Sains Malaysiana 47(2)(2018): 319-322
http://dx.doi.org/10.17576/jsm-2018-4702-14

Chabrolene, a Novel Norditerpene from the Bornean Soft Coral *Nephthea* sp.

(Chabrolene, Sebatian Novel Norditerpene daripada Karang Lembut Bornean *Nephthea* sp.)

TAKAHIRO ISHII, TAKASHI KAMADA, CHIN-SOON PHAN & CHARLES S. VAIRAPPAN*

ABSTRACT

Soft corals are often the research interest organism due to their structural diverse secondary metabolites with wide spectrum of biological activities range from antibacterial to anticancer potentials. The Borneo Island is rich in diversity of marine organisms including soft coral. Therefore, a population of Bornean soft coral belongs to genus Nephthea *was collected from Mantanani Island (Sabah, Malaysia) which led to the isolation of a novel norditerpene, chabrolene* **(1)** *together with three known compounds* **(2-4)**. *The chemical structure of 1 was determined by NMR and HREIMS data. Compound* **1** *exhibited repellent activity against the maize weevil* Sitophilus zeamais.

Keywords: Nephthea *sp.; norditerpene; repellent activity;* Sitophilus zeamais*; soft coral*

ABSTRAK

Karang lembut sering dikenali sebagai organisma yang penting dalam penyelidikan disebabkan oleh metabolit sekunder yang pelbagai struktur. Selain itu, ia juga mempunyai pelbagai aktiviti biologi terdiri daripada potensi antibakteria hingga anti-kanser. Pulau Borneo kaya dengan kepelbagaian organisma marin termasuk karang lembut. Oleh itu, satu populasi karang lembut Bornean genus Nephthea *dikumpulkan dari Pulau Mantanani (Sabah, Malaysia) dan mendapati satu norditerpene baru, chabrolene* **(1)** *dan tiga sebatian yang telah dikenal pasti* **(2-4)**. *Struktur kimia untuk keempat-empat sebatian ini dikenal pasti berdasarkan analisis data spektroskopi NMR dan HREIMS. Sebatian* **1** *didapati mempunyai aktiviti penolak terhadap kutu jagung* Sitophilus zeamais.

Kata kunci: Aktiviti penolak; karang lembut; Nephthea *sp.; norditerpene;* Sitophilus zeamais

INTRODUCTION

Soft corals are well known to be a rich source of secondary metabolites with a variety of chemical structures and biological activities (Amir et al. 2012a, 2012b; Cheng et al. 2009; Hu et al. 2011; Wang et al. 2013) and they are widely distributed in the tropical waters of Sabah, Malaysia. Cembranoids and their cyclized derivatives have been frequently found in soft corals (Chen et al. 2012; Tello et al. 2009; Zhang et al. 2001; Zhao et al. 2013). Our previous studies on the chemical constituents from the Bornean soft coral genus *Nephthea* have discovered a series of interesting metabolites including sterols (Ishii et al. 2010a, 2009a), sesquiterpenes (Ishii et al. 2010a, 2009b) and cembrane diterpenes (Ishii et al. 2010b). As part of our continuing search for other types of metabolites from this genus, we have further isolated a novel 14-membered ring norditerpene, chabrolene **(1)**, along with two known cembranoids, nephthenol **(2)** (Januar et al. 2010; Schmitz et al. 1974; Zhang et al. 2001) and 2-hydroxynephthenol **(3)** (Januar et al. 2010; Zhang et al. 2001) and a known 4α-methyl sterol, 4α-methyl-3β,8β-dihydroxy-5α-ergost-24(28)-en-23-one **(4)** (Bortolotto et al. 1977; Cheng et al. 2007). The isolation and structure elucidation of these compounds are discussed herein. Meanwhile, the maize weevil *Sitophilus zeamais* is a species of beetle belong to family Curculionidae that usually found in tropical habitats around the world. They have been known to be a major pest of maize as well as wheat, rice, oats and barley. This led to loss of agriculture revenues. Therefore, the isolated compounds were evaluated for repellent activity against the maize weevil, *S. zeamais*.

MATERIALS AND METHODS

BIOLOGICAL MATERIALS

The specimen of *Nephthea* sp. was collected from Mantanani Island, Sabah (6°70’245’’N, 116°31’930’’E). The voucher specimen (MAR37789BOR) was deposited in the BORNEENSIS Collection of Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

EXTRACTION AND ISOLATION

The fresh soft coral (891 g wet wt.) was extracted with MeOH at room temperature (24°C) for 5 days. The crude extract was evaporated under reduced pressure and the residue was partitioned between EtOAc/H_2O. The EtOAc layer was further partitioned with *n*-hexane/90% aqueous MeOH. The *n*-hexane extract (1.20 g) was chromatographed on a silica gel column using gradient of *n*-hexane/EtOAc in an order of increasing polarity as eluent to yield five fractions. A total of 24.0 mg of fraction 2 eluted with *n*-hexane/EtOAc (8:2) was submitted to repeated preparative TLC with $CHCl_3$ and toluene to give compounds **1** (5.6 mg) and **2** (8.9 mg). On the other hand, the 90% methanol extract (1.20 g) was also subjected to a silica gel column eluted with gradient solvent system of *n*-hexane and EtOAc to afford five fractions. A portion of fraction 3 (24.1 mg) eluted with *n*-hexane/EtOAc (7:3) was separated by preparative TLC with *n*-hexane/EtOAc (3:1) to yield compound **4** (9.6 mg). Fraction 4 (20.0 mg) eluted with *n*- hexane/EtOAc (1:1) was purified by preparative TLC with *n*-hexane/EtOAc (65:35) to yield compound **3** (14.9 mg).

REPELLENT ACTIVITY

The repellent activities of all isolated compounds against the maize weevils *Sitophilus zeamais* were evaluated using the filter paper impregnation method, as previously described (Ishii et al. 2010c). The numbers of adult beetle present in each Petri dish were recorded after 24 h of exposure. Each treatment was repeated three times. The range of concentrations were 250, 125, 100, 50, 25, 12.5, 6.3, 3.1 and 1.6 μg/cm^2. Pyrethrin standard was used as positive control.

RESULTS AND DISCUSSION

Chabrolene (**1**) was obtained as a colorless oil, $[\alpha]_D^{28}$ –7.0 (*c* 1.3, $CHCl_3$). The molecular formula of **1** was elucidated as $C_{17}H_{26}$ with five degrees of unsaturation by HR-EI-MS [*m/z* 230.2036 $[M]^+$ (calcd. for $C_{17}H_{26}$, 230.2035)]. The ^{1}H and ^{13}C NMR spectra of 1 are summarized in Table 1. The ^{13}C NMR, DEPT-135 and HSQC data showed the presence of three vinyl methyl (δ_C 16.0, 15.5, and 14.8), six sp^3 methylene (δ_C 40.4, 39.6, 39.1, 29.4, 26.0, and 25.3), five sp^2 methine (δ_C 131.9, 128.2, 127.7, 127.6, and 124.4) and three quaternary olefinic carbons (δ_C 135.2, 132.6, and 131.8). The above functionalities could account for four of the five degrees of unsaturation, suggesting a monocyclic structure in **1**.

TABLE 1. ^{1}H and ^{13}C NMR data of **1** (600 and 150 MHz in $CDCl_3$, δ in ppm, *J* in Hz)

Position	δ_C	δ_H
1	131.9 (CH)	5.24 (1H, *dt*, *J* = 15.0, 7.2)
2	127.6 (CH)	5.91 (1H, *dd*, *J* = 15.0, 10.8)
3	127.7 (CH)	5.58 (1H, *d*, *J* = 10.8)
4	132.6 (C)	
5	39.1 (CH_2)	2.08 (2H, *m*)
6	26.0 (CH_2)	2.14 (2H, *m*)
7	124.4 (CH)	4.85 (1H, *t*, *J* = 7.2)
8	135.2 (C)	
9	39.6 (CH_2)	2.13 (2H, *m*)
10	25.3 (CH_2)	2.14 (2H, *m*)
11	128.2 (CH)	4.79 (1H, *brs*)
12	131.8 (C)	
13	40.4 (CH_2)	2.04 (2H, *m*)
14	29.4 (CH_2)	2.12 (2H, *m*)
15	16.0 (CH_3)	1.62 (3H, *s*)
16	14.8 (CH_3)	1.46 (3H, *s*)
17	15.5 (CH_3)	1.49 (3H, *s*)

The planar structure for **1** could be assigned by the ^{1}H-^{1}H COSY and HMBC spectra as shown in Figure 2. On the basis of the ^{1}H-^{1}H COSY spectrum, three consecutive spin systems, H_2-14/H-1/H-2/H-3/H_3-15, H_2-6/H-7/H_3-16 and H_2-10/H-11/H_3-17 were established, as well as the interpretation of long range correlations were observed between H-3/H_3-15, H-7/H_3-16 and H-11/H_3-17. However, the ^{1}H-^{1}H COSY experiment provided limited information due to severe overlapping of methylene signals. Despite that, the 14-membered ring was connected based on HMBC correlations from H_3-15 to C-3, C-4 and C-5; H_3-16 to C-7, C-8 and C-9; H_3-17 to C-11, C-12 and C-13; H_2-5 to C-6 and C-7; H_2-9 to C-10; and H_2-13 to C-1 and C-14. On the basis of these findings, the planar structure of **1** was established unambiguously (Figure 1).

The geometries of the four olefins in **1** were deduced from the coupling constant in the ^{1}H NMR spectrum and the ^{13}C NMR chemical shifts as well as the NOESY experiment. The *E*-configuration for the double bond between C-1 and C-2 was established by the characteristic coupling constant (*J* = 15.0 Hz). The ^{13}C NMR chemical shifts of three exocyclic vinyl methyls [C-15 (δ_C 16.0), C-16 (δ_C 14.8), and C-17 (δ_C 15.5)] suggested that all three double bonds had the *E* configurations (Blackman et al. 1982; Kamada et al. 2016). Furthermore, the lack of NOESY correlations between H-3/H_3-15, H-7/H_3-16, and H-11/H_3-17 supported this deduction. Therefore, compound **1** was identified as (1*E*,3*E*,7*E*,11*E*)-1,7,11-trimethylcyclotetradeca-1,3,7,11-tetraene with a trivial name as chabrolene. The structures of the compounds **2-4** were identified by the comparison of their spectral data with those reported in the literature (Cheng et al. 2007; Schmitz et al. 1974; Zhang et al. 2001).

The *Sitophilus zeamais* is a serious pest of stored grains worldwide. The repeated use of insecticides has led to pest resurgence and resistance, lethal effect on non-target organisms, the risk of users' contamination, food residues, and environmental pollution (Chu et al. 2012; Moreira et al. 2007). All compounds were evaluated for repellent activity against *S. zeamais* by a modified filter paper impregnation method, as previously described (Ishii et al. 2010c). Compound **1** showed repellent activity at 25 μg/cm^2, however, the remaining compounds were all inactive at 250 μg/cm^2. Some of metabolites in soft corals were reported to have a wide range of biological activities such as antibacterial, antifungal, antifouling and insecticidal activities, and play an important role in the protection from microorganisms, fishes and animals (Chen et al. 2012; Hu et al. 2011; Lai et al. 2013; Núñez-Pons et al. 2013; Zhang et al. 2013). Similar insecticidal activity was also reported by Handayani et al. (1997) from oxygenated sesquiterpene (hydroxycolorenone)

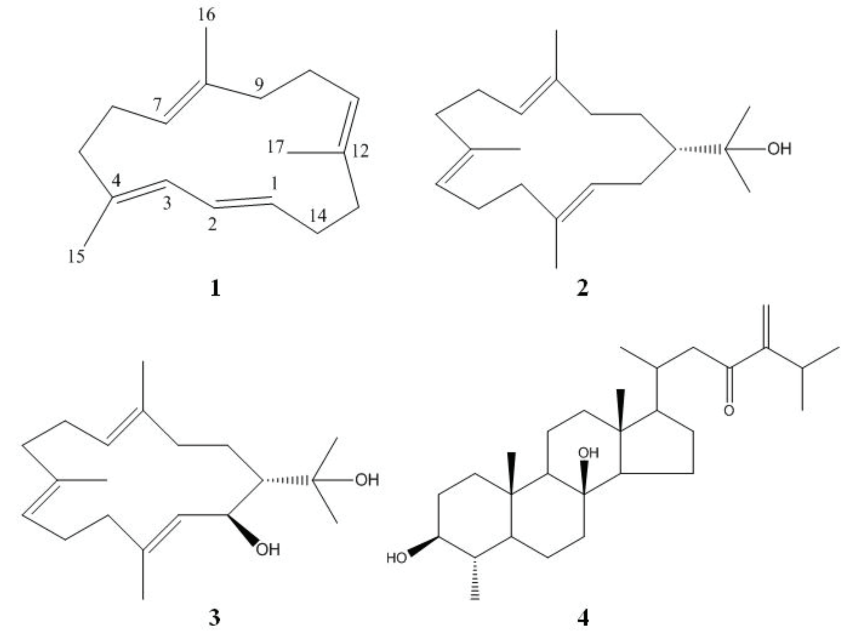

FIGURE 1. Structures of compounds 1-4

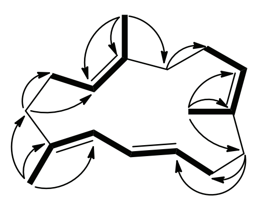

FIGURE 2. ^{1}H-^{1}H COSY correlations (▬) and the selected HMBC correlations (→) of **1**

isolated from the Indonesian soft coral *N. chabrolii* towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis*. In addition, compound **1** could also be considered to have the potential to act as a chemical defensive substance.

To the best of our knowledge, C17 compound with 14-membered cyclic tetraene is very rare natural product. Only one analog compound, 3,7,11-trimethyl-1,3,6,10-cyclotetradecatetraene was previously obtained from the essential oil of roasted tobacco (Hong et al. 1992). In addition, another C17 norditerpene, chabrolol A possessing trimethyl cyclotetradecatriene ring system was isolated from *Nephthea chabrolii* (Zhang et al. 2001). Structure of compound **1** was very similar to chabrolol A, in which the hydroxyl group at C-1 and C-4 of chabrolol A was eliminated in **1** (Zhang et al. 2001). This is the second report in discovery of C17 norditerpene with 14-membered ring from the marine organisms. These findings suggested that this type of compound seem to be one of characteristic metabolites in the soft coral genus *Nephthea*.

Soft corals have been well recognized as a rich source of a variety of bioactive secondary metabolites. In this paper, a new norditerpene, chabrolene (**1**) was isolated from *Nephthea* sp. collected from Mantanani Island, Sabah, along with three known compounds, nephthenol (**2**), 2-hydroxynephthenol (**3**), and 4α-methyl-3*β*,8*β*-dihydroxy-5α-ergost-24(28)-en-23-one (4). Their structures were established on the basis of spectral analysis. Compound **1** showed repellent activity against the maize weevil, *Sitophilus zeamais*, at a concentration as low as 25 μg/cm^2. To our knowledge, this is the second report on the presence of C17 compound with 14-membered ring in the marine organisms. The results of this study will lead us to find further unusual secondary metabolites in Bornean soft corals.

ACKNOWLEDGEMENTS

This work was supported by the Sabah Biodiversity Centre [GL0070] and Fujiwara Natural History Foundation. The authors are grateful to the Sabah Park for the support and assistance during field survey. Thanks are also due to Mr. Takafumi Ishikawa, Hokkaido University, for his kind assistance during data comparison.

REFERENCES

Amir, F., Koay, Y.C. & Yam, W.S. 2012a. Chemical constituents and biological properties of the marine soft coral *Nephthea*: A Review (Part 1). *Tropical Journal of Pharmaceutical Research* 11(3): 485-498.

Amir, F., Koay, Y.C. & Yam, W.S. 2012b. Chemical constituents and biological properties of the marine soft coral *Nephthea*: A Review (Part 2). *Tropical Journal of Pharmaceutical Research* 11(3): 499-517.

Blackman, A.J., Bowden, B.F., Coll, J.C., Frick, B., Mahendran, M. & Mitchell, S.J. 1982. Studies of Australian soft corals. XXIX several new cembranoid diterpenes from *Nephthea brassica* and related diterpenes from a *Sarcophyton* species. *Australian Journal of Chemistry* 35(9): 1873-1880.

Bortolotto, M., Braekman, J.C., Daloze, D. & Tursch, B. 1977. Chemical studies of marine invertebrates. XXIX. 4α-methyl-3β,8β-dihydroxy-5α-ergost-24(28)-en-23-one, a novel polyoxygenated sterol from the soft coral *Litophyton viridis* (Coelenterata, Octocorallia, Alcyonacea). *Steroids* 30(2): 159-164.

Chen, W.T., Li, Y. & Guo, Y.W. 2012. Terpenoids of *Sinularia* soft corals: Chemistry and bioactivity. *Acta Pharmaceutica Sinica B* 2(3): 227-237.

Cheng, S.Y., Dai, C.F. & Duh, C.Y. 2007. New 4-methylated and 19-oxygenated steroids from the Formosan soft coral *Nephthea erecta*. *Steroids* 72(8): 653-659.

Cheng, S.Y., Huang, K.J., Wang, S.K., Wen, Z.H., Hsu, C.H., Dai, C.F. & Duh, C.Y. 2009. New terpenoids from the soft corals *Sinularia capillosa* and *Nephthea chabroli*. *Organic Letters* 11(21): 4830-4833.

Chu, S.S., Liu, Z.L., Du, S.S. & Deng, Z.W. 2012. Chemical composition and insecticidal activity against *Stiphilus zeamais* of the essential oils derived from *Artemisia giraldii* and *Artemisia subdigitata*. *Molecules* 17(6): 7255-7265.

Handayani, D., Edrada, R.A., Proksch, P., Wray, V., Witte, L., van Ofwegen, L. & Kunzmann, A. 1997. New oxygenated sesquiterpenes from the Indonesian soft coral *Nephthea chabrolii*. *Journal of Natural Products* 60(7): 716-718.

Hong, T., Ma, Y., Sun, S. & Ma, C. 1992. Study on chemical constituents of essential oil of Henan roasted tobacco. *Journal of Instrumental Analysis* 11: 64-65.

Hu, J., Yang, B., Lin, X., Zhou, X., Yang, X., Long, L. & Liu, Y. 2011. Chemical and biological studies of soft corals of the Nephtheidae family. *Chemisty & Biodiversity* 8(6): 1011-1032.

Ishii, T., Matsuzawa, H. & Vairappan, C.S. 2010c. Repellent activity of common spices against the rice weevil, *Sitophilus zeamais* Motsch (Coleoptera, Curculionidae). *Journal of Tropical Biology and Conservation* 7: 75-80.

Ishii, T., Matsuura, H., Zhaoqi, Z. & Vairappan, C.S. 2009a. A new 4α-methylated sterol from a *Nephthea* sp. (Nephtheidae) Bornean soft coral. *Molecules* 14(9): 3360-3366.

Ishii, T., Matsuura, H., Zhaoqi, Z. & Vairappan, C.S. 2009b. A new norsesquiterpenoid from a Bornean soft coral genus *Nephthea*. *Molecules* 14(11): 4591-4596.

Ishii, T., Ueoka, R., Matsunaga, S. & Vairappan, C.S. 2010a. Bioactive secondary metabolites from the Borneon soft corals of the genus *Nephthea*. *Malaysian Journal of Science* 29(3): 262-268.

Ishii, T., Zhaoqi, Z. & Vairappan, C.S. 2010b. A new cembrane diterpene from the Bornean soft coral *Nephthea* sp. *Molecules* 15(6): 3857-3862.

Januar, H.I., Chasanah, E., Motti, C.A., Tapiolas, D.M., Liptrot, C.H. & Wright, A.D. 2010. Cytotoxic cembranes from Indonesian specimens of the soft coral *Nephthea* sp. *Marine Drugs* 8(7): 2142-2152.

Kamada, T., Phan, C.S., Tin, H.S., Vairappan, C.S. & Muhammad, T.S.T. 2016. 16-Hydroxycembra-1,3,7,11-tetraene, a new cembrane diterpene from Malaysian soft coral genus *Sarcophyton*. *Natural Product Communications* 11(8): 1077-1078.

Lai, D., Geng, Z., Deng, Z., van Ofwegen, L., Proksch, P. & Lin, W. 2013. Cembranoids from the soft coral *Sinularia rigida* with antifouling activities. *Journal of Agricultural and Food Chemistry* 61(19): 4585-4592.

Moreira, M.D., Picanço, M.C., Barbosa, L.C.A., Guedes, R.N.C., Campos, M.R., Silva, G.A. & Martins, J.C. 2007. Plant compounds insecticide activity against Coleoptera pests of stored products. *Pesquisa Agropecuária Brasileira* 42(7): 909-915.

Núñez-Pons, L., Carbone, M., Vázquez, J., Gavagnin, M. & Avila, C. 2013. Lipophilic defenses from Alcyonium soft corals of Antarctica. *Journal of Chemical Ecology* 39(5): 675-685.

Schmitz, F.J., Vanderah, D.J. & Ciereszko, L.S. 1974. Marine natural products: Nephthenol and epoxynephthenol acetate, cembrene derivatives from a soft coral. *Journal of the Chemical Society, Chemical Communication* 1974(10): 407-408.

Tello, E., Castellanos, L., Arevalo-Ferro, C. & Duque, C. 2009. Cembranoid diterpenes from the Caribbean Sea whip *Eunicea knighti*. *Journal of Natural Products* 72(9): 1595-1602.

Wang, S.K., Puu, S.Y. & Duh, C.Y. 2013. New steroids from the soft coral *Nephthea chabrolii*. *Marine Drugs* 11(2): 571-580.

Zhang, J., Li, L.C., Wang, K.L., Liao, X.J., Deng, Z. & Xu, S.H. 2013. Pentacyclic hemiacetal sterol with antifouling and cytotoxic activities from the soft coral *Nephthea* sp. *Bioorganic & Medicinal Chemistry Letters* 23(4): 1079-1082.

Zhang, W.H., Williams, I.D. & Che, C.T. 2001. Chabrolols A, B and C, three new norditerpenes from the soft coral *Nephthea chabroli*. *Tetrahedron Letters* 42(28): 4681-4685.

Zhao, M., Yin, J., Jiang, W., Ma, M., Lei, X., Xiang, Z., Dong, J., Huang, K. & Yan, P. 2013. Cytotoxic and antibacterial cembranoids from a South China Sea soft coral, *Lobophytum* sp. *Marine Drugs* 11(4): 1162-1172.

Institute for Tropical Biology and Conservation
Universiti Malaysia Sabah
88400 Kota Kinabalu, Sabah
Malaysia

*Corresponding author; email: csv@ums.edu.my

Received: 6 July 2017
Accepted: 7 September 2017