Biosynthetic Origin of C-37 and C-38 in the Polyether Toxins Okadaic Acid and Dinophysistoxin-1

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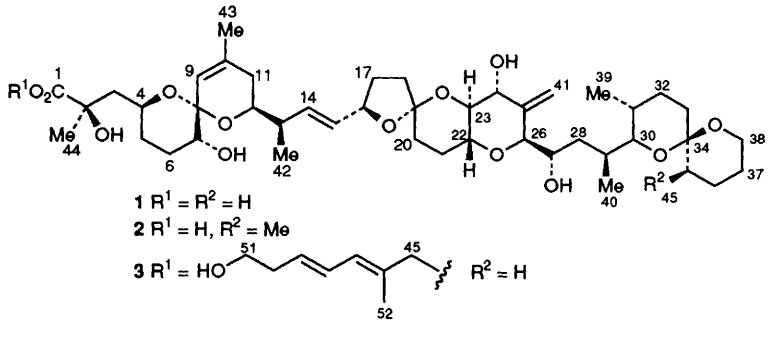
Stable isotope incorporation experiments have shown that carbons 37 and 38 in okadaic acid and dinophysistoxin-1 are derived from glycolate.

Okadaic acid, the dinophysistoxins, and their derivatives are a group of related polyether toxins that are responsible for Diarrhetic Shellfish Poisoning (DSP) in many parts of the world.[1] These toxins are produced by some species of marine dinoflagellates belonging to the genera *Dinophysis* and *Prorocentrum*. Preliminary biosynthetic studies by Yasumoto *et al.* on okadaic acid (OA) **1** showed that 39 of the 44 carbon atoms in OA were labelled with ^{13}C on feeding [1-^{13}C], [2-^{13}C] and [1,2-$^{13}C_2$]acetate.[2] Recently, Norte *et al.* have demonstrated that all the carbons except C-37 and C-38 in dinophysistoxin-1 (DTX-1) **2** are derived from acetate.[3] Previous biosynthetic studies in our laboratory using [1,2-$^{13}C_2$]acetate have indicated that *all* the carbons in OA and DTX-1 are labelled with ^{13}C, but that the incorporation of ^{13}C into C-37 and C-38 was not as an intact acetate unit.[4] Our studies also showed that the ^{13}C isotope concentration at each carbon site in the molecule was essentially the same (12% total ^{13}C) except for C-37 and C-38 which were significantly lower (4% total ^{13}C). The incorporation of ^{13}C-labelled acetate in our studies indicated that the biosynthetic flow in OA and DTX-1 is from C-38 to C-1, and the lower level of incorporation of scrambled singly labelled precursor into C-37 and C-38 suggests that these carbons, assumed to be the starter unit, are not derived from an intact acetate unit but from an alternative C_2 source. Possible sources of C_2 units include alanine, glycerate, glycine, glycolate, lactate, pyruvate, and succinate. A stable isotope incorporation study was therefore conducted to determine the biosynthetic origin of the C-37/C-38 starter unit in OA and DTX-1.

Glycine was considered a likely candidate to provide the C-37/C-38 starter unit in OA, DTX-1, and their derivatives. Cultures of *Prorocentrum lima* (Mahone Bay strain Pa[5]) were grown in 48 × 2.8 l Fernbach flasks containing 1 l of an enriched seawater medium,[6] and supplemented with 2.0 g of [1,2-$^{13}C_2$, 99%]glycine (CIL, Andover, MA) in two pulses at 14 and 21 days after inoculation (final glycine concentration 0.56 mmol dm^{-3}), and the cultures were harvested by centrifugation after a total of 28 days. Interestingly, the biomass of *P. lima* at the end of the glycine experiment was approximately double that previously observed in labelling experiments with acetate. The *P. lima* cells were extracted with methanol and the okadaic acid diol ester **3** (6.3 mg) was isolated from the ether soluble fraction of the methanol extract as previously described.[7] In this experiment **3** was the most abundant of the three polyether compounds (**1–3**) produced. The extent of incorporation of ^{13}C into **3** was calculated by a refinement of methods previously described.[8]

The 125 MHz ^{13}C NMR spectrum of **3** labelled with glycine displayed small doublets ($J_{C\text{-}37/C\text{-}38}$ = 34.9 Hz), arising from ^{13}C–^{13}C coupling, flanking a central singlet for C-37 and C-38 (both 1.2% total ^{13}C). Small doublets were also observed around all the carbon resonances that were previously shown to be derived from acetate (≤1.2% total ^{13}C). Owing to the small amount of ^{13}C enrichment above natural abundance (≤0.1%) in this experiment, the amount of incorporation of intact doubly labelled units and/or scrambled precursor in **3** could not be determined by quantitative measurements.[8] The incorporation of ^{13}C at carbon atoms known to be derived from acetate was unexpected and suggests that *P. lima* may use glycine as a carbon source and that it is metabolized to acetate before incorporation into **3**. However, glycine is known to be a source of glyoxylate, acetate, and one-carbon units; for example, it is metabolized by *Pseudomonas fluorescens* to acetate which is incorporated into the polyketide portion of the molecule during the biosynthesis of andrimid.[9] In the present experiment, the low incorporation of ^{13}C into C-37 and C-38 of **3** suggests that glycine may not be a direct precursor in the biosynthesis of OA, DTX-1 and **3**, but is rapidly metabolized by the dinoflagellate cells. This led us to investigate glycolate as an alternative C_2 source. Glycolate has been reported as a source of C_2 units in the biosynthesis of geldanamycin.[10]

Following the same protocol used for the glycine experiment, cultures of *P. lima* were fed 3.0 g of [1,2-$^{13}C_2$, 99%]calcium glycolate (final glycolate concentration 0.66 mmol dm^{-3}), which was synthesized according to the literature from [1,2-$^{13}C_2$, 99%]bromoacetic acid[11] (CIL, Andover, MA). The 125 MHz ^{13}C NMR spectrum of the resulting OA (3.5 mg) labelled with glycolate contained two enhanced resonances corresponding to C-37 and C-38 (both 1.8% total ^{13}C). In this spectrum the C-37 and C-38 resonances both appeared as doublets, arising from ^{13}C–^{13}C coupling ($J_{C\text{-}37/C\text{-}38}$ = 35.1 Hz) in doubly labelled molecules, flanking a central singlet which arises from the natural abundance ^{13}C in unlabelled molecules. Similarly, the ^{13}C NMR spectrum of DTX-1 (6.3 mg) isolated from the same biomass contained two enhanced resonances corresponding to C-37 and C-38 (both 2.2% total ^{13}C) which also appeared as doublets ($J_{C\text{-}37/C\text{-}38}$ = 34.8 Hz) flanking a central singlet. Quantitative measurements of the %^{13}C at C-37 and C-38 in OA and DTX-1 showed that the enrichment of ^{13}C above natural abundance at these positions (OA: C-37 and C-38 both 0.7%; DTX-1: C-37 and C-38 both 1.1%) is due entirely to the incorporation of intact doubly labelled units. These results



provide evidence for the direct incorporation of an intact glycolate unit into C-37 and C-38 of OA and DTX-1. Furthermore, there was no evidence for the incorporation of the labelled precursor at any other carbon in these molecules.

We have previously observed the incorporation of scrambled singly labelled precursor into C-37 and C-38 of OA and DTX-1 during biosynthetic studies using [1,2-$^{13}C_2$]acetate.[4] The doubly labelled acetate may be scrambled to a singly labelled C_2 unit, presumably glycolate, by its participation in the tricarboxylic acid (TCA) cycle. One passage of doubly labelled acetate through the TCA cycle would lead to two different labelling patterns in oxaloacetate, which may be converted into pyruvate. Subsequent conversion of pyruvate to hydroxypyruvate and decarboxylation, as suggested by Omura *et al.*,[12] would lead to [1-^{13}C] and [2-^{13}C]glycolate.

In conclusion, stable isotope feeding experiments have shown that an intact unit of glycolate provides the C-37/C-38 C_2 starter unit of OA and DTX-1. Glycolate as a source of C_2 units is rare,[10] and to the best of our knowledge this is the first time glycolate has been shown to be the starter unit in polyketide biosynthesis. Since glycolate is required to trigger the biosynthesis of the acetate derived chain in the DSP toxins, it is interesting to speculate that the C-2 hydroxy of glycolate is essential for the construction of the first spiroketal ring system. The incorporation of a small amount of ^{13}C from labelled glycine into C-37 and C-38 of the okadaic acid diol ester **3**, as well as in the carbons known to be derived from acetate supports the conversion of glycine into glycolate and acetate.

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