remains similar to those in the other β-$(ET)_2X$ salts.

In conclusion, the high-T_c superconducting phase, β^*-$(ET)_2I_3$, is completely ordered although it is not isostructural with other known β-$(ET)_2X$ materials. Furthermore, there appears to be a correlation between the degree of structural ordering and T_c in these compounds. That is, β-$(ET)_2I_2Br$ does not become superconducting apparently because of the random I/Br occupancy of the terminal *anion* position. The ambient-pressure modulated β-$(ET)_2I_3$ structure is ordered, but the periodicity of the modulation is incommensurate with the average lattice. This may result in suppression of T_c to 1.4 K. Finally, the pressure-induced structural phase transition from the modulated β-$(ET)_2I_3$ to the completely ordered β^*-$(ET)_2I_3$ produces a dramatic rise in T_c to 8 K. This value is now in agreement with band calculation predictions of increasing T_c's for the IBr_2^-, AuI_2^-, and I_3^- salts, in that order,[1a] which is also the order of increasing anion size.

Note Added in Proof. In a very recent report[14] (in Russian), an X-ray diffraction study of β-$(ET)_2I_3$ at room temperature and P = 9.5 kbar is described. Although hydrogen atoms were not located, the reported crystal structure is isostructural with the β^*-phase presented here. Thus, the β^* *structural* phase, characterized with the sample in its high-T_c superconducting state, exists at room temperature if sufficient pressure is applied.

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Supplementary Material Available: Tables of positional and thermal parameters for β^*-$(ET)_2I_3$ at 4.5 K and 1.5 kbar and at 6.1 K and 4.6 kbar (2 pages); table of structure factors for β^*-$(ET)_2I_3$ (5 pages). Ordering information is given on any current masthead page.

(14) Molchanov, V. N.; Shibaeva, R. P.; Kachinskii, V. N.; Yagubskii, E. B.; Simonov, V. N.; Vainshtein, B. K. *Dokl. Akad. Sci. USSR* **1986**, *286*, 637.

Biosynthetic Origins and Assignments of ^{13}C NMR Peaks of Brevetoxin B

Min S. Lee, Daniel J. Repeta, and Koji Nakanishi*

Department of Chemistry, Columbia University New York, New York 10027

Michael G. Zagorski

Department of Biochemistry and Molecular Biophysics College of Physicians and Surgeons Columbia University, New York, New York 10032

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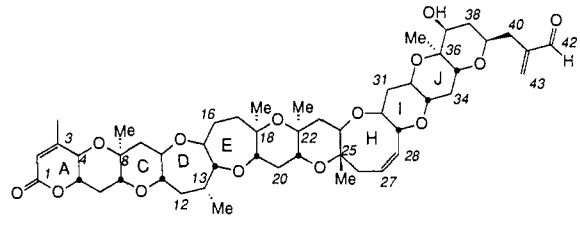
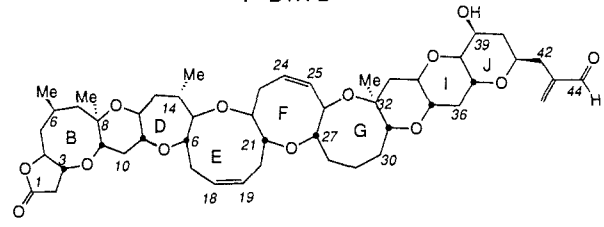
The explosive growth of dinoflagellates, *Gymnodinium breve* Davis (*Ptychodiscus brevis* Davis), the "red tide", has led to massive fish kills, mollusk poisoning, and human food poisoning along the Florida coast and the Gulf of Mexico.[1] The potent neurotoxins responsible for this are the brevetoxins (BTX) with linear polycyclic ether structures represented by brevetoxin B (BTX-B) (**1**) $C_{50}H_{70}O_{14}$ and congeners;[2] recently, the structure of BTX-A (**2**) having a different carbon skeleton has been elucidated.[3] Here we report the assignment of ^{13}C NMR peaks and biosynthetic origins of carbon atoms in BTX-B by applying the 2D NMR INADEQUATE sequence[4] to BTX-B biosynthesized from sodium [1,2-$^{13}C_2$]acetate. This led to clarification of C–C connectivities of 14 acetate units by using 1.5 mg of sample (Figure 1).



1 BTX-B



2 BTX-A

The NMR assignment of hydrogens and carbons in nonlabeled BTX presents a challenge, due to limited sample amount and repetition of similar moieties, i.e., 10 cyclic ethers or 17 –O–CH– groups, 12 methylenes, and 6 angular methyls. The methyl, methylene, and methine carbons were identified by the DEPT technique,[5] while quaternary carbons were identified by a quaternary-only sequence[6] under conditions of suppressed nuclear Overhauser enhancement. A combination of ^1H–^1H COSY[7] and ^1H–^{13}C COSY (HETCOR)[8] via one-bond coupling allowed assignment of most protonated carbons and many protons, especially the severely overlapping methylene protons. These experiments resulted in assignments of 35 carbons. Of the remaining 15 carbons, eight were quaternary, all of which could be assigned by the COLOC sequence[9] designed to detect ^1H–^{13}C two- and three-bond coupling; namely, it allowed one to correlate all methyl protons to quaternary carbons ($^2J_{CH}$) and methylene carbons ($^3J_{CH}$).

The biogenesis and NMR assignments of carbon atoms were next carried out by measurements of samples enriched with [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]acetates and methyl-^{13}C-methionine. In a typical labeling experiment, artificial saltwater medium (NH-15)[10] is inoculated with *G. Breve* culture and 10 days later treated with penicillin G and streptomycin sulfate. On day 11 the culture medium is treated with 0.68 mM labeled sodium acetate, the culture is grown for a further 7 days at 20 °C under constant illumination, and the BTX's are isolated. Namely, *G. breve* is extracted with ether and the crude extract is purified by preparative TLC, 5:1 hexane/isopropyl alcohol. The BTX's are further purified by normal-phase HPLC by using 6:1 isooctane/isopropyl alcohol; 18 L of *G. breve* culture usually yielded 1–2 mg of BTX-B and 0.5–0.8 mg of BTX-A. However, in some

(1) (a) Shimizu, Y. In *Marine Natural Products*; Scheuer, P. J. Ed.; Academic: New York, 1978; Vol. 1, Chapter 1. (b) Nakanishi, K. *Toxicon* **1985**, *23*, 473–479 and references cited therein.

(2) (a) Lin, Y. Y.; Risk, M.; Ray, S. M.; Van Engen, D.; Clardy, J.; Golik J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773–6775. (b) Golik, J.; James, J. C.; Nakanishi, K.; Lin, Y. Y. *Tetrahedron Lett.* **1982**, *23*, 2535–2538. (c) Chou, H. N.; Shimizu, Y. *Tetrahedron Lett.* **1982**, *23*, 5521–5524. (d) Chou, H. N.; Shimizu, Y.; Van Duyne, G.; Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 2865–2868. (e) Alam, M.; Sanduja. R.; Hossain, M. B.; van der Helm, D. *J. Am. Chem. Soc.* **1982**, *104*, 5232–5234. (f) DiNovi, M.; Trainor, D. A.; Nakanishi, K.; Sanduja, R.; Alam, M. *Tetrahedron Lett.* **1983**, *24*, 855–858.

(3) Shimizu, Y.; Chou, H. N.; Bando, H.; Van Duyne, G.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 514–515.

(4) Turner, D. L. *J. Magn. Reson.* **1982**, *49*, 175–178. (b) Mareci, T. H.; Freeman, R. *J. Magn. Reson.* **1982**, *48*, 158–163.

(5) Pegg, D. T.; Doddrell, D. M.; Bendall, M. P. *J. Chem. Phys.* **1982**, *77*, 2745–2752.

(6) Bendall, M. R.; Pegg, D. T. *J. Magn. Reson.* **1983**, *53*, 272–296.

(7) (a) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229–2246. (b) Bax, A.; Neuman, R. *J. Magn. Reson.* **1981**, *44*, 542–561.

(8) (a) Maudsley, A. A.; Kumar, A.; Ernst, R. R. *J. Magn. Reson.* **1977**, *28*, 463–469. (b) Bodenhausen, G.; Freeman, R. *J. Magn. Reson.* **1977**, *28*, 471–476.

(9) (a) Kessler, H.; Griesingie, C.; Zarboch, J.; Foosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331–336. (b) Kessler, H.; Griesingie, C.; Lauty J. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 444–445.

(10) Gates, E. J.; Wilson, W. B. *Limnol. Oceanogr.* **1960**, *5*, 171–174.

0002-7863/86/1508-7855$01.50/0

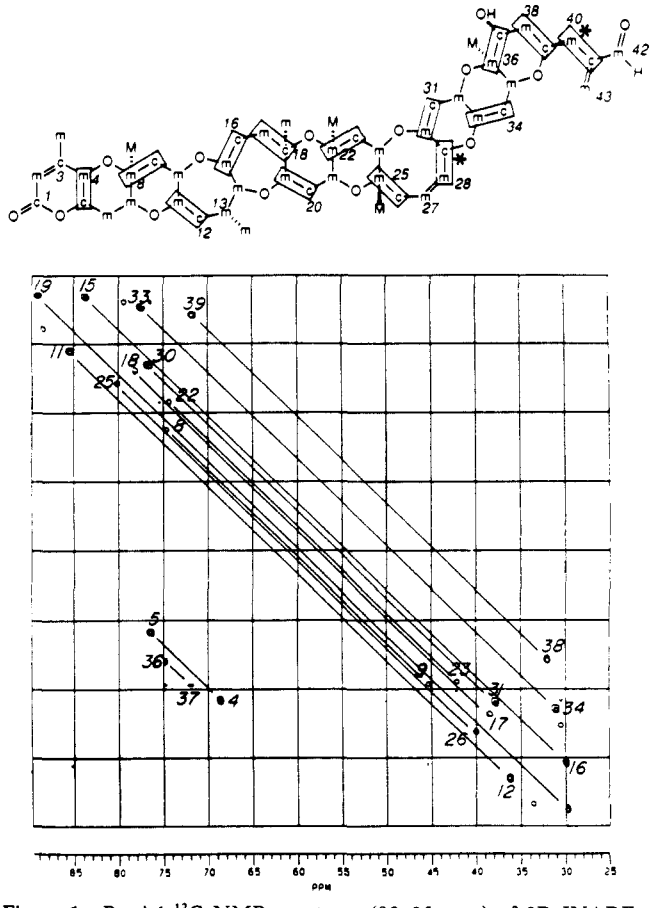


Figure 1. Partial ^{13}C NMR spectrum (90–25 ppm) of 2D INADEQUATE of $^{13}CH_3{}^{13}CO_2Na$ labeled BTX-B (3.2 mM) in C_6D_6, 125.13 MHz Bruker AM-500. The C_2 units that originate from the same acetates are enclosed in squares. The asterisked carbon pairs 28/29 and 40/41 are not in the figure because they fall out of the range shown, but the connectivities were evident. m = $^{13}CH_3CO_2Na$, c = $CH_3{}^{13}CO_2Na$, and M = $^{13}CH_3SCH_2CH_2CH(NH_2)CO_2H$.

labeling experiments the yield of BTX-B is frequently less then 1 mg. The ^{13}C NMR peak heights showed the incorporation level of acetates in BTX-B to be 2.0–2.5%. The erratic growth of these dinoflagellates has been a major obstacle in obtaining the toxins. This was especially true with respect to labeling experiments. In some instances the addition of sodium acetate to the *G. breve* culture led to the destruction of the entire culture, whereas in some instances it actually enhanced the growth of the culture.

Of the 50 carbons of BTX-B, 16 carbons showed enrichment from [1-^{13}C]acetate, 30 carbons from [2-^{13}C]acetate, and 4 carbons from methyl-^{13}C-methionine (Figure 1), thus accounting for the origin of all carbons. The doubly labeled BTX-B molecule is an ideal model for demonstrating the INADEQUATE ^{13}C NMR technique[11] in biosynthetic studies. Namely, its single carbon chain contains many contiguous carbon pairs, e.g., C-8/C-9 in **1**, in which one carbon (C-9) is linked only to hydrogens and carbon (C-10) thus appearing around 35 ppm, whereas the other (C-8) is linked to an oxygen and appears around 80 ppm. This large difference in chemical shifts gives a spectrum in which the carbons correlated by connectivity are clearly separated (Figure 1).[12] These 2D INADEQUATE measurements[13] on BTX-B

(11) (a) Chan, J. K.; Moore, R. N.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 3334–3336. (b) Carter, G. T.; Fantini, A. A.; James, J. C.; Borders, D. B.; White, R. J. *Tetrahedron Lett.* **1984**, *25*, 255–258. (c) Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakashima, T. T. Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3694–3701.

(12) Cross peaks for the acetate connectivity patterns of C-1, C-2, C-3, and 3-Me were absent from the 2D INADEQUATE spectrum, presumably due to longer t_1's of the carbons within this ene lactone moity.

(13) For the INADEQUATE experiment, 125.13 MHz Bruker AM-500, internal reference C_6D_6 at 25 °C (128.0 ppm) was used;[4] the residual artifacts in 2D matrix were removed by symmetrization.[14]

Table I. Carbon Assignments of BTX-B[a]

carbon	ppm	carbon	ppm	carbon	ppm
1	162.51	18	78.04	35	63.65
2	116.54	19	89.02	36	74.92
3	159.43	20	29.76	37	71.93
4	68.63	21	74.85	38	32.01
5	76.47	22	74.25	39	71.70
6	30.53	23	42.16	40	32.34
7	79.37	24	75.39	41	148.79
8	74.69	25	80.03	42	193.53
9	45.39	26	40.01	43	134.40
10	83.89	27	126.94	3-Me	16.70
11	85.43	28	136.41	8-Me	15.84
12	36.24	29	80.35	13-Me	18.33
13	33.59	30	76.73	18-Me	22.12
14	88.41	31	37.85	22-Me	20.53
15	83.59	32	69.78	25-Me	18.33
16	29.95	33	77.48	36-Me	14.00
17	38.48	34	31.09		

[a] Measurements were performed with Bruker AM-500, 125.13 MHz, and Bruker WM-250, 62.89 MHz, in C_6D_6 at 25 °C.

incorporating [1,2-$^{13}C_2$]acetate enabled one to assign remaining carbons and also to confirm assignments of many other carbons. This led to the assignments of all carbons (Table I) as well as elucidation of their biosynthetic origins. It should be noted that the single carbon chain which constitutes the backbone of the ladder-like oxacyclic skeleton is not a simple polyketide. Indeed there are six m–m moieties and even two contiguous m–m–m moieties, one of which is extended by an additional Me group (13-Me). Further studies of this unprecedented biosynthetic pattern are being carried out.

Acknowledgment. This work was supported by NIH grant AI 10187; financial support from Merck Sharpe and Dohme is also gratefully acknowledged. We thank J. C. Cesarelli for technical assistance.

(14) Baumann, R.; Kumar, A.; Ernst, R. R.; Wuthrich, K. *J. Magn. Reson.* **1981**, *44*, 76–83.

Thiol as an Electron Donor in Molybdenum Oxo-Transferase Analogue Reaction Systems: Observations by ^{19}F NMR Spectroscopy and Biological Implications

John P. Caradonna,[1] Edgar W. Harlan, and R. H. Holm*

Department of Chemistry, Harvard University Cambridge, Massachusetts 02138

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The molybdenum oxo-transferases (hydroxylases)[2] catalyze the two-electron oxidation or reduction of substrates X/XO in processes which may be formally represented as $X + H_2O \rightleftharpoons XO + 2H^+ + 2e^-$. Our recent investigations have demonstrated that (i) the complexes MoO_2(L-NS_2) (**1**) and MoO(L-NS_2)(DMF) (**2**) are reasonable structural representations of certain enzyme sites;[3] (ii) stoichiometric reaction 1 occurs with a variety of substrates, including sulfoxides and *N*-oxides that are enzyme substrates;[4,5] (iii) the catalytic cycle 2 (XO = R_2SO) operates

(1) NIH Postdoctoral Fellow, 1985–1987.

(2) (a) Bray, R. C. *Adv. Enzymol.* **1980**, *51*, 107. (b) *Molybdenum and Molybdenum-Containing Enzymes*; Coughlan, M. P., Ed.; Pergamon Press: New York, 1980. (c) *Molybdenum Enzymes*; Spiro, T. G., Ed.; Wiley-Interscience: New York, 1985. (d) Rajagopalan, K. V. *Biochem. Elem.* **1984**, *3*, 149.

(3) Berg, J. M.; Holm, R. H. *J. Am. Chem. Soc.* **1985**, *107*, 917.

(4) Berg, J. M.; Holm, R. H. *J. Am. Chem. Soc.* **1985**, *107*, 925.

0002-7863/86/1508-7856$01.50/0