Pergamon

Tetrahedron Letters, Vol. 35, No. 9, pp. 1441-1444, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0086-D

Studies on the Biosynthesis of the Polyether Marine Toxin Dinophysistoxin-1 (DTX-1)

Manuel Norte, Agustín Padilla and José J. Fernández.

Institute of Bioorganic Chemistry, University of La Laguna. 38206 La Laguna, Tenerife, Spain.

Abstracts: The biosynthetic origin of DTX-1 is establish on the basis of the NMR spectral data of samples obtained by addition of labelled [1-$^{13}C_1$], [2-$^{13}C_1$] and [1,2-$^{13}C_2$] sodium acetate to artificial cultures of the dinoflagellate *Prorocentrum lima*.

Dinophysistoxin-1 (DTX-1)[1] belongs, together with okadaic acid (OA)[2] and the dinophysistoxins-2[3] and 3, to a class of toxins responsible for a red tide phenomenon known as Diarrhetic Shellfish Poisoning (DSP) (Fig. 1a). These toxins are produced by the dinoflagellates *Prorocentrum lima, Dinophysis fortii* and *Dinophysis acuminata,* and it has been established that they are potent tumour-promoting agents and protein phosphatase inhibitors.

The structure of this group of toxins somewhat resembles the polyether ionophores from terrestrial microorganisms, which are characterized by possessing oxolane and oxane rings. Conversely, other groups of polyether marine toxins isolated from red tide dinoflagellates, the brevetoxins (BTXs) produced by *Phtychodiscus brevis*[4], maitotoxin and ciguatoxins isolated from *Gambierdiscus toxicus*[5,6] are characterized by the presence of 6/7/8/9 ether rings trans-fused in a ladder-like manner.

Although extensive ^{13}C-NMR biosynthetic studies have been carried out of the polyether antibiotics produced by terrestrial microorganisms, in the case of polyether marine toxins they are restricted to brevetoxins A and B, probably because of the difficulties in obtaining artificial cultures of the dinoflagellates and also because these organisms seem to be highly selective, using exogenous labelled precursors. The feeding experiments using ^{13}C-labelled acetates for BTXs showed that the carbon skeletons are biosynthesized by a quite different mechanism in comparison with the polyether antibiotics from terrestrial sources. On the basis of these results it has been proposed that perhaps this constitutes one of the most important distinctions between the metabolites from terrestrial and marine sources as a consequence of their different evolutions[7]. In view of the above results and the resemblance between DTX-1 and the terrestrial polyethers, we decided to investigate its biogenesis in order to progress in the knowledge of the biosynthesis of these fascinating molecules.

The carbon signal assignment of the ^{13}C NMR chemical shifts in DTX-1[8] was made by a combination of 2D-NMR techniques and by comparison of the spectral data with those observed for OA[9]. A combination of COSY and HMQC experiments allowed the assignment of most protonated carbons in DTX-1, all the remainder being assigned by the HMBC experiment. The biogenesis and the confirmation of the assigned NMR carbon chemical shifts were next carried out by measurements of labelled samples.[10].

The ^{13}C-NMR spectrum of DTX-1 labelled from [2-$^{13}C_1$] sodium acetate showed significant enrichment of 27 carbons, whereas those enriched by [1-$^{13}C_1$] sodium acetate were 16. These results accounted for the origin of all carbons except C-37 and C-38, which did not show distinctive enrichment. The ^{13}C-NMR spectrum of DTX-1 labelled from [1,2-$^{13}C_2$] sodium acetate showed most of the enriched carbon signals flanked by two strong satellite signals which, in accordance with the carbon-carbon coupling constant values, established the definite incorporation of 16 acetate units. These results are summarized in Fig. 1b.

The most prominent feature in these results was that in the ^{13}C-NMR spectrum of the sample enriched by [2-$^{13}C_1$] sodium acetate, several signals showed distinct splittings due to spin-spin coupling between the

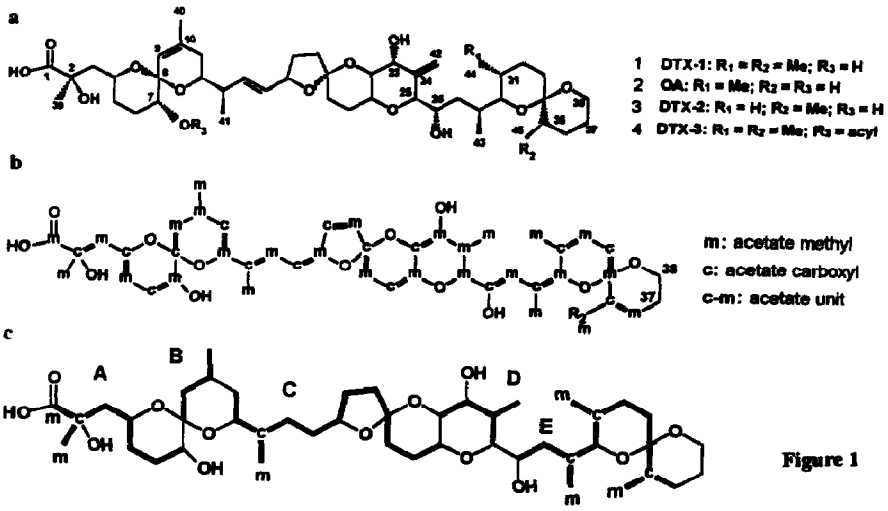

Figure 1

adjacent carbons. Thus, a detailed analysis of these splittings showed that in fragment C-8/Me-40, carbon signals from C-9, and Me-40 appear flanked by two weak satellite signals, whereas the carbon signal from C-10 was flanked by four, with constant coupling values identical with those observed for C-9 and Me-40. A reverse situation was observed in the carboxyl-acetate labelled sample, where only the signal from C-8 was enriched. The results in the [1,2-$^{13}C_2$] sodium acetate feeding experiment showed that the carbon signals from C-10 and Me-40 still showed the same labelling pattern, while the C-8 and C-9 carbon signals were flanked by two strong satellite signals, thus establishing the presence of an intact acetate unit between them and the presence of an "m-m-m-c" moiety in this fragment of the molecule (Fig. 2). A similar situation was observed for fragment C-23/C-26. However, in the methyl acetate-labelled sample, only two weak satellite signals could be observed around the C-25 carbon signal, due to the fact that the coupling constants values between C-24/C-25 and C-25/C-26 were very similar. As observed for fragment B, for fragment D the only change in the sample enriched by [1,2 $^{13}C_2$] sodium acetate was the presence of an acetate unit between C-23 and C-24, thus establishing the presence of a "c-m-m(m)-m" moiety in this fragment (Fig. 2).

In DTX-1, all branching methyl groups C-39, C-41, C-43, C-44 and C-45 as well as the carboxyl carbon C-1 showed distinctive single enrichment in the sample enriched by [2,$^{13}C_1$] sodium acetate. However, in the sample enriched by [1,2-$^{13}C_1$] sodium acetate, each carbon signal was flanked by two weak satellite signals. This result clearly showed the simultaneous presence of methyl and carboxyl ^{13}C-enriched vicinal carbons in some molecules, although they did not belong to an intact acetate unit.

In accordance with these results, DTX-1 is biosynthesized through the condensation of three kinds of ketogenic fragments (Fig. 1c). Fragments A, C and E are classical polyketides derived as a result of the condensation of four-, six- and five-acetate units, respectively. All of them showed the presence of several methyl branches derived from the C-2 carbon of an acetate and attached to carbons in a linear carbon chain that has been derived from C-1 carbon of an acetate, which is rarely encountered in polyketide biosynthesis. However, similar situations were observed in the biosynthesis of BTXs[7] as well as for the antibiotic virginiamicin M, the macrolide myxoveriscin A and the seawater bacterial metabolite oncorhyncolide[11], where the methylation by an "m" of acetate with a subsequent loss of one C-1 unit has been proposed.

With regard to the building blocks B and D, the proposed biosynthetic origin is as follows. Fragment B, C-8/C-9/C-10/Me-40 contains the "c-m-m-m" moiety and it seems reasonable that this fragment is directly or indirectly derived from succinate after [1,2-$^{13}C_2$] acetate, two rounds through the TCA cycle, as has been observed in the biosynthesis of the BTXs [7]. With respect to fragment D, its structure seems to be derived from 3-hydroxyl-3-methyl-glutarate, although the absence of an intact acetate unit between C-25 and C-42 clearly discards its formation from acetate as established in the isoprenoid biosynthesis. On the other hand, the

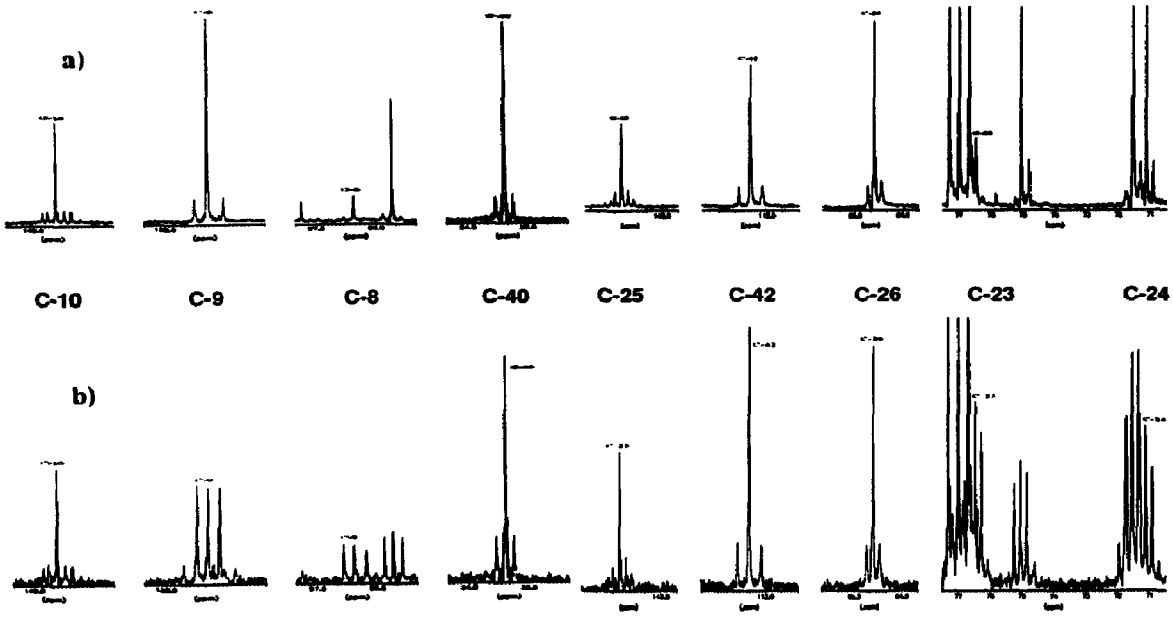

Figure 2: ^{13}C-NMR carbon signals of fragments B and D resulting from a) feeding of [2-$^{13}C_1$] sodium acetate and b) feeding of [1,2-$^{13}C_2$] sodium acetate

possibility has been proposed of amino acid participation, by using their metabolized small fragments obtained after deamination, in the formation of secondary metabolites in dinoflagellates. It has been also observed during the biosynthetic studies of the terrestrial polyether monensin A in *Streptomyces cinnamonensis*, where L-valine was the source of n-butyrate[12]. Taking into account that the L-valine metabolism yields isobutyrate, this result establish the capability of this organism to carry out the bioconversion of isobutyrate into n-butyrate. A similar situation could be hypothesized in order to explain the observed labelling pattern in fragment D. Thus, all L-valine carbons derive from pyruvate which, in turn, could be obtained from oxaloacetate by decarboxylation through the C_4 pathway. As observed for L-valine metabolism in *S. cinnamonensis*[13], the n-butyrate should be formed and its β-oxidation will give acetoacetate. Taking into account the labelling pattern of oxaloacetate after the second TCA cycle using [1,2 ^{13}C] acetate, it could be explained that all carbons from the acetoacetate were methyl-acetate labelled. This acetoacetate should be used to build the HMGA as required by the labelling pattern in fragment D .

Recently the preliminary results in the biosynthesis of okadaic acid by Yasumoto et al. have been published[12]. In the experiment with labelled ^{13}C-sodium acetate they did not observe distinctive enrichment of carbons C-1, C-2 and Me-39 or carbons C-37 and C-38 nor did they did establish the carbon-carbon connectivity between carbons C-24 and C-25. In consequence, their advanced hypothesis of the origin of the okadaic acid backbone carbon-chain differs from our proposal for DTX-1, although it is logical to think of a common biosynthetic origin.

The results we have obtained clearly showed that the heavy involvement of the dicarboxyclic acids from the TCA cycle observed in the biosynthesis of BTXs is not a general rule for the biosynthetic origin of polyethers from marine microorganisms. In DTX-1, most of the backbone carbon chain is built up by the direct condensation of acetate units, as observed in classical polyketides. Moreover, taking into account that the involvement of aminoacids in the biogenesis of terrestrial polyethers has been demonstrated, the biosynthetic origin of DTX-1 is not far from that of the ionophores from terrestrial microorganisms. Studies to identify the biosynthetic origin of carbons C-37 and C-38 as well as to evaluate L-valine catabolism to acetoacetate are currently in progress.

Acknowledgments: M.N. acknowledges financial support from the Plan Nacional de Investigaciones Farmacéuticas Far 91-0827. A. P. thanks M.E.C. for a fellowship. Authors thanks I. Bravo from IEO (Vigo) for a *P. lima* culture.

References and Notes.-

1.- Tachibana, K.; Scheuer, P.J.; Kikuchi, H.; Tsukitani, Y.; Van Engen, D.; Clardy, J.; Gopichand, Y.; Schmitz, F.J.; *J. Am. Chem Soc.*, **1981**, *103*, 2469-2471

2.- Yasumoto, T.; Murata, M.; Oshima, Y.; Sano, M.; Matsumoto, G.K.; Clardy, J.; *Tetrahedron* **1985**, *41*, 1019-1025

3.- Tingmo, H.; Doyle, J.; Jackson, D.; Marr, J.; Nixon, E.; Pleasance, S.; Quilliam, M.; Walter, J.; Whright, J. J. *Chem. Soc. Chem. Commun.*, **1992**, 39-41

4.- a) Shimizu, Y.; Chou, H.N.; Bando, H.; Van Duyne, G.; Clardy, J.C., *J. Am. Chem. Soc.*, **1986**, *108*, 514-515, and references cited therein. b) Lee, M.S.; Tempesta, M.S.; Nakanishi, K.; Golik, J.; Pawlak, J.; Zagorski, M.G.; Iwashita, T.; Gross, M.L.; Tomer, K.B., *J. Am. Chem. Soc.* **1987**, *109*, 1144-1150, and references cited therein.

5.- Murata, M.; Naoki, H.; Iwashita, T.; Matsunaga, S.; Sasaki, M.; Yokoyama, A.; Yasumoto, T.; *J. Am. Chem. Soc.* **1993**, *115*, 2060-2062

6.- a) Murata, M.; Legrand, A.M.; Ishibashi, Y.; Fukui, M.; Yasumoto, T., *J. Am. Chem. Soc.*, **1990**, *112*, 4380-4386. b) Satake, M.; Murata, M.; Yasumoto, T. *Tetrahedron Letters*, **1993**, *34*, 1975-1978

7.- Lee, M.S.; Qin, G.; Nakanishi, K.; Zagorski, M.G.; *J. Am. Chem. Soc.*, **1989**, *111*, 6234-6241. b) Repeta, D.J.; Nakanishi, K.; Zagorski, M.G., *J. Am. Chem. Soc.*, *108*, 7855-7856. c) Chou, H.N.; Shimizu, Y., *J. Am. Chem. Soc.*, **1987**, *109*, 2184-2185.

8 .- **DTX-1**: ^{13}C-NMR (100 MHz, $CDCl_3$) of sample labelled from [1,2-$^{13}C_2$] sodium acetate δ: 11.13 (C-44); 16.29 (C-41); 16.44 (C-43); 17.16 (C-45); 23.48 (C-40); 26.37 (C-33, J=45.1 Hz); 26.77 (C-37); 26.87 (C-32, J=41.2 Hz); 27.01 (C-6, J=36.5 Hz); 27.53 (C-21, J=36.9 Hz); 27.72 (C-39); 27.83 (C-31 J=41.5 Hz); 27.88 (C-36, J=31.8 Hz); 31.06 (C-17, J=32.8 Hz); 31.59 (C-29, J=40.1 Hz); 32.01 (C-5, J=35.3 Hz); 33.24 (C-20, J=44.9 Hz); 33.63 (C-11 J=37 Hz); 35.66 (C-28, J=40.2 Hz); 38); 65.11 (C-27, J=40.3 Hz); 69.69 (C-4 J=35.7 Hz); 70.18 (C-22, J=37.1 Hz); 71.52 (C-24, J=39.9 Hz); 71.93 (C-12, J=36.6 Hz); 71.98 (C-7, J=36.2 Hz); 75.23 (C-30, J=39.8 Hz); 76.87 (C-23, J=40 Hz); 77.59 (C-2, J=37.3 Hz); 79.62 (C-16, J=50.4 Hz); 85.34 (C-26); 96.94 (C-8, J=55.8 Hz); 98.31 (C-34, J=44.8 Hz); 106.11 (C-19, J=43 Hz); 112.94 (C-42); 121.88 (C-9, J=56 Hz); 131.71 (C-15, J=50 Hz); 136.82 (C-14, J=43 Hz); 139.80 (C-10); 145.06 (C-25); 177.26 (C-1).

9.- Norte, M.; González, R.; Fernández, J.J., Rico, M. *Tetrahedron*, **1991**, 7437-7446.

10.- In a typical labelling experiment, 10 x 3l flasks of bacteria-free unialgal culture of *P. lima* were grown at 25 °C under constant illumination in Guillard K medium. Ten days after inoculation, each flask was fed with labelled [1-^{13}C] or [2-^{13}C] or [1,2-^{13}C] sodium acetate (0.67 mmol/l). The culture was grown for a further 10 days, the cells were filtered, then extracted with $CHCl_3$:MeOH (1:1) and 2 mg of DTX-1 were isolated.

11.- a)Kingston, D.G.I.; Kolpak, M.X.; Lefevre, J.W.; Borup-Grochtmann, I., *J. Am. Chem. Soc.*, **1983**, *105*, 5105-5110 and references cited therein. b)Trowitzch, W.; Gerth, K.K.; Wray, V.; Höfle, G.J.; *J. Chem. Soc. Chem. Commun.*, **1983**, 1174-1175. c) Needham J., Andersen R. J., Kelly M. T. *J. Chem. Soc. Chem. Comm.*, **1992**, 1367-1369

12.- Reynolds K.A., O'Hagan D., Gani D., Robinson J.A., *J. Chem. Soc. Perkin Trans I.*, **1988**, 3195-32078

13.- Yasumoto T., Torigoe, K., J. Nat. Prod., **1991**, *54*, 1487

(*Received in UK* 15 *November* 1993; *revised* 31 *December* 1993; *accepted* 7 *January* 1994)