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Biosynthesis of Tolytoxin. Origin of the Carbons and Heteroatoms

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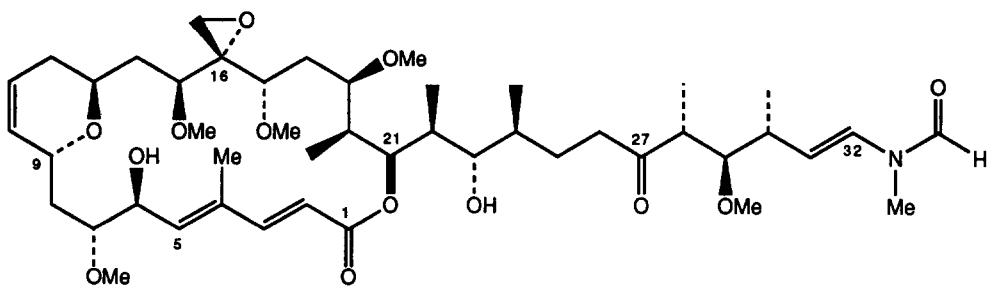
Abstract. Preliminary studies on the biosynthesis of tolytoxin (**1**) in the terrestrial blue-green alga *Scytonema mirabile* BY-8-1 are reported. Incorporation experiments with [1,2-^{13}C]acetate, [1-^{13}C,^{18}O]acetate, [1,2-^{13}C]glycine, [2-^{13}C,^{15}N]glycine and [*methyl*-^{13}C]methionine indicate that the carbon chain of tolytoxin is a polyketide assembled from a glycine starter unit and 15 acetate units. The one-carbon branches on the polyketide chain originate from the tetrahydrofolate C_1 pool.

Tolytoxin (**1**),[1] a potent microfilament-depolymerizing agent,[2] is the most frequently encountered scytophycin-type compound[3] associated with certain blue-green algae (cyanobacteria) belonging to the Scytonemataceae.[4] This 22-membered macrolide was first isolated from terrestrial *Tolypothrix conglutinata* var. *colorata* found at Fanning Island in 1977.[5] Curiously several marine secondary metabolites from sponges, nudibranchs, and gastropod mollusks (sea hares), viz. swinholides,[6] bistheonellides (misakinolides),[7] ulapualides,[8] kabiramides,[9] halichondramides,[10] mycalolides,[11] sphinxolide,[12] and aplyronine,[13] possess structures that are biogenetically closely related to the scytophycins. We present here evidence that tolytoxin is a polyketide and its biosynthesis utilizes glycine and acetate in the construction of the C_{32} carbon chain and the tetrahydrofolate C_1 pool for the attachment of the seven one-carbon branches on this acetogenic chain.



1

Scytonema mirabile (Dillwyn) Bornet (strain BY-8-1) was cultured in A_3M_7 medium in 20 L bottles as previously described[1] and feeding experiments were carried out with sodium [1,2-^{13}C]acetate, [1,2-^{13}C]glycine, [2-^{13}C,^{15}N]glycine and [*methyl*- ^{13}C]methionine. The alga was harvested from each run and the labeled **1** was isolated and analyzed by ^{13}C NMR spectroscopy in acetone-d_6. An INADEQUATE spectrum of **1** produced from feeding sodium [1,2-^{13}C]acetate (600 mg, diluted threefold with unlabeled acetate) to the alga in one portion about 10 days after inoculation and harvesting the alga 4 days later showed that intact acetate is incorporated into C1-C2 ($^1J_{CC}$ = 76.3 Hz), C3-C4 (50.9), C5-C6 (46.7), C7-C8 (39.0), C9-C10 (43.2), C11-C12 (40.7), C13-C14 (41.5), C15-C16 (50.9), C17-C18 (38.2), C19-C20 (39.0), C21-C22 (39.0), C23-C24 (38.2), C25-C26 (37.3), C27-C28 (42.5), C29-C30 (39.0), but not into C31-C32 (Fig. 1 and 2).

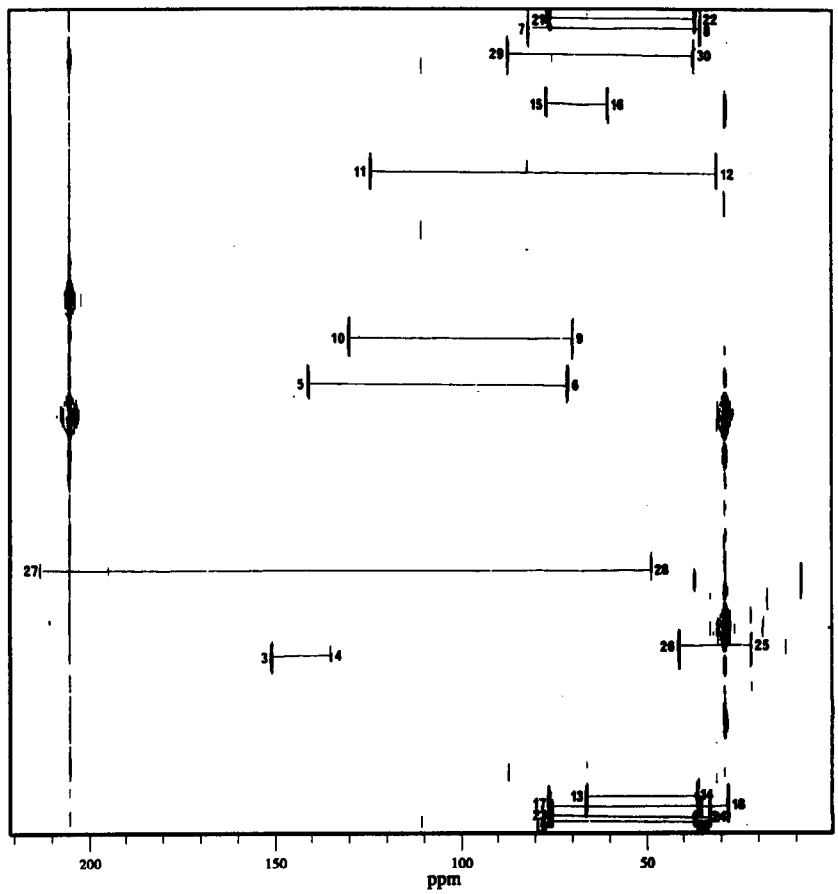


Fig. 1. The 125 Mz 2D-INADEQUATE spectrum of tolytoxin labeled from sodium [1,2-$^{13}C_2$]acetate feeding experiment in acetone-d_6. Acquisition data contained 4096 data points along t2 with 32 t1 increments and 2048 scans per increment. The experiment was optimized by setting the t1 dwell time equal to the dwell time, resulting in the folding of data in the F1 dimension. The final data matrix contained 4096 x 128 complex points. Due to the larger coupling constant, cross peaks were not observed between C1 and C2 signals in this particular spectrum.

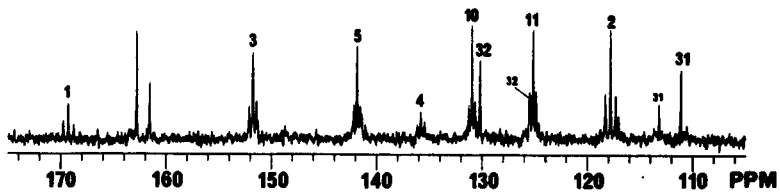
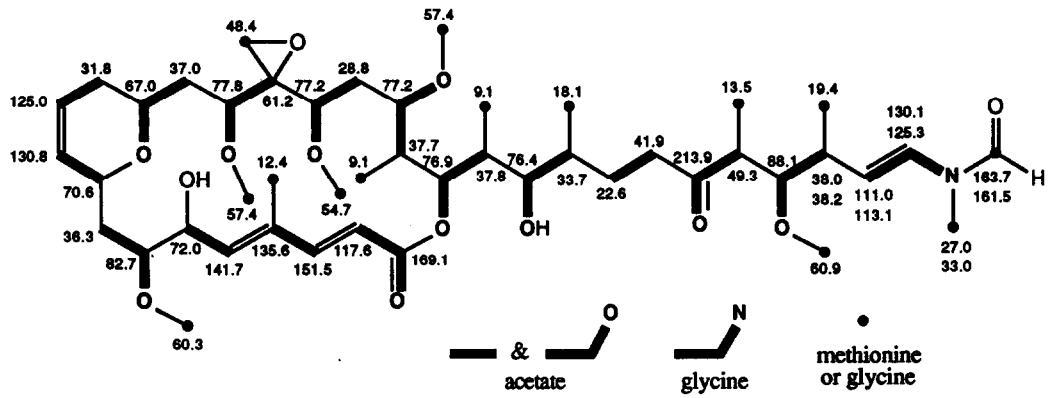


Fig. 2. The 105-175 ppm region of the 500 MHz ^{13}C NMR spectrum of [1,2-$^{13}C_2$]acetate-labeled tolytoxin in acetone-d_6. Note that the coupling between C1 and C2 is much larger than any other coupling, e.g. between C3 and C4. Note also that the signals for C31 and C32 are singlets, but are doubled because of conformational isomerism around the formamide bond.

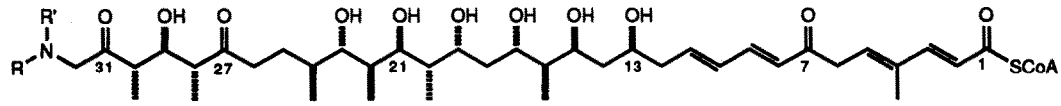
Analysis of the ^{13}C spectrum of [1-^{13}C,^{18}O]acetate-labeled tolytoxin, produced from feeding sodium [1-^{13}C,^{18}O]acetate (220 mg) to the alga in one portion 7 days after inoculation and harvesting the alga 5 days later, strongly suggested that C2-C1=O of acetate is incorporated intact into C2-C1=O, C8-C7-O, C14-C13-O, C16-C15-O, C18-C17-O, C20-C19-O, C22-C21-O, C24-C23-O, C28-C27=O, and C30-C29-O. The signals for C1, C3, C5, C7, C9, C11, C13, C15, C17, C19, C21, C23, C25, C27, and C29 were all enriched and ^{13}C-^{18}O peaks were found at higher field for C1 ($\Delta\delta_C$ -0.035 ppm), C7 (-0.025), C13 (-0.026), C15 (-0.028), C17 (-0.028), C19 (-0.028), C21 (-0.023), C23 (-0.029), C27 (-0.051) and C29 (-0.027) in addition to the normal ^{13}C-^{16}O peaks.[14]

^{13}C NMR analysis of **1** produced from feeding either [1,2-^{13}C]glycine or [2-^{13}C,^{15}N]glycine in 2 x 250 mg portions on days 7 and 9 after inoculation and harvesting 7 days later strongly suggested that C1-C2-N of glycine is incorporated intact into C31-C32-N, since C1-C2 of [1,2-^{13}C]glycine was taken up intact into C31 and C32 ($J_{C31-C32}$ = 80.8 Hz) and C2-N of [2-^{13}C,^{15}N]glycine was taken up intact into C32 and the nitrogen on C32 (J_{C32-N} = 15.0 Hz) (5% enrichment). Moreover, the signals for methyl carbons on C4, C20, C22, C24, C28, and C30, the epoxy methylene carbon on C16, the five O-methyl carbons, the N-methyl, and the formamide carbon were as intense as the one for C32, which meant that these 14 carbons were all derived from the tetrahydrofolate C_1 pool. The C-methyl, O-methyl, and N-methyl carbons were also labeled extensively by [*methyl*- ^{13}C]methionine, but ^{13}C was incorporated only slightly into the formamide carbon with this C_1 precursor.



Scheme 1. Labeling pattern from incorporation of [1,2-^{13}C]acetate, [1-^{13}C,^{18}O]acetate, [1,2-^{13}C]glycine, [2-^{13}C,^{15}N]glycine and [*methyl*- ^{13}C]methionine into tolytoxin.

In the first step of the biosynthesis of **1**, a polyketide synthetase (PKS) presumably assembles a hexadecaketide, possibly one having structure **2**, by successive Claisen condensations of 15 acetate units (via malonyl CoA) with a glycine starter unit. After the addition of each acetate unit to the growing polyketide chain, the PKS processes the intermediate β-enzyme-bound β-ketoacyl thiolester, either partially or fully, using reduction-dehydration-reduction steps that are analogous to those carried out in fatty acid biosynthesis. One-carbon branches are attached to the polyketide chain at carbon sites derived from C2 of acetate by methylation with S-adenosyl-L-methionine. Although hard evidence is lacking, C-methylation may occur after the condensation step on the α-carbon of the enzyme-bound β-ketoacyl thiolester, prior to processing. If that is the case then the methylation enzyme would have to be a component of the PKS multienzyme complex.



2 R,R' = H,H or CHO,CH_3

After construction of the hexadecaketide, modification (post-assembly processing) and cyclization would lead to **1**. For example, the dihydropyran ring might be formed as follows: A *trans,trans*-dienone system on C7-C11 isomerizes to the corresponding *trans,cis*-8,10-dien-7-one. A hydroxy group on C13 then adds to the α,β double bond of the dienone to give the dihydropyran ring.[14a] The keto group is reduced and the resulting alcohol methylated to give the methoxy group on C7.

It is interesting that the stereochemistry of the oxygens on C13, C15, C21 and C29 of **1** is the same as that noted in fatty acid biosynthesis,[15] but opposite to the stereochemistry of the oxygens on C17, C19 and C23. It is possible that the β-ketoacyl thiolester reductase exhibits a different stereospecificity in producing these three chiral centers during pre-assembly processing; however, the fatty acid stereochemistry could be introduced at C17, C19 and C23 during pre-assembly processing and inverted during post-assembly processing.[16] The C-methylation also proceeds with mixed stereochemistry, but the factors which control the stereochemical outcome are unknown.

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