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(7E, 13ξ, 15Z)-14,16-DIBROMO-7,13,15-HEXADECATRIEN-5-YNOIC ACID. A NOVEL DIBROMO ACETYLENIC ACID FROM THE MARINE SPONGE *XESTOSPONGIA MUTA*

Francis J. Schmitz* and Y. Gopichand

Department of Chemistry
The University of Oklahoma
Norman, Oklahoma 73019, U.S.A.

In earlier papers[1] we have described the isolation from a sea hare of two halogenated acetylenic C_{15} straight-chain ethers, one of which exhibits the interesting pharmacologic property of markedly potentiating the activity of pentobarbital in mice.[2] In our continuing search[3] for bioactive compounds from marine organisms we have isolated a dibrominated straight-chain C_{16} acetylenic acid, the first compound of this type to be reported, and we describe herein its structure elucidation.

The new acid was derived from the sponge *Xestospongia muta*, the crude extracts of which showed both *in vivo* tumor-inhibitory activity and central nervous system activity.[3b] Fresh, wet sponges collected near Summerland Key, Florida, were macerated in isopropyl alcohol, and the resulting concentrated alcohol extract was diluted with water and extracted continuously with dichloromethane. The dichloromethane solubles were partitioned according to the procedure of Kupchan,[4] and the resulting carbon tetrachloride fraction was chromatographed over silica gel to give the crude, unstable, oily acid 1 (~0.13% of wet sponge wt.). Esterification (CH_2N_2) gave the somewhat more stable ester 2, which was obtained as a clear oil by further chromatography over silica gel.

A molecular formula of $C_{17}H_{22}O_2Br_2$ was established for 2 from a combination of high resolution mass spectral data (M^+ - Br: 339.07901, calcd for $C_{17}H_{22}O_2Br$, 339.07827) and combustion analysis.[5] Infrared spectral data[6] indicated a saturated ester (1740 cm^{-1}) and a disubstituted acetylene group (2222 cm^{-1}, no C≡C-H stretch). The ultraviolet spectrum[6] showed absorption (225 nm, ε 17, 290) characteristic of a conjugated diene and/or a conjugated enyne[1] group. The ^{13}C NMR spectrum[6] contained signals indicative of a disubstituted acetylene (δ 81.5, 88.5, singlets) and three double bonds (δ 112.0 - 144.7, one singlet, five doublets) which account for all of the carbon-carbon unsaturation in 2.

Catalytic hydrogenation of 2 gave methyl palmitate in good yield (ir, ms, gc). Since no signals were evident in the ^1H NMR spectrum of 2 for a terminal or vinyl methyl group, an unsaturated chain-terminating group was inferred for the natural product, but a terminal methylene group was ruled out by the ^{13}C NMR data[6].

The partial structure $-CH_2-CH_2CO_2Me$ in 2 was indicated by the infrared data,[6] a triplet at

δ 2.44 (overlapping a more complex two proton multiplet) in the ^{1}H NMR spectrum, and intense peaks at m/e and 87, 88 in the mass spectrum. The ^{1}H NMR spectrum also showed signals for two methylene units not deshielded by any factors (δ 1.41), three methylene groups in the region δ 1.7-2.2 and one methylene group (δ 2.38) adjacent to an acetylene unit. The lack of signals in the region δ 2.6-5.3 except for the methyl ester singlet indicated that both bromine atoms are attached to unsaturated carbons.

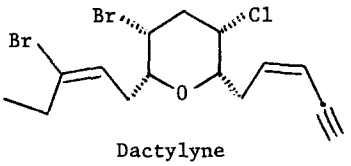
Five olefinic proton signals occur in the ^{1}H NMR spectrum[6] of 2, two of which interact in one spin system, while the other three are interrelated in another. A broad doublet at δ 5.43 (J = 16) and a doubled triplet at δ 6.03 (J = 16,7), each of which was converted to a fairly sharp doublet by irradiation in the allylic region (δ 2.06), provided evidence for a $-CH_2-CH=CH-C\equiv C-CH_2-$ unit with an E double bond configuration. Of the remaining three olefinic proton signals, a sharp doublet at δ 6.39 (J = 7.7) and a complex doublet at δ 6.70 (J = 7.7, small) indicated a Z disubstituted double bond. The final olefinic signal (δ 6.06) was a complex triplet (J = 7.7, 1.5 Hz) corresponding to a proton on a trisubstituted double bond joined on the monosubstituted side to a methylene group. Irradiation in the allylic region (δ 2.06) converted the δ 6.06 broad triplet to a slightly broadened singlet and removed some of the fine splitting from the δ 6.70 signal, which indicated that the last two double bonds are connected as follows: $BrCH=CH-CBr=CH-CH_2-$.

Conclusive evidence regarding the location and orientation of the conjugated acetylene group in the carbon chain of 2 was obtained by reducing this ester ($LiAlH_4$, -70°) and analyzing the ^{1}H NMR spectrum of the resulting alcohol 3[7] in the presence of $Eu(fod)_3$ shift reagent. At 0.21 mole ratio of shift reagent to 3, four methylene group signals were well separated: (δ 8.97, t; 4.84, quint.; 3.96, quint.; 3.52, t; all J = 7 Hz). Decoupling confirmed that these signals were due to an uninterrupted methylene chain, and irradiation of the δ 3.52 signal not only collapsed the δ 3.96 resonance to a triplet, but also eliminated much of the fine splitting in the δ 5.72 one-proton, broad doublet (J = 16) which corresponds to the δ 5.42 broad doublet in 2. Thus the acetylenic group was revealed to be at C-5,6 in 3, and the overall structures 1 and 2 are confirmed for the acid and ester, respectively. The configuration of the 13,14 double bond has not been determined.

$$BrCH=CH-CBr=CH-(CH_2)_4-CH=CH-C\equiv C-(CH_2)_3-R$$

1, R = CO_2H (7E, 13ξ, 15Z)
2, R = CO_2CH_3 "
3, R = CH_2-OH "

In view of the potentiation of pentobarbital activity noted for the halogenated acetylenic ether dactylyne,[1b] the acid 1 and ester 2 were evaluated for central nervous system activity, but neither showed activity. Both 1 and 2 showed a slight degree of cytotoxicity. The effective doses for 50% inhibition (ED_{50}) in the National Cancer Institute's *in vivo* PS and L1210 cellculture evaluations[8] were respectively: 1, 24, 34 mcg/ml; 2, 29, 34 mcg/ml. Neither 1 nor 2 showed *in vivo* PS tumor inhibitory activity.



Dactylyne

Acetylenic acids are well-known constituents of terrestrial plants,[9] but to our knowledge no such acids have been isolated previously from marine organisms. Among marine natural products the acetylene group has been found in carotenoids,[10] in two sterols,[11] and in a group of C_{15} straight chain ethers isolated from algae[12] and a sea hare.[1]

Although the occurrence of halogen is common in marine natural products,[10,12c,] the number of halogenated acids reported to date is still small.[12c,13,14] The only other halogenated long-chain acids of marine origin that we are aware of are the chlorohydrins of palmitic and stearic acid found in the lipids of an edible jellyfish.[14]

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6. IR(film) 2222, 1740, 1600, 1440, 1230, 960 cm^{-1}: UV (95% EtOH) 225, 212 (ε 17,290, 16,235) 250, 264, 281 (ε 6235, 4353, 2470); ^1H NMR (100 MHz, $CDCl_3$) 1.41 (4 H, m), 1.70-2.20 (6 H, m) 2.46 (2 H, t, J = 7 Hz, H_2), 2.37 (2 H, dt, J = 7, 2), 3.66 (3 H, s, OCH_3), 5.43 (1 H, brd d, J = 16, H_7), 6.03 (1 H, dt, J = 16, 7 Hz, H_8), 6.06 (1 H, dt, J = 1.5, 7.7 Hz, H_{13}), 6.39 (1 H, d, J = 7.7 Hz, H_{16}), 6.70 (1 H, brd d, J = 7.7 Hz, H_{15}); ^{13}C NMR

(25.2 MHz, $CDCl_3$) δ 19.5(t), 25.3(t), 29.2, 29.6, 32.2, 33.8, 52.4(q), 81.5(s), 88.5(s), 112.0(d), 114.1(d), 115.3(s), 133.0(d), 138.2(d), 144.7(d), 176.4(s).

7. IR(film) 3350, 2220 cm^{-1}; MS, 311, 309 (M^+-Br); ^1H NMR (100 MHz, $CDCl_3$) 1.20-1.80 (8 H, m) 1.80-2.50 (6 H, m), 2.60 (1 H, s, OH), 3.58 (2 H, brd t), low field signals same as in 2; 0.21 mole ratio $Eu(fod)_3$/3: δ 1.53 (4 H, m), 2.20 (4 H, m), 3.52 (2 H, t, J = 7 Hz, H_4), 3.96 (2 H, quint., H_3), 4.84 (2 H, quint., H_2), 5.72 (1 H, brd d, J = 16 Hz, H_7), 6.0-6.35 (2 H, m, H_8, H_{13}), 6.40 (1 H, d, J = 8 Hz, H_{16}), 6.72 (1 H, d, J = 8 Hz, H_{15}), 8.97 (2 H, t, J = 7 Hz, H_1).

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