Biosynthesis of DTX-4: Confirmation of a Polyketide Pathway, Proof of a Baeyer−Villiger Oxidation Step, and Evidence for an Unusual Carbon Deletion Process

J. L. C. Wright,*,† T. Hu,† J. L. McLachlan,‡
J. Needham,† and J. A. Walter†

Institute for Marine Biosciences
National Research Council of Canada
1411 Oxford Street, Halifax
Nova Scotia, Canada B3H 3Z1
Department of Biology, Acadia University
Wolfville, Nova Scotia, Canada B0P 1X0

Received May 20, 1996

The diarrhetic shellfish poisoning (DSP) toxins include the phosphatase inhibitors okadaic acid (**1**), DTX-1,[1] and DTX-2,[2] found in extracts of shellfish and marine dinoflagellates from the genera *Prorocentrum* and *Dinophysis*. These lipid-soluble toxins appear to exist within the dinoflagellate cell as the much less active water-soluble derivatives DTX-4 (**2**) in *Prorocentrum lima*[3] and DTX-5a/5b in *Prorocentrum maculosum*.[4] The biosynthesis has some unusual features:[5−8] (a) glycolate is used as a starter unit in the okadaic acid and diol segments of **2**;[7,8] (b) the successive addition of intact acetate units to the nascent polyketide chain is occasionally interrupted by a single carbon derived from the methyl group of a cleaved acetate unit;[5−8] (c) the pendant methyl groups and the exomethylene group are derived from the methyl groups of acetate units,[5−8] a feature unique to some bacterial,[9] blue-green algal,[10] and dinoflagellate[11] products; (d) the starter unit for the sulfated acyl chain is derived from the carboxyl carbon of a cleaved acetate unit.[8] Nevertheless, the extensive use of acetate units certainly points to a polyketide-like biosynthetic pathway, and we sought further evidence of the mechanism of biosynthesis of **1** and **2** through ^{13}C NMR studies of these compounds labeled from [2-$^{13}CD_3$]-acetate. In the usual polyketide process, acetate units successively added to the nascent polyketide chain may undergo β-keto reduction, dehydration, and enoyl reduction, resulting in a variety of functionality along the chain.[12] Thus backbone carbons derived from the methyl group of [2-$^{13}CD_3$]acetate contain up to two deuteriums if no dehydration step occurs, and only one deuterium if it does. The degree of deuterium retention at pendant methyl groups would also provide clues to their origin.

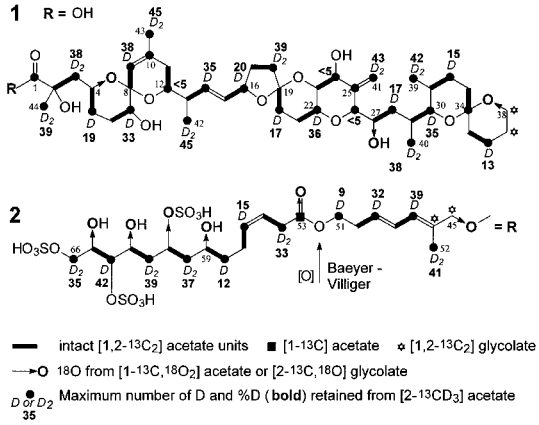


Figure 1. Distribution of ^{13}C, ^{18}O, and D, following the incorporation of labeled precursors into **1** and **2**. Deuterium retention (R %)[14] is shown for **2**. The Baeyer−Villiger insertion of O between C-51 and C-53 is illustrated schematically, although the state of modification of the chain at the time of this reaction has not been determined.

In two separate labeling experiments, [2-^{13}C D_3]acetate was administered in the usual way[8] to cultures of *P. lima*. Compounds **1** and **2** were isolated in the first experiment,[13] while in the second only **2** was obtained. Both experiments showed a pattern of ^{13}C labeling[14] (Figure 1) fully consistent with our previous observations.[7,8] Absolute % ^{13}C measurements at positions of **2** labeled from the methyl group of acetate were uniform in the okadaic acid, diol, and sulfated chain moieties.[15] The {^{1}H,D}-decoupled ^{13}C NMR spectra of the labeled compounds[14] showed a series of isotopically-shifted resonances denoting carbons bearing deuterium. The same pattern of deuterium retention (R %)[14] was observed in both labeling experiments, the similar values of R being averaged for each position in Figure 1. In the okadaic acid portion of **2**, most of the backbone carbons derived from intact acetate units retained a single deuterium atom (Figure 1). Among the exceptions, C-3 and C-18 retained two deuteriums, indicating that no dehydration occurred at the preceding positions (C-4, C-19) in the biosynthetic process. Retention of ^{18}O at C-4 following incorporation of [1-^{13}C,$^{18}O_2$]acetate[8] supported this although no ^{18}O was retained at C-19, suggesting that ^{18}O was completely exchanged or substituted during formation of the spiroketal system. Loss of deuterium occurred at the oxymethine carbons C-12, C-24, and C-26, presumably during oxidative formation of the ring systems.

In the C_8 diol portion of **2**, C-47 and C-49 derived from the methyls of intact acetate units contained a single deuterium (R = 39% and 32%), as did C-51 which is derived from a cleaved acetate group, although in this case R was greatly reduced (9%). The pattern of isotope incorporation in the sulfated acyl chain of **2** was also consistent with a polyketide pathway (Figure 1). Resonances of carbons 54, 60, 62, and 66 each displayed peaks for approximately equal proportions of CHD and CD_2, with average R = 36% ± 3% SD, indicating that dehydration does not occur at these positions following β-keto reduction, con-

† Institute for Marine Biosciences.
‡ Acadia University.

(1) Yasumoto, T.; Murata, M.; Oshima, Y.; Sano, M.; Matsumoto, G. K.; Clardy, J. *Tetrahedron* **1985**, *41*, 1019−1025.
(2) Hu, T.; Doyle, J.; Jackson, D.; Marr, J.; Nixon, E.; Pleasance, S.; Quilliam, M.; Walter, J. A.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1992**, 39−41.
(3) Hu, T.; Curtis, J. M.; Walter, J. A.; Wright J. L. C. *J. Chem. Soc., Chem. Commun.* **1995**, 597−599.
(4) Hu, T.; Curtis, J. M.; Walter, J. A.; McLachlan J. L.; Wright J. L. C. *Tetrahedron Lett.* **1995,** *36,* 9273−9276.
(5) Yasumoto, T.; Torigoe, K. *J. Nat. Prod.* **1991**, *54*, 1486−1487.
(6) Norte, M.; Padilla, A.; Fernandez, J. J. *Tetrahedron Lett.* **1994**, *35*, 1441−1444.
(7) Needham, J.; McLachlan, J. L.; Walter, J. A.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1994**, 2599−2600.
(8) Needham, J.; Hu, T.; McLachlan, J. L.; Walter, J. A.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1995**, 1623−1624.
(9) (a) Kingston, D. G. I.; Kolpak, M. X.; LeFevre, J. W.; Borup-Grotchmann, I. *J. Am. Chem. Soc.* **1983**, *105*, 5106−5110. (b) Trowitzsch, W.; Gerth, K.; Wray, V.; Höfle, G. *J. Chem. Soc., Chem Commun.* **1983**, 1174−11775. (c) Kohl, W.; Irschik, H.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1984**, 1088−1093. (d) Nakagawa, A.; Konda, Y.; Hatano, A.; Harigaya, Y.; Onda, M.; Omura, S. *J. Org. Chem.* **1988**, *53*, 2660−2661. (e) Needham, J.; Andersen, R. J.; Kelly, M. T. *J. Chem. Soc., Chem Commun.* **1993**, 1367−1368. (f) Priestley, N. D.; Gröger, S. *J. Org. Chem.* **1995**, *60*, 4951−4953.
(10) Bobzin, S. C.; Moore, R. E. *Tetrahedron* **1993**, *49*, 7615−7626.
(11) Kobayashi, J.; Takahashi, M.; Ishibashi, M.*; J. Chem. Soc., Chem. Commun.* **1995**, 1639−1640.
(12) Simpson, T. J. *Chem. Ind.* **1995**, 407−411 and references therein.
(13) The second labeling experiment was performed to increase the yield and ^{13}C enrichment of **2**.
(14) ^{13}C NMR spectra (125 MHz): CD_3OD solution, Bruker AMX-500 spectrometer, {^{1}H}- or {^{1}H, D}-broadband decoupling, suppression of NOE for 5 s between acquisition periods (1 s). Details of the calculation of isotope enrichment at labeled positions are described in the supporting information.
(15) Respectively, in these moieties % ^{13}C was 3.9%, 3.9%, and 3.7% (SD *ca.* ± 0.6%) in the first experiment; 7.8%, 7.6%, and 7.3% (SD *ca.* ±0.8%) in the second.

S0002-7863(96)01715-5 CCC: $12.00 Published 1996 by the American Chemical Society

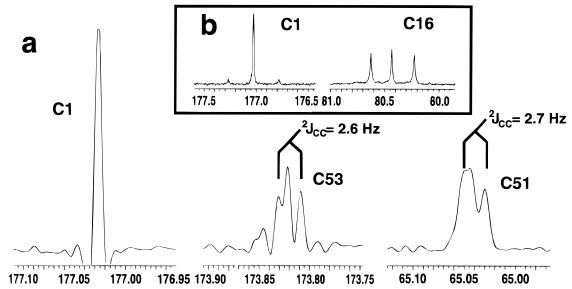


Figure 2. Portions of the ^{13}C NMR spectrum of **2** labeled from [1,2-$^{13}C_2$]acetate: (a) resolution-enhanced central components of C-51 and C-53 resonances showing intense satellites due to 2-bond ^{13}C-O-^{13}C coupling and correlation of carbon labeling at these positions, compared to C-1 on the same scale; (b) C-1 resonance showing low-intensity $^1J_{C1,C2}$ satellites resulting from uncorrelated incorporation of ^{13}C at C-2, compared with intense satellites of the C-16 resonance due to incorporation as part of a doubly labeled unit with C-15.

sistent with our earlier observation of ^{18}O retention at C-61 and C-65.[8] At C-62 and C-66, as well as C-18, separate CHD peaks of equal intensity were resolved for each enantiomer, revealing nonstereospecific exchange at these positions.

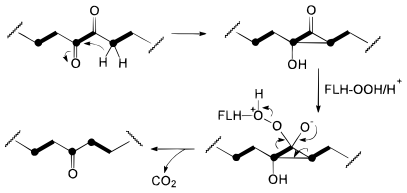
The high average retention (42% ± 3% SD) at the exo-methylene and pendant methyl groups suggests that the acetate (or malonate) precursor is drawn directly from the acetate pool with little opportunity for D-exchange. All the pendant methyl groups of the okadaic acid and diol moieties contained approximately equal proportions of CH_2D and CHD_2 species but no CD_3. This is compatible with an aldol-type condensation between a backbone carbonyl and malonate which has undergone partial deuterium exchange. Comparable patterns of deuterium incorporation have been observed in a bacterial[9e] and blue-green algal product[10] where a similar condensation has been proposed. Slightly lower retention levels (36% ± 3%) were found in the backbone carbons and retention at some positions e.g. C-36 was further reduced, perhaps due to a longer residence time on the enzyme surface as a β-keto acyl derivative during which D-exchange could occur. A similar trend has been found in the biosynthesis of microalgal fatty acids.[16]

The combined ^{13}C-, D- and ^{18}O-labeling data indicate that aside from the two glycolate starter units, **2** is assembled from acetate units drawn from the same biosynthetic pool in a manner typical of other polyketides, and the D- and ^{18}O-labeling data provides some important clues as to how this is achieved. For example, although the terminal carbons in the diol (C-51) and sulfated acyl chain (C-66) both arise through cleavage of an acetate unit, the retention of deuterium at C-51 is substantially lower, suggesting that the cleavage mechanism in each case is different. The ester carbonyl C-53 derived from the carboxyl of a cleaved acetate unit is part of an ester link with C-51. Significantly, ^{18}O was retained at the ester carbonyl C-53 and *not* at the diol oxygen (C-51) following incorporation of [1-^{13}C,$^{18}O_2$]acetate. These observations suggest that C-51 and C-53 were once part of an intact acetate unit that is cleaved in a Baeyer−Villiger oxidation step (Figure 1). This hypothesis was confirmed by resolution enhancement (Figure 2) of the ^{13}C NMR spectrum of **2** labeled from [1,2-$^{13}C_2$]acetate, which revealed narrow satellites corresponding to a $^2J_{COC}$ coupling of 2.6 Hz[17] around the central resonances of both C-51 and C-53. The proportional intensity P (0.58 ± 0.08) of these satellites relative to the total intensity of each peak is, within error, the same as that (P = 0.66 ± 0.04) for carbons which are incorporated as intact acetate units (for which $^1J_{CC}$ is larger, e.g. C-16) and can only be explained if the two carbons arose from the same acetate unit.

(16) McInnes, A. G.; Walter, J. A.; Wright, J. L. C. *Tetrahedron* **1983**, *39*, 3515−3522.

(17) Krivdin, L. B.; Della, E. W. *Prog. Nucl. Magn. Reson. Spectrosc.* **1991**, *23*, 301−610.

Scheme 1. Proposed Mechanism for the Generation of Isolated Carbons Derived from the Methyl Group of Acetate in the Polyketide Chains of **1** and **2**



Baeyer−Villiger oxidation of a polyketide chain is rare but biosynthetic precedents exist,[18] and such reactions may be mediated by flavin monooxygenases.[19] The discovery that the ester link between C-51 and C-53 in DTX-4 (**1**) arises through Baeyer−Villiger oxidation of an extended polyketide chain, presumably the product of a polyketide synthase (PKS), has important ramifications. Firstly, only two polyketide chains, both with glycolate starter units, are required in the assembly of **1**. Secondly, a similar monooxygenase-mediated reaction could be invoked in which a single carbon is ultimately eliminated from the polyketide chain as carbon dioxide. Thus, oxidation of a methyl-derived carbon to yield an α diketide followed by a Favorski-type rearrangement,[20] peroxide attack, and collapse of the cyclopropanone[21] would yield a shortened polyketide chain containing an oxidized methyl-derived carbon (Scheme 1). Such a process explains how the backbone carbons C-10, C-25, and C-26 in the polyketide chain of okadaic acid each arise from the methyl group of a cleaved acetate unit.[22] This carbon deletion step accounts for the interrupted pattern of acetate units in the chain, and is consistent with the uniform enrichment observed in all our labeling experiments as it does not require the intervention of other intermediate precursors. Even more compelling, the deletion step would not interrupt the flow of the polyketide assembly process through detachment and reassembly of the nascent polyketide chain, yet it could also occur after the completed polyketide chain has been released from the PKS. This oxidation and deletion process also explains the labeling pattern reported for amphidinolide J, another dinoflagellate product.[11]

Acknowledgment. We thank Pat LeBlanc for culturing and purification, Don Leek and Ian Burton for NMR spectroscopy, and Richard E. Moore for useful discussion. Work supported in part by a Natural Sciences and Engineering Research Council operating grant to J.L.McL. Issued as NRCC publication 39721.

Supporting Information Available: Details of the calculation of isotope enrichment at labeled positions (2 pages). See any current masthead page for ordering and Internet access instructions.

JA961715Y

(18) (a) Lee, J. J.; Lee, J. P.; Keller, P. J.; Cottrel, C. E.; Chang, C-J.; Zähner, H.; Floss, H. G. *J. Antibiot.* **1986**, *39*, 1123−1134. (b) Carter, G. T.; Goodman, J. J.; Torrey, M. J.; Borders, D. B.; Gould, S. J. *J. Org. Chem.* **1989**, *54*, 4321−4323. (c) Bockholt, H.; Udvarnoki, G.; Rohr, J.; Mocek, U.; Beale, J. M.; Floss, H. G. *J. Org. Chem.* **1994**, *59*, 2064−2069. (d) Amegadzie, A. K.; Ayer, W. A.; Sigler, L. *Can. J. Chem.* **1995**, *73*, 2119−2125. (e) Watanabe, C. M. H.; Townsend, C. A. *J. Org. Chem.* **1996**, *61*, 1990−1993.

(19) (a) Schwab, J. M.; Li, W.; Thomas, L. P. *J. Am. Chem. Soc.* **1983**, *105*, 4800−4808 and references therein. (b) Ryerson, C. C.; Ballou, D. P.; Walsh, C.; *Biochemistry* **1982**, *21*, 2644−2655 and references therein.

(20) Other Favorski-type mechanisms can be envisaged invoking a 3-halo or 3,4 epoxide intermediate of a β diketone that would yield an olefinic or hydroxy ketide, respectively.

(21) Baldwin, J. E: Cardellina J. *J. Chem. Soc., Chem. Commun.* **1968**, 558−559.

(22) A similar decarbonylation or decarboxylation step has been suggested in the biosynthesis of the xanthones simaomicin[18b] and the lysilipins.[18c]