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HIGH MOLECULAR WEIGHT POLYACETYLENES FROM THE NUDIBRANCH *Peltodoris atromaculata* AND THE SPONGE *Petrosia ficiformis*[1]

D. Castiello, G. Cimino, S. De Rosa, S. De Stefano and G. Sodano

Istituto per la Chimica di Molecole di Interesse Biologico del C.N.R.
Via Toiano n. 2, 80072 - Arco Felice, Naples, Italy.

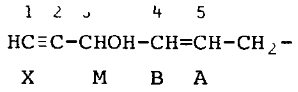
Abstract.- Two series of high molecular weight straight chain polyacetylenes (C_{46}*,* C_{49} *and* C_{49}*,* C_{52}*,* C_{55}*) have been isolated from two marine invertebrates and partially characterized.*

The chemistry of predator-prey relationship is of value for understanding some basic biological problems linked to symbiosis[2], including chemical defence mechanisms[3].

We have previously shown that the nudibranch *Peltodoris atromaculata* is able to find its prey, the sponge *Petrosia ficiformis*, by chemotaxis and to store metabolites taken up during the predation[4]. We wish to report now the isolation and chemical characterization of some unusual high molecular weight polyacetylenes from both invertebrates.

Acetone extraction of the gut content of the nudibranchs and of the whole sponges, followed by concentration of the solvent and extraction with diethyl ether of the remaining water yielded extracts showing two major spots in t.l.c. (apart from sterols and fatty acids) which have been trivially separated by silica gel column chromatography (light petroleum and increasing amounts of diethyl ether). The more polar component, constituted of a very complex mixture of polyacetylenic acids, was not further investigated. The less polar component was further splitted in two series of homologoues (fractions 1 and 2) by HPLC (μBondapak C_{18}; CH_3OH-H_2O, 9:1).

Fraction 1 (0.007% dry weight of the sponge) which does not give interpretable E.I., C.I. or F.D. mass spectra, showed an average molecular weight of 650 ± 10% (osmometric). The i.r. spectrum suggested the presence of a terminal acetylene (ν_{max} 3295 and 2110) and of other acetylenic linkages (ν_{max} 2205). The p.m.r. spectrum is dominated by an intense signal at δ 1.26 (linear $-CH_2-$ chain) a broad signal at δ 2.2 (allylic $-CH_2$'s) and a complex set of signals between δ 4.80 and 6.20; methyl groups are lacking. Decoupling experiments showed the presence of the partial structure *a*.



$$\underset{X}{\overset{1}{H}C}\equiv\overset{2}{C}-\underset{M}{\overset{3}{C}HOH}-\underset{B}{\overset{4}{C}H}=\underset{A}{\overset{5}{C}H}-CH_2-$$

a

Irradiation at δ 2.2 discloses a clear ABMX system displaying signals at δ 2.58 (H_X, d, J_{XM} 2Hz), 4.84 (H_M, dd, J_{MX} 2Hz, J_{MB} 5Hz), 5.60 (H_B, dd, J_{AB} 15Hz, J_{BM} 5Hz) and 5.92 (H_A, d, J_{AB} 15Hz); withouth irradiation at δ 2.2 the A part of the ABMX system resonates as a double triplet (J 15 and 6 Hz).

The assignment was confirmed by comparison of the above data with those[5] of the model compound *3* synthesized following a known route[6] by reaction between *trans*-hex-2-en-1-al and ethynylmagnesiumbromide.

$CH_3-CH_2-CH_2-CH=CH-CHOH-C{\equiv}CH$

3

Besides the ABMX system, the δ 2.2 irradiated p.m.r. spectrum showed the presence of an AB system at δ 6.20 and 5.40 (J_{AB} 15Hz; δ 145.5 and 109.0 in the c.m.r. spectrum), the δ 5.40 signal being further coupled (J 2Hz); without irradiation the A part resonates as a double triplet. The p.m.r. and c.m.r. chemical shifts make this AB system assignable to a double bond conjugated with an acetylenic function. The u.v. spectrum (λ_{max} 232) is in agreement with this assignment. Furthermore the c.m.r. spectrum displays a signal (doublet in the off resonance spectrum) at δ 52.5 (δ 5.2 in the p.m.r. spectrum, d, J 2Hz) shifted in the spectrum of the triacetate (acetic anhydride, py; r.t.) at δ 54.1 (δ 6.2 in the p.m.r. spectrum); therefore this signal is due to a CHOH, which must be localized between two acetylenic functions because of its very high field resonance. In the c.m.r. spectrum there is only one signal attributable to a CH_2 linked to an acetylenic function (δ 19.1) and, besides the terminal acetylenic carbon, there are only five signals of acetylenic carbons (δ 77.8, 82.5, 83.4, 85.2 and 85.6); assembling all the above evidences the partial structure *b* can be inferred.

$-CH_2-CH=CH-C{\equiv}C-CHOH-C{\equiv}C-CH_2-$

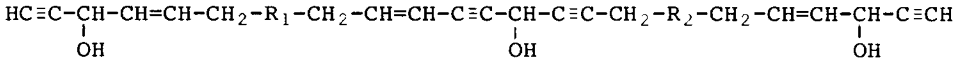
b

MnO_2 oxidation (C_6H_6; 0.5 hours; r.t.) of *fraction 1* yielded a product which, besides the i.r., u.v. and p.m.r. properties shown also by the MnO_2 oxidation product of the model compound *3* (ν_{max} 3290, 2100, 1645 cm^{-1}; λ_{max} 240 nm; δ 3.18, 1H, s; 6.20, 1H, d, J 16Hz and 7.25, 1H, dt, J 16 and 6Hz), displays spectral data attributable to the oxidized partial structure *b* (ν_{max} 2180, 1625 and 1610 cm^{-1}; λ_{max} 287 and 298 nm; δ 5.64, 1H, d, J 15Hz and 6.56, 1H, dt, J 15 and 6Hz).

Therefore *fraction 1* have at one end the partial structure *a* and in the centra part of the molecule the partial structure *b*. However the lack of methyl signals and of other possible terminal groups in the NMR spectra suggested that the partial structure *a* constitutes the end of the molecule on both sides.

In fact the integrated areas of the CHOH groups at δ 4.84 and 5.20 are in a 2:1 ratio; similarly careful examination of the ABMX system (partial structure *a*) and of the AB system (partial structure *b*) showed that the ratio of the integrated areas between the two A parts (isolated signals) is 2:1.

Finally the p.m.r. and c.m.r. spectra show signals for three isolated double bonds (p.m.r. δ 5.36, 6H; c.m.r. δ 128.1, 129.6, 130.1, 130.2, 130.9). The structure complexity prevented an unequivocal double bond location. The reported data suggested the following general formula:



$$HC\equiv C-\underset{OH}{CH}-CH=CH-CH_2-R_1-CH_2-CH=CH-C\equiv C-\underset{OH}{CH}-C\equiv C-CH_2-R_2-CH_2-CH=CH-\underset{OH}{CH}-C\equiv CH$$

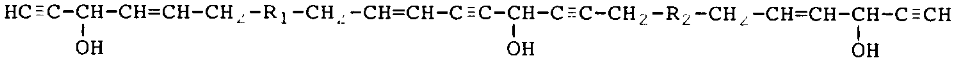
$$R_1 + R_2 = C_nH_{2n-6}; \quad n = 25,\ 28$$

1

The size of $R_1 + R_2$ was established by hydrogenolysis to the parent hydrocarbons. Hydrogenation of *fraction 1* (acetic anhydride, sodium acetate, W2 Raney Ni, 1.5 hours, 40°C) yielded two straight chain hydrocarbons $C_{46}H_{94}$ (42%) and $C_{49}H_{100}$ (58%) identified by g.l.c. data (1% Dexil on Gas-Chrom Q 100-120; 0.5m x 3mm glass column, 295°C); thus *fraction 1* is constituted of two homologoues of general formula *1* having n = 25 and 28 respectively.

The stereochemistry of the double bonds reported in the general formula *1* must be *trans*, because of the coupling constant values in the p.m.r. spectrum; accordingly in the c.m.r. spectrum there are only three signals (δ 31.6, 31.9, 33.1) attributable to CH_2's allylic to *trans* double bonds[7]. On the contrary, the isolated double bonds must have a *cis* stereochemistry because the CH_2's allylic to these double bonds resonate in the 26.2-27.6 δ zone.

Fraction 2 (0.01% dry weight of the sponge) have the same spectral features of *fraction 1*, including stereochemical details, the only difference being the number of isolated double bonds which are two (p.m.r. δ 5.36, 4H; c.m.r. δ 128.1, 129.5, 130.0, 130.8). Hydrogenolysis of *fraction 2* yielded three straight chain hydrocarbons $C_{49}H_{100}$ (5%), $C_{52}H_{106}$ (30%) and $C_{55}H_{112}$ (65%). The general formula of *fraction 2* is therefore *2*.



$$HC\equiv C-\underset{OH}{CH}-CH=CH-CH_2-R_1-CH_2-CH=CH-C\equiv C-\underset{OH}{CH}-C\equiv C-CH_2-R_2-CH_2-CH=CH-\underset{OH}{CH}-C\equiv CH$$

$$R_1 + R_2 = C_nH_{2n-4}; \quad n = 28,\ 31,\ 34$$

2

The polyacetylenes isolated from *Peltodoris atromaculata* and *Petrosia ficiformis* add to a very small group of acetylenic compounds isolated from marine organisms[8] Acetylenic compounds are widespread[9], however in our knowledge these polyacetylenes are the highest molecular weight naturally occurring acetylenic derivatives so far isolated.

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5. Spectral data for 3:
p.m.r. δ (CDCl$_3$): 2.56 (1H, d, J 2Hz; $-C\equiv C\underline{H}$); 4.82 (1H, bd, J 5Hz; $C\underline{H}OH$); 5.62 (1H, dd, J 5 and 15Hz; $-CH=C\underline{H}-CHOH-$); 6.0 (1H, dt, J 6 and 15Hz; $-C\underline{H}=CH-CHOH-$).
I.r. ν_{max} (CHCl$_3$): 3570, 3280, 2100 cm^{-} .
c.m.r. δ (CDCl$_3$): C-1, 73.8; C-2, 83.8; C-3, 62.5; C-4, 133.6; C-5, 129.1; C-6, 34.1; C-7, 22.2; C-8, 13.8.
c.m.r. data for *1* (part structure *a*) δ (CDCl$_3$): C-1, 73.9; C-3, 62.9; C-4, 134.3; C-5, 129.1.

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