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FURTHER ACETYLENIC ACIDS FROM THE MARINE SPONGE *XESTOSPONGIA TESTUDINARIA*

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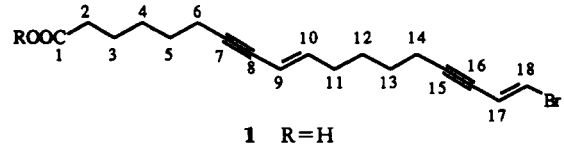
ABSTRACT.—Two novel brominated acetylenic acids, methyl 18-bromooctadeca-(9*Z*,17*E*)-diene-7,15-diynoate [**3**] and methyl 18-bromooctadeca-(9*E*,17*E*)-diene-5,7,15-triynoate [**4**] have been isolated from *Xestospongia testudinaria*. Nmr spectroscopic studies of these and related acetylenic acids are reported.

The fatty acids of sponges have attracted considerable interest because of their unique characteristics such as increased chain length, branching, and unusual unsaturation patterns and because of the implications these structural variations may have, when present in phospholipids, for membrane function (1–3). Sponges of the family Nepheliospongiidae have been found to be a source of novel fatty acids. We have previously reported (4) the occurrence of the novel brominated bis-acetylenic acid **1** in *Xestospongia testudinaria* Lamarck (Nepheliospongiidae), and the dibromo monoacetylenic acid **2** has been isolated from *Xestospongia muta* (5). Compound **1** and four brominated polyenynic acids and a dibromo unsaturated acid have been reported from a *Xestospongia* sp. from the Red Sea (6).

During our investigation of the minor components of the $CHCl_3$ extract of *X. testudinaria* we have isolated a number of related compounds, including the 9*Z* isomer of **1** as its methyl ester. A complete assignment of the ^{13}C-nmr spectra of these two isomers has been made, partly on the basis of a comparison of their spectra which provided information on the chemical shifts of the conjugated enyne systems in the molecules. Although considerable work has been done on the assignments of the carbon



1 R=H
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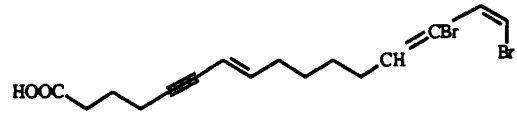

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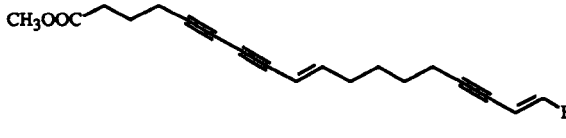

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spectra of alkenoic and alkynoic acids (7–9), the literature concerning conjugated enyne systems is somewhat limited. [We are only aware of five reports (10–14) in which the carbon resonances of an enyne system are assigned, and in each case the chemical environment of the enyne fragment is sufficiently different from that in **1** to produce substantial differences in the ^{13}C-nmr spectra.] As well as adding to the existing body of knowledge, the assignment of the ^{13}C-nmr spectrum of **1** has proven valuable in the structural eluciation of related compounds extracted from *X. testudinaria*. In this paper we report the isolation of the 9*Z* isomer (as its methyl ester **3**), and another novel brominated acid as its ester, methyl 18-bromooctadeca-(9*E*,17*E*)-diene-5,7,15-triynoate [**4**]. All methyl esters were unstable, requiring spectra to be run immediately upon isolation.

The $CHCl_3$ extract of *X. testudinaria* was separated into MeOH-soluble and MeOH-insoluble (mainly sterol) fractions. Reversed-phase medium pressure liquid chromatography (mplc) of the MeOH-soluble fraction followed by repeated reversed-phase hplc gave the esters **3** and **5**. The isolation procedure was modified in subsequent extractions to facilitate chromatography. The crude extract was methylated (15), allowing easier separation of the methyl esters from the steroidal fraction using normal phase mplc. Repeated normal phase hplc then led to the isolation of another methyl ester **4** as well as **3** and **5** and a number of mono-unsaturated esters which were not further investigated.

Ester **5** was assigned as the methyl ester of the previously reported acid **1** as it had very similar ^{1}H- and ^{13}C-nmr spectra (Table 1). This was confirmed by a comparison of spectral data with a sample of ester prepared by methylation (15) of **1**. Acid **1** was shown to undergo facile methylation by warming a sample with MeOH for 2 h at 40°. ^{1}H nmr revealed a singlet at δ 3.6 corresponding to 9% conversion to ester. It seems likely that **3** and **5** were not present in the sponge in that form but are artifacts from the separation method, which involved warming the crude extract with MeOH.

TABLE 1. ^{13}C-nmr Shifts in C_6D_6 Downfield from TMS.

Carbon	Compound			
	Acid **1**	Ester **5**	Ester **3**	Ester **4**
OMe		50.9	50.9	50.9
C-1	179.3	173.2	173.2	172.5
C-2	33.8	33.9	33.9	32.6
C-3	24.4	24.7	24.7	23.7
C-4	28.5	28.6	28.6	18.9
C-5	28.8	28.8	28.8	82.8
C-6	19.5	19.5	19.6	67.1
C-7	89.0	89.0	94.7	74.7
C-8	80.0	80.0	78.1	74.2
C-9	111.3	113.3	110.7	109.5
C-10	142.4	142.4	141.5	147.5
C-11	32.5	32.5	29.7	32.5
C-12	28.2	28.2	28.2	27.8
C-13	28.0	8.0	28.0	27.7
C-14	19.3	19.3	19.3	19.3
C-15	93.0	93.1	93.2	92.9
C-16	78.0	78.0	78.0	78.0
C-17	118.5	118.4	118.4	118.4
C-18	117.3	117.3	117.2	117.4

Ester **3** had a ^{13}C-nmr spectrum which was very similar to that of **5** except that small shifts had occurred for two of the vinyl carbons, two acetylenic carbons, and the allylic methylene carbon (C-11) (Table 1). The shift to higher field of the allylic carbon is consistent with the shift due to a γ-gauche effect (16) experienced by substituents on a *Z* double bond. This can also explain the upfield shift of one of the acetylenic carbons (C-8). Another of the acetylenic carbons (C-7) is shifted to lower field due to a δ-gauche effect (16). The magnitudes of the γ- and δ-gauche effects are similar to those found (12) for the *E* and *Z* isomers of hex-3-en-1-yne and hept-3-en-1-yne (Table 2). The presence of a *Z*-Δ^9 double bond in **3** was confirmed by the ^{1}H-nmr spectrum, as the coupling constant between the two vinyl protons is 10.9 Hz (cf. 15.8 Hz in **5**).

TABLE 2. Chemical Shifts of the Acetylenic Carbons for Three Pairs of *E*-*Z* Isomeric Enynes (in ppm downfield from TMS).

Compound/Carbon	δ*Z*	δ*E*	δ*Z* − δ*E*
Esters **3** and **5**			
C-7	94.7	89.0	5.7
C-8	78.1	80.0	−1.9
Hex-3-en-1-yne[a]			
C-1	81.2	75.7	5.5
C-2	80.5	82.5	−2.1
Hept-3-en-1-yne[a]			
C-1	81.3	75.7	5.6
C-2	80.5	82.6	−2.1

[a]Values from Hearn (10).

It was possible to assign many of the carbon resonances of **5** by comparison with spectra of known compounds (9). The peaks at δ 33.9, 24.7, and 32.5 can be assigned to C-2, C-3 (α and β to carboxyl), and C-11 (α to double bond), respectively. The signals from δ 27–29 are assigned as chain methylenes and δ 19.3, 19.5 as methylenes α to a triple bond. The individual assignments of most of these peaks (Table 1) were made from ^{13}C-^{1}H correlation 2D-nmr experiments. This required assignment of the ^{1}H-nmr spectrum, which was achieved using a COSY-45 experiment. These 2D nmr experiments were run on **1**, as resolution in the ^{1}H-nmr spectrum was superior to that observed for the corresponding ester **5**. The ^{1}H-nmr spectrum of **1** in pyridine-d_5 was resolved with the exception of two multiplets (δ 1.52 and 1.38), each of which consists of two methylene groups. Spectra run in other solvents showed greater overlap of the multiplets as well as other signals in the spectrum. The COSY-45 experiment revealed complete connectivity from the methylene adjacent to the acid group (δ 2.48) through to the proton on the terminal double bond (δ 6.95). Assignments of the protonated carbon resonances then followed from observation of the directly bonded protons in the ^{13}C-^{1}H correlation 2D nmr experiments, except for C-4 and C-5, both of which correlate to the ^{1}H signal at δ 1.54, and C-12 and C-13, both of which correlate to the signal at δ 1.38. In C_6D_6 the ^{1}H-nmr signals of these four unresolved methylenes shifted so that one methylene occurred at δ 1.28 (overlapped with the methylene on C-3) while the other three methylenes were at δ 1.16. A COSY-45 showed that the downfield methylene was due to the protons on C-5; i.e., a correlation peak was observed with the propargylic methylene at δ 2.09 (C-6) which, in turn, correlated with the vinylic proton on C-9. The ^{13}C-^{1}H correlation 2D spectrum then showed that the protons on C-5 directly correlated to the carbon signal at δ 28.8, C-4, must then be the δ 28.5 signal. The assignments for C-12 and C-13 were deduced using substituent parameters calculated by Bus *et al.* (7). C-12 (β to a double bond and γ to a triple bond) is calculated to be 0.3 ppm downfield from C-13. Although this is only an approximation, as both the double and triple bonds in this partial structure are further conjugated whereas the parameters are for isolated double and triple bonds, the agreement with the measured difference of 0.2 ppm

between the two possible carbon resonances is quite good. Assignment of the acetylenic resonances became possible with the isolation of **3**. The upfield shift relative to the corresponding peak in **5**) of the signal at δ 78.1 in **3** indicates that it is C-8 next to the *Z* double bond, while the downfield shift of the signal at 94.7 identifies it as C-7, revealing that the outer acetylenic carbon in an enyne with a substitution pattern such as in **5** occurs at lower field than the inner carbon. By analogy, of the remaining pair of acetylenic resonances (assigned to the brominated enyne moiety) the lowest field signal (δ 93.1) is assigned to C-15, at the end of the conjugated system. Thus a complete assignment of the ^{13}C-nmr spectrum of esters **3** and **5** is possible (Table 1). The enyne system of the dibromo monoacetylenic acid **2** had not been assigned. From this work the enyne of the methyl ester of **2** can be assigned as follows: δ 88.5 (C-5), 81.5 (C-6), 112.0 (C-7), 144.7 (C-8).

The structure of ester **4** was deduced from its ^{13}C-nmr spectrum, which showed 19 carbon resonances. Eight of these were almost identical in chemical shift to those assigned to C-11–C-18 in **5**, suggesting that this portion of the molecule was common to both esters. The major difference was the presence of two additional resonances in the region 70–90 ppm assignable to acetylenic carbons and the absence of two signals occurring around 28 ppm in the spectrum of **5**. This implies that two of the methylene carbons from the chain of five methylenes (C-2–C-5) in **5** have been replaced by a triple bond in **4**. Because there are signals at δ 32.6 and 23.7, the methylenes α and β to the carboxyl group must still be present, and as they are only shifted to a small extent the extra triple bond must be separated from them by another methylene carbon. This leads to the structure **4** for the ester. This is confirmed by the ^{1}H-nmr spectrum. The signals for the vinyl protons are almost identical to those of **5**, with the exception that the signal for H-10 is shifted downfield and the coupling (1.8 Hz) between the proton on C-9 and the two protons on C-6 in **5** is absent in **4**, resulting in a much sharper doublet for H-9, with only slight broadening due to coupling (through two triple bonds) between H-9 and the protons on C-4. In the upfield region, one of the propargylic methylenes, which both resonate as a broad triplet of doublets in the case of **5**, is a broad triplet at δ 2.41. Irradiation at δ 1.87 causes this triplet to collapse and also decouples the triplet at δ 2.46 due to the methylene next to the carboxyl function, confirming the partial structure $CH_3OOC—CH_2—CH_2—CH_2—C\equiv C—$.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Bruker CXP-300 spectrometer. 2D nmr spectra were run on a Bruker WM-250 spectrometer. Rplc was carried out (with a flow rate of 9 ml/min) using a Corning teflon and glass column (400 × 50 mm) packed with octadecylsilylated Si gel, prepared (17) from Merck Si gel (40–63 μm). Normal phase mplc was done using an Amicon stainless steel column (500 × 50 mm) packed with Amicon Matrex silica (25–40 μm) at a flow rate of 100 ml/min. Rainin Dynamax Macro hplc C18 and SiO_2 (250 × 22 mm, 8 μm) columns were used for preparative hplc, at a flow rate of 10 ml/min.

BIOLOGICAL MATERIAL.—The sponge *X. testudinaria* was collected by scuba at a depth of 5 m from Pandora Reef, 18° 49′S, 146° 25′E near Townsville, Queensland, Australia on 2 July 1985. A voucher specimen (FN 58) has been deposited in the Griffith University collection.

TYPICAL ISOLATION PROCEDURE.—The freeze-dried sponge (240 g) was twice extracted with $CHCl_3$ in a stainless steel column for 8 h at room temperature. Evaporation of the extract yielded a dark green oil (11 g). The MeOH-soluble fraction (4.9 g) was fractionated by two passes through the reversed-phase mplc column, using MeOH and 80% MeOH as solvents. The longest retained fraction (500 mg) (eluting in 7–8 column volumes with 80% MeOH) was chromatographed twice by reversed-phase hplc (solvent 90% MeOH) to yield methyl 18-bromooctadeca-(9*Z*,17*E*)-diene-7,15-diynoate [**3**] (21 mg) (eluting in 6.3 column volumes) and methyl 18-bromooctadeca-(9*E*,17*E*)-diene-7,15-diynoate [**5**] (60 mg) (eluting in 7.0 column volumes).

ALTERNATIVE ISOLATION PROCEDURE.—Freeze-dried sponge (ca. 250 g) was extracted with CH_2Cl_2-MeOH (1:1) (3 liters) for 20 h to yield a green oil (14.5 g), which was stirred with MeOH (500 ml) and chlorotrimethylsilane (50 ml) under argon for 18 h. The CH_2Cl_2-soluble portion of the product was chromatographed by normal phase mplc with CH_2Cl_2 as solvent. (The percentage of CH_2Cl_2 soluble material in the methylated extract can be increased by using CH_2Cl_2 only rather than CH_2Cl_2/MeOH for the extraction. This was done for all subsequent extractions.) The second and third fractions from mplc, eluting in 1.2–3.0 column volumes, yielded a colorless oil (2.5 g) that contained a mixture of methyl esters. Repeated normal phase mplc allowed isolation of methyl 18-bromooctadeca-(9*E*,17*E*)-diene-5,7,15-triynoate [**4**] (10 mg), eluting in 7.2 column volumes in 40% CH_2Cl_2/cyclohexane.

METHYL 18-BROMOOCTADECA-(9*Z*,17*E*)-DIENE-7,15-DIYNOATE [**3**].—^{1}H nmr ($CDCl_3$) δ 6.53 (1H, d, J = 13.9 Hz, H-18), 6.14 (1H, dt, J = 13.9, 2.3 Hz, H-17), 5.76 (1H, dt, J = 10.6, 7.3 Hz, H-10), 5.42 (1H, dbrt, J = 10.6, 1.3 Hz, H-9), 3.65 (3H, s, OMe), 2.31 (2H, t, J = 7.3 Hz, H-2), 2.30 (6H, m, H-6, -11, -14), 1.65 (2H, qnt, J = 7.3 Hz, H-3), 1.53 (8H, m, H-4, -5, -12, -13).

METHYL 18-BROMOOCTADECA(9*E*,17*E*)-DIENE-7,15-DIYNOATE [**5**].—^{1}H nmr ($CDCl_3$) δ 6.54 (1H, d, J = 13.9 Hz, H-18), 6.14 (1H, dt, J = 13.9, 2.3 Hz, H-17), 5.98 (1H, dt, J = 15.8, 7.1 Hz, H-10), 5.43 (1H, dt, J = 15.8, 1.8 Hz, H-9), 3.65 (3H, s, OMe), 2.31 (2H, t, J = 7.5 Hz, H-2), 2.26 (4H, m, H-6, -14), 2.09 (2H, m, H-11), 1.64 (2H, qnt, J = 7.4 Hz, H-3), 1.50 (8H, m, H-4, -5, -12, -13); eims *m/z* 332, 301, 285, 253, 225, 211, 183, 169, 155, 141, 129, 119, 105, 91 (100%); exact mass $[M-Br]^+$ 285.184 ($C_{19}H_{25}O_2$ requires 285.185).

METHYL 18-BROMOOCTADECA-(9*E*,17*E*)-DIENE-5,7,15-TRIYNOATE [**4**].—^{1}H nmr ($CDCl_3$) δ 6.56 (1H, d, J = 13.9 Hz, H-18), 6.24 (1H, dt, J = 15.8, 7.1 Hz, H-10), 6.15 (1H, dt, J = 13.9, 2.3, H-17), 5.48 (1H, brd, J = 15.8 Hz, H-9), 3.68 (3H, s, OMe), 2.46 (2H, t, J = 7.3 Hz, H-2), 2.41 (2H, t, J = 7.3 Hz, H-4), 2.27 (2H, m, H-14), 2.15 (2H, m, H-11), 1.87 (2H, qnt, J = 7.3 Hz, H-3), 1.52 (4H, m, H-12, -13).

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