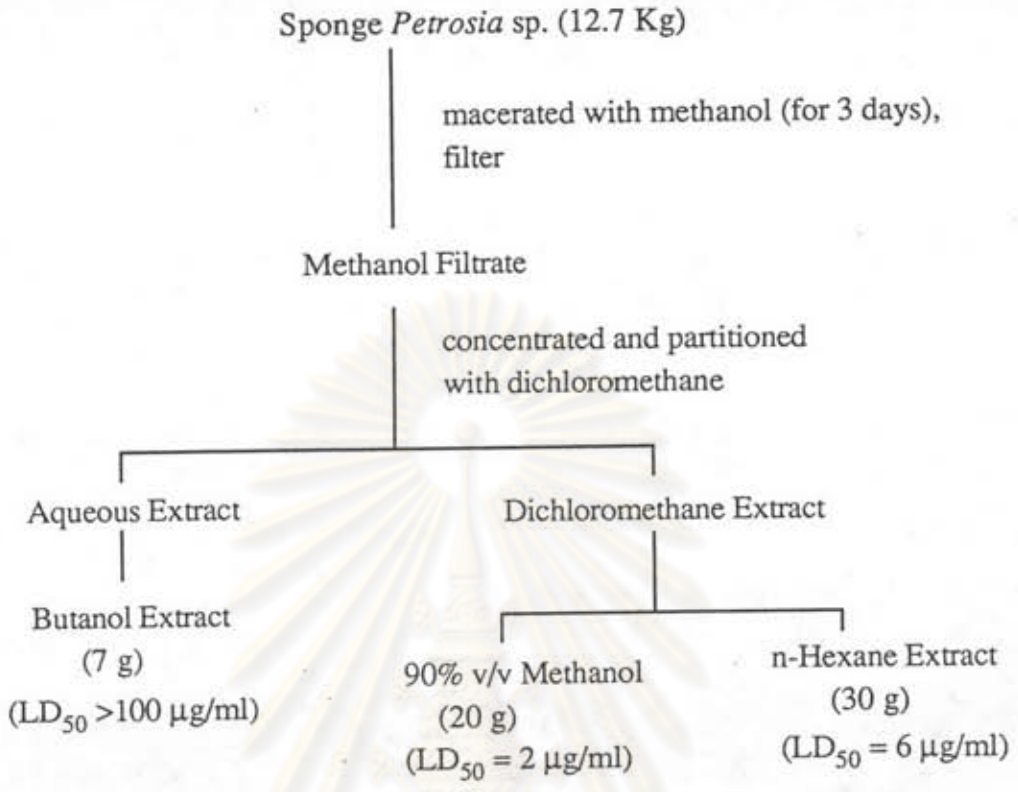
CHAPTER IV

DISCUSSION

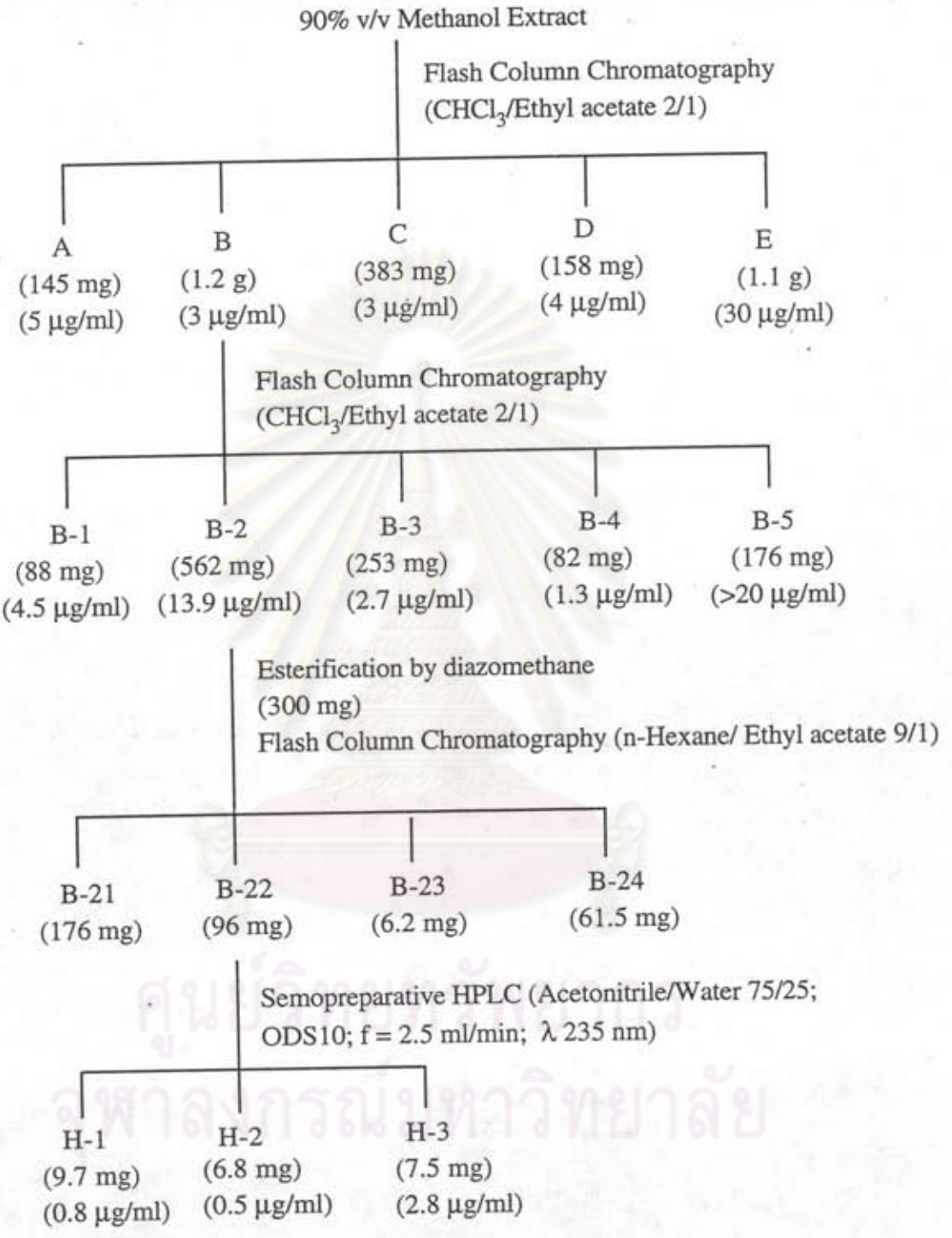
A collection of the sponge *Petrosia* sp. from Sichang Island, Chonburi Province, Thailand was pursued because its dichloromethane extract was active in bioassay pre-screens of both cytotoxic and brine shrimp assays. This extract was later partitioned with n-hexane and 90% v/v methanol in order to obtain its polar and non-polar parts. The 90% v/v methanol extract showed better activity in brine shrimp lethality assay than the n-hexane extract (Scheme 1). This result led us to investigate the bioactive compounds from the 90% v/v methanol extract. The crude 90% v/v methanol extract was purified by silica gel flash column chromatographies to yield pools B-1, B-2, B-3, B-4, and B-5. Pool B-2 which yielded the highest amount, was selected to further purify, instead of its lowest activity against the brine shrimp bioassay. Preliminary separation on reversed phase HPLC of the pool B-2 did not succeed. The infrared spectrum of B-2 (Figure 7) showed the carboxylic acid functional group (strong band of O-H stretching at 3,425 cm^{-1} and C-O stretching at 1,709 cm^{-1}). Attempt to esterify pool B-2 by diazomethane was carried out. This pool B-2 was subsequently fractionated by silica gel flash column chromatography and reversed-phase HPLC (acetonitrile/water, 75/25 as mobile phase) to furnish compounds **H-1** and **H-2**. The nuclear magnetic resonance spectra indicated **H-1** was the mixture of two compounds, in the approximate ratio 2:1, **H-1A** and **H-1B**. The isolation procedure of these compounds are shown in Scheme 2. The characterization of the isolated compounds , were achieved by the analyses of ultraviolet, infrared, nmr and mass spectra. The structure elucidation of three compounds are described as follows.

The pattern of UV absorption spectrum of **H-1** (Figure 11) showed λ_{max} at 254(17,084), 269(19,735), and 284(17,313) nm, respectively . The spectrum indicated that **H-1** consisted of ene-diyne chromophore (Bohlmann, Burkhardt, and Zdero, 1973). The infrared spectrum of **H-1** (Figure 10) exhibited C-H aliphatic stretching at 2,931 cm^{-1}, C $\equiv$ C stretching at 2,361 cm^{-1}, and the carbonyl ester stretching at 1,734 cm^{-1}. The EI mass spectrum of **H-1** (Figure 12) did not show the



Note: LD_{50} (µg/ml) for brine shrimp lethality assay

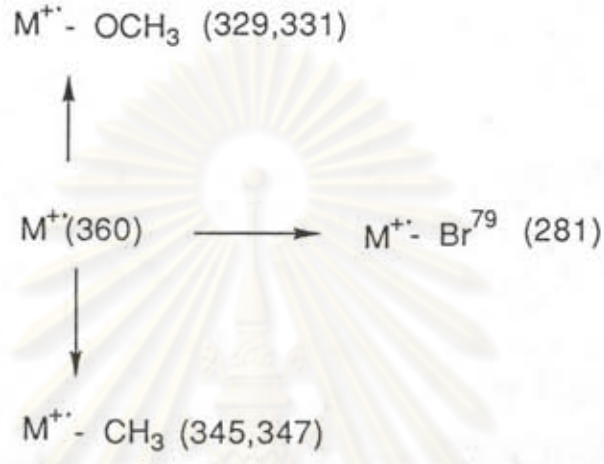
Scheme 1 The extraction scheme of a Thai marine sponge *Petrosia* sp.



Note: LD_{50} (µg/ml) for brine shrimp lethality assay

Scheme 2 The isolation scheme of bioactive compounds from the 90% v/v methanol extract of the sponge *Petrosia* sp.

molecular ion peak. However, fragment ion peak at *m/e* 345/347 (ratio 1:1) indicated the presence of one bromine atom in **H-1**. The base peak at *m/e* 281 supported the loss of one bromine from the molecular ion. Therefore **H-1** was proposed to have the molecular weight of 360 implying the tentative molecular formula of $C_{19}H_{21}BrO_2$. The fragmentations of **H-1** may occur as shown below:

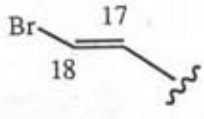


However, the ^{1}H-NMR (Figure 13) and ^{13}C-NMR (Figure 24) spectra showed that **H-1** consisted of two inseparable compounds in the approximate ratio 2:1. The different intensities of NMR signals led us to separate signals of the major **H-1A** from the minor **H-1B**.

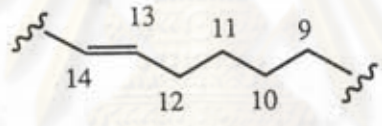
1. Structure Elucidation of **H-1A**

The 1-D ^{1}H-NMR (Figures 13, 14) and 2-D ^{1}H-^{1}H COSY (Figures 15, 18) spectral part of **H-1** for the major **H-1A** showed the *trans* relationship between two adjacent olefinic protons at δ 6.64 ppm (d, J = 14.1 Hz, H-18) and δ 6.30 ppm (dd, J = 14.1, 2.2 Hz, H-17) with the coupling constant of 14.1 Hz. The small coupling constant 2.2 Hz of H-17 and the correlation of H-17 and H-14 (δ 5.56 ppm, dq, J = 15.9, 2.2 Hz) in ^{1}H-^{1}H COSY spectra indicated their long-range coupling through an acetylenic bond. The expanded signal of the olefinic proton H-14 at δ 5.56 ppm presented as a doublets of quartet (J = 15.9, 2.2 Hz) (Figure 14). The larger coupling constant 15.9 Hz indicated its additional *trans* relation to H-13 (δ 6.17 ppm, dt, J = 15.9, 7 Hz) and the smaller coupling constant 2.2 Hz further indicated its long-range coupling to two methylene protons at δ 2.14 ppm (H-12, qd, J = 7, 1.5 Hz). The homonuclear decoupling experiment confirmed the long-range correlations of the olefinic proton H-14 (δ 5.56 ppm) to H-17 (δ 6.30 ppm) and to the methylene protons (δ 2.14 ppm). The irradiation at H-14 (δ 5.56 ppm) (Figure 20) caused the H-17 doublets of doublet at δ 6.30 ppm collapse to a doublet (J = 14 Hz) and the H-12

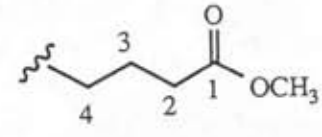
doublets of quartet at δ 2.14 ppm to a quartet (J = 7 Hz). Further, H-12 showed vicinal couplings to H-13 and to two methylene protons at H-11(δ 1.53 ppm, m) with the coupling constant of 7 Hz. The methylene protons H-9 (δ 2.26 ppm, brt, J= 7 Hz) were coupled to the methylene protons H-10 which were overlapped with H-11. The high field region of ^{1}H-^{1}H COSY spectrum showed the correlations among three contiguous methylene protons, H-2 (δ 2.45 ppm, t, J = 7.2 Hz), H-3 (δ 1.85 ppm, quintet, J = 7.2 Hz) and H-4 (δ 2.34 ppm, brt, J = 7.2 Hz). The chemical shift of H-2 was rather down field compared to other methylenes, thus H-2 should connect to the ester carbonyl (C-1). The signal of methyl ester was presented at δ 3.68 ppm (s, C-19). Therefore, the bromine atom must be at the other end of the terminated methyl ester and it was connected to the olefinic proton H-18. The broad signals of H-9 and H-4 originated from their long-range couplings which were confirmed by selective proton decoupling experiment (Figure 21). Irradiation of methylene protons at δ 2.26 ppm caused the H-4 broad triplet at δ 2.34 ppm collapse to a sharp triplet (J = 7 Hz). These NMR data suggested the following fragments:



Fragment A

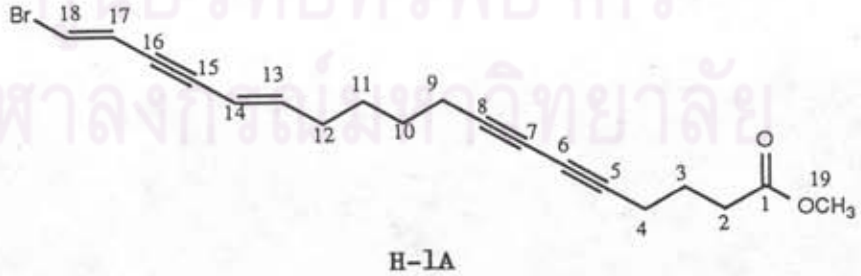


Fragment B



Fragment C

These NMR data accounted for $C_{13}H_{21}BrO_2$. The remaining six carbons must be assigned for three acetylene groups which should be placed among these three fragments. Thus, **H-1A** was proposed as methyl 18-bromo-(13*E*,17*E*)-octadeca-13,17-diene-5,7,15-triynoate. The structure of **H-1A** is shown below:



H-1A

The ^{13}C-NMR spectrum of **H-1A** (Figure 24) indicated nineteen carbon signals. The methyl ester functional group was confirmed by an ester carbonyl carbon at δ 173.37 ppm (C-1) and a methoxy carbon at δ 51.62 ppm (C-19). The signals of the protonated carbons were unambiguously assigned by the HMQC spectrum (Figure 23). The four ethylenic carbons were at δ 117.64 ppm (C-18), δ 117.71 ppm (C-17),

δ 109.61 ppm (C-14), and δ 145.25 ppm (C-13). The five methylene carbons were at δ 32.71 ppm (C-12), δ 27.60 ppm (C-11), δ 27.65 ppm (C-10), δ 18.98 ppm (C-9), δ 18.67 ppm (C-4), δ 23.50 ppm (C-3), and δ 30.08 ppm (C-2).

With the aid of the HMBC spectrum (Figure 22), the carbon assignments and the placement of three acetylenic groups were completely established. C-16 (δ 84.64 ppm) showed long-range correlation to both H-18 and H-14. C-15 (δ 90.49 ppm) exhibited correlations to H-13 and H-14. C-8 (δ 77.47 ppm) showed correlation to H-9. C-7 (δ 65.46 ppm) showed long-range correlation to H-9. C-6 (δ 66.11 ppm) showed long-range correlation to H-4. C-5 (δ 76.15 ppm) showed correlations to both H-4 and H-3. These data hinted us to replace one acetylene group between the two *trans* double bonds and two conjugated acetylene groups between the methylene carbons at C-9 and C-4. The relationship of these acetylenic carbons and neighbouring protons are shown in Figure 1.

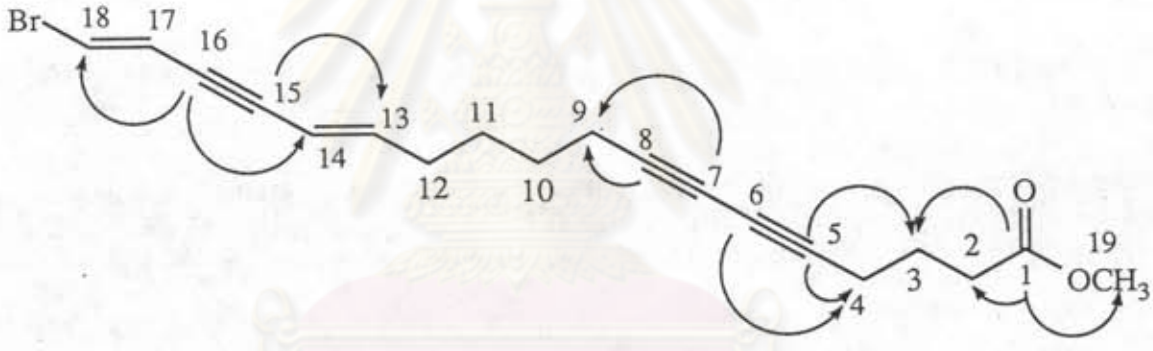


Figure 1 The C-H correlations from HMBC spectrum of compound **H-1A**

Consequently, the structure of compound **H-1A** was, then, identified as methyl 18-bromo-(13*E*,17*E*)-octadeca-13,17-diene-5,7,15-triynoate. This compound has been reported by Fusetani et al., 1993 from sponge *Petrosia volcano*. The assignments of carbon and proton signals of **H-1A** are summarized in Table 7. The EI mass spectrum of **H-1** showed the characteristic fragmentations which confirmed the structure of **H-1A**. The patterns of fragmentation were shown in Figure 2.

Table 7 Carbon and proton assignments of **H-1A** and long-range correlation between carbon and proton by HMBC spectrum.

Position	δ C (ppm)	δ H (ppm) (multiplicity, *J* Hz)	long-range correlation from C on H observed in HMBC spectrum
1	173.37	-	H-2, H-3, H-19
2	30.08	2.45 (t, *7.2*)	H-3, H-4
3	23.50	1.85(quintet, *7.2*)	H-2, H-4
4	18.67	2.34(brt, *7.2*)	H-2, H-3
5	76.15	-	H-3, H-4
6	66.11	-	H-4
7	65.46	-	H-9
8	77.47	-	H-9
9	18.98	2.26(brt, *7*)	-
10	27.65	1.53(m)	H-9, H-11
11	27.60	1.53(m)	H-10, H-12
12	32.71	2.14(qd, *7*, *1.5*)	H-13, H-14
13	145.25	6.17(dt, *15.9*,*7*)	H-12
14	109.61	5.56(dq, *15.9*, *2.2*)	H-12
15	90.49	-	H-13
16	84.64	-	H-14, H-18
17	117.71	6.30(dd, *14.1*, *2.2*)	H-18
18	117.64	6.64(d, *14.1*)	H-17
19	51.62	3.68(s)	-

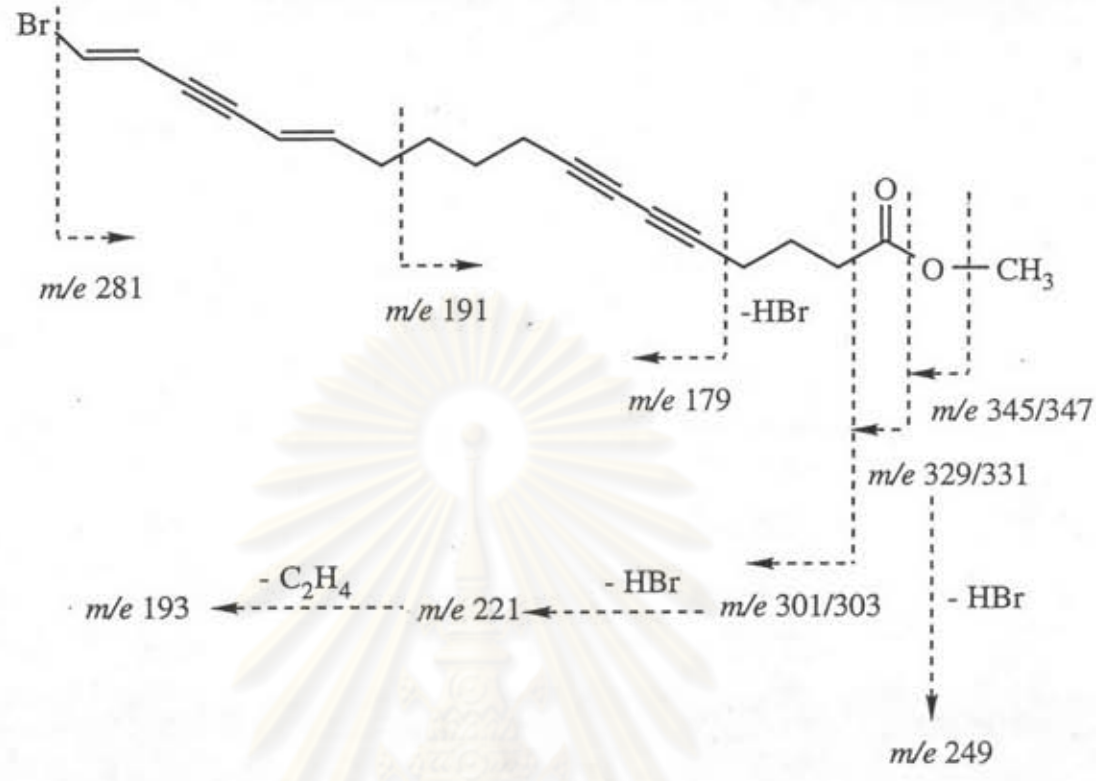


Figure 2 The proposed fragmentation patterns of **H-1A** from the EI mass spectrum

2. Structure Elucidation of **H-1B**

The ^{13}C-NMR spectrum of the mixture **H-1**(Figure 36) exhibited the remaining nineteen carbon signals belong to the minor **H-1B**. They composed of one ester carbonyl (δ 173.33 ppm), four ethylenic carbons(δ 147.18, 117.98, 117.07, 108.56 ppm), six acetylenic carbons(δ 92.78, 83.42,78.16, 77.32, 72.27,66.00 ppm), one methoxy carbon (δ 51.60 ppm), two shielded signals of methylenes adjacent to acetylenic carbons (δ 19.22, 19.07 ppm), and five methylene carbons (δ 32.63, 29.69, 27.87, 27.72, 23.45 ppm). The EI mass spectrum of the mixture **H-1** (Figure 12) indicated the same fragment ion peak at *m/e* 345/347 (ratio 1:1), and the same base peak at *m/e* 281. Therefore, **H-1B** must be the isomer of **H-1A** and have the molecular formula of $C_{19}H_{21}BrO_2$.

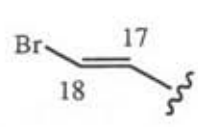
The 1-D ^{1}H-NMR (Figure 25) and 2-D ^{1}H-^{1}H COSY (Figure 27) spectral part of **H-1** for the minor **H-1B** exhibited two olefinic protons at δ 6.58 ppm (d, J = 14.0 Hz, H-18) and δ 6.18 ppm (dt, J = 14.0, 2.0 Hz, H-17), the large coupling constant 14.0 Hz indicated their *trans* relationship. The down field chemical shift of H-18 at δ 6.58 ppm suggested the bromine substitution at this terminal as in compound **H-1A**. The small coupling constant 2.0 Hz of H-17 and the correlation, in 2D ^{1}H-^{1}H COSY spectrum, between H-17 and the methylene protons H-12 (δ 2.30 ppm, td, J = 7, 2 Hz) implied their long-range coupling through the acetylenic bonds. The selective proton decoupling experiment was, then, designed to confirm their relationships. Upon irradiation of the olefinic proton H-17 at δ 6.18 ppm(dt), the doublet H-18 at δ 6.58 ppm collapsed to a singlet and the triplets of doublet H-12 at δ 2.30 ppm collapsed to a triplet (J = 7 Hz) (Figure 31). The cross peak in ^{1}H-^{1}H COSY spectrum showed that H-12 were further coupled to the methylene protons H-11 (δ 1.53 ppm, m). The methylene protons H-10, which were superimposed with H-11, were connected to the methylene protons H-9 (δ 2.36 ppm, qd, J = 7, 1.2 Hz). The protons H-9 showed vicinal coupling (J = 7 Hz) to the olefinic proton H-8 at δ 6.03 ppm (dt, J = 10.8, 7 Hz). The coupling constant of 10.8 Hz showed that H-8 was *cis* to the olefinic proton H-7 at δ 5.49 ppm (doublet of quintet, J = 10.8, 1.2 Hz).

The NMR data for three contiguous methylene protons, H-2 (δ 2.46 ppm, t, J = 7 Hz), H-3 (δ 1.88 ppm, quintet, J = 7 Hz), and H-4 (δ 2.42 ppm, td, J = 7, 0.9 Hz) showed similar connection to those of **H-1A**. The chemical shift of H-2 was rather down field, thus H-2 should connect to the terminated carbonyl ester (C-1).

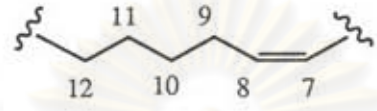
The long-range coupling between H-4 and H-7 (J≈1 Hz) was supported by the selective proton decoupling experiment (Figure 32). Upon irradiation of H-7 at δ 5.49

ppm, the triplets of doublet H-4 collapsed to a sharp triplet (J = 7 Hz). This experiment also showed the long-range coupling between H-7 and H-9. The small cross peaks in 2D ^{1}H-^{1}H COSY spectrum between H-4 and H-7, and between H-9 and H-7(Figure 28) further supported these phenomena.

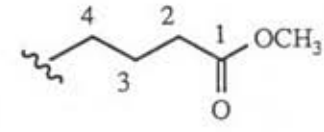
The above ^{1}H-NMR data led to the proposed fragments as shown below. These fragments were counted for $C_{13}H_{21}BrO_2$.



Fragment A



Fragment B



Fragment C

The remaining six carbons were then assigned for three acetylene groups as in **H-1A**. The placements of them were established by the aid of HMBC spectrum (Figure 34) as follows. C-16 (δ 78.16 ppm) showed correlation to H-18. C-15 (δ 72.27 ppm) showed correlation to H-17. C-14 (δ 66.00 ppm) and C-13 (δ 77.32 ppm) showed correlation to H-12. C-6 (δ 92.78 ppm) showed correlation to H-4. C-5 (δ 83.42 ppm) showed correlations to both H-4 and H-3. The relationships of these acetylenic carbons and neighbouring protons are shown in Figure 3.

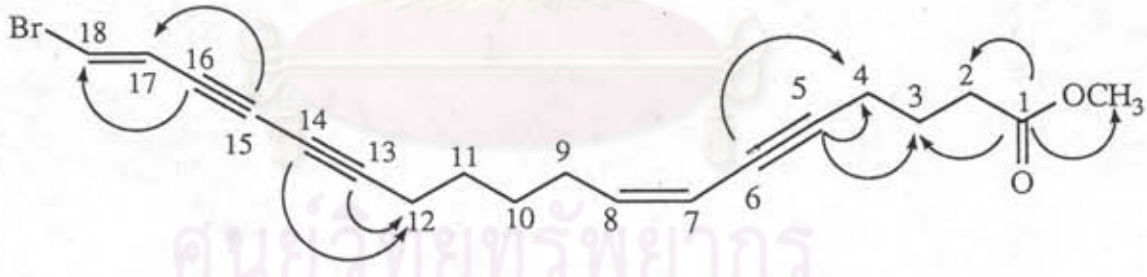
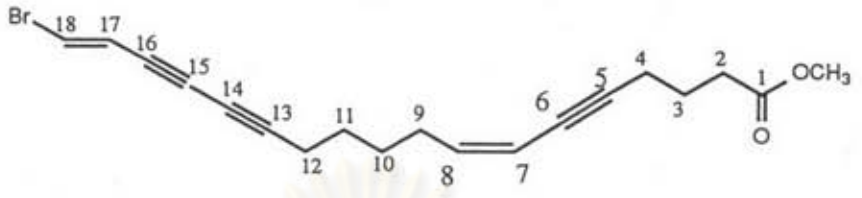


Figure 3 The C-H correlations from HMBC spectrum of **H-1B**

The signals of the other carbons were unambiguously assigned by the HMQC spectrum (Figure 35). The ester carbonyl carbon was at δ 173.33 ppm (C-1) and the methoxy carbon was at δ 51.60 ppm (C-19). The four ethylenic carbons were at δ 117.98 ppm (C-18), δ 117.07 ppm (C-17), δ 147.18 ppm (C-8), and δ 108.56 ppm (C-7). The five methylene carbons were at δ 19.22 ppm (C-12), δ 27.87 ppm (C-11), δ 27.72 ppm (C-10), δ 29.69 ppm (C-9), δ 19.07 ppm (C-4), δ 23.45 ppm (C-3), and δ 32.63 ppm (C-2). Thus, **H-1B** is identified as methyl 18-bromo-(7*Z*, 17*E*)-

octadeca-7,17-diene, 5,13,15-triynoate, which is the methyl ester of a new member of brominated polyacetylenic acids. The structure of **H-1B** is shown below:



H-1B

The summary of the assignments of carbon and proton signals is shown in Table 8. The EI mass spectrum of **H-1B** showed the characteristic fragmentations which were confirmed the structure of **H-1B**. The patterns of fragmentation were shown in Figure 4.

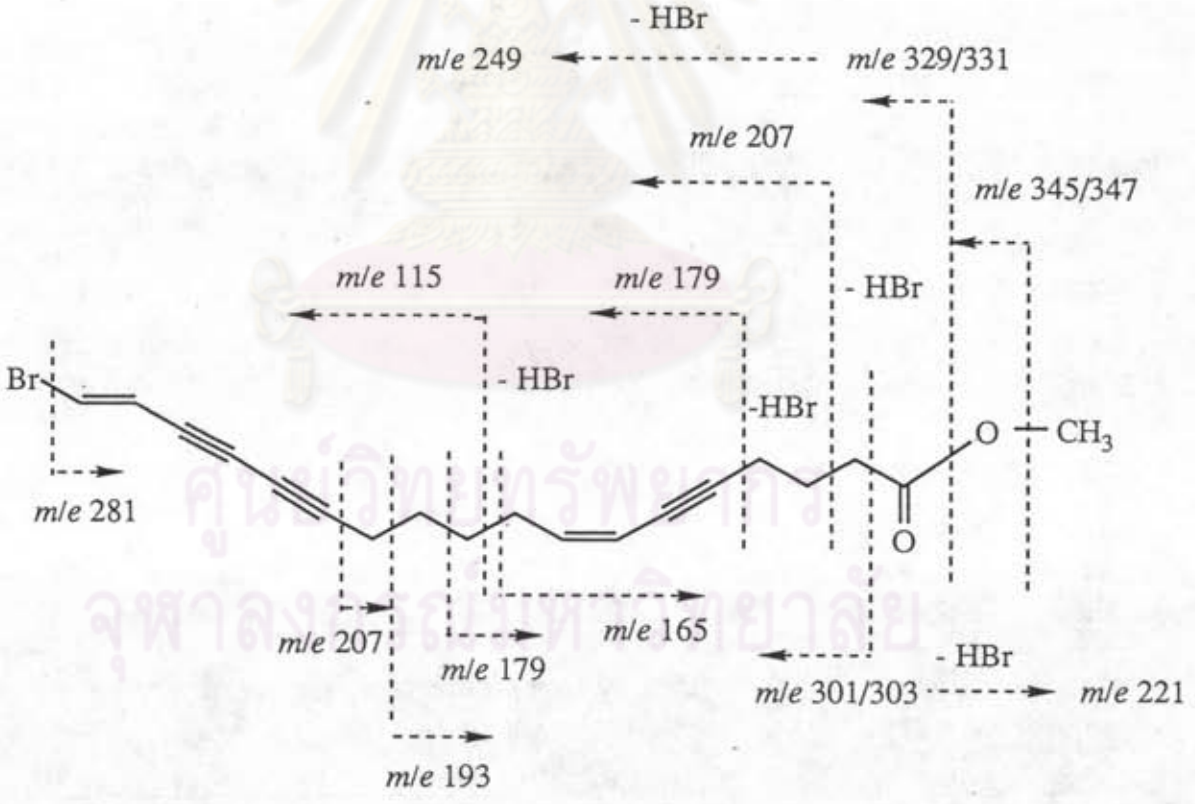


Figure 4 The proposed fragmentation patterns of **H-1B** from the EI mass spectrum

Table 8 Carbon and proton assignments of **H-1B** and long-range correlation between carbon and proton by HMBC spectrum

Position	δ C (ppm)	δ H (ppm) (Multiplicity, *J* Hz)	long-range correlation from C on H observed in HMBC spectrum
1	173.33	-	H-2, H-3, H-19
2	32.63	2.46 (t, *7*)	H-3, H-4
3	23.45	1.88(quintet, *7*)	H-2, H-4
4	19.07	2.42(td, *7*, *0.9*)	H-2, H-3
5	83.42	-	H-3, H-4
6	92.78	-	H-4
7	108.56	5.49(dqt, *10.8*, *1.2*)	H-9
8	147.18	6.03(dt, *10.8*, *7*)	H-9
9	29.69	2.36(qd, *7*, *1.2*)	H-7
10	27.72	1.53(m)	H-9, H-11
11	27.87	1.53(m)	H-10, H-12
12	19.22	2.30(td, *7*, *2*)	-
13	77.32	-	H-12
14	66.00	-	H-12
15	72.27	-	H-17
16	78.16	-	H-18
17	117.07	6.18(dt, *14.0*, *2*)	H-18
18	117.98	6.58(d,*14.0*)	H-17
19	51.60	3.70(s)	-

3. Structure Elucidation of **H-2**

The infrared absorption spectrum of **H-2** (Figure 37) suggested aliphatic C-H stretching at 2,934 cm^{-1}, C ≡ C stretching at 2,355 cm^{-1}, and the carbonyl ester stretching at 1,732 cm^{-1}. The ultravioet spectrum (Figure 38) showed the maximum absorption at 242 nm (15,698) indicating an enyne system (Bohlmann, Burkhardt, and Zdero, 1973). The EI mass spectrum of **H-2** (Figure 39) exhibited the molecular ion peak at *m/e* 362/364 (ratio 1:1) and the fragment ion peak at *m*/e 283 implying the presence of one bromine atom in **H-2**. Therefore, **H-2** was shown to have the molecular weight of 362 implying the tentative molecular formula of $C_{19}H_{23}BrO_2$.

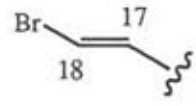
The decoupled ^{13}C-NMR (Figure 44) and DEPT 135° (Figures 45, 46) spectra confirmed nineteen carbon signals which consisted of one ester carbonyl (δ 173.38 ppm), two olefinic carbons (δ 117.97, 117.02 ppm), six acetylenic carbons (δ 92.98, 77.72, 77.32, 75.99, 66.14, 65.20 ppm), one methoxy carbon (δ 51.60 ppm), three shielded signals of methylenes adjacent to acetylenic carbons (δ 19.34, 19.10, 18.65 ppm), and six methylene carbons (δ 32.69, 28.25, 28.25, 28.15, 28.07, 23.49 ppm).

The ^{1}H-NMR data of **H-2** (Figure 40) exhibited signals for two *trans* ethylenic protons at δ 6.56 ppm (d, J = 14.0 Hz, H-18) and at δ 6.16 ppm (dt, J = 14.0, 2.0 Hz, H-17) with the coupling constant of 14.0 Hz. The ^{1}H-^{1}H COSY spectrum (Figure 42) also confirmed the correlation of H-18 and H-17. The small coupling constant 2.0 Hz indicated that H-17 long-range coupled to H-14 (δ 2.25 ppm, td, J = 7.0, 2.0 Hz) through acetylenic bond. The long range ^{1}H-^{1}H COSY spectrum (Figure 43) confirmed this relationship. The down field chemical shift of H-18 suggested the bromine substitution at this terminal as in compounds **H-1A** and **H-1B**.

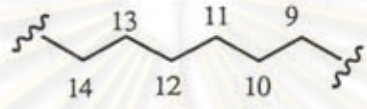
The ^{1}H-^{1}H COSY spectra (Figure 42) in high field region indicated contiguous methylenes at δ 2.24 ppm , δ 1.52 ppm, and δ 1.38 ppm. The integration of ^{1}H-NMR signals of these methylene protons (Figure 40) showed that each signal was composed of four protons. Thus, these NMR data suggested the connection of six methylene protons as follows. H-14 (δ 2.25 ppm, td, J = 7.0, 2.0 Hz) connected to H-13 (δ 1.52 ppm, broad quintet, J = 7.0 Hz). H-13 coupled to H-12 which was superimposed with H-11(δ 1.38 ppm, m). H-11 coupled to H-10 (1.52, quintet, J = 7 Hz). H-10 coupled to H-9 (δ 2.24 ppm, brt, J = 7.0 Hz).

The long range ^{1}H-^{1}H COSY spectrum (Figure 43) indicated the long-rang coupling through acetylenic bond between H-9 and H-4 (δ 2.33 ppm, brt, J = 7 Hz). The high field ^{1}H-^{1}H COSY spectrum (Figure 41) showed the three contiguous methylenes at δ 2.33 ppm (brt, J = 7 Hz, H-4), δ 1.84 ppm (quintet, J = 7 Hz, H-3), and δ 2.44 ppm (t, J = 7 Hz, H-2). The down field chemical shift of H-2 indicated its connection to the ester carbonyl.

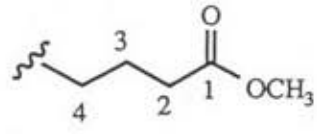
The above ^{1}H-NMR data led to the proposed fragments as shown below. These fragments were counted for $C_{13}H_{23}BrO_2$.



Fragment A

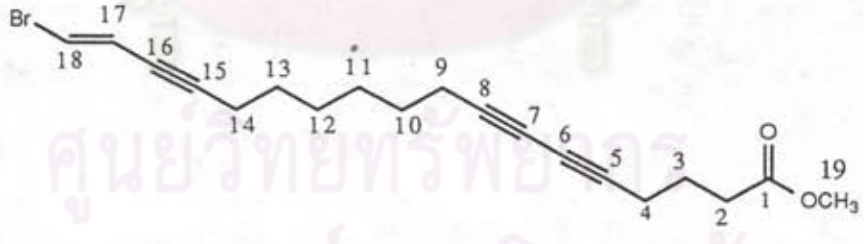


Fragment B



Fragment C

The remaining six acetylenic carbons should correlate between these fragments. The ^{1}H-NMR data of **H-2** corresponded with the published values of the methyl ester of xestospongic acid extracted from *Xestospongia testudinaria* (Bourguet-Kondracki et al., 1992) and *Petrosia volcano* (Fusetani et al., 1993). Thus, it was proposed to be methyl 18-bromo-(17*E*)-octadeca-17-ene-5,7,15-triynoate. The structure of **H-2** is shown below:



H-2

Furthermore, the carbon signals in decoupled ^{13}C-NMR (Figure 44) and DEPT 135° (Figure 45) corresponded with those signals of the published values of the methyl ester of xestospongic acid. Consequently, these carbon signals were assigned by comparing with those values. The methyl ester was confirmed by the carbonyl carbon at δ 173.38 ppm (C-1) and the methoxy carbon at δ 51.60 ppm (C-19). Two ethylenic carbons were at δ 117.02 (C-18) and δ 117.97 ppm (C-17). Six acetylenic carbons were at δ 77.32 ppm (C-16), δ 92.98 ppm (C-15), δ 77.72 ppm (C-8),

δ 65.20 ppm (C-7), δ 66.14 ppm (C-6), δ 75.99 ppm (C-5). Three shielded signals of methylenes adjacent to acetylenic carbons were at δ 19.34 ppm (C-14), δ 19.10 ppm (C-9), and δ 18.65 ppm (C-4). Six methylene carbons were at δ 28.15 ppm (C-13), δ 28.25 ppm (C-11, C-12), δ 28.07 ppm (C-10), δ 23.49 ppm (C-3), and δ 32.69 ppm (C-2). The summary of carbon and proton assignments including the correlations between protons and protons by ^{1}H-^{1}H COSY spectrum are shown in Table 9. The EI mass spectrum of **H-2** showed the characteristic fragmentations which confirmed the structure of **H-2**. The patterns of fragmentation were shown in Figure 5.

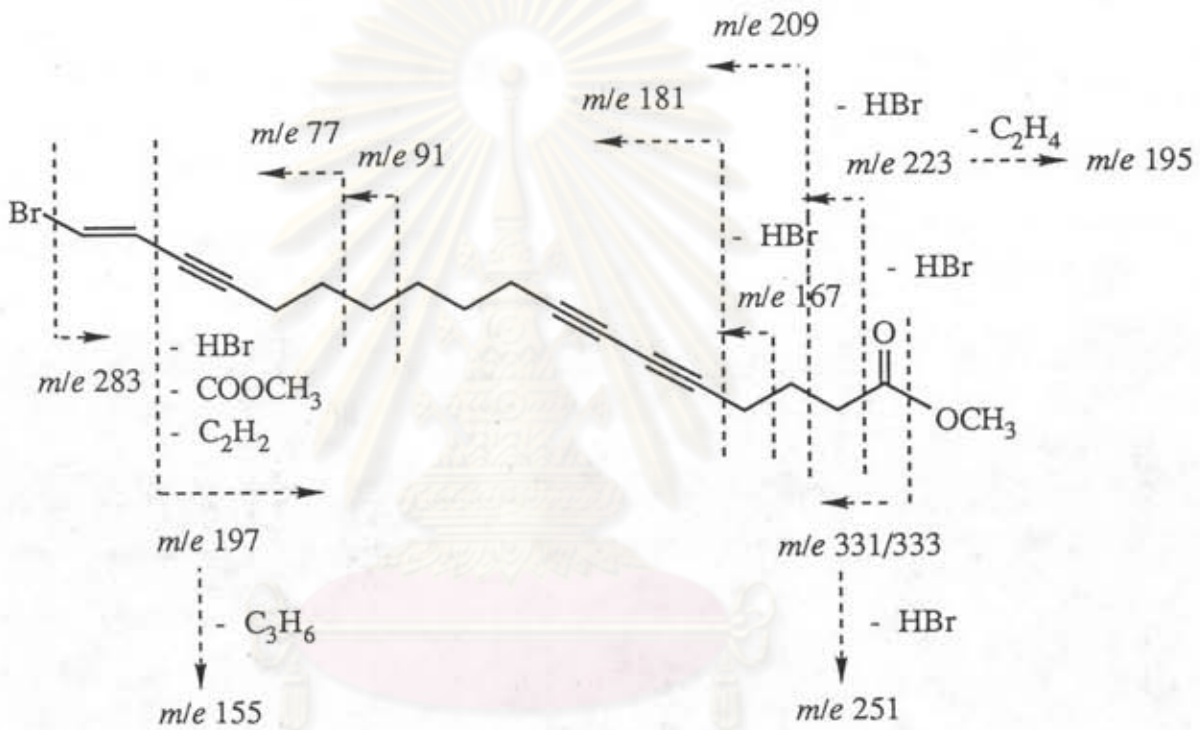


Figure 5 The proposed fragmentation patterns of **H-2** from the EI mass spectrum

Table 9 Carbon and proton assignments of **H-2** and correlations between proton and proton in 2D ^{1}H-^{1}H COSY spectrum

Position	δ C (ppm)	δ H (ppm) (multiplicity, *J* Hz)	^{1}H,^{1}H-COSY spectrum
1	173.38	-	-
2	32.69	2.44(t, *7*)	H-3, H-4
3	23.49	1.84(quintet, *7*)	H-2, H-4
4	18.65	2.33(brt, *7*)	H-2, H-3, H-9*
5	75.99	-	-
6	66.14	-	-
7	65.20	-	-
8	77.72	-	-
9	19.10	2.24(brt, *7*)	H-10
10	28.07	1.52(quintet, *7*)	H-11
11	28.25	1.38(m)	H-10
12	28.25	1.38(m)	H-13
13	28.15	1.52(brqt, *7*)	H-14
14	19.34	2.25(td, *7*, *2*)	H-13, H-17*
15	92.98	-	-
16	77.32	-	-
17	117.97	6.16(dt, *14*, *2*)	H-18
18	117.02	6.56(d, *14*)	H-17
19	51.60	3.67(s)	-

* The signals are observed in long-range ^{1}H,^{1}H-COSY spectrum (Figure 43).

4. Biological Activities of the Isolated Compounds

The compounds **H-2** and the mixture **H-1** showed potent biological activity against brine shrimp lethality bioassay at LD_{50} = 0.5 and 0.8 μg/ml, respectively. Xestospongic acid and its methyl ester of xestospongic acid (**H-2**) were reported about antimicrobial activity against *Staphylococcus aureus*: diameter inhibition 12 mm at 100 and 500 μg/disc, respectively (Bourguet-Kondracki et al., 1992). **H-1A** was reported about antifungal activity against *Mortierella ramannianus* (Fusetani et al., 1993).