Reniera* sp. collected from the vicinity of Isla Grande, Mexico, and renieramycin G [**42**] was found in *Xestospongia caycedoi*. Renieramycins from both sources contain angelate ester moiety substituted at position 22.

The chemical structures of saframycin-type isoquinoline quinones were shown in Table 4.

**Table 4** Chemical structures of saframycin-type isoquinoline quinones

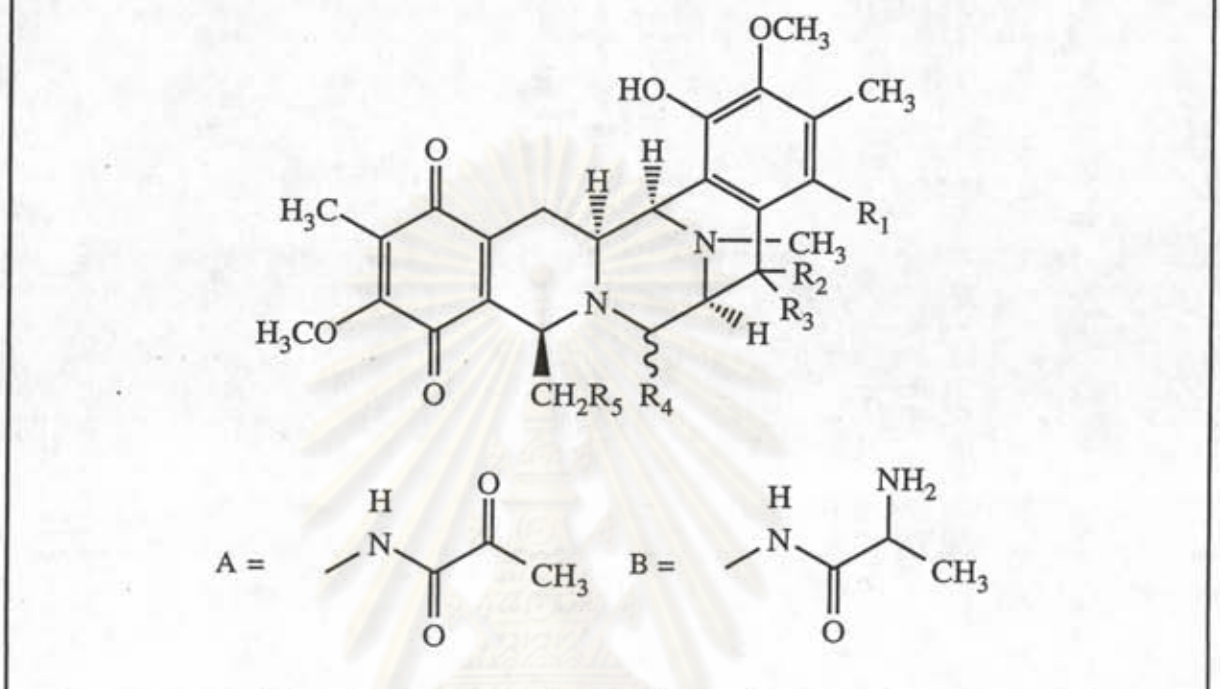


| compounds | $R_1$ | $R_2$ | references |
|---|---|---|---|
| **27** saframycin A | -CN | -H | Arai, Takahashi, Nakahara, and Kubo, 1980 |
| **28** saframycin B | -H | -H | Arai *et al.*, 1979 |
| **29** saframycin C | -H | -OH | Arai *et al.*, 1979 |
| **30** saframycin S | -OH | -H | Arai, Takahashi, Ishiguro, and Yazawa, 1980 |



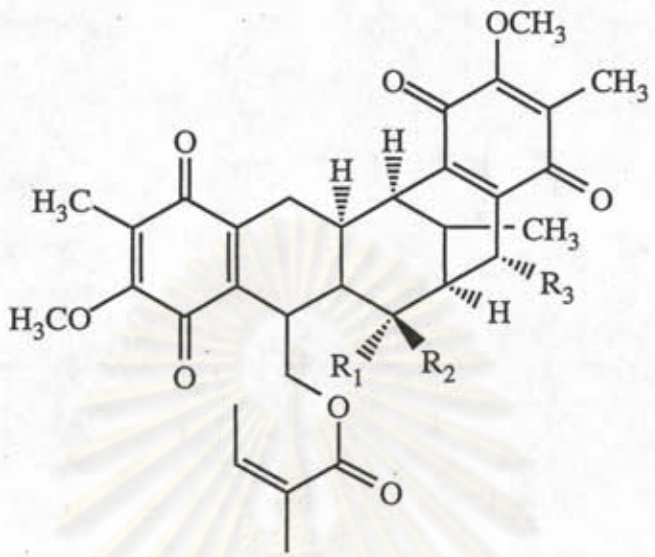
**31** saframycin R
(Lown, *et al.*,1983)

**Table 4 (cont.)**



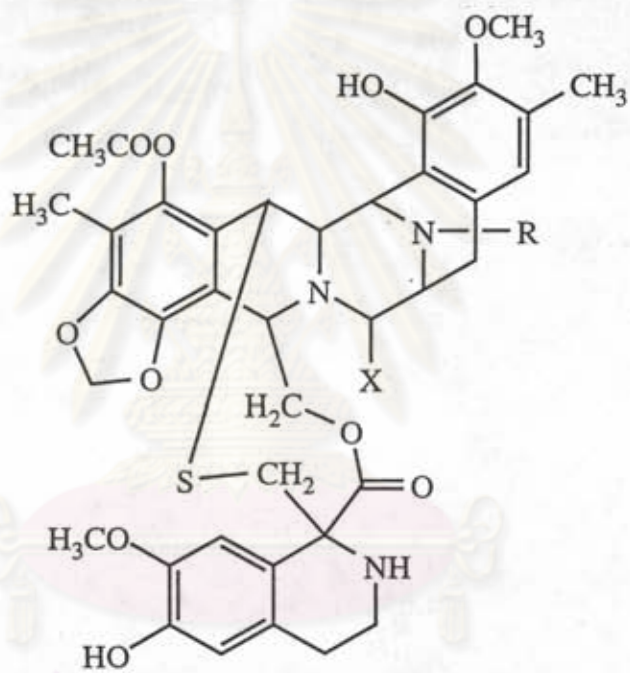

| compounds | R1 | R2 | R3 | R4 | R5 | references |
|---|---|---|---|---|---|---|
| **32** saframycin D | -OH | =O | | -H | A | Arai and Kubo, 1983 |
| **33** saframycin F | -OH | =O | | -CN | A | Arai and Kubo, 1983 |
| **34** safracin A | -H | -H | -H | -H | B | Arai and Kubo, 1983 |
| **35** safracin B | -H | -H | -H | -OH | B | Arai and Kubo, 1983 |

**Table 4 (cont.)**




| compounds | $R_1$ | $R_2$ | $R_3$ | references |
|---|---|---|---|---|
| **36** renieramycin A | -H | -H | -OH | Frincke and Faulkner, 1982 |
| **37** renieramycin B | -H | -H | -OEt | Frincke and Faulkner, 1982 |
| **38** renieramycin C | =O | | -OH | Frincke and Faulkner, 1982 |
| **39** renieramycin D | =O | | -OEt | Frincke and Faulkner, 1982 |
| **40** renieramycin E | -OH | -H | -H | He and Faulkner, 1989 |
| **41** renieramycin F | -OH | -H | -OMe | He and Faulkner, 1989 |
| **42** renieramycin G | =O | | -H | Davidson, 1992 |

There are some tetrahydroisoquinolines exhibiting the related structures to those of saframycin-type isoquinoline quinones isolated from other marine organisms. These compounds are ecteinascidins and were able to be isolated from a tunicate, *Ecteinascidia turbinata* (Wright *et al.*, 1990; Rinehart *et al.*, 1990 b; Sakai *et al.*, 1992). This group of compounds contains 3 units of the tetrahydroisoquinoline nucleus. Two of these tetrahydroisoquinoline rings are linked in the same pattern as those of the saframycins and provide the similar stereochemistry. Ecteinascidins exhibit a high antitumor activity against a number of tumor cell lines, both *in vitro* and *in vivo*. The structures of some ecteinascidins are shown below.




| | R | X |
|---|---|---|
| **43** ecteinascidin 729 | -H | -OH |
| **44** ecteinascidin 743 | -$CH_3$ | -OH |
| **45** ecteinascidin 745 | -$CH_3$ | -H |
| **46** ecteinascidin 770 | -$CH_3$ | -CN |

**Figure 7** Chemical structures of some ecteinascidins

## 2. Synthesis of Isoquinoline Quinones

There were a number of publications demonstrating synthetic procedures of isoquinoline quinones. These procedures included total synthesis and semisynthesis. Stereocontrolled total synthesis of the dimeric isoquinoline quinones, saframycins and renieramycins, which had been already assigned their configurations, were also reported.

List of isoquinoline quinones which had been totally synthesized was shown in Table 5.

To increase production, isoquinoline quinones bearing cyano group were also semisynthesized. The treatment of the culture filtrates of *Streptomyces lavendulae* no. 314, the saframycin-production strain, with 1 mM sodium cyanide at 27°C increased production of saframycin A [**27**]. It was proved that the precursor of saframycin A [**27**], and maybe other saframycins, was saframycin S [**30**] (Arai, Takahashi, Ishiguro, *et al.*, 1980). The interconvertion between saframycins A [**27**] and S [**30**] is shown in Figure 8 (Arai and Kubo, 1983).

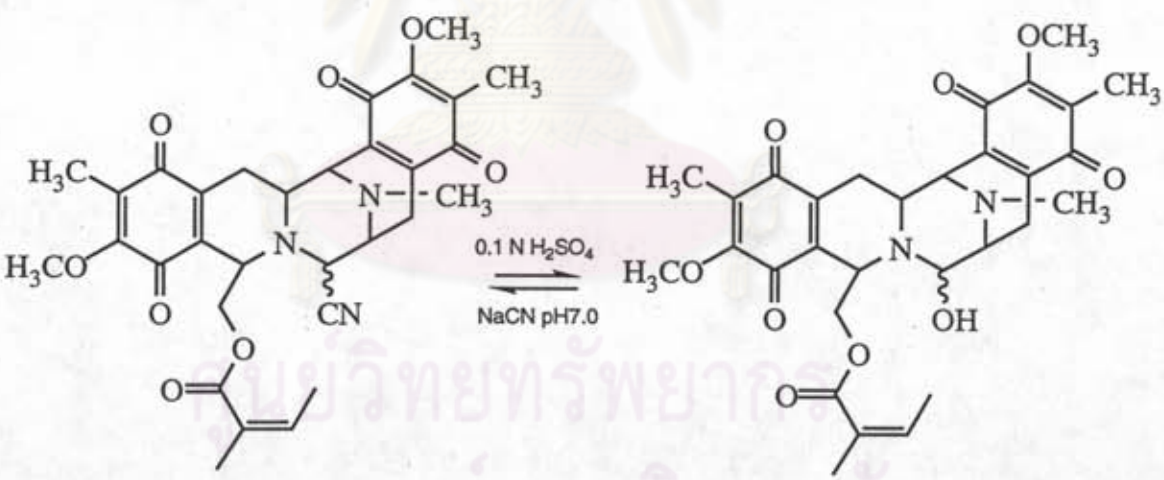



**Figure 8** Interconvertion between saframycins A [**27**] and S [**30**]

**Table 5** Isoquinoline quinones which were able to be synthesized

| compounds | references |
|---|---|
| **16** mimosamycin | Fukumi *et al.*, 1977; Fukumi, Kurihara, and Mishima, 1978; McKillop and Brown, 1987; Parker and Casteel, 1988 |
| **17** renierone | Danishefsky *et al.*,1980; Kubo and Nakahara, 1981; Kubo *et al.*, 1986 |
| **18** mimocin | Kubo *et al.*, 1980 |
| **19** *N*-formyl-1,2-dihydrorenierone | Kubo *et al.*, 1985, Kubo *et al.*, 1986 |
| **21** 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione | Kubo *et al.*, 1985; Kubo *et al.*, 1986 |
| **24** renierol propionate | Kubo *et al.*, 1989 |
| **25** *N*-formyl-1,2-dihydrorenierol acetate | Kubo *et al.*, 1989 |
| **26** *N*-formyl-1,2-dihydrorenierol propionate | Kubo *et al.*, 1989 |
| **27** saframycin A | Fukuyama *et al.*, 1990 |
| **28** saframycin B | Fukuyama and Sachleben, 1982; Kubo *et al.*, 1988 |
| **36** renieramycin A | Fukuyama, Linton, and Tun, 1990 |

Addition of a cyano group was also carried out in naphthyridinomycin [**12**]. In order to increase stability of naphthyridinomycin, it was converted to a more stable compounds, cyanonaphthyridinomycin, by using the same condition as that of saframycin A [**27**] (Zmijewski and Goebel, 1982). Cyanonaphthyridinomycin was later isolated from *Streptomyces flavogriseus* no.40 and named as cyanocycline A [**15**] (Hayashi *et al.*, 1982).

## 3. The Bioactivities

All of the isoquinoline quinones exhibit antimicrobial activity. They are highly to moderately active against gram positive bacteria and slightly active against gram negative bacteria, but less active against fungi and yeasts. There are also some reports described the activities of antiviral and cytotoxic in some isoquinoline quinones.

### 3.1 The Bioactivities of Naphthyridinomycin-type Isoquinoline Quinones

Kluepfel *et al.* (1975) reported the activity of naphthyridinomycin [**12**] as antibiotic against gram positive bacteria including *Staphylococcus aureus* penicillin resistant and sensitive strains and *Streptococcus faecalis*. The minimum inhibitory concentration (MIC) was less than $2.5 \times 10^{-5}$ mg/ml. Naphthyridinomycin [**12**] was also active against gram negative bacteria but the MIC was higher. It did not show activity against pathogenic yeasts and dermatophytes.

Other members of this class also showed antimicrobial activity in the same pattern as naphthyridinomycin [**12**]. The MIC against *S. aureus* of SF-1739 HP [**13**] and naphthocyanidine [**14**] was $2 \times 10^{-4}$ mg/ml and the MIC of cyanocycline A [**15**] was $5 \times 10^{-6}$ mg/ml (Itoh *et al.*, 1982; Hayashi *et al.*, 1982). They were slightly active against gram negative bacteria and inactive in fungi and yeasts.

Cytotoxicity of these compounds was also reported. In 1982, Zmijewski and Goebel semisynthesized cyanonaphthyridinomycin, which were later proved to be identical to cyanocycline A [**15**], from naphthyridinomycin [**12**]. These two compounds were compared their cytotoxic activity against Hela cells in cell culture at the concentration of $1 \times 10^{-3}$ mg/ml. Numbers of cell counts at 24 hours after treatment with naphthyridinomycin [**12**] and cyanonaphthyridinomycin [**15**] were 6.2 and $2.9 \times 10^{-5}$ cells, respectively. Itoh *et al.* (1982) reported the cytotoxicity of SF-1739 HP [**13**] and

naphthocyanidine [**14**] against mouse P388 leukemia at the dose of 8 mg/kg/day. Increases of life span of test animals for these two antibiotics were over 368.6 and 183.8 %, respectively.

It was reported that the intraperitoneal injection of an aqueous solution of naphthyridinomycin [**12**] (3.125 mg/kg) killed mice in 24 to 48 hours (Kluepfel *et al.*, 1975).

### 3.2 The Bioactivities of Mimosamycin-type Isoquinoline Quinones

Arai *et al.* (1976) found that mimosamycin [**16**] was mainly active against mycobacteria including streptomycin sensitive and resistant strains of human tubercle bacilli. This antibiotic was also reported to be active against gram positive bacteria and *Candida albicans* using disc method at the concentration of 0.05 mg/disc (Frincke and Faulkner, 1982). However, it showed no antitumor activity (Arai *et al.*, 1976).

Renierone [**17**] and its derivatives found in *Reniera* sp. and mimocin [**18**] were also reported their antimicrobial activity. The determination was carried out by disc method, using compounds to be tested at concentrations of 0.1, 0.05, and 0.01 mg/ disc. The test microorganisms were *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Vibrio anguilarium*, and B-392, a marine *Pseudomonas* (Kubo *et al.*, 1980, Frincke and Faulkner, 1982).

Renierol [**22**], an isoquinoline quinone of this class, showed anti-microbial activity against *S. aureus* at the concentration of 0.1 mg/disc. It was mildly cytotoxic against the L1210 cell lines ($IC_{50}$ 3 x $10^{-3}$ mg/ml), too (McKee and Ireland, 1987).

### 3.3 The Bioactivities of Saframycin-type Isoquinoline Quinones

Arai *et al.* (1977) reported the antibiotic activities of saframycins against a number of bacteria. Saframycins A - F [**27-29** and **32-33**] were active against gram positive bacteria, particularly *Corynebacterium*, slightly active against gram negative bacteria, and inactive in fungi. Among these antibiotics, saframycin A [**27**] is the most active one (MIC against *Corynebacterium diptheriae* 3 x $10^{-6}$ mg/ml).

Saframycin A [**27**] also exhibited cytotoxicity against a number of cell lines. It inhibited Ehrlich ascites carcinoma in mice at the dose of 1 mg/kg/day from days 1 to 4 or from days 1 to 6, and the percentages of 60-day survivors were 60 and 70 %, respectively (Arai and Kubo, 1983). The maximum increase of life span in mice bearing P388 leukemia and receiving saframycin A [**27**] intraperitoneally at the doses of 0.75 - 1.0 mg/kg for 10 consecutive days was 119 % (Arai, Takahashi, Nakahara, and Kubo, 1980). It was also effective on leukemia L1210 and B16 melanoma (Arai, Takahashi, Ishiguro, and Yazawa, 1980).

The acute toxicity of saframycin A [**27**] was determined with mice. Its $LD_{50}$ for *ddY* mice by single injection were 4.9 mg/kg intraperitoneally, and 3.3 mg/kg intravenously. No sign of toxicity of saframycin C [**29**] was observed in *ddY* mice up to 15 mg/kg by intraperitoneal administration (Arai and Kubo, 1983).

The antimicrobial activity of renieramycins A-D [**36-39**] was determined by disc method using the concentrations of 0.1, 0.05, and 0.01 mg/disc. These compounds were active against only gram positive bacteria (Frincke and Faulkner, 1982).

### 3.4 Sites and Mechanisms of Actions

A number of reports discussed in the mechanisms and sites of actions of isoquinoline quinones. Among these isoquinoline quinones, saframycin A [**27**] has been the most studied compounds.

It was indicated that saframycin A [**27**] was a potent inhibitor of nucleic acid synthesis (Ishiguro *et al.*, 1978). The quinone moiety had to be reduced to be hydroquinone. Then, the cyano residue, the active site of this compound, converted to be immonium ion or α-carbinolamine moiety. These two species was proposed to involve in the interaction with nucleic acids by the covalent binding to the base pairs (Ishiguro *et al.*, 1981; Lown, Joshua, and Lee, 1982).

The change in pH of the medium can cause the change in mode of actions. It was found that the binding between immonium ion of saframycins and nucleic acid was the mechanism of action of saframycins in the lower pH condition. In contrast, at pH 7.0, reduction of quinone moiety played an important role of action. The reduced forms of saframycins reacted with the dissolved oxygen, and generated the reactive

species, including hydrogen peroxide, superoxide anion, and hydroxyl free radical. The latter radical caused the strand scission of DNA template.

There was no publication about the sites and mechanisms of actions of other isoquinoline quinones. However, it can be deduced that their mechanisms would be like those of saframycins.

Danishefsky *et al.* (1980) suggested that the quinonoid ring of renierone [**17**] recognized the substructural unit of mitomycin B. So, the mechanisms of actions would be explained in the same way, by the bioreductive alkylation models of Moore (1977). In the model, the quinone moiety of isoquinoline quinone will act as an electron sink which can be reduced to be hydroquinone, an electron releasing unit. After reduction of quinone ring, the electron drives can cause the loss of the leaving group. The newly formed species will be a potent alkylating agent and react with the nucleophiles such as nucleic acid.