

CHAPTER II

HISTORICAL

Sponges, considered as a rich source for the discovery of natural compounds, are the most primitive of the multicellular animals. All of them are sessile and exhibit little detectable movement. This combination of characteristics convinced Aristotle, Pliny, and other ancient naturalists that sponges were plants. In fact, it was not until 1765, when internal water currents were first observed, that the animal nature of sponges became clearly established (Barnes, 1974). Except for about 150 freshwater species, the approximately 5,000 described species are marine sponges. They are abundant in all seas, wherever rocks, shells, submerged timbers, or coral provide a suitable substratum. Some species even live on soft sand or mud bottoms. Most sponges prefer relatively shallow water, but some groups live in deep water (Ruppert and Barnes, 1994). They feed on their food by filtering the microplanktons from sea water passing through the small holes on their body (Brusca and Brusca, 1990).

Because of their pore-bearing bodies, marine sponges contain many symbionts (bacteria, unicellular algae), epibionts (algae) and endobionts (crustaceans) which can produce secondary metabolites (Hirata and Uemura, 1986; Rutzler, 1986). In addition, the marine sponges, particularly those without spicules, frequently produce large quantities of compounds in order to deter potential predators and inhibit the growth of fouling organisms (Faulkner, 1984). These characters contribute to sponges being one of the most abundant sources of secondary metabolites.

Acanthella cavernosa Dendy

1. Taxa and description

The taxa and description of the genus *Acanthella*, according to those given by Bergquist (1978) are shown below.

Kingdom	Animalia
Subkingdom	Metazoa
Phylum	Porifera
Class	Demospongia
Subclass	Tetractinomorpha
Order	Axinellida
Family	Axinellidae
Genus	<i>Acanthella</i>

Sponges, which constitute the phylum Porifera of the animal kingdom, are classified by their microscopic mineral skeletal structures into 4 classes: Calcarea, Hexactimellida, Sclerospongiae and Demospongia (Bergquist and Wells, 1983; Brusca and Brusca, 1990). Characteristics of the sponges of the class Demospongia are as follows:

Mineral skeleton is siliceous, and megasclere and microsclere elements are present; a strong fibrous skeleton augments the mineral skeleton in many groups and, in some, displaces it entirely; a few genera have neither mineral nor fibrous skeleton.

Description of *Acanthella cavernosa*

Subspherical to flabellate growth form, digitate or globular, cavernous honeycomb - reticulate construction. Live colour variable from bright orange, pale orange or light brown; pale brown in ethanol. Texture rubbery, slightly compressible, tough. Oscules large, located between surface conules, surrounded by membrane flush with surface, membrane collapses upon preservation leaving porous - reticulate, cavernous construction. Surface with prominent conules, sharp, interconnected by ridges. Ectosomal skeleton membraneous, without specialized skeleton but with tips of styles from ascending extra-axial chonosomal skeleton protruding, singly, regularly spaced. Chonosomal skeleton clearly divided into axial and extra-axial regions. Axial skeleton with compressed fibres, lightly invested with spongin, nearly fully cored by strongyles. Extra-axial skeleton consists of individual styles embedded in and perpendicular to axial skeleton, ascending to surface. Mesophyl only slightly collagenous. Megascleres axial strongyles, large

and small, sinuous or simply curved (287-609 × 2-11.5 micrometres); extra-axial styles straight or slightly curved at centre, hastate points, evenly rounded bases (271-453 × 3-2 micrometres).

2. Terpenoids from marine sponges in the genus *Acanthella*

Studies on the chemical constituents of the sponges in the genus *Acanthella* began about 20 years ago. Similar to other sponges, abundant metabolites from this genus are terpenoids (Ireland *et al.*, 1988).

An earlier study on terpenoids from this genus yielded acanthellin-1 (1), an isocyanide sesquiterpene that was isolated from *Acanthella acuta* (Minale, Riccio and Sodano, 1974), together with the corresponding isothiocyanate (2) and formamide (3) and a second isocyanide-isothiocyanate-formamide trio [(4)-(5)-(6)]. A third isocyanide-isothiocyanate-formamide trio [(7)-(8)-(9)] and bicyclogermacrene (10) were later found in the same extract (Ciminiello *et al.*, 1987). The isocyanide (11) and isothiocyanate (12) were obtained in high yield from *Acanthella acuta*, which also contained a 2:3 mixture of axisonitrile-3 (13) and 1-isocyanoaromadendrane (14), the stereochemistry of which was based on its chemical interconversion with palustrol (15) (Braekman *et al.*, 1987). Additionally, Mayol, Piccialli and Sica (1987) reported three isonitrile-isothiocyanate pairs (16)-(21) found as minor metabolites of the same species. Investigation of the Great Barrier Reef sponge, *Acanthella klethra*, resulted in the isolation of two isomeric isothiocyanates [(22) and (23)] (Konig *et al.*, 1992). A sesquiterpene thiocyanate, cavernothiocyanate (24), and two isonitriles, cavernoisnitrile (25) and 10 α -isocyno-4-amorphene (26), were isolated from the marine sponge *Acanthella cf cavernosa* (Fusetani *et al.*, 1992). Acanthenes A (27) and B (28) are sesquiterpenes that were isolated from a British Columbia species of *Acanthella*, while a related sesquiterpene, acanthenes C (29), was obtained from skin extracts of the nudibranch *Cadlina luteomarginata*, which feeds on *Acanthella* (Burgoyne, Dumdei and Andersen, 1993).

Another major type of terpenoids found from the genus *Acanthella* is diterpenes. All of *Acanthella* diterpenes that have been reported so far contain nitrogen elements. The first group of constituents found from the marine sponge, *Acanthella* sp. is 5 diterpene isocyanides, kalihinol A (30) (Chang *et al.*, 1984), kalihinol B (31), kalihinol C (32), kalihinol E (33) and kalihinol F (34)

(Patra *et al.*, 1984). Three years later, five known metabolites (30)-(34) and six new compounds (35)-(40), were isolated from two specimens of *Acanthella* by Chang and his co-workers (1987). Kalihinols D (35), G (36) and H (37) were trace components of a species of *Acanthella* from Guam while kalihinols X (38), Y (39) and Z (40) were obtained from a Fijian species of *Acanthella*. All kalihinols in this group inhibited the growth of 3 test organisms: *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* (Chang *et al.*, 1984; 1987). One additional diterpene isonitrile, isokalihinol F (41), was obtained from a Fijian specimen of *Acanthella cavernosa* (Omar *et al.*, 1988). Two cytotoxic diterpene isonitriles, kalihinene (42) and isokalihinol B (43), were isolated from *Acanthella klethra* (Fusetani *et al.*, 1990). Investigation of *Acanthella cavernosa* collected from Thailand resulted in the isolation of four diterpene isothiocyanates, kalihinols I (44), J (45), X (38) and Y (39) (Alvi, Tenenbaum and Crews, 1991). Furthermore, six diterpene isonitriles, 10-*epi*-isokalihinols F (46) and H (47), 1-*epi*-kalihinene (48), 15-isothiocyanato-1-*epi*-kalihinene (49), 1,10-*diepi*-kalihinene (50) and kalihipyran (51), were isolated from a specimen of *Acanthella cavernosa* from the Seychelles (Trimurtula and Faulkner, 1994). Two of these compounds, 1,10-*diepi*-kalihinene (50) and 1-*epi*-kalihinene (48), were also reported as kalihinene-A and kalihinene-B, respectively, from the same sponge collected in the same general location by Braekman and his co-workers (1994), although (50) was found to be racemic in this study. *Acanthella cavernosa* from Fiji yielded seven more diterpenes, (52)-(58), most of which are formamides, together with some known metabolites (Rodriguez *et al.*, 1994). Recently, Okino *et al.* (1995) also reported the occurrence of an interesting group of kalihinenes from *Acanthella cavernosa*, kalihinenes X-Z (59)-(61). These compounds exhibited potent antifouling activities against the barnacle larvae of the barnacle *Balanus amphitrite*. These distributions of the terpenoids are summarized in Table 1.

Table 1. Terpenoids from marine sponges in the genus *Acanthella*

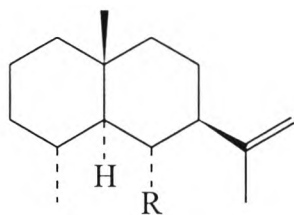
Sources	Compounds	References
Sesquiterpenes		
<i>Acanthella acuta</i>	Acanthellin-1 (1)	Minale <i>et al.</i> , 1974
	(2)-(6)	Minale <i>et al.</i> , 1974
	(7)-(10)	Ciminiello <i>et al.</i> , 1984
	(11)-(12)	Braekman <i>et al.</i> , 1987
	Axiosonitrile (13)	Braekman <i>et al.</i> , 1987
	1-Isocyanoaromadendrane (14)	Braekman <i>et al.</i> , 1987
	(16)-(21)	Mayol <i>et al.</i> , 1987
<i>Acanthella klethra</i>	(22)-(23)	Konig <i>et al.</i> , 1992
<i>Acanthella cf cavernosa</i>	Cavernothiocyanate (24)	Fusetani <i>et al.</i> , 1992
	Cavernoisonyrile (25)	Fusetani <i>et al.</i> , 1992
	10 α -Isocyano-4-amorphene (26)	Fusetani <i>et al.</i> , 1992
<i>Acanthella</i> sp.	Acanthene A (27)	Burgoyne <i>et al.</i> , 1993
	Acanthene B (28)	Burgoyne <i>et al.</i> , 1993
	Acanthene C (29)	Burgoyne <i>et al.</i> , 1993

Table 1. Terpenoids from marine sponges in the genus *Acanthella* (continued)

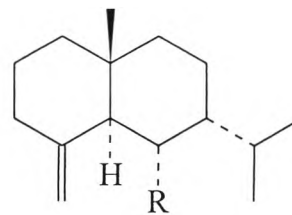
Sources	Compounds	References
Diterpenes		
<i>Acanthella</i> sp.	Kalihinol A (30)	Chang <i>et al.</i> , 1984
	Kalihinol B (31)	Patra <i>et al.</i> , 1984; Chang <i>et al.</i> , 1987
	Kalihinol C (32)	Patra <i>et al.</i> , 1984; Chang <i>et al.</i> , 1987
	Kalihinol D (35)	Chang <i>et al.</i> , 1984
	Kalihinol E (33)	Patra <i>et al.</i> , 1984; Chang <i>et al.</i> , 1987
	Kalihinol F (34)	Patra <i>et al.</i> , 1984; Chang <i>et al.</i> , 1987
	Kalihinol G (36)	Chang <i>et al.</i> , 1984
	Kalihinol H (37)	Chang <i>et al.</i> , 1984
	Kalihinol X (38)	Chang <i>et al.</i> , 1984
	Kalihinol Y (39)	Chang <i>et al.</i> , 1984
	Kalihinol Z (40)	Chang <i>et al.</i> , 1984
<i>Acanthella cavernosa</i>	Kalihinol I (44)	Alvi <i>et al.</i> , 1991
	Kalihinol J (45)	Alvi, <i>et al.</i> , 1991
	Kalihinol X (38)	Alvi <i>et al.</i> , 1991

Table 1. Terpenoids from marine sponges in the genus *Acanthella* (continued)

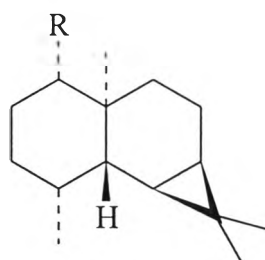
Sources	Compounds	References
<i>Acanthella klethra</i>	Kalihinol Y (39)	Alvi <i>et al.</i> , 1991
	Isokalihinol F (41)	Omar <i>et al.</i> , 1988
	10- <i>Epi</i> -isokalihinol F (46)	Trimurtula and Faulkner, 1994
	10- <i>Epi</i> -isokalihinol H (47)	Trimurtula and Faulkner, 1994
	1- <i>Epi</i> -kalihinene (kalihinene-B) (48)	Trimurtula and Faulkner, 1994; Braekman, 1994
	15-Isothiocyano-1- <i>epi</i> - kalihinene (49)	Trimurtula and Faulkner, 1994
	1,10-Diepi-kalihinene (kalihinene-A) (50)	Trimurtula and Faulkner, 1994; Braekman, 1994
	Kalihipyran (51)	Trimurtula and Faulkner, 1994
	(52) - (58)	Rodriguez <i>et al.</i> , 1994
	Kalihinene X (59)	Okino <i>et al.</i> , 1995
	Kalihinene Y (60)	Okino <i>et al.</i> , 1995
	kalihinene Z (61)	Okino <i>et al.</i> , 1995
	kalihinene (42)	Fusetani <i>et al.</i> , 1990
isokalihinol B (43)	Fusetani <i>et al.</i> , 1990	



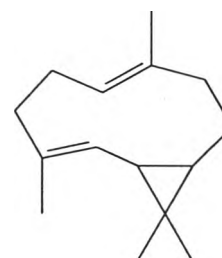
- (1) R = -NC
 (2) R = -NCS
 (3) R = NHCHO



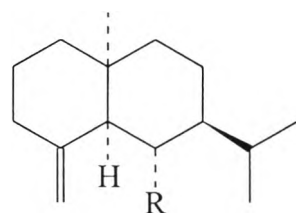
- (4) R = -NC
 (5) R = -NCS
 (6) R = NHCHO



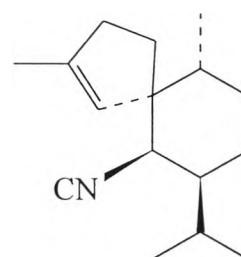
- (7) R = -NC
 (8) R = -NCS
 (9) R = NHCHO



(10)

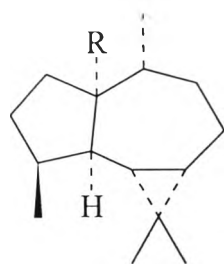


- (11) R = -NC
 (12) R = -NCS



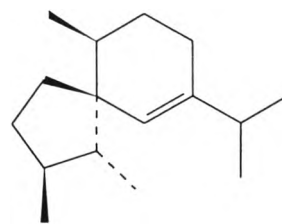
(13)

Figure 1. Terpenoids from marine sponges in the genus *Acanthella*



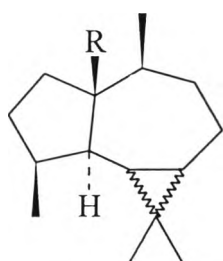
(14) R = -NC

(15) R = -OH



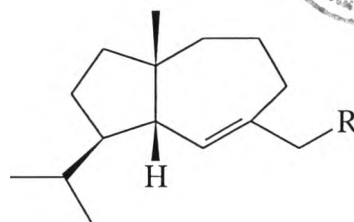
(16) R = -NC

(17) R = -NCS



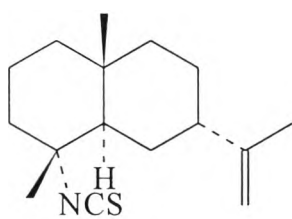
(18) R = -NC

(19) R = -NCS

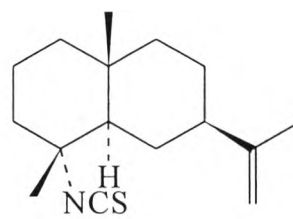


(20) R = -NC

(21) R = -NCS

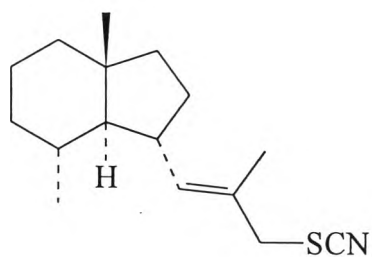


(22)

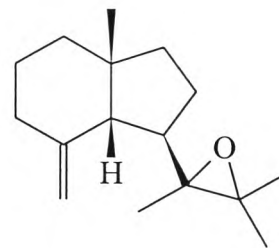


(23)

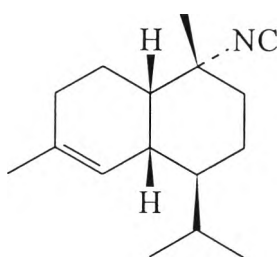
Figure 1. Terpenoids from marine sponges in the genus *Acanthella* (continued)



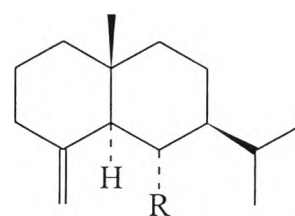
(24)



(25)



(26)

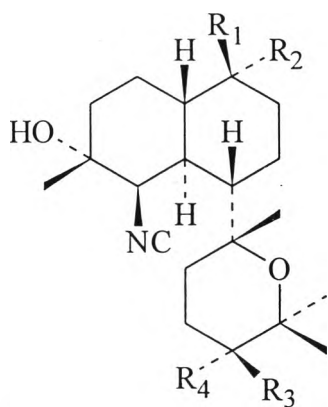


(27) R = Cl

(28) R = -NCS

(29) R = -NHCHO

Figure 1. Terpenoids from marine sponges in the genus *Acanthella* (continued)



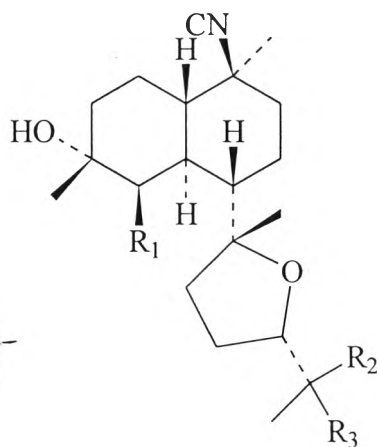
(30): $R_1 = -NC$, $R_2 = Me$, $R_3 = H$, $R_4 = Cl$

(33): $R_1 = -NC$, $R_2 = Me$, $R_3 = Cl$, $R_4 = H$

(38): $R_1 = Me$, $R_2 = -NCS$, $R_3 = Cl$, $R_4 = H$

(39): $R_1R_2 = CH_2$, $R_3 = Cl$, $R_4 = H$

(40): $R_1 = Me$, $R_2 = -NC$, $R_3 = Cl$, $R_4 = H$



(31): $R_1 = -NC$, $R_2 = Me$, $R_3 = Cl$

(32): $R_1 = -NC$, $R_2R_3 = CH_2$

(34): $R_1 = -NC$, $R_2 = Me$, $R_3 = -NC$

(35): $R_1 = Cl$, $R_2 = Me$, $R_3 = -NC$

(36): $R_1 = -NC$, $R_2 = Me$, $R_3 = -NCS$

Figure 1. Terpenoids from marine sponges in the genus *Acanthella* (continued)

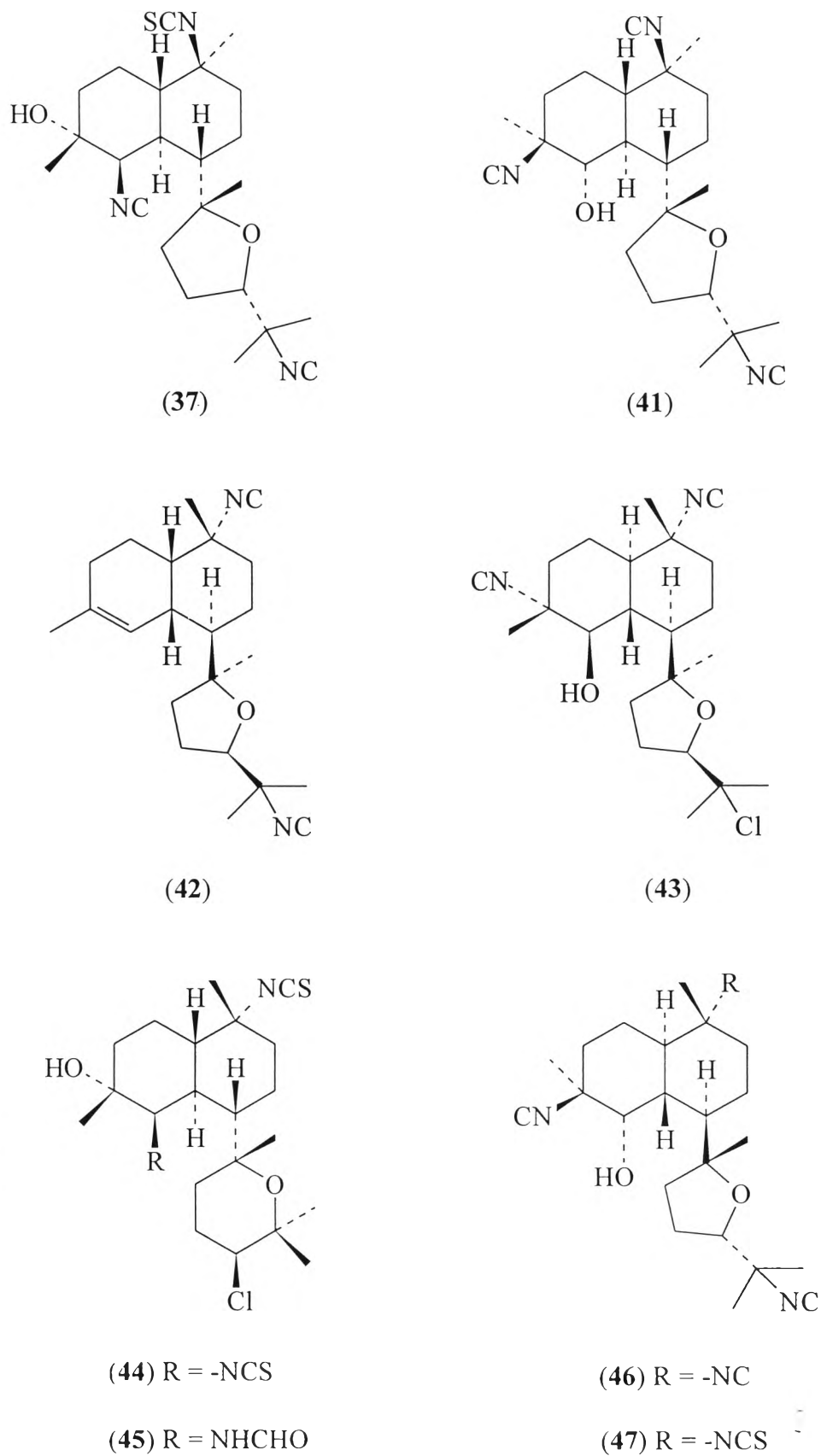
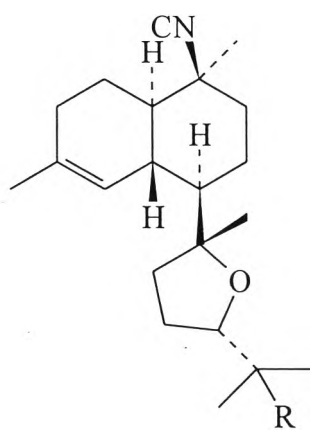
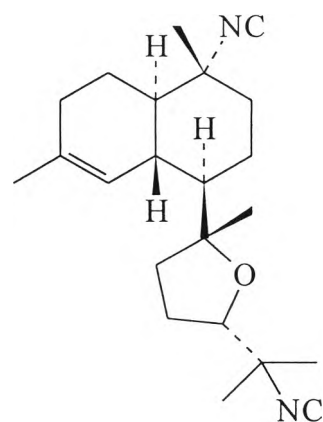


Figure 1. Terpenoids from marine sponges in the genus *Acanthella* (continued)

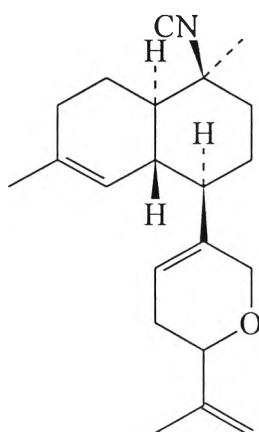


(48) R = -NC

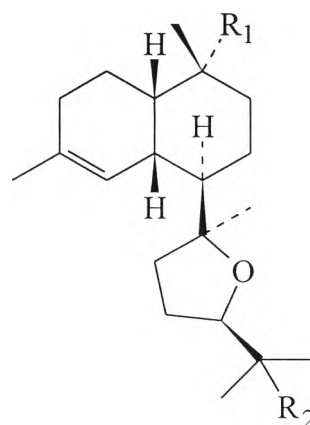
(49) R = -NCS

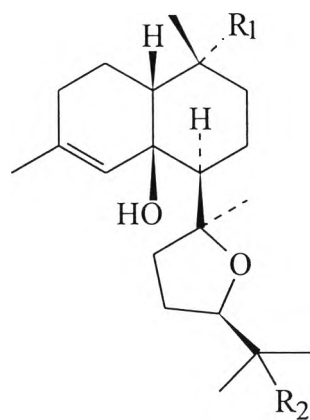


(50)



(51)

(52) R₁ = NHCHO, R₂ = -NC(53) R₁ = -NC, R₂ = NHCHO(54) R₁ = R₂ = NHCHOFigure 1. Terpenoids from marine sponges in the genus *Acanthella* (continued)

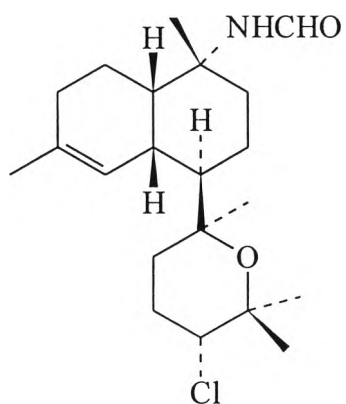


(55) $R_1 = R_2 = -NC$

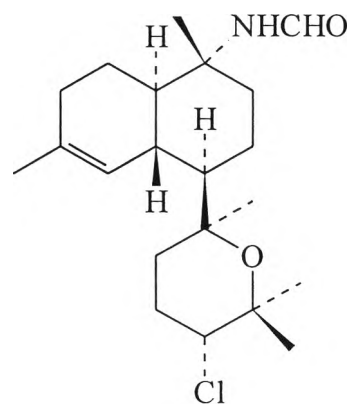
(56) $R_1 = -NC, R_2 = NHCHO$

(57) $R_1 = NHCHO, R_2 = -NC$

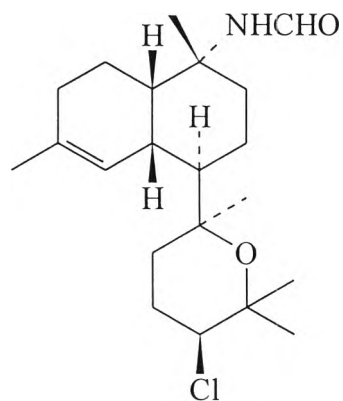
(58) $R_1 = NHCHO, R_2 = -NCS$



(59)



(60)



(61)

Figure 1. Terpenoids from marine sponges in the genus *Acanthella* (continued)

3. Nitrogen - containing diterpenes from marine organisms

Marine organisms continue to provide a wide range of structural types of diterpenoids. Nitrogen-containing diterpenes have been found in higher plants (Luckner, 1990) and also some marine organisms (sponges, coelenterates and ascidians). The distribution of this type of diterpenoids is summarized in Table 2.

Table 2. Nitrogen - containing diterpenes from marine organisms

Sources	Compounds	References
Sponges		
<i>Acanthella</i> sp.	Kalihinol A (30)	Chang <i>et al.</i> , 1984
	Kalihinol B (31)	Patra <i>et al.</i> ,1984; Chang <i>et al.</i> ,1987
	Kalihinol C (32)	Patra <i>et al.</i> ,1984; Chang <i>et al.</i> ,1987
	Kalihinol D (35)	Chang <i>et al.</i> , 1984
	Kalihinol E (33)	Patra <i>et al.</i> ,1984; Chang <i>et al.</i> ,1987
	Kalihinol F (34)	Patra <i>et al.</i> ,1984; Chang <i>et al.</i> ,1987
	Kalihinol G (36)	Chang <i>et al.</i> , 1984
	Kalihinol H (37)	Chang <i>et al.</i> , 1984
	Kalihinol X (38)	Chang <i>et al.</i> , 1984
	Kalihinol Y (39)	Chang <i>et al.</i> , 1984
	Kalihinol Z (40)	Chang <i>et al.</i> , 1984
<i>Acanthella cavernosa</i>	Kalihinol I (44)	Alvi <i>et al.</i> ,1991
	Kalihinol J (45)	Alvi <i>et al.</i> ,1991
	Kalihinol X (38)	Alvi <i>et al.</i> ,1991

Table 2. Nitrogen - containing diterpenes from marine organisms (Continued)

Sources	Compounds	References
<i>Acanthella klethra</i>	Kalihinol Y (39)	Alvi <i>et al.</i> ,1991
	Isokalihinol F (41)	Omar <i>et al.</i> ,1988
	10- <i>Epi</i> -isokalihinol F (46)	Trimurtula and Faulkner, 1994
	10- <i>Epi</i> -isokalihinol H (47)	Trimurtula and Faulkner, 1994
	1- <i>Epi</i> -kalihinene (kalihinene-B) (48)	Trimurtula and Faulkner, 1994; Braekman, 1994
	15-Isothiocyano-1- <i>epi</i> - kalihinene (49)	Trimurtula and Faulkner, 1994
	1,10-Diepi-kalihinene (kalihinene-A) (50)	Trimurtula and Faulkner, 1994; Braekman, 1994
	Kalihipyran (51)	Trimurtula and Faulkner, 1994
	(52) - (58)	Rodriguez <i>et al.</i> , 1994
	Kalihinene X (59)	Okino <i>et al.</i> ,1995
	Kalihinene Y (60)	Okino <i>et al.</i> ,1995
	Kalihinene Z (61)	Okino <i>et al.</i> ,1995
	Kalihinene (42)	Fusetani <i>et al.</i> , 1990
Isokalihinol B (43)	Fusetani <i>et al.</i> , 1990	
<i>Agelas</i> sp.	Ageline A (62)	Carpon and Faulkner, 1984

Table 2. Nitrogen - containing diterpenes from marine organisms (Continued)

Sources	Compounds	References
<i>Agelas clathrodes</i>	Ageline B (63)	Carpon and Faulkner, 1984
	Agelasine G (64)	Ishida <i>et al.</i> , 1992
	Agelasidine C (65)	Morales And Rodriguez, 1992
	Agelasidine D (66)	Morales And Rodriguez, 1992
<i>Agelas mauritiana</i>	Agelasimine A (67)	Fathi - Afshar and Allen, 1988
<i>Agelas nakamura</i>	Agelasimine B (68)	Fathi - Afshar and Allen, 1988
	Agelasine A (69)	Wu <i>et al.</i> , 1984; Carpon and Faulkner, 1984
	Agelasine B (70)	Wu <i>et al.</i> , 1984; Carpon and Faulkner, 1984
	Agelasine C (71)	Wu <i>et al.</i> , 1984; Carpon and Faulkner, 1984
	Agelasine D (72)	Wu <i>et al.</i> , 1984; Carpon and Faulkner, 1984
	Agelasine E (73)	Wu <i>et al.</i> , 1984; Carpon and Faulkner, 1984
	Ageline A (62)	Wu <i>et al.</i> , 1984; Carpon and Faulkner, 1984
	Agelasidine B (74)	Nakamura <i>et al.</i> , 1985
Agelasidine C (75)	Nakamura <i>et al.</i> , 1985	
	Nakamurol D (76)	Shoji <i>et al.</i> , 1996

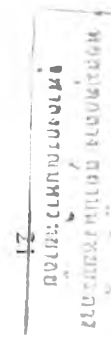


Table 2. Nitrogen - containing diterpenes from marine organisms (Continued)

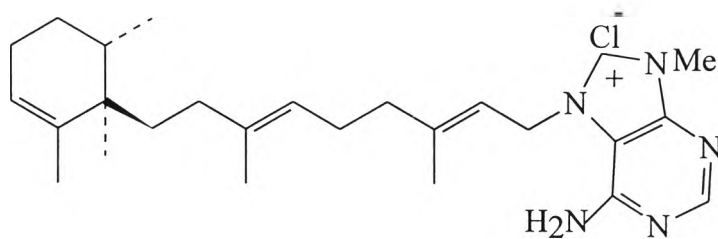
Sources	Compounds	References
<i>Amphimedon</i> sp.	(77) - (82)	Kazlauskas <i>et al.</i> , 1980
	7,20 - Di - isocyanoadociane (83)	Garson ,1986; Corey and Megriotis, 1987
<i>Halichondria</i> sp.	(3S*, 4R*, 7S*, 8R*, 11S*, 12R*, 13S*) -7 -	Molinski <i>et al.</i> , 1987
	Isocyano-15,20-cycloamphilect-1-ene (84)	
	(1S*, 3S*, 4R*, 7S*, 8R*, 13R*) -7 -Isocyano-	Molinski <i>et al.</i> , 1987
	15,20-cycloamphilect-11-ene (85)	
	8-Isocyanoamphilect-10,14-diene (86)	Molinski <i>et al.</i> , 1987
(3S*, 4R*, 7S*, 8R*, 13R*) -8-Isocyano-15,20-	Kazlauskas <i>et al.</i> , 1980	
cycloamphilect-1(12)-ene (87)		
<i>Hymeniacidon amphilecta</i>	8,15-Diisocyanoamphilect-11(20)-ene (88)	Wratten <i>et al.</i> , 1978
	(89)	Wratten <i>et al.</i> , 1978
<i>Sinularia mayi</i>	Sinulamine I (90)	Kobayashi <i>et al.</i> , 1987; Iguchi <i>et al.</i> , 1992
	Sinulamine II (91)	Kobayashi <i>et al.</i> , 1987; Iguchi <i>et al.</i> , 1992

Table 2. Nitrogen - containing diterpenes from marine organisms (Continued)

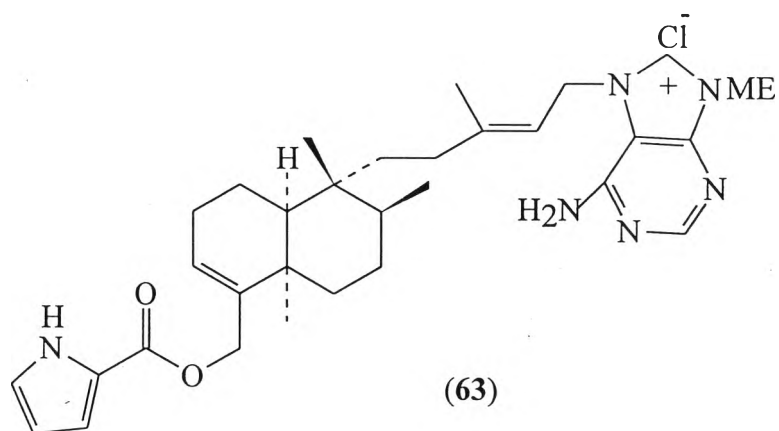
Sources	Compounds	References
<i>Spongia</i> sp.	Sinulamine III (92)	Kobayashi <i>et al.</i> , 1987; Iguchi <i>et al.</i> , 1992
	Haumanamide (93)	Pham <i>et al.</i> , 1992
Coelenterates		
<i>Pseudoterogorgia acerosa</i>	Tobagolide (94)	Chan <i>et al.</i> , 1991
	(95)	Tinto <i>et al.</i> , 1991
<i>Sarcodictyon roseum</i>	Sarcodictyin A (96)	D' Ambrosio, Guerriero and Pietra, 1987; 1988
	Sarcodictyin B (97)	D' Ambrosio <i>et al.</i> , 1987; 1988
	Sarcodictyin C (98)	D' Ambrosio <i>et al.</i> , 1987; 1988
	Sarcodictyin D (99)	D' Ambrosio <i>et al.</i> , 1987; 1988
	Sarcodictyin E (100)	D' Ambrosio <i>et al.</i> , 1987; 1988
	Sarcodictyin F (101)	D' Ambrosio <i>et al.</i> , 1987; 1988

Table 2. Nitrogen-containing diterpenes from marine organisms (continued)

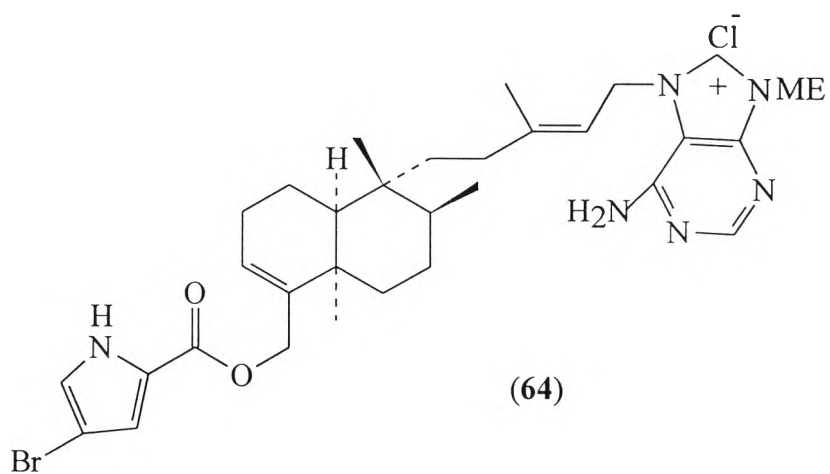
Sources	Compounds	References
Ascidian <i>Lissoclinum voeltzkowi</i>	Chlorolissoclimide (102) Dichlorolissoclimide (103)	Biard <i>et al.</i> , 1994 Biard <i>et al.</i> , 1994



(62)

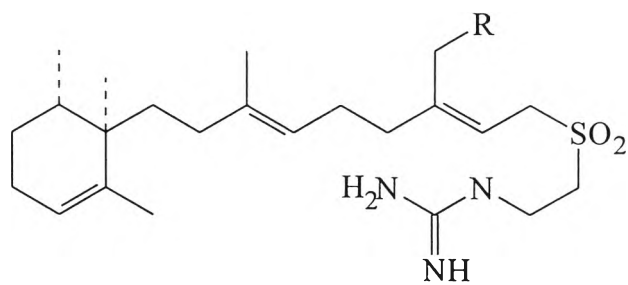


(63)



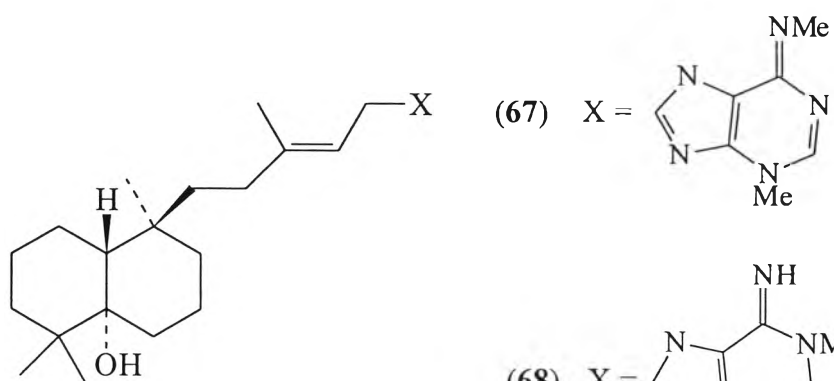
(64)

Figure 2. Nitrogen - containing diterpenes from marine organisms



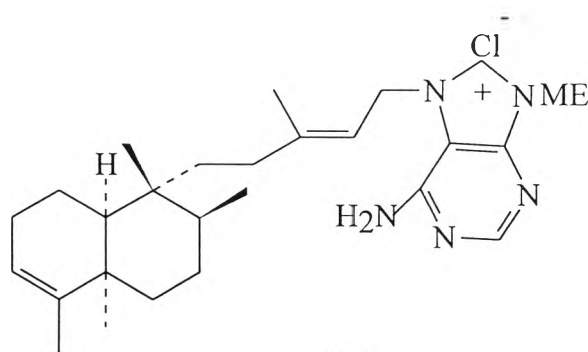
(65) R = H

(66) R = OH



(67) X =

(68) X =



(69)

Figure 2. Nitrogen - containing diterpenes from marine organisms (Continued)

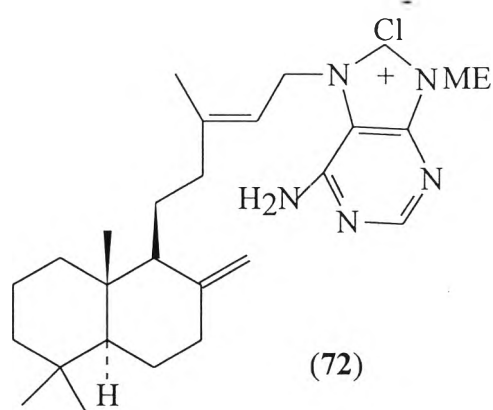
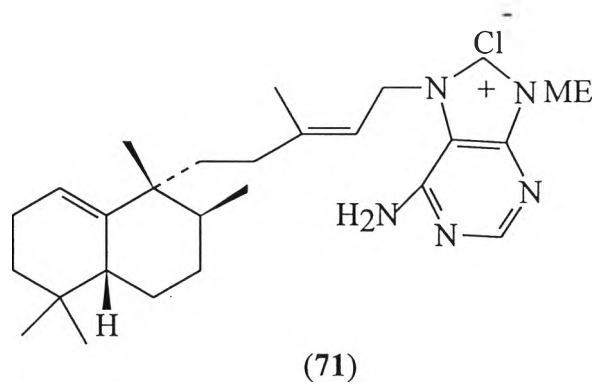
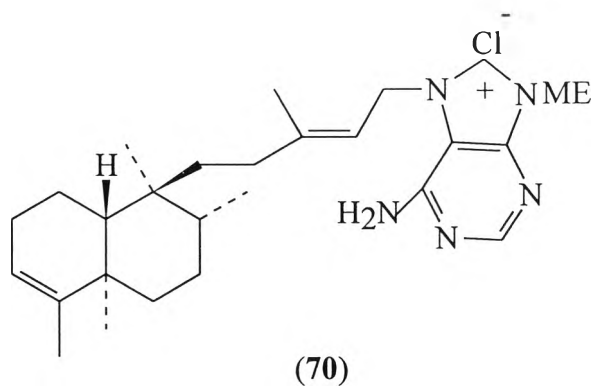


Figure 2. Nitrogen - containing diterpenes from marine organisms (Continued)

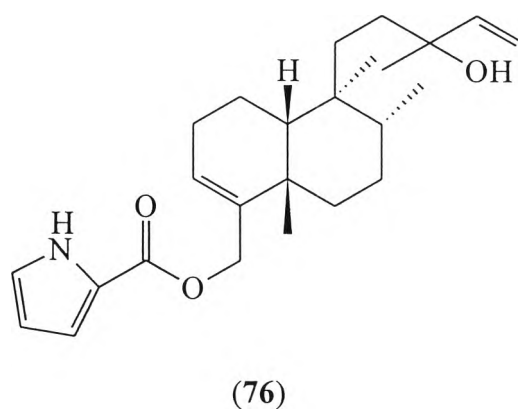
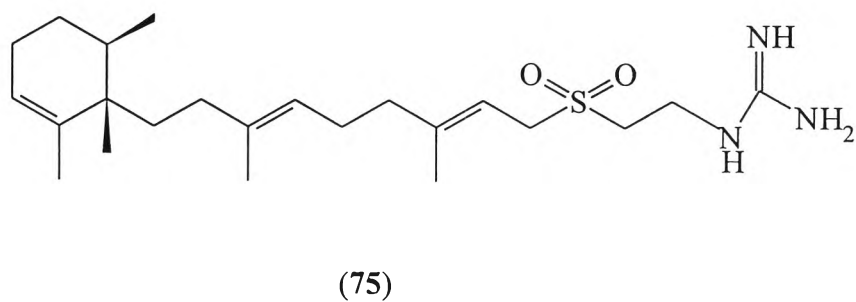
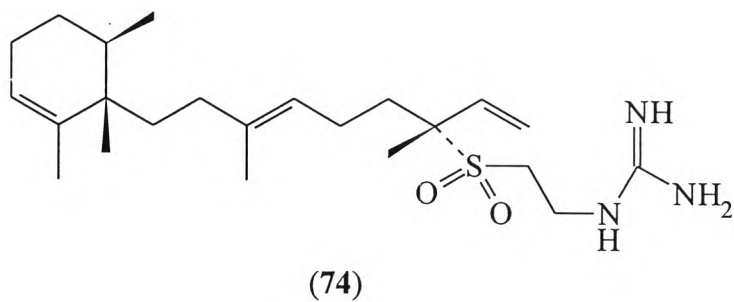
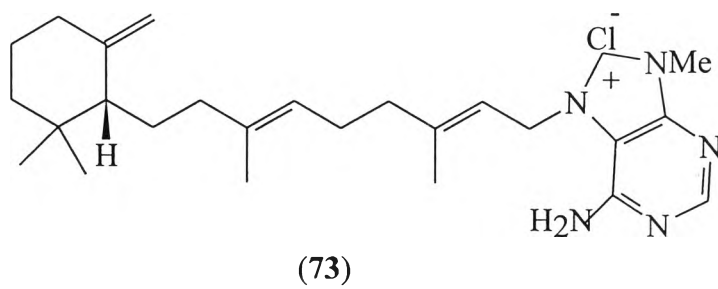
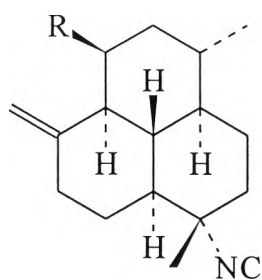


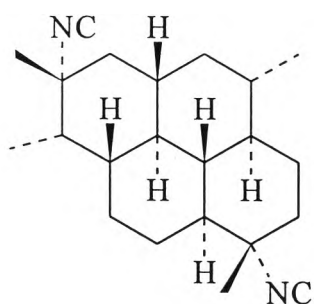
Figure 2. Nitrogen - containing diterpenes from marine organisms (Continued)



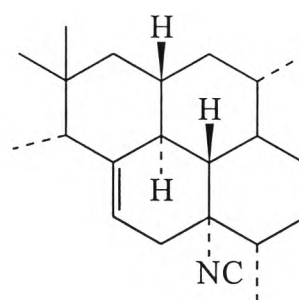
(77) $R = \text{CH} = \text{CMe}_2$

(78) $R = \text{CH}_2\text{C}(\text{Me}) = \text{CH}_2$

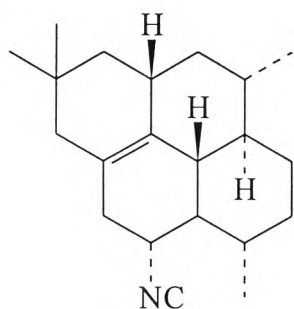
(79) $R = \text{CH}_2\text{C}(\text{NC})\text{Me}_2$



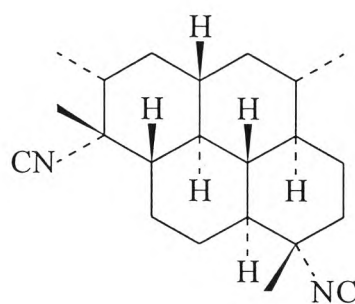
(80)



(81)

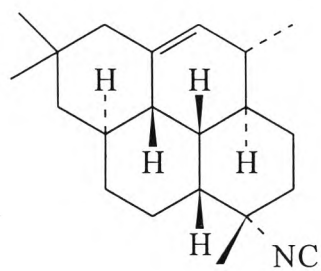


(82)

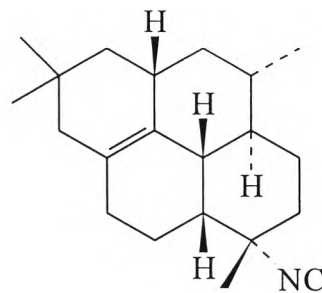


(83)

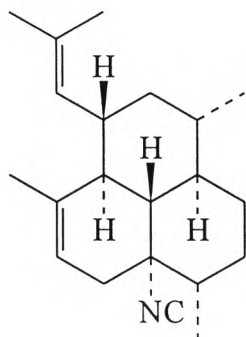
Figure 2. Nitrogen - containing diterpenes from marine organisms (Continued)



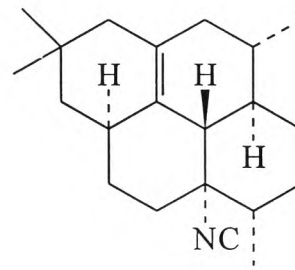
(84)



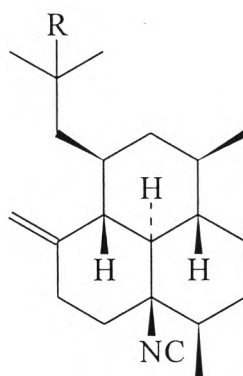
(85)



(86)



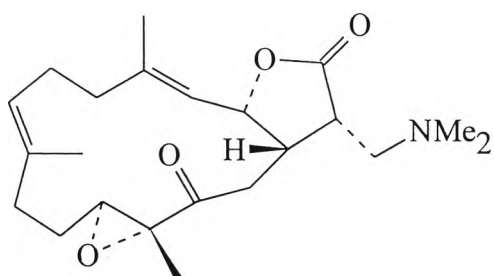
(87)



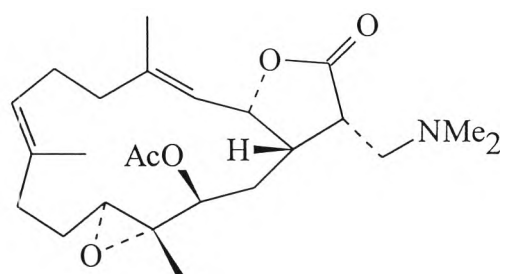
(88) R = -NC

(89) R = -NHCHO

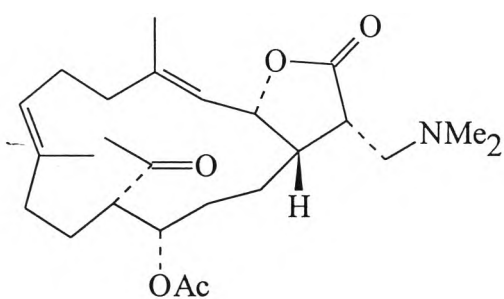
Figure 2. Nitrogen - containing diterpenes from marine organisms (Continued)



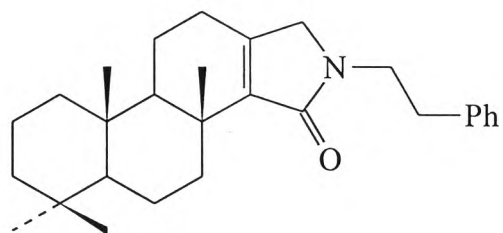
(90)



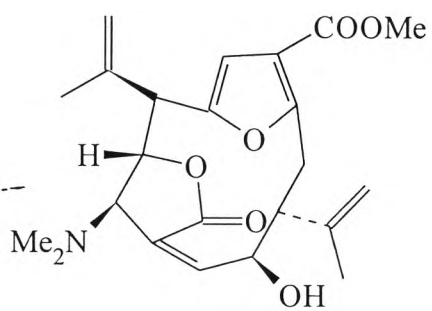
(91)



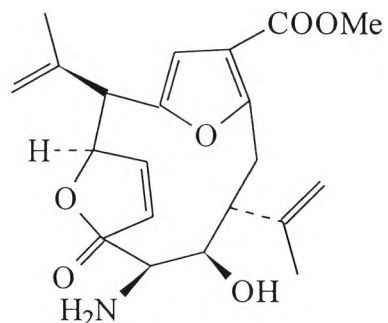
(92)



(93)

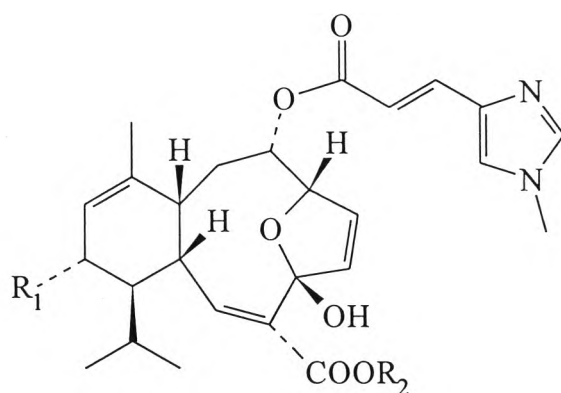


(94)



(95)

Figure 2. Nitrogen - containing diterpenes from marine organisms (Continued)

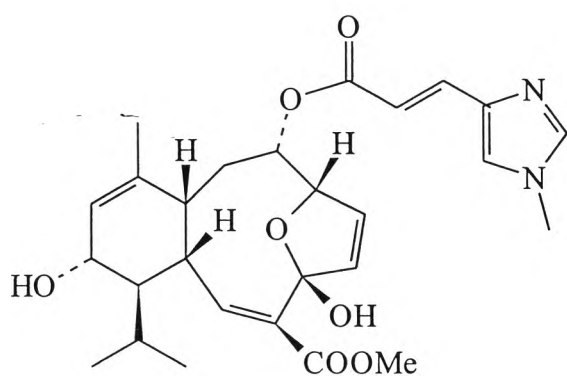


(96) $R_1 = H, R_2 = Me$

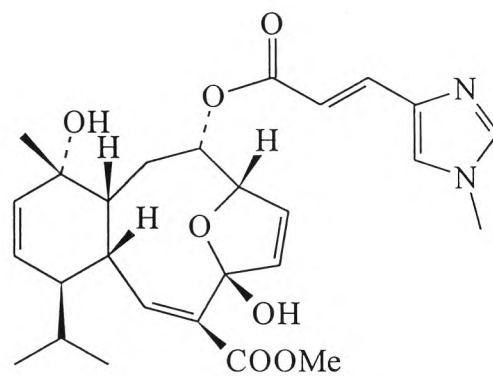
(97) $R_1 = H, R_2 = Et$

(98) $R_1 = OH, R_2 = Me$

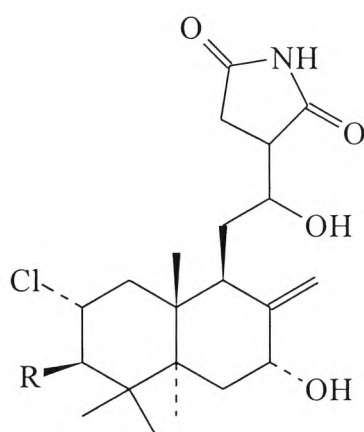
(99) $R_1 = OAc, R_2 = Me$



(100)



(101)



(102) $R = H$

(103) $R = Cl$

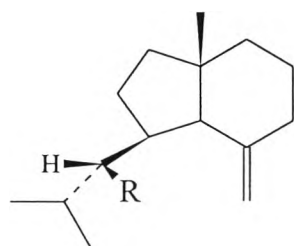
Figure 2. Nitrogen - containing diterpenes from marine organisms (Continued)

4. Biosynthesis of isocyanate, isothiocyanate and formamide diterpenes in marine sponges

The key precursor in the formation of most diterpenes is geranylgeranyl diphosphate. Important reactions in its transformation are hydrolysis of the diphosphate group, reduction of double bonds and cyclization to form different types of diterpenes (Luckner, 1990).

Secondary metabolites containing the isocyano function are rare in nature. Marine sponges are sources of isonitriles, which usually co-occur with isothiocyanates, formamides and terpene hydrocarbons, occasionally with isocyanates, ureas, thioureas, dichlorocarbimines and free amines - all presumably derived from an isocyano precursor (Gulavita *et al.*, 1986).

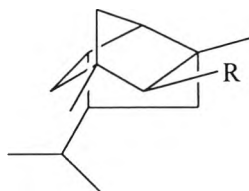
The biosynthetic origin of isocyanide carbon in the diterpene isocyanides from marine sponges is one of the most controversial and intriguing problems in marine natural product chemistry. Initially, the co-occurrence of the formamide-isocyanide pair was claimed as circumstantial evidence that the formamide might act as a biogenetic precursor to yield the isocyanide, but this hypothesis was disproved by Sodano and his co-workers' experiment (Iengo, Santacroce and Sodano, 1979) in which ^{14}C -labelled axamide-1 (104) was supplied to *Axinella cannabina* [which produces both 104 and axisonitrile-1 (105)] over a period of five days. No conversion of radioactivity into axisonitrile-1 was found.



(104) axamide-1, R = NHCHO

(105) axisonitrile-1, R = -NC

In another pioneering research, Hagadone, Scheuer and Holm (1984) studied the origin of isocyano groups in the metabolite 2-isocyanopupukeanane (106) from a species of *Hymeniacidon*. They suggested that ^{13}C -labelled 2-isocyanopupukeanane was converted into its co-metabolites, *i.e.* the formamide 107 and isothiocyanate 108 while the latter was not converted into 106. Therefore, their experiment confirmed Sodano's earlier finding that formamide was not a precursor of the isocyanide and also showed that the isothiocyanate was not transformed into the isocyanide. Furthermore, they successfully demonstrated that, contrary to previous hypothesis, both formamide and isothiocyanate were biosynthesized from isocyanide.

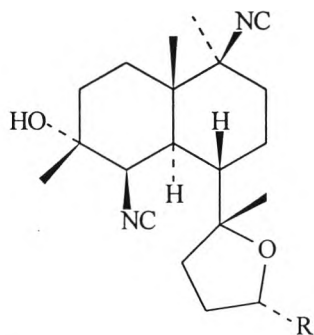


(106) 2-Isocyanopupukeanane, R = -NC

(107) R = NHCHO

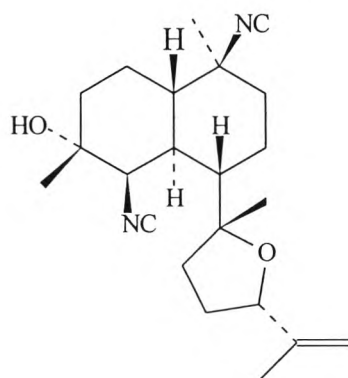
(108) R = -NCS

The most intriguing aspect of the isocyanide-containing diterpenes is the biochemical origin of the isocyanide substituent itself. Kalihinols B (109), C (110) and F (111), isolated from the Guam sponge *Acanthella* sp., containing a halogen, an olefinic and an isocyanide substituent, respectively, indirectly suggest a likely mechanism for the biosynthesis of diterpene isocyanides, in which the ambident nucleophile isocyanide is captured by a carbonium ion or an equivalent intermediate (Garson, 1989).



(109) Kalihinol B, R = CMe₂ Cl

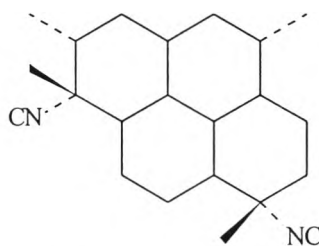
(111) Kalihinol F, R = CMe₂ NC



(110) Kalihinol C

The incorporation of sodium [¹⁴C] cyanide into a marine sponge, *Amphimedon* sp., which produces the tetracyclic diterpene di-isocyanide, di-isocyanoadociene (112), had been

investigated. The incorporation of cyanide into the metabolite at the level of 0.2 - 1.8%, together with chemical degradation, revealed that the isocyanide carbons were equally and selectively labelled by cyanide. The result was of interest for a number of reasons; first, isocyanide substituents were conventionally believed to derive from the addition of a single carbon atom from the C_1 -tetrahydrofolate pool to an amine precursor, followed by dehydration. Secondly, the utilization of cyanide by a marine invertebrate was of interest in view of the known role of cyanide as an inhibitor of cytochrome oxidase (Vennesland *et al.*, 1981). A complication was that, similar to many other sponges, the *Amphimedon* sp. contains several bacterial symbionts, and it was not yet clear whether the animal host or the bacterial symbiont was responsible for the uptake of cyanide. Many strains of bacteria were known to have developed cyanide-insensitive respiratory chains or to generate inorganic cyanide from amino acids. On the other hand, some isocyanide-producing sponges were known to contain negligible amounts of symbionts, which suggested that the uptake might occur directly into sponge cells. The source of cyanide *in situ* was believed, by analogy with terrestrial metabolism, to be an amino acid, but may not be glycine, leucine, alanine or arginine since these did not appear to be incorporated into (112) although they were readily metabolized by the sponge (Garson, 1989).



(112) Di-isocyanoadociane

