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BIOLOGICAL PROPERTIES AND CHEMICAL DIVERSITY OF *Sinularia flexibilis,* AN ALCYONACEAN SOFT CORAL

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Abstract: Soft corals of the genus *Sinularia* (order Alcyonacea) are one of the most widespread sessile organisms in the Indo-Pacific waters. Unlike the hard corals, the major portion of soft coral is made up of inorganic skeletons consisting of calcareous spicules surrounded by a thin layer of tissue. This layer of tissue, made up of fleshy colonies with organic matter, is responsible in diversity of secondary metabolites. In order to survive the outburst of algal bloom, dynamic of microbial growth and predation of marine organisms, these soft corals synthesize unique chemicals as part of defense mechanism that makes them unpalatable to most marine life. As such, members of the genus *Sinularia* exhibits a diversity of secondary metabolites ranging from sesquiterpenes, diterpenes, polyhydroxylated steroids, and polyamine compounds. These metabolites had been shown to possess various biological activities such as antimicrobial, anti-inflammatory and cytotoxicity. This present paper reviews the chemistry and highlights the potential biological activities of metabolites from *Sinularia flexibilis* and provides a perspective for future research.

Keywords: *Sinularia flexibilis,* soft coral, secondary metabolites, biological activities.

Introduction

The status of marine natural products has been a popular subject for reviews (Kelecom, 2002; Proksch *et al.,* 2002; Haefner, 2003; Capon, 2010; Blunt *et al.,* 2015) as this field has resulted in the development of a substantial number of bioactive metabolites for the betterment of mankind. Scheuer (1983) and Faulkner (2002) contributed tremendously in creating a platform in the field of marine natural product chemistry through comprehensive reviews pertaining to chemical and biological perspectives of secondary metabolites. The coral reef represents an extraordinary diverse ecosystem in tropical environments with soft corals often constituting a dominant part of the reef. *Sinularia* is a genus in phylum Cnidaria classified in class Alcyonaria, in the family Alcyoniidae and is widely distributed from the waters of east Africa to the western Pacific, inhabiting the coral reefs or rocks up to depths of 30 meters (Piccinetti *et al.,* 2017). This genus is known to consist approximately 90 species, of which more than 50 have been chemically evaluated (Chen *et al.,* 2012). Researchers from Australia, India and Japan have isolated and elucidated an extensive range of secondary metabolites constituting sesquiterpenes, diterpenes, polyhydroxylated steroids and polyamine skeletons. Metabolites isolated from the genus *Sinularia* is reported to display potential bioactivities such as antimicrobial, anti-inflammatory and cytotoxic activities closely related to the high interaction in the marine environment (Palaniveloo & Vairappan, 2014). To date, numerous publications are currently available pertaining to the chemistry and pharmacology of metabolites isolated from *Sinularia flexibilis* found in the waters of Australia, Japan and India (Kamel *et al.,* 2005; Chen *et al.,* 2010; Anjeneyulu *et al.,* 1997) as well as extensive research on *S. flexibilis* from the Formosan waters (Hu *et al.,* 2013; Duh *et al.,* 1998; Lin *et al.,* 2009; 2013; Lo *et al.,* 2009; 2010) is documented. Over the past 30 years, more than 15 000 novel secondary metabolites have been discovered from this organism (Li *et al.,* 2006). Since 1995, various research papers have been published on investigations of the chemical constituents

of the soft coral genus *Sinularia* reporting new, novel terpenoids and their pharmacological potentials, globally. Hence, this comprehensive review focuses on the chemistry and biological activities of the chemical constituents from *S. flexibilis* (Fig. 1) which has been a subject of interest in the area of marine drug discovery.

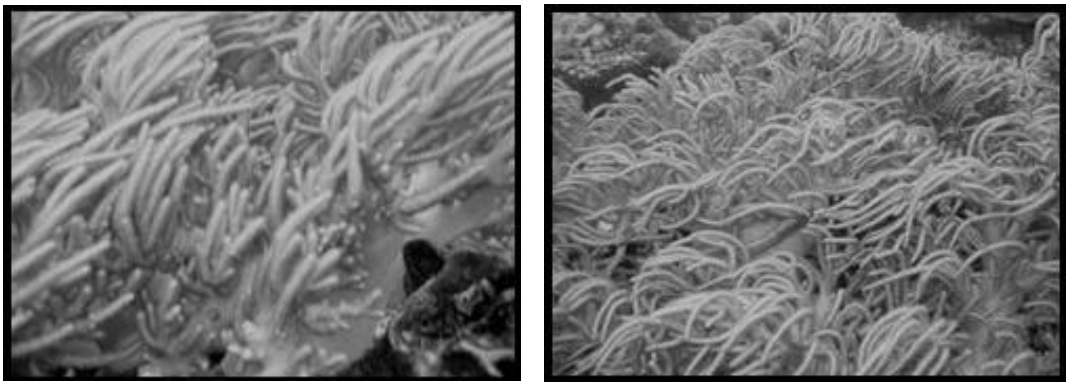


Figure 1: Underwater photographs of *S. flexibilis* found in the waters of Mantanani Island, Sabah, Malaysia.

Terpenoids of Alcyonacean

Investigations involving soft corals started as early as in 1972 with the isolation of prostaglandin [(15R)-PGA2] from *Plexaura homomalla* Esper, a gorgonian whereas the attention on isolation of secondary metabolites from Alcyonacean started in 1970s. Coll (1992) reported patterns in chemical distribution across the various soft coral genera where terpenoids have been confirmed as the dominating cluster of secondary metabolites in Alcyonacean.

According to Coll (1992), the presence of diterpenes in *Sinularia* spp and *Sarcophyton* spp is made up of cembrane-type skeleton while *Lobophytum* spp was reported to produce a mixture of 70 % cembrane and 30% of germacrene-type skeleton, respectively. The *Cladiella* spp was found to produce cladiellane-type, cyclized cembrane compounds and only sesquiterpenes were isolated from *Parerythropodium* spp. In the family Nephtheiidae, the genus *Nephthea* spp comprised of sesquiterpenes or diterpenes of which 70% are cembrane-type skeleton producers. Interestingly, *Litophyton* spp have only been reported to contain cembranoid-type diterpenes while the genera *Lemnalia, Paralemnalia,* and *Capnella* spp produces sesquiterpenes. Across the family Xeniidae, the genera *Efflatounaria, Cespitularia* and *Xenia, Anthelia* spp have been studied with the earlier two genera producing diterpenes, sesquiterpenes and cembrane-type diterpene. In the latter two genera, 90% of the compounds isolated from *Xenia* spp and *Anthelia* spp belong to the xenicane-type skeleton group. Shown below in Figure 2 (1-19) is the highlight of diverse skeletal pattern across soft coral genera.

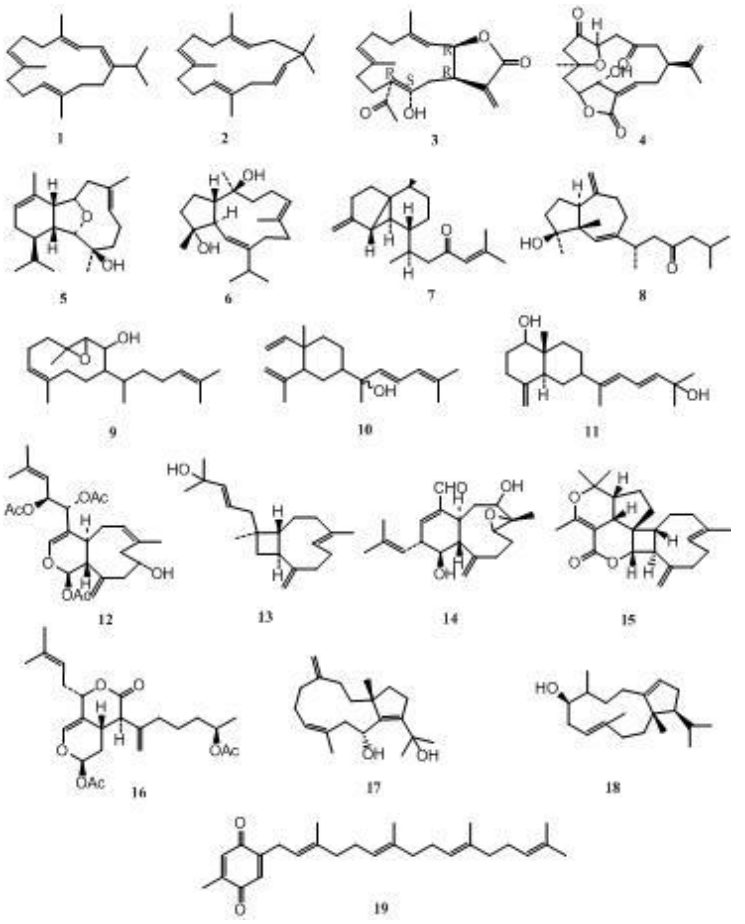


Figure 2: Diversity of isolated chemical skeletal across soft corals genera.

Chemical diversity of *Sinularia flexibilis*

Genus *Sinularia* is placed under the soft coral family Alcyoniidae. In total, 90 species of *Sinularia* have been identified worldwide and close to 50 species have been chemically studied (Chen *et al.,* 2012). Numerous publications currently exists pertaining to this genus from Australian, Japanese and Indian waters (Coll *et al.,* 1985; Anjeneyulu *et al.,* 1997; Kamel *et al.,* 2005). The secondary metabolites isolated commonly includes sesquiterpenes, diterpenes, polyhydroxylated steroids and polyamine which displays a wide array of biological properties such as antimicrobial, anti-inflammatory and cytotoxic activities (Anjeneyulu *et al.,* 1997; Duh *et al.,* 1998; Kamel *et al.,* 2005; Khalesi *et al.,* 2008; Lakshmi *et al.,* 2009; Chen *et al.,* 2010; Hu *et al.,* 2013; Lin *et al.,* 2013; 2009; Chen *et al.,* 2012) making them valuable for biomedical research. The secondary metabolites isolated from soft corals, apart from having biomedical potentials, it is also serves as chemotaxonomical markers for *Sinularia* species identification (Veseveldt, 1980; Palaniveloo & Vairappan, 2014).

Sesquiterpenes

Among earliest sesquiterpenes discovered from *Sinularia* was furanosesquiterpenoid acid (20) by Coll *et al.* and co-workers (1977) from *Sinularia gonatodes* followed by isolation of furanoquinol (21) from *Sinularia lochomodes* in 1978.

Furanosesquiterpenoids are commonly known to exhibit a wide range of biological potentials such as anti-microbial, anti-inflammatory and cytotoxic activities (Kamel *et al.*,2005; Chen *et al.*, 2010; Yang *et al.*, 2013). However, only one furano-type sesquiterpene have been reported from *S. flexibilis* to date. The compound (+)-β-elemene (22) was isolated along with the diterpene lobatriene from *S. flexibilis* from waters of Okinawa, Japan (Kusumi *et al.*, 1992). This same sesquiterpene skeleton, was initially isolated from the soft coral genus *Lobophytum* by Dunlop and Well (1979) and *Sinularia dissecta* (Reddy *et al.*, 1993; Ramesh *et al.*, 1999; Reddy *et al.*, 2002). Figure 3 displays the chemical structures of furanosesquiterpenoid acid (20), furanoquinol (21) and (+)-β-elemene (22).

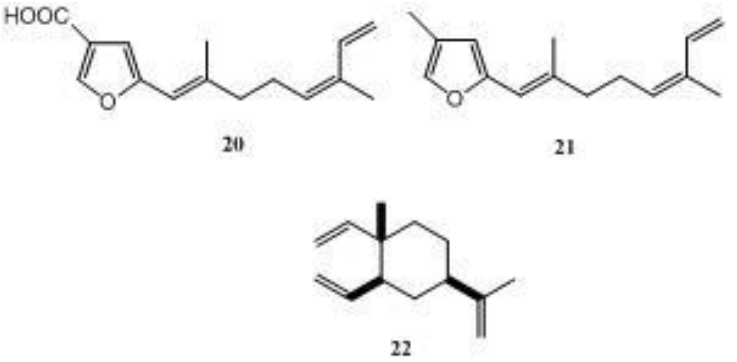


Figure 3: The structures of furanosesquiterpenoid acid (20), furanoquinol (21) and (+)-β- elemene (22) from the genus Sinularia.

Diterpenes

Cembranes are the most common secondary metabolites isolated from soft corals. This class of diterpenes possesses a 14-membered ring skeleton. As such, cembranes can be considered a chemotaxonomical marker for soft corals. The earliest of cembrane-type skeleton isolated is pukalide (23), consisting of a butenolide, epoxide, isopropenyl and a α,α-disubstituted furan-β-carboxylate moiety derived from Sinularia abrupta (Missakian *et al.*, 1975). As for *S. flexibilis*, sinulariolide (24) was the first diterpene isolated (Tursch *et al.*, 1975). Following the isolation of sinulariolide (24), its derivatives comprising of 11-dehydrosinulariolide (25), 11-epi-sinulariolide (26), 11-epi-sinulariolide acetate (27) (Fig. 4) was isolated from specimens collected from Red Sea water (Kashman *et al.*, 1997) along with 11-epoxysinulariolide (28) (Mori *et al.*, 1983). These compounds were also isolated from a Taiwanese specimen of *S. flexibilis* with additional diterpenes; sinuladiterpenes A (29) - F (34) (Lo *et al.*, 2009). Sinuladiterpenes A (29) and B (30) had an interesting oxygen moiety bearing hydroxyl group (-OOH) represented by a low field chemical shifts of δ 7.60 – 7.80 ppm. Lo and co-workers also isolated more cembranoid-type diterpenes, sinuladiterpenes G (35) - I (37) (Fig. 4) as a continuation of their previous work documented in 2009 (Lo *et al.*, 2010).

The compound flexibilene (38) is among the first few cembranes isolated and reported from *S. flexibilis* followed by the isolation of sinularin (39) and dihydrosinularin (40) reported by Weinheimer *et al.* (1977). These compounds were then revised as flexibilide (39) and dihydroflexibilide (40) prior being isolated from specimens extracted from Australian waters (Kazlauskas *et al.*, 1978). Next, the Philippines specimen led to the isolation of sinulariolone (41) with a lactone ring. However, sinulariolone (41) as reported to be highly oxygenated, bearing a total of six oxygen atoms (Guerrero *et al.*, 1995).

In addition, the Indian Ocean specimen led to the isolation of cembranolide-type δ-lactones,

flexibilolide (42) and dihydroflexibilolide (43) from the ethyl acetate extract (Anjaneyulu *et al.*, 1996). Along with these compounds, cembrene A (44), flexibilene (38) sandensolide (45) as well as the ϵ-lactone sandensolide monoacetate (46) (Anjaneyulu *et al.*, 1996; 1997) were also discovered. Bioassay-guided isolation of secondary metabolites from crude extracts of the Chinese specimen led to the isolation of cembranoid diterpenes, sinuflexolide (47), dihydrosinuflexolide (48), sinuflexibilin (49) and the bicembranoid sinuflexin (50) (Duh *et al.*, 1998) (Fig. 5).

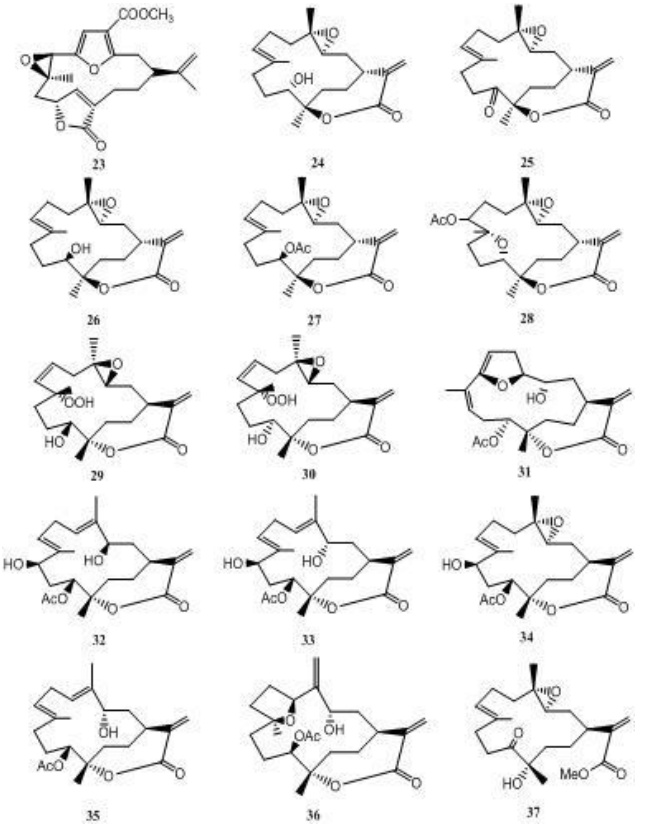


Figure 4: Chemical structures of pukalide (23), sinulariolide (24), its derivatives and Sinuladiterpenes A (29) - I (37).

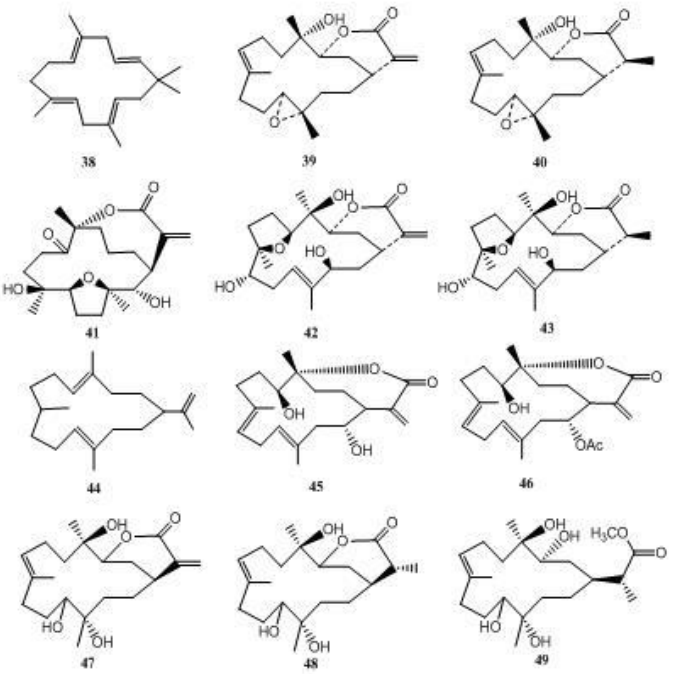


Figure 5: Chemical structures of cembrane diterpenes and its derivatives (38-49) isolated from the Indian Ocean *S. flexibilis.*

S. flexibilis have yielded several other bicembranoids as well. A study on the Chinese specimens of this soft coral has led to the isolation of a highly oxygenated bicembranoid-type sinulaflexiolide A (51). In addition to the isolation of sinuflexin (50) and sinulaflexiolide A (51), Chen and co-workers (Chen *et al.*, 2010) were able to isolate and elucidate a sulphated biscembranoid called thioflexibilolide A (52). Uniquely, this compound comprised of structures chemically identical to sinularin (49) and dihydrosinularin (40) and is linked with a sulphur atom. Figure 6 displays the chemical structures of bicembranoids isolated from *S. flexibilis.*

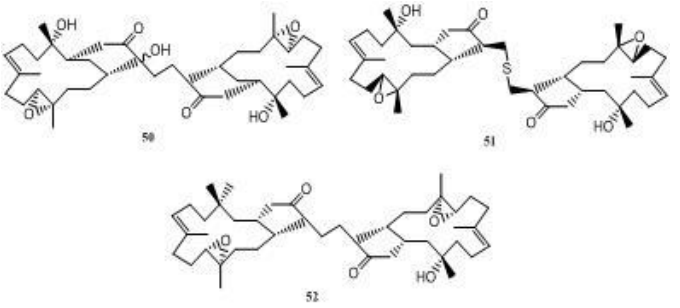


Figure 6: Bicembranoids sinuflexin (50), sinulaflexiolide A (51) and thioflexibilolide A (52) isolated from Chinese *S. flexibilis.*

In-depth study on the Chinese specimen of S. flexibilis, had contributed to the collection of cembranoid-type diterpenes, where the isolation of 10 more sinulaflexiolides via bioassay-guided techniques were named sinulaflexiolides B (53) - K (62). Most of these sinulaflexiolides are

structurally, highly oxygenated. The chemical structures of sinulaflexiolides B (53) - K (62) along with its derivatives, acetylsinuflexolide (63), 11-acetyldihydrosinuflexolide (64), 5-dehydrosinulariolide (65), capillolide (66), 5,8-epoxy-9-acetoxysinulariolide (67) and the enantiomer of 14-deoxycrassin (68) (Mori *et al.*, 1983) were isolated. Capillolide (66) have been previously reported being isolated from Sinularia capillosa (Su *et al.*, 2000) and Sinularia microclavata (Zhang *et al.*, 2008), however this is the first record of Capillolide (66) being isolated from *S. flexibilis.* The structures of these compounds are also shown in Figure 7.

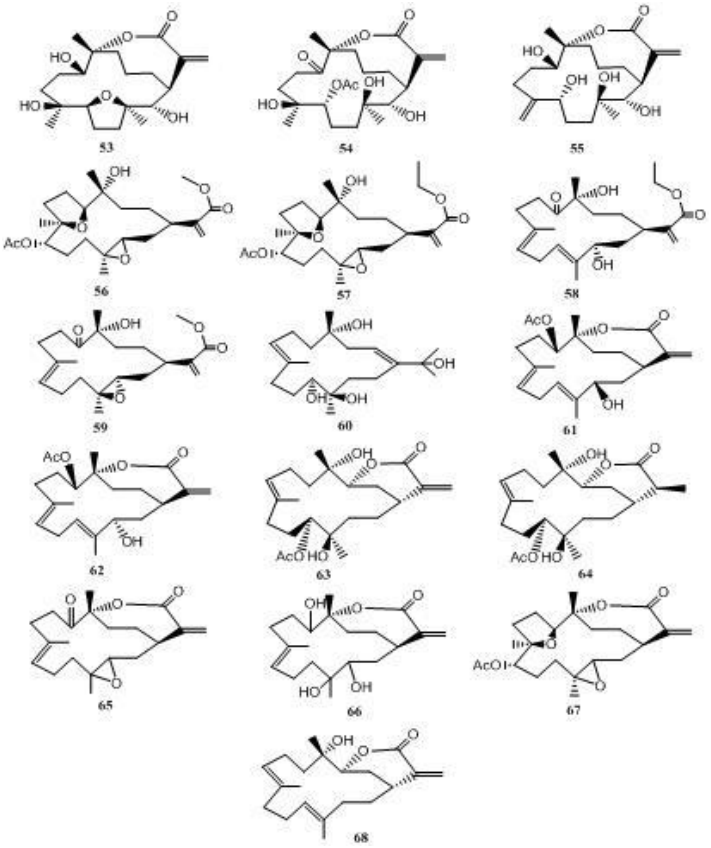


Figure 7: The collection of cembranoid-type diterpenes (53-68) isolated from the Chinese *S. flexibilis.*

Investigation upon Taiwanese *S. flexibilis* produced four cembrane-type diterpenes, flexibilins A (69), B (76), C (71) and D (72) (Fig. 8) along with sandensolide (Hu *et al.*, 2013). This same group of researchers was also successful in isolating flexibilisolides A (73) and B (74), flexibilisins A (75) and B (76) together with several other pre-isolated compounds. Flexibilisin C (77) was later isolated alongside 11,12-secoflexibilin (78). The analogue of flexibilisolides and flexibilisin is distingushable by the presence of acetate instead of hydroxyl moiety in the latter's structure. As a continuation of chemical analysis conducted on this specimen, Shih and co-workers (2012) further isolated flexibilisolides C (79) - G (83) (Fig. 8). *Sinularia flexibilis* was also reported to be a source for flexilarin where a total of 10 cembranoid diterpenes flexilarins A (84) - J (93) (Fig. 9) were isolated.

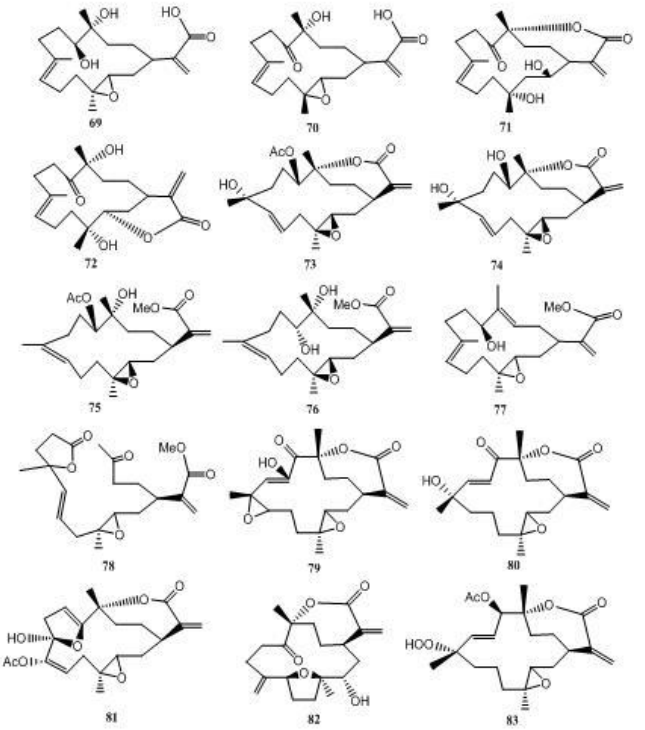


Figure 8: Chemical structures of flexibilin (69-72), flexibilisin (75-77) and flexibilisolide (73-83) derivatives from Taiwanese *S. flexibilis.*

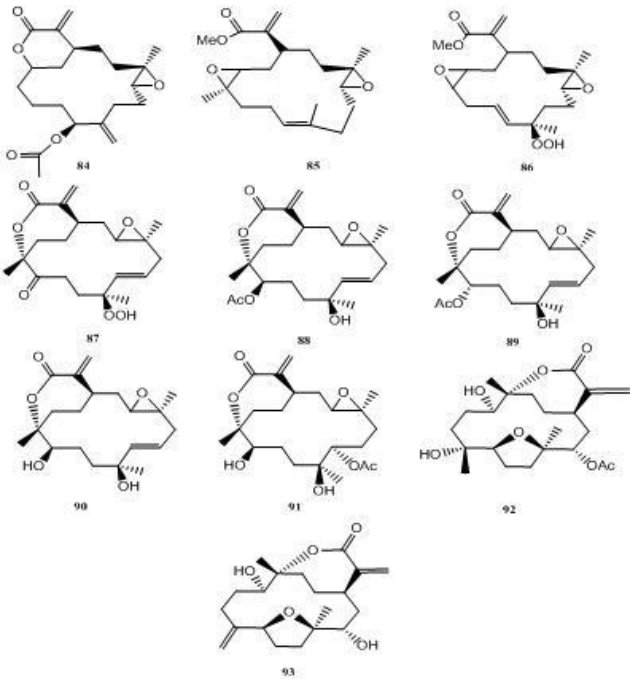


Figure 9: Diversity of flexilarins A (84) - J (93) from the Taiwanese *S. flexibilis.*

Apart from cembrane-type diterpenes, Japanese researchers successfully isolated lobane-type diterpenes; lobatrienol (94) and lobatriene (95) from the specimens from Okinawa (Hamada *et al.,* 1992). The relative and absolute stereochemistry of lobatriene (95) was determined *via* a modified Mosher method (Kusumi *et al.,* 1992). The cultured *S. flexibilis* from Taiwan was found to produce a quinone derivative, named flexibilisquinone (96), which possessed anti-inflammatory properties. However, it is still debatable whether the bioactive compound isolated is produced as a chemical adaptation in a cultured environment. The production of certain metabolite could possibly be a natural response of the organism to survive the environmental stress to sustain the evolution of species (Coll & Sammarco, 1992). Apart from this, a cytotoxic cladiellene-type diterpene named alcyonin (97) was also reported from *S. flexibilis* of the Okinawan waters along with litophynin A (98) and cladiellin (99) (Kusumi *et al.,* 1988). Lithophynin A (98) was previously reported from the soft coral, *Litophyton* sp. (Ochi *et al.,* 1987). This was the only record of cladiellene-type metabolite from this species of soft coral up to 2013. Structures of these compounds are shown in Figure 10.

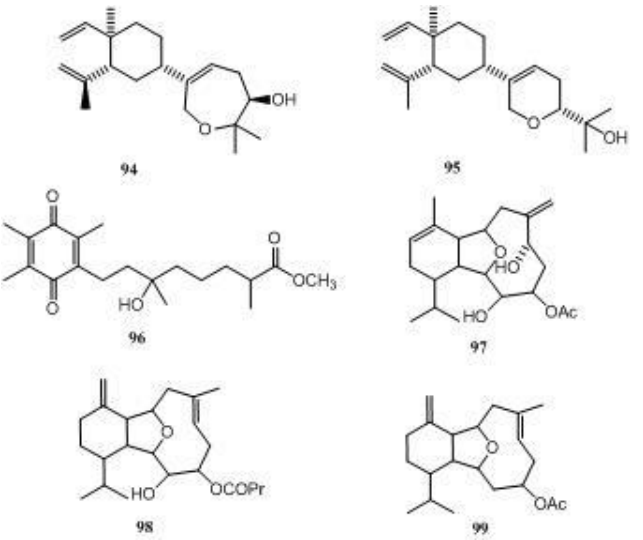


Figure 10: Chemical skeletons of lobane (94-95), quinine (96) and cladiellene (97-99) type diterpenes.

Since 2013, eight additional diterpenes were reported from the *S. flexibilis* of South China Sea. In 2014, Palaniveloo and Vairappan reported the isolation of ten cembrane-type diterpene from a single specimen from the waters of Kota Kinabalu, Malaysia. However, all the reported metabolites were known analogues. In a separate investigation, the *S. flexibilis* from Malaysia also contained two isopropyl (ene)-type cembrene diterpenes. These metabolites, (3S,4S,11S,12S,1E,7E)-3,4:11,12-bisepoxycembra-1,7-diene (100) and (1E,3E,7E)-11,12-epoxycembra-1,3,7-triene (101) were isolated and elucidated based on 1D and 2D-NMR spectroscopic measurement. Tsai *et al.* (2015), reported the isolation of five new diterpenes from the soft coral *S. flexibilis* and *Sinularia sandensis*, marking a total of six new records for *S. flexibilis.* The diterpenes diepoxycembrene B (102), dihydromanaarenolide I (103) and isosinulaflexiolide K (104) were isolated along with 3,4:811-bisepoxysinuacetoxycembra-15(17)-en-1,12-olide (105), dendronpholide F (106) and dendronpholide G (107). On the other hand, (Chen *et al.,* 2015) reported the isolation of eight new metabolites comprising of six α-methylene-δ-lactone-bearing cembranoids, a 15-membered macrocyclic diterpenoid, and a biscembranoid. The six

α-methylene-δ-lactone-bearing cembranoids reported were 9α-hydroxy-flexibilede (108), 15(17)-dehydromanaarenolide (109), 8-dehydroxy- 15(17)-dehydromanaarenolide E (110), 15, 17-dedihydromanaarenolide A (111), 15,17-dedihydromanaarenolide C (112), epi-flexilarin A (113), epoxyflexibilene (114) and sinulaflexiolide L (115). The isolation of epoxyflexibilene (114) represents the second 15-membered macrocyclic diterpenoid being discovered from a marine source, whereas sinulaflexiolide L (115) is the third member of the extremely rare cembrane dimers connected through C–C single bond (Chen *et al.,* 2015). The chemical structures of new metabolites reported from *S. flexibilis* throughout 2014 to the present is highlighted in Figure 11.

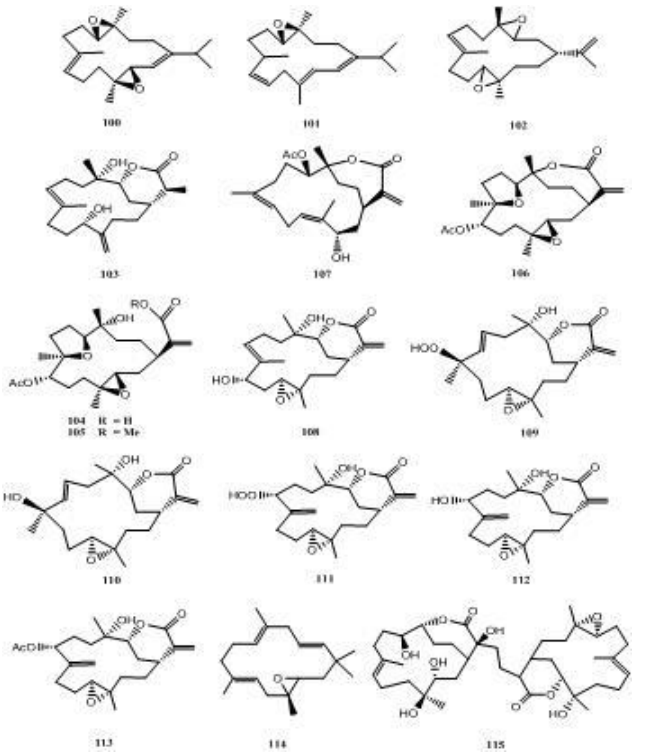
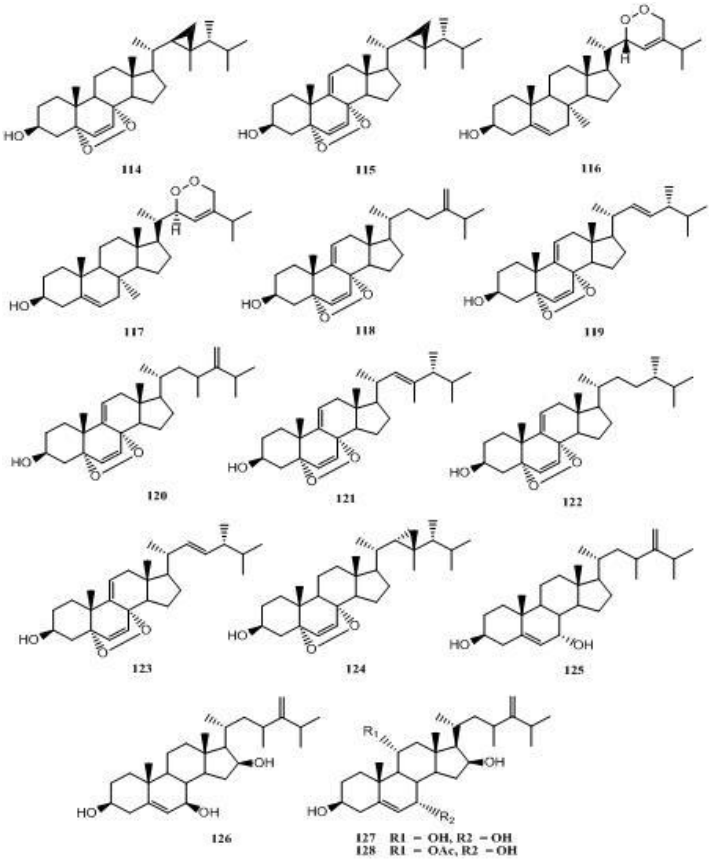


Figure 11: Chemical structures of recent compounds from *S. flexibilis.*

Sterols

Soft corals synthesize a wide range of sesquiterpenes and diterpenes that are crucial for the survival of these organisms in harsh marine environment. However, sterols are also equally vital for the development of neural system of these soft-bodied invertebrates. Sterols are metabolically active compounds and biosynthesis of these chemicals is important and could cause major disorder when a mismatch occurs in the production and physiological needs (Sarma *et al.,* 2009). Among the earliest records, a total of four sterols, 5α,8α-epidioxygorgosta-6-en-3β-ol (114), 5α,8α-epidioxygorgosta-6,9(11)-dien-3β-ol (115), 22α,28-epidioxycholesta-5,23(E)-dien-3β-ol (116) and 22β,28-epidioxycholesta-5,23(E)-dien-3β-ol (117) (Yu *et al.,* 2006) and ergosterol peroxide (118) isolated from the Chinese *S. flexibilis* were also found in marine sponges (Arepalli *et al.,* 2009). This unique group of sterol, 5,8-epidioxy-24-methylcholesta-6,24(28)-dien-3β-ol (119), 5,8-epidioxy-24-methylcholesta-6,9(11),24(28)-trien-3β-ol (120), 5,8-epidioxy-24-methylcholesta-6,9(11),22-trien-3β-ol (121) from the terrestrial mycorrhizal species, *Rhizoctonia repens* (Gunatilaka *et al.,* 1981)

and 5,8-epidioxy-24-methylcholesta-6-en-3β-ol (122) and 5,8-epidioxy-23,24-dimethylcholesta-6,22-dien-3β-ol (123) were previously isolated from an edible mushroom *Grifola frondosa* (Yaoita *et al.,* 1998) were also found in *S. flexibilis.* However, 5,8-epidioxy-22,23-methylene-24-methylcholesta-6-en-3β-ol (124) and 24-methylcholesta-5,24(28)-dien-3β-ol (125) were reported from an unidentified *Sinularia* species (Venkateswarlu *et al.,* 1999) and *Cladiella* species. Luo and co-workers (2007), reported the isolation of 5,8-epidioxy-24-methylcholesta-6,9(11),24(28)-trien-3β-ol (120), 5,8-epidioxy-24-methylcholesta-6,9(11),22-trien-3β-ol (121) and 24-methylcholesta-5,24(28)-diene-3,7-diol (126), which were originally reported from the bark of *Amoora yunnanensis* were found in the Taiwanese *S. flexibilis.* Finally, the most recent additions in the list of sterols of *S. flexibilis* is the isolation of two new polyhydroxylated sterols 7a-hydroxy-crassarosterol A (127) and 11-acetoxy-7a-hydroxy-crassarosterol A (128) (Chen *et al.,* 2015). Figure 12 shows the chemical structures of sterols isolated from *S. flexibilis.*



Figures 12: Chemical structures of sterols (114-128) isolated from S. flexibilis.

Biological properties of *Sinularia flexibilis*

Investigation upon biological potentials of metabolites isolated from this soft coral is still limited compared to other marine organisms. Nevertheless, a number of compounds isolated from *S. flexibilis* are reported to be biologically active. Sinulariolide (24) was reported to display antifouling properties and cytotoxicity against cancer cell lines (Tursch *et al.,* 1975; Wang *et al.,* 2017). Its derivative, 11-*epi*-sinulariolide acetate (27) inhibited iNOS and COX-2 protein of LPS stimulated on RAW264.7 macrophage

cells (Lin *et al.,* 2013; Hsu *et al.,* 2013). On the other hand, extract of cultured *S. flexibilis* revealed anti-migration, invasion effects and isolated 11-epi-sinulariolide acetate (27) displays toxicity towards HA22T cells. It was discovered that 11-*epi*-sinulariolide acetate (27) displayed a concentration-dependent inhibitory effect on the migration of human HCC HA22T (hepatocellular carcinoma) cells (Wu *et al.,* 2015). This compound suppressed protein levels of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9) and urokinase-type plasminogen activator (uPA) in HA22T cells. Introduction of the cembrane-type diterpene also marks tissue inhibition of metalloproteinase-1 (TIMP-1) and metalloproteinase-2 (TIMP-2) in a concentration-dependent manner, suppression of phosphorylation of ERK1/2 and p38MAPK leading to successful suppression of the phosphorylation of FAK/PI3K/AKT/mTOR pathways (Lin *et al.,* 2014).

Sinularin (39) and dihydrosinularin (40) were revised and renamed as flexibilide (39) and dihydroflexibilide (40) by Weinheimer *et al.* (1977). The compounds flexibilide and dihydroflexibilide were reported to display anti-inflammatory and antiarthritic potential as well as antimicrobial properties against the Gram-positive *Bacillus subtilis* and *Staphylococcus aureus* (Buckle *et al.,* 1980). On the contrary, 11-*epi*-sinulariolide (26) and its acetate (27) only displayed mild inhibition towards these bacteria. Meanwhile, Flexibilide (39), dihydroflexibilide (40) and sinulariolide (24) were found to exhibit vascular contractions when tested against rat cardiac muscles (Aceret *et al.,* 1998).

The crude extracts of *S. flexibilis* were also reported to display cytotoxicity towards A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), KB (human epidermoid carcinoma) and P-388 (mouse lymphocytic leukaemia) cell lines. Bioassay-guided isolation of cembranoid-type diterpenes, sinuflexolide (47), dihydrosinuflexolide (48) and sinuflexibilin (49) (Duh *et al.,* 1998) displayed potential as anticancer agents except dihydrosinuflexolide (48) exhibited mild potency against the P-388 (murine leukemia) cell line. Sinuflexin (50), a bicembranoid isolated inhibits the P-388 cell line at the IC_{50} value of 1.32 µg/ml. Meanwhile, sinulaflexiolides B (53) - K (62) displayed inhibitory effects against HL-60 (human promyelocytic leukemia cells) at 87%, HeLa (cervical carcinoma) at 92%, BGC-823 (human gastric carcinoma) at 93%, Bel-7402 (human hepatocarcinoma) at 52%, PC-3M-IE8 (human prostate carcinoma) at 83% and Hep-2 (human epithelial type-2 carcinoma) with 83% cell inhibition, constantly at a concentration of 100 µg/ml (Wen *et al.,* 2008; Guo *et al.,* 2016).

The quinone-type diterpene isolated from Taiwanese S. flexibilis displayed anti-inflammatory properties. Sharing the similar chemical skeleton with diferrent functional moiety, flexibilisquinone (96) was able to inhibit the accumulation of iNOS and COX-2 protein of LPS stimulated RAW264.7 macrophage cells (Lin *et al.,* 2013). In addition, the cembranoid diterpenes from the same specimen, flexilarin D (87) displayed an extremely impressive 50% inhibition of Hep-2 cell line as compared to the commercial anti-bladder cancer drug, Mitomycin C at 0.07 µg/mL. This compound was also found to suppress the proliferation of HeLa, DAOY (medullablasto carcinoma) and MCF-7 (human breast adenocarcinoma) cells by requiring 0.41, 1.24, 1.24 µg/mL to inhibit 50% of the cell lines, respectively (Lin *et al.,* 2009). This same group of researchers evaluated cytotoxicity of flexibilisolides A (73), B (74), flexibilisins A (75) and B (76), where all of these compounds exhibited cytotoxicity towards the same set of cell lines with IC_{50} values of less than 20 µg/mL, respectively (Dunlop & Well, 1979).

Flexibilisolide C (79) together with several known compounds, 11-dehydrosinulariolide (25), 11-*epi*-sinulariolide (26), 11-epoxysinulariolide (28) and 14-deoxycrassin (68) were reported to display cytotoxicity of 50% cell inhibition at a concentration of less then 15 µg/mL against HeLa and B16 cell lines as well as reduction of LPS induced protein, iNOS and COX- 2 in RAW 264.7 macrophage cells (Shih *et al.,*

2012) whereas thioflexibilolide A (52) was found to reduce LPS stimulated protein, iNOS in macrophage cells. Similarly, the compound 3,4:8,11-bisepoxysinuacetoxycembra-15(17)-en-1,12-olide (103) was reported to suppress the production of the iNOS and COX-2 pro-inflammatory cytokines (Tsai *et al.,* 2015). Thioflexibilolide A (52) displayed neuroprotective activity at 0.01 µM with values of 73.2% suggesting it to have therapeutic potential for neurodegenerative disease (Chen *et al.,* 2010). The widely studied sterol from *S. flexibilis*, ergosterol peroxide (118) is known to exhibit a range of biological potential including as immunosuppressive, anti-inflammatory, antiviral, antiplasmodial and antitumor properties (Wang *et al.,* 2004). In addition to that, the polyhydroxylated sterol 7a-hydroxy- crassarosterol A (127) exhibited a moderate protein tyrosine phosphatase 1B (PTP1B) inhibitory activity with an IC_{50} value of 33.05 µM while 11-acetoxy-7a-hydroxy-crassarosterol A (128) displayed weak in vitro cytotoxicities against the tumor cell lines K562 (myelogenous leukemia) and HL-60 (Chen *et al.,* 2014; Mayer *et al.,* 2003). The summary of compound bioactivity from *S. flexibilis* is displayed in Table 1.

Table 1: Highlights of bioactivities displayed by metabolites isolated from *S. flexibilis* globally.

Carcinoma cells	Metabolites	IC 50 Activity
A549 (human lung cell)	Crude extracts	-
HT-29 (human colon cell)	Crude extracts	-
KB (human epidermoid cell)	Crude extracts	-
P388	Crude extracts	-
(mouse lymphocytic leukaemia)	Sinuflexolide	-
	dihydrosinuflexolide	-
	Sinuflexibilin	-
	Sinuflexin	50 % at 1.32 µg/ml
HL-60	sinulaflexiolides B - K	87 % at 100 µg/ml
(human promyelocytic leukemia)	flexilarin D	50 % at 0.41 µg/ml
	flexibilisolides A	-
	flexibilisolides B	-
	flexibilisins A	50 % at < 20 µg/ml
	flexibilisins B	-
HeLa (cervical carcinoma)	sinulaflexiolides B – K	92 % at 100 µg/ml
	Flexibilisolide C	-
	11-dehydrosinulariolide	-
	11-*epi*-sinulariolide	50 % at < 15 µg/ml
	11-epoxysinulariolide	-
	14-deoxycrassin	-
BGC-823 (human gastric cell)	sinulaflexiolides B – K	93 % at 100 µg/ml
Bel-7402 (human hepatocarcinoma)	sinulaflexiolides B – K	52 % at 100 µg/ml
PC-3M-IE8 (human prostate cell)	sinulaflexiolides B – K	83 % at 100 µg/ml
Hep-2 (human epithelial type-2 cell)	sinulaflexiolides B – K	83 % at 100 µg/ml
	flexilarin D	50 % at 0.07 µg/ml

Carcinoma cells	Metabolites	IC 50 Activity
	flexibilisolides A	-
	flexibilisolides B	-
	flexibilisins A	50 % at < 20 µg/ml
	flexibilisins B	-
DAOY (medullablasto cell)	flexilarin D	50 % at 1.24 µg/ml
	flexibilisolides A	-
	flexibilisolides B	-
	flexibilisins A	50 % at < 20 µg/ml
	flexibilisins B	-
MCF-7 (human breast cell)	flexilarin D	50 % at 1.24 µg/ml
	flexibilisolides A	-
	flexibilisolides B	-
	flexibilisins A	50 % at < 20 µg/ml
	flexibilisins B	-
B16 (melanoma cell)	Flexibilisolide C	-
	11-dehydrosinulariolide	-
	11-epi-sinulariolide	50 % at < 15 µg/ml
	11-epoxysinulariolide	-
	14-deoxycrassin	-
RAW264.7 macrophage cells	flexibilisquinone	-
	Flexibilisolide C	-
	11-dehydrosinulariolide	iNOS and COX-2
	11-*epi*-sinulariolide	-
	11-epoxysinulariolide	-
	14-deoxycrassin	-
	3,4:8,11-bisepoxysinuacetoxy-cembra-15(17)-en-1,12-olide	-
	thioflexibilolide A	iNOS
Neuroprotective activity	thioflexibilolide A	73.2 % at 0.01 µM
PTP1B	7α-hydroxy-crassarosterol A	50 % at 33.05 µM

Conclusion

The diversity of chemical analogues and the discovery of new metabolites from marine organisms are signs that these organisms adapt to survive in extreme environments. Along with these adaptations, more diversified metabolites with a wide array of bioactive potentials are produced. As of today, quite a handful of secondary metabolites originating from the marine environment were obtained from marine invertebrates. Sea slug, tunicates, sponges are amongst the most active contributors. Hence, this review paper is aimed to discuss about the Alcyonaria chemistry that has been rapidly developed over the past 25 years and is still progressing. With the diverse chemical scaffolds isolated, the biological and pharmacological properties associated with these soft corals, particularly, terpenoids is highly promising and excites further investigations. Cembranoid diterpenes isolated from *S. flexibilis* are known

to exhibit impressive cytotoxic, antitumor and anti-inflammatory activities. In depth investigation on the differential activity of these metabolites, their mechanisms of action as well as structure-related activity of marine-derived metabolites are extremely necessary to develop potent pharmaceuticals for mankind. More future research in these aspects should be considered. Recent hybridization and indoor cultivation of soft corals could also contribute to chemical variation as compared to naturally growing *S. flexibilis* in the marine environment.

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