Pergamon

Tetrahedron Letters 39 (1998) 6395–6398

TETRAHEDRON
LETTERS

Aztèquynol A, the first clearly defined, C-branched polyacetylene and the analogue Aztèquynol B. Isolation from the tropical marine sponge *Petrosia* sp.

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Received 14 April 1998; accepted 23 June 1998

Abstract

Aztèquynol A (**1**), isolated from the nepheliospongid sponge, *Petrosia* sp., from the Banc Aztèque off New Caledonia, represents the first case of a structurally defined C-branched polyacetylene based on high-energy collisionally-activated decomposition tandem mass spectrometry of lithium adducts which may have wide application in natural product structural analysis.

Keywords: Marine metabolites; Acetogenins; Mass spectrometry; Sponges

1. Introduction

The top of the sea mounts on the Banc Aztèque off New Caledonia is normally under violent turbulence of the waters and whirlpools, which prevent access to the diver. On a rare occasion, a few hours rest in September 1989 allowed us scuba diving at the highest sea mount, at a depth of 55 m, to collect a demosponge (620M = R1499), identified as *Petrosia* sp. (Nepheliospongida). Lipophilic extracts of this sponge proved cytotoxic against KB and P388 human tumour cell lines, as well as active in the *Artemia salina* toxicity assay. Among the unusual products in the sponge extract, we rapidly identified two methyl-branched long-chain polyacetylene alcohols ending in a carboxylic acid functionality, aztèquynols A (**1**) and B (**8**). However, determination of the position of the methyl group along the chain defied both high-field NMR spectrometry and ordinary MS techniques and the aztèquynols were left aside as chemically undefined compounds. The problem

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PII: S0040-4039(98)01319-7

has been recently reopened and the structure of aztèquynol A fully solved - except for the absolute configuration at the branching centre - with the aid of high-energy collisionally-activated decomposition tandem mass spectrometry, as described here.

2. Sponge collection, product isolation, and structure elucidation

Immediately after collections the sponge was frozen, then freeze-dried (450 g) and extracted with ethanol. The extract was subjected to SiO_2 flash chromatography (FC) under gradient elution with petroleum ether / Et_2O / AcOEt / EtOH. The fractions eluted from AcOEt to EtOH were combined and evaporated to give a residue, 4.5 g, which was subjected to RP18 reverse-phase FC, gradient-elution from 1:1 H_2O-MeOH to MeOH. The fractions eluted with MeOH gave a mixture, 285 mg, containing aztèquynol A (**1**) and aztèquynol B (**8**) in 94:6 ratio, as evaluated from NMR integration of the acetylenic proton signal, besides fatty acids. One third of this mixture was subjected to RP18 reverse-phase HPLC with 99:1 MeOH-HCOOH to give pure aztèquynol A (**1**) (t_R = 10.7 min, 42 mg). The remaining mixture was treated with ethereal diazomethane for 10 min at r.t. followed by SiO_2 HPLC with 94:6 *n*-hexane-AcOEt to give aztèquynol B methyl ester (**9**) (t_R = 14 min, 3.5 mg).

In order to increase the solubility in non-polar solvents and improve the resolution of some signals, aztèquynol A (**1**)[2] was treated briefly with CH_2N_2 to give aztèquynol methyl ester (**2**), whose composition $C_{33}H_{54}O_3$ rests on NMR[3] and MS data. 1D- and 2D-(^1H-^1H- and ^1H-^{13}C) NMR spectra support the structure of fragments C1-C6, C15-C21 and C25-C31 while ^{13}C NMR data indicate a methyl-branched, central polymethylene chain. DDQ oxidation of **2** gave enynone **5**[4],

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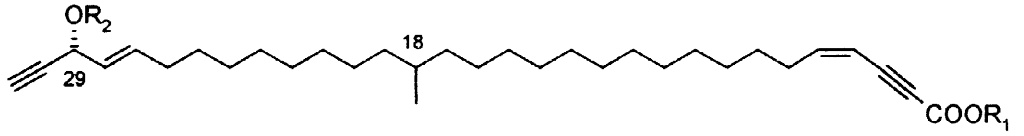
[2] Data for **1**. δ_H ($CDCl_3$, 299.94 MHz): 5.49 (dt, *J* = 10.9, 1.3, H-4), 5.99 (dt, *J* = 10.9, 7.5, H-5), 2.31 (br.dt, *J* = 7.5, 7.5, H_2-6), 1.3 (submerged, assigned from COSY, 18-H), 2.05 (br.dt, *J* = 6.8, 6.6, H_2-26), 5.89 (ddt, *J* = 15.3, 1.2, 6.8, H-27), 5.61 (ddt, *J* = 15.3, 6.0, 1.5, H-28), 4.83 (br.ddd, *J* = 6.0, 2.1, 1.2, H-29), 2.55 (d, *J* = 2.1, H-31), 0.83 (d, *J* = 6.4, Me-18), remaining protons between δ_H 1.1 and 1.5 ppm; δ_C ($CDCl_3$, 75.43 MHz): 160.71 (s, C-1), 90.55 (s, C-3), 107.70 (d, C-4), 146.93 (d, C-5), 30.75 (t, C-6), 27.07 (t, C-16 or C-20), 37.06 (t, C-17), 32.72 (d, C-18), 37.06 (t, C-19), 27.05 (t, C-20 or C-16), 31.96 (t, C-26), 134.36 (d, C-27), 128.48 (d, C-28), 62.65 (d, C-29), 83.54 (s, C-30), 73.83 (d, C-31), 19.76 (q, Me-18), remaining methylene carbons between 28.8 and 30.1 ppm.

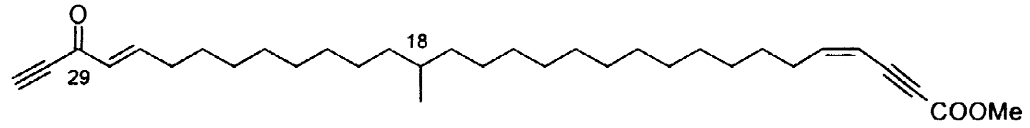
[3] Data for **2**. δ_H ($CDCl_3$): 5.55 (dt, *J* = 10.8, 1.5, H-4), 6.25 (dt, *J* = 10.8, 7.5, H-5), 2.37 (ddt, *J* = 7.5, 1.5, 7.5, H_2-6), 1.32 (submerged, assigned from COSY, 18-H), 2.06 (br.dt, *J* = 6.8, 6.8, H_2-26), 5.92 (ddt, *J* = 15.3, 1.2, 6.8, H-27), 5.61 (ddt, *J* = 15.3, 6.0, 1.5, H-28), 4.84 (br.m, H-29), 2.56 (d, *J* = 2.1, H-31), 0.83 (d, *J* = 6.3, Me-18), 3.79 (s, MeO), remaining protons between 1.1 and 1.5 ppm; δ_C ($CDCl_3$): 154.55 (s, C-1), 83.35 (s, C-2), 84.13 (s, C-3), 106.39 (d, C-4), 151.70 (d, C-5), 30.97 (t, C-6), 30.05 (t, C-15 or C-21), 27.11 (t, C-16 or C-20), 37.11 (t, C-17 or C-19), 32.77 (d, C-18), 37.10 (t, C-19 or C-17), 27.10 (t, C-20 or C-16), 30.01 (t, C-21 or C-15), 31.95 (t, C-26), 134.66 (d, C-27), 128.34 (d, C-28), 62.84 (d, C-29), 83.83 (s, C-30), 73.95 (d, C-31), 19.73 (q, Me-18), 52.65 (q, MeO), remaining, unassigned methylene triplets at δ_C 29.75, 29.71, 29.69, 29.65, 29.63, 29.57, 29.49, 29.42, 29.20, 29.11, 28.84 and 28.59 ppm.

[4] Data for **5**. δ_H ($CDCl_3$): 5.55 (dt, *J* = 10.8, 1.5, H-4), 6.25 (dt, *J* = 10.8, 7.5, H-5), 2.37 (ddt, *J* = 7.5, 1.5, 7.5, H_2-6), 1.3 (submerged, assigned from COSY, 18-H), 2.31 (ddt, *J* = 6.9, 6.8, 1.5, H_2-26), 7.25 (dt, *J* = 15.6, 6.9, H-27), 6.18 (dt, *J* = 15.6, 1.5, H-28), 3.21 (s, H-31), 0.83 (d, *J* = 6.5, Me-18), 3.79 (s, MeO), remaining protons between 1.1 and 1.5 ppm; δ_C ($CDCl_3$): 83.83 (s, C-2), 84.31 (s, C-3), 106.39 (d, C-4), 151.70 (d, C-5), 30.97 (t, C-6), 28.59 (t, C-7), 30.06 (t, C-15 or C-21), 27.11 (t, C-16 or C-20), 37.11 (t, C-17 or C-19), 32.77 (d, C-18), 37.09 (t, C-19 or C-17), 27.06 (t, C-20 or C-16), 29.97 (t, C-21 or C-15), 29.38 (t, C-24), 29.21 (t, C-25), 27.85 (t, C-26), 32.71 (t, C-26), 155.98 (d, C-27), 131.89 (d, C-28), 78.88 (d, C-31), 19.72 (q, Me-18), 52.65 (q, MeO), besides unassigned methylene triplets at δ_C 29.75, 29.71, 29.69, 29.65, 29.57, 29.55, 29.42 and 29.11 ppm.

which showed Yb(fod)$_3$-induced deshielding effects on protons at positions 28 > 27 = 31 > MeO



1 $R_1=R_2=H$
2 $R_1=Me$, $R_2=H$
3 $R_1=Me$, $R_2=(S)$-MTPA
4 $R_1=Me$, $R_2=(R)$-MTPA



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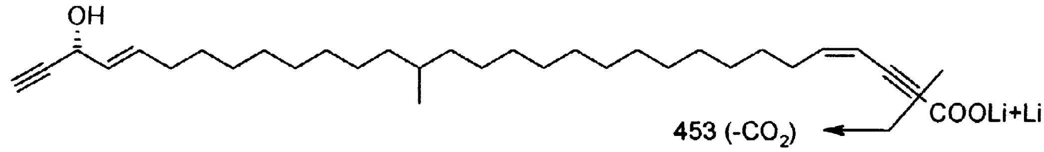

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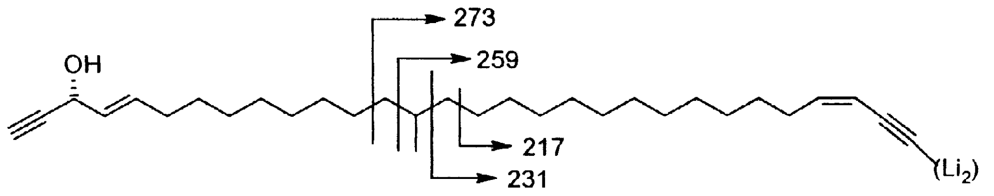
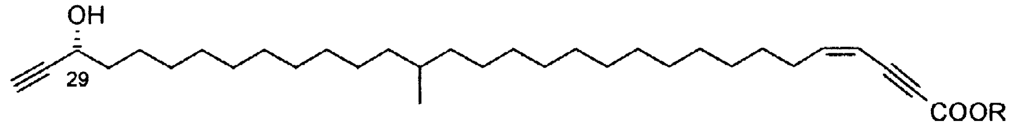


7 (*m/z* = 453)



8 R=H
9 R=Me

> 26 > 25 > 4 > 24 > 5 > 6 > 23 > 7, decreasing in this order in a range from 0.05 to 0.4 eq of added shift reagent. This allows us to extend the assignment of the resonances from the C1-C6 fragment to the C1-C-7 fragment and from the C31-C25 fragment to the C31-C23 fragment. EIMS of aztèquynol A (**1**) revealed peaks at *m/z* 466 and 440 for the loss of H_2O and CO_2, respectively, from the molecular ion, which could not be detected. Ionspray mass spectra of aztèquynol A (**1**) with added ammonium salts revealed peaks at *m/z* 507 ($[M + Na]^+$) and 502 ($[M + NH_4]^+$), while low-energy CAD [1] (20 eV) on *m/z* 502 revealed positive ions for loss of H_2O, unusually preceding loss of NH_3, which gave fragment ion *m/z* 467. Loss of CO from the latter gave a prominent peak at *m/z* 439, while a series of peaks for fragment ions deriving from charge-remote fragmentation of the polymethylene chain was observed, not extending, however, to the position of methyl branching. Recourse was then made to high energy CAD on a VG ZAB-T four-sector tandem mass spectrometer. Thus, FAB of aztèquynol A (**1**), in a *m*-NBA matrix with added LiI, gave signals at *m/z* 497 ($[M - H + 2Li]^+$), 491 ($[M + Li]^+$) and 453 ($[M - H - CO_2 + 2Li]^+$). High-energy CAD [2,3] on *m/z* 497, BpI 141, gave, via ion **6**, a prominent peak at *m/z* 453 for **7**, which underwent charge-remote fragmentation over the whole polymethylene chain. Lack of a 14 unit fragment between peak *m/z* 259 (more intense) and peak *m/z* 231 (less intense) locates the methyl branching at C-18. This was confirmed by a similar CAD on the lithium adduct of the

molecular ion at m/z 491, by which lack of a 14 unit fragment could be noticed between peak m/z 297 (more intense, for C18-C19 breaking) and peak m/z 269 (less intense, for C17-C18 breaking).

In order to assign the configuration at the carbinol centre, Mosher's MTPA esters **3** and **4**[5] were prepared by a methodology already reported in detail for other compounds [4]. A consistent trend $\Delta\delta = \delta(S) - \delta(R) = -13, +34, +20$ and $+12$ Hz for H-31, H-28, H-27 and H_2-26, respectively, and only slight deviation, $\Delta\delta = -3$, from the ideal Mosher's value for the proton at the ester centre, allows us to assign with confidence configuration (R) at C-29.

The minor analogue, aztèquynol B (**8**), was analysed as the methyl ester, **9**[6], although neither MS-MS nor chiroptical experiments were carried out. Therefore, the position of methyl branching and the absolute configuration at the carbinol centre in both **8** and **9** are only drawn as in aztèquynol A (**1**) for analogy owing to co-occurrence, and thus likely similar biogenesis.

3. Perspective

Sponges in the orders Nepheliospongida and Haplosclerida are typically rich in polyacetylenes, normally straight-chain compounds. Those structurally most closely related to the aztèquynols, albeit unbranched, are the petroformynes A-B [5]. These have the opposite configuration at the carbinol centre with respect to aztèquynol A (**1**). While the aztèquynols were structurally undefined compounds, methyl-branched polyacetylenes appeared in the literature - as the only other example of C-branching in this compound class, though undefined as to the position of branching - as isolates from a haplosclerid sponge, *Haliclona* sp., collected at Palau [6].

Neither aztèquynol A (**1**) nor aztèquynol B (**2**) (showing < 20% cytotoxicity at 10µg/ml) account for the cytotoxicity of the sponge extracts on KB tumour cell lines.

We thank the ORSTOM diving team for the sponge collection, C. Levi for the taxonomy of the sponge, as well as MURST and CNR, Roma, for financial support. This work was carried out within the collaborative ORSTOM-CNRS Program on Marine Substances of Biological Interest.

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[5] Relevant data for ester **3**, δ_H ($CDCl_3$): 2.59 (d, J = 2.2, H-31), 6.01 (br.d, J = 6.0, H-29), 5.60 (ddt, J = 15.3, 7.0, 1.5, H-28), 6.07 (br.dt, J = 15.3, 7.0, H-27), 2.08 (ddt, J = 7.0, 7.0, H_2-26); for ester **4** δ_H ($CDCl_3$): 2.62 (d, J = 2.2, H-31), 6.02 (br.d, J = 6.0, H-29), 5.49 (ddt, J = 15.2, 6.9, 1.5, H-28), 6.00 (br.t, J = 15.2, 7.0, H-27), 2.04 (ddt, J = 7.0, 7.0, H_2-26).

[6] Data of **9**. δ_H ($CDCl_3$): 5.53 (dt, J = 10.7, 1.5, H-4), 6.24 (dt, J = 10.7, 7.5, H-5), 2.36 (ddt, J = 7.5, 1.5, 7.5, H_2-6), 1.3 (submerged, 18-H), 1.65 (br.m, H_2-28), 4.36 (br.t, H-29), 2.45 (d, J = 2.1, H-31), 0.82 (d, J = 6.4, Me-18), 3.78 (s, MeO), remaining protons between δ_H 1.1 and 1.5 ppm; δ_C ($CDCl_3$): 154.53 (s, C-1), 83.81 (s, C-2), 84.27 (s, C-3), 106.35 (d, C-4), 151.70 (d, C-5), 30.96 (t, C-6), 30.04 (t, C-15 or C-21), 27.08 (t, C-16 and C-20), 37.09 (t, C-17 and C-19), 32.74 (d, C-18), 30.02 (t, C-21 or C-15), 25.00 (t, C-27), 37.66 (t, C-28), 62.35 (d, C-29), 85.00 (s, C-30), 72.80 (d, C-31), 19.71 (q, Me-18), 52.65 (q, MeO), remaining, unassigned methylene triplets at δ_C 29.73, 29.70 (2C), 29.67, 29.64 (2C), 29.55 (2C), 29.51, 29.41, 29.23, 29.09 and 28.57 ppm. EIMS m/z (%) 468 (0.3, $[M - H_2O]^{+}$), 442 (4.6, $[M - CO_2]^{+}$), 427 (1.2), 414 (1.4), 55 (100).