NEW ACETYLENIC COMPOUNDS FROM THE SPONGE RENIERA FULVA

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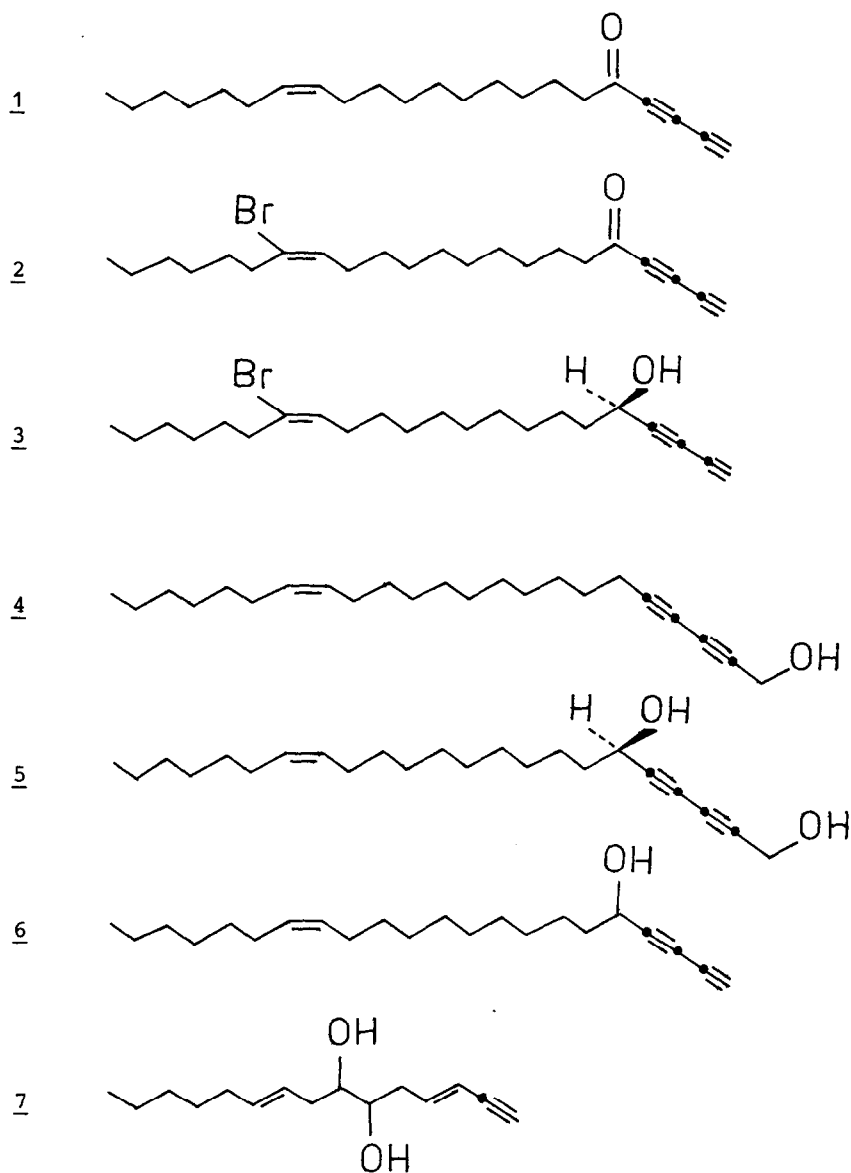
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Very few acetylenic compounds from marine organisms have been reported so far. Algae have proved to be a good source of halogenated products[1] among which a small group of ethers, characterized by a straight-chain C-15 carbon skeleton and a terminal enyne function[2], has been isolated. In the case of sponges, acetylenic functions have been found only in some arylcarotenoids isolated from Reniera japonica[3].

In the course of our research for new metabolites from marine sponges[4], Reniera fulva was found to contain a new group of substances characterized by unsaturated and in some cases, brominated, long alkyl chains containing, also, acetylenic bonds.

Fresh tissues of the sponge, collected in the bay of Naples, were exhaustively extracted with acetone. Ether-water partition of the residue and silica gel column chromatography (light-petroleum and increasing amounts of ether) of the ether-soluble fraction (5,9 g from 100 g of the dry weighted animal), followed by preparative SiO_2 t.l.c., gave five closely related new acetylenic compounds named, in order of polarity, debrorenierin-1 ($\underline{1}$; 0,04% based on the dry weight of the animal), renierin-1 ($\underline{2}$; 0,4%), 18-dihydrorenierin-1 ($\underline{3}$; 0,06%), renierin-2 ($\underline{4}$; 0,15%), 18-hydroxyrenierin-2 ($\underline{5}$; 0,06%). Three of these new substances were oils, the other two crystalline. All compounds were homogeneous on SiO_2 t.l.c. and g.l.c. (Se 30; 3%). Scarceness of material and the instability of most of them did not allow extensive chemical investigation and the structures were mainly assigned on the basis of spectral data. The molecular formulas were derived from accurate mass measurements.

Debromorenierin-1 ($\underline{1}$), $C_{22}H_{34}O$; the i.r., u.v. and n.m.r. spectra of $\underline{1}$ suggested the presence of a terminal $-\overset{\text{O}}{\underset{\text{||}}{C}}-C\equiv C-C\equiv C-H$ group; $\lambda_{max}^{CH_3OH}$ 236, 246, 262, 276 (ε 5800, 9320, 12900, 8700); ν_{max}^{film} 3300 (terminal $-C\equiv C-H$) 2920, 2850, 2210 (conjugated $-C\equiv C-$), 1670 (conjugated $-\overset{\text{O}}{\underset{\text{||}}{C}}-$), cm^{-1}; $\delta_{TMS}^{CCl_4}$ 2.5 (1H, acetylenic proton); this chromophore was confirmed by reduction of $\underline{1}$ with $NaBH_4$ which gave the conjugated diyne alcohol $\underline{6}$; $\lambda_{max}^{CH_3OH}$ 227, 240, 254 (ε 950, 800, 380). The n.m.r. spectrum of $\underline{1}$ also provided additional information on the structure showing triplets at δ 2.48 (2H, J = 6Hz; $-CH_2-CH_2-CO-C\equiv C-$) and 5.38 (2H, J = 6Hz; $-CH_2-CH=CH-CH_2-$) and a quartet at δ 1.98 (4H; $-CH_2-CH_2-CH=CH-CH_2-CH_2-$). These assignments were confirmed by spin decoupling experiments. The n.m.r. spectrum was completed by a distorted triplet at δ 0.9 (3H) and by a large signal at δ 1.26 corresponding to a normal non-branched aliphatic chain. Assignment of the cis configura-

tion of the isolated double bond was based on the absence of infrared absorption at 970 cm^{-1} [5].

The location of the double bond in the chain was established by ozonolysis of $\underline{1}$ in CH_3OH[6](4 min.)

which gave enanthic aldehyde, the methyl ester of enanthic acid and undecanoic dicarboxylic acid

as dimethyl ester. The structure $\underline{1}$ proposed for debromorenierin-1 fits in with all the above da-

ta. The mass spectral fragmentation added further confirmatory evidence: M^+/e 314 (30%), 299 $M^+ -$

$-CH_3$; 7%), 285 ($M^+ - C_2H_5$; 15%), 271 ($M^+ - C_3H_7$; 25%), 257 ($M^+ - C_4H_9$; 50%), 243 ($M^+ - C_5H_{11}$; 100%),

229 ($M^+ - C_6H_{13}$; 65%).

<u>Renierin-1</u> (<u>2</u>), $C_{22}H_{33}BrO$, M^+/e 394 (1,5%), 392 (1,5%), 313 (100%; $M^+- Br$). The spectral data are quite similar to those of debromorenierin-1: $\lambda_{max}^{CH_3OH}$ 236, 246, 262, 276 (ε 5880, 9400, 12150, 8920); ν_{max}^{film} 3260, 2920, 2850, 2210, 1675 cm^{-1}; $\delta_{TMS}^{CDCl_3}$ 0.9 (t; 3H) and 1.26 (bs) signals of a normal non-branched aliphatic chain; 2.36 (t, J = 6Hz, $-CH_2-CH_2-\underset{\delta}{C}-C\equiv C-$), 2.6 (s, 1H, acetylenic proton). Furthermore, the n.m.r. spectrum displayed signals at δ 5.8 (t, J = 6Hz, 1H; $-CH_2-CH=$ $=CBr-CH_2-$), δ 1.98 (q, J = 6Hz; 2H, $-CH_2-CH_2-CH=CBr-CH_2-CH_2-$), δ 2.5 (t, J = 6Hz; 2H, $-CH_2-CH_2-$ $-CH=CBr-CH_2-CH_2-$). These data agree with the partial structure: $-CH_2-CH_2-CBr=CH-CH_2-CH_2$ confirmed by double resonance experiments: in fact, irradiation at δ 5.8 produced a triplet at δ 1.98 and sharpened the lines of the δ 2.5 triplet.

The chromophore was confirmed by reduction with $NaBH_4$, which gave the conjugated diyne alcohol [6] <u>3</u>. The double bond was localized by ozonolysis[6] which gave, in very good yield, enanthic acid as methyl ester and a minor amount of undecanoic-dicarboxylic acid as dimethyl ester. Renierin-1 differs from debromorenierin-1 only in the presence of bromine on the double bond.

We localized the bromine on the basis of the absence of enanthic aldehyde among the products obtained by ozonolysis[6]. No evidence has been obtained to establish beyond all doubt the stereochemistry of the double bond; but the chemical shift of the olefinic proton (δ 5.8)[2] suggested a hydrogen-bromine cis relation.

<u>18-Dihydrorenierin-1</u> (<u>3</u>), $C_{22}H_{35}BrO$; M^+/e 396 (3,5%), 394 (3,5%), 315 ($M^+- Br$ 100%); $[\alpha]_D^{CHCl_3}=$ $- 5.4$ (c = 5.4); $\lambda_{max}^{CH_3OH}$ 227, 240, 254 (ε 1200, 880, 430); $\nu_{max}^{CHCl_3}$ 3580, 3330, 2920, 2850; $\delta_{TMS}^{CDCl_3}$ 0.9 (t, J = 6Hz; 3H) and 1.26 (bs) normal non-branched aliphatic chains signals, 1.6 (m, $-CH_2-$ $-CH_2-CHOH-C\equiv C-$), 1.7 (s, $CH_2-\underset{OH}{CH}-C\equiv C-$), 1.98 (q, J = 6Hz; 2H, $-CH_2-CH_2-CH=CBr-CH_2-CH_2-$), 2.15 (s, 1H, acetylenic proton), 2.4 (t, J = 6Hz; 2H, $-CH_2-CH_2-CH=CBr-CH_2-CH_2-$), 4.36 (t, J = 6Hz; 1H, $-CH_2-\underset{OH}{CH}-C\equiv C-$). Irradiation at δ 1.98 gave a singlet at δ 5.2; and at δ 1.6 a singlet at δ 4.36. 18-Dihydrorenierin-1, by ozonolysis in CH_3OH[6] (4 min.) gave a large amount of enanthic acid as methyl ester.

By analogy to <u>2</u>, an E configuration was suggested for the isolated double bond[2]. The chirality of the sole asymmetric center is assigned to be R (+ 1.4%) on the basis of a gas-chromatographic modification of Horeau's method[7]. The above results and the correlation with compound <u>2</u>, agree with the proposed structure <u>3</u>.

<u>Renierin-2</u> (<u>4</u>), $C_{23}H_{38}O$ mp 35°. $\lambda_{max}^{CH_3OH}$ 231, 242, 256 (ε 354, 330, 400); $\nu_{max}^{CHCl_3}$ 3620, 3000, 2920, 2850, 2260, 1465, 1380, 1020; M^+/e 330 (8%), 329 (8%), 315 ($M^+- CH_3$; 9%), 312 ($M^+- H_2O$; 9%), 299 ($M^+- CH_2OH$; 32%), 241 ($M^+- H_2O - CH_3(CH_2)_5-$; 100%); $\delta_{TMS}^{CDCl_3}$ 0.92 (t, J = 6Hz; 3H),1.26 (bs), 1.66 (s, $-OH$), 2 (q, J = 6Hz; 4H, $-CH_2-CH_2-CH=CH-CH_2-CH_2-$), 2.28 (t, J = 6Hz; 2H, $-CH_2-$ $-CH_2-C\equiv C-$), 4.31 (s, 2H, $C\equiv C-CH_2-OH$), 5.36 (t, J = 6Hz; 2H, $CH_2-CH=CH-CH_2-$). Irradiation at δ 2 produced a singlet at δ 5.36. Ozonolysis[6] (4 min.) gave enanthic aldehyde, enanthic acid as methylester and dodecanoic dicarboxylic acid as dimethylester. A <u>cis</u> configuration is suggested[5].

On the basis of these data we suggested for renierin-2, the structure $\underline{4}$, which was confirmed by hydrogenation with Ni raney W2 in $(CH_3CO)_2O$ and traces of fresh fused $NaOCOCH_3$, at 50° for an hour, and this gave the perhydrorenierin-2 acetate: $C_{22}H_{43}CH_2OCOCH_3$, M^+/e 382 (18%), 322 (M^+ – CH_3COOH, 100%); $\nu_{max}^{CHCl_3}$ 2900, 2840, 1720, 1460, 1365, 1210 cm^{-1}; $\delta_{TMS}^{CCl_4}$ 0.85 (t, 3H), 1.26 (bs), 1.95 (s, 3H, $\underline{CH_3}CO-$), 3.95 (t, 2H, $-CH_2-\underline{CH_2}-OCOCH_3$). The normal chain of $\underline{4}$ was confirmed because the logarithm of the retention time of its perhydroacetate versus number of carbon atoms fails on the straight line that can be built,analogously,using several normal chain primary acetilated alcohols (g.l.c.).

18-Hydroxyrenierin-2 ($\underline{5}$), $C_{23}H_{38}O_2$; mp 32°; $[\alpha]_D^{CH_3OH}$ – 38 (c = 1.6); $\lambda_{max}^{CH_3OH}$ 230, 243, 256 (ε =354, 330, 200); ν_{max}^{film} 3580, 3350, 2920, 2850, 2250, 2160, 1450, 1370, 1010 cm^{-1}; M^+/e 346 (8%), 328 (M^+ – H_2O; 20%), 315 (M^+ – CH_2OH; 50%), 243 (100%); $\delta_{TMS}^{C_6H_6}$ 0.95 (t, 3H); 1.4 (bs); 2.09 (q, J = 6Hz; 4H); 3.74 (s, 2H); 4.21 (t, J = 6Hz; 1H); 5.46 (t, J = 6Hz; 2H); $\delta_{TMS}^{CDCl_3}$ 3.8 (s, 2H exchangeable with D_2O). Ozonolysis[6] (4 min.) gave enanthic aldehyde and enanthic acid as methylester. As in the case of $\underline{1}$ a cis configuration is suggested and as for $\underline{3}$ tne configuration of 18 hydroxilated carbon is proposed to be R (+ 1.5%). The non terpenic acetylenic compounds, from algae mentioned above[2], appear to be derived from a common C_{15} precursor $\underline{7}$ which is in all probability derived from a C_{16} fatty acid. Accordingly, for the studied compounds,we can suggest a nC_{23} fatty acid precursor. This hypothesis seems to be substantiated by the finding that, by gas-chromatography, the nC_{23} fatty acid can be identified. No nC_{22} seems, on the contrary, to be present.

ACKNOWLEDGEMENT

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