Biosynthesis of Armentomycin: A Chlorinated Nonprotein Amino Acid

Sir:

Although over 1,500 halogenated natural products have been identified in animals, plants, and microorganisms,[1,2] only two biological routes for their formation are known: the direct methylation of halide ions,[3] and haloperoxidase-catalyzed halogenation.[2,3] The biosynthesis of armentomycin (L-2-amino-4,4-dichlorobutanoic acid, **6**), a nonprotein amino acid with antibiotic properties[4] is of particular interest because no mechanism is known for the chloroperoxidase-catalyzed insertion of chlorine substituents remote from other functional groups.[2] We present here the initial results from our investigation of armentomycin biosynthesis in *Streptomyces armentosus* var. *armentosus*.

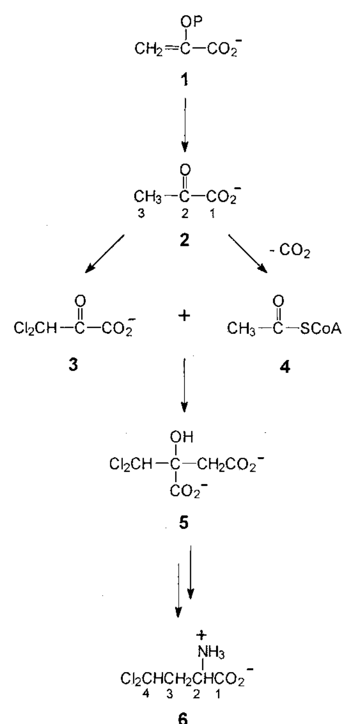
A starch, L-lysine and KCl medium (100 ml/500-ml Erlenmeyer flask) was inoculated with a 48-hour seed culture of *S. armentosus* var. *armentosus* UC2862 and incubated at 27°C with rotary shaking (220 rpm).[5] ^{13}C-Labelled substrates (1.8 ~ 2.7 mmol) were added in four equal portions at *ca.* 48, 72, 96, and 120 hours, and the cultures were harvested 24 hours after the last addition. Armentomycin was isolated by cation-exchange chromatography (Amberlite IR-120, H^+ form, 0.3 M pyridine elution) and derivatized with *p*-toluenesulfonyl chloride and ethereal diazomethane. *N*-Methyl-*N*-tosylarmentomycin methyl ester was purified by preparative-layer chromatography (silica gel, developed sequentially in hexane - EtOAc, 80 : 20 v/v, and benzene - MeOH, 99 : 1 v/v). Isotopic enrichments were measured by ^{13}C NMR spectroscopy (62.9 MHz). The extent of isotopic labelling at each carbon atom, expressed as % ^{13}C enrichment above the 1.1% natural abundance of this isotope, was calculated from ^{13}C NMR peak heights normalized to the natural abundance signals provided by the derivatization reagents.[6] The standard deviation of these measurements is ≤ 0.2.

N-Methyl-*N*-tosylarmentomycin methyl ester from cultures fed [1-^{13}C]acetate was labelled efficiently and exclusively at C-1 (15% enrichment); cultures fed [2-^{13}C]acetate gave armentomycin highly enriched at C-2 (14%). Enriched (7%), coupled ($^1J_{CC}$ = 61 Hz) signals for C-1 and C-2 of *N*-methyl-*N*-tosylarmentomycin methyl ester demonstrated the intact incorporation of [1,2-$^{13}C_2$]acetate. In the cultures fed [2-^{13}C]- or [1,2-$^{13}C_2$]acetate, smaller but equal enrichments (< 4%) at C-3 and C-4 of armentomycin indicated that C-2 of acetate was incorporated into this half of the molecule *via* intermediates of the citric acid cycle.[6] Since the methylene carbon of oxaloacetate is a possible site for chloroperoxidase-catalyzed chlorination,[2] labelled oxaloacetate was supplied by feeding DL-[3-^{13}C]aspartic acid. The small and approximately equal enrichment (1 ~ 1.5%) of all carbon atoms in the armentomycin obtained indicated that oxaloacetate was not a direct precursor. The pattern of incorporation suggested equilibration *via* a symmetrical intermediate of the citric acid cycle; both C-1, C-2 and C-3, C-4 of armentomycin could then be derived from phosphoenolpyruvate (**1**) or pyruvate (**2**) formed from oxaloacetate. Acetyl-CoA (**4**) derived from oxaloacetate labelled at C-2 and C-3 would be equally labelled at both carbons, and would account for enrichment of C-1 and C-2 of armentomycin. Concurrent incorporation of the C-2, C-3 labelled phosphoenolpyruvate or pyruvate into C-3 and C-4 of armentomycin would explain the similar enrichment of all four carbons.

The postulated precursor role of phosphoenolpyruvate and pyruvate was tested by feeding [2-^{13}C]glycerol and [2-^{13}C]pyruvate. The similar enrichments (8 and 7%) at C-1 and C-3 of armentomycin derived from glycerol confirmed the hypothesis, and supported the idea that C-1, C-2 and C-3, C-4 of armentomycin are derived from a common precursor. In the [2-^{13}C]pyruvate experiment, ^{13}C enrichments at both C-1 (5%) and C-3 (4%) of armentomycin indicated that pyruvate rather than phosphoenolpyruvate was the last intermediate common to the two C_2 units of armentomycin.

In the biosynthetic pathway proposed (Fig. 1), pyruvate (**2**) is converted to acetyl-CoA (**4**) and a chlorinated intermediate, such as dichloropyruvate (**3**); subsequent condensation of these two units provides an intermediate (**5**) that can be converted to armentomycin (**6**) by dehydration/hydration, oxidation/decarboxylation, and transamination reactions analogous to those in the chain-extension sequences of the biosynthetic

Fig. 1. Hypothesis for the biosynthesis of armentomycin (**6**) from pyruvate (**2**).



pathways to lysine and leucine.[7] Armentomycin is a structural analogue of leucine, and dichloropyruvate is the corresponding analogue of the leucine precursor, α-ketoisovalerate. However, the dehydration/hydration and oxidation/decarboxylation sequence is similar to that catalyzed by citric acid cycle enzymes in the pathway to phosphinothricin, the nonprotein amino acid residue in the herbicidal tripeptide bialaphos.[8] Whether enzymes in the citric acid cycle or the leucine pathway, or a distinct set of secondary metabolism enzymes, are used for armentomycin biosynthesis must now be addressed.

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