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BIOSYNTHESIS OF THE 17-MEMBERED CARBOCYCLIC RING OF LANKACIDIN ANTIBIOTICS

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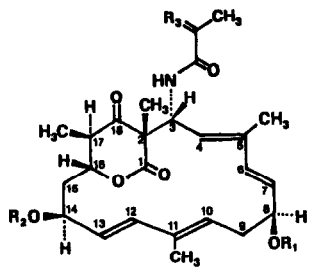
Abstract: A mechanism involving contraction of an 18-membered polyketide ring was proposed for the biosynthesis of the 17-membered carbocyclic ring of lankacidin antibiotics based on the feeding experiments of deuterated glycine and ^{2}H-NMR spectroscopy.

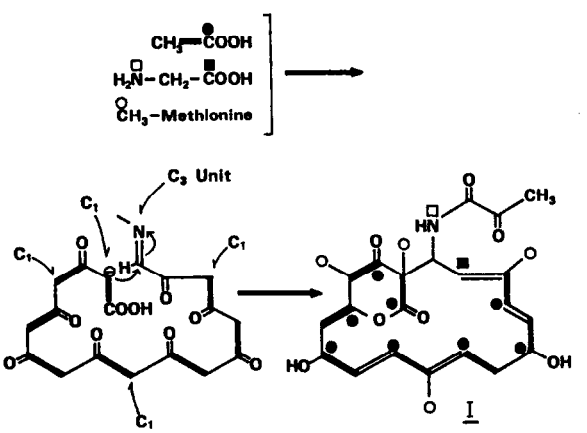
Lankacidins constitute a structurally unique class of antibiotics due to their 17-membered carbocyclic ring, which distinctly differs from the macrocyclic rings of regular macrolide antibiotics and ansamycins. Biosynthesis of lankacidins was previously studied by labeling experiment using ^{13}C- and ^{15}N-enriched precursors and the 17-membered ring I was elucidated to be formed from a linear polyketide II consisting of a glycine starter and eight molecules of acetate.[1] The four methyl groups on the ring were also determined to be derived from methyl group of methionine. Mechanism of this macrocyclic ring formation was discussed in some extent and a route suggested was through attack by a terminal nucleophilic center on an electrophilic imine carbon at the glycine unit,[1] as shown in Fig. 1.

Lankacidin Antibiotics

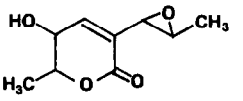


R_1	R_2	R_3	
H	H	O	Lankacidin C (Bundlin A, T-2636C)
H	$COCH_3$	O	Lankacidin A (Bundlin B, T-2636A)
$COCH_3$	$COCH_3$	O	Lankacidin C diacetate
H	H	H,OH	Lankacidinol (T-2636F)
H	$COCH_3$	H,OH	Lankacidinol A (T-2636D)

Fig. 1 ^{13}C- and ^{15}N Labeling Pattern



However, the δ-lactone system fused to the carbocyclic ring in lankacidins seemed to be quite characteristic and suggestive as to the ring formation, since δ-lactone structures of aspyrone and vulgamycin were proposed to be formed from their linear polyketide precursors via Favorskii-type rearrangements.[2,3,4,5] This implied an alternative mechanism for the formation of lankacidin skeleton I, that is, first formation of a regular 18-membered polyketide ring III including a glycine starter, followed by a similar Favorskii-type rearrangement with extrusion of a carboxyl group ends up with formations of the δ-lactone as well as the 17-membered carbocyclic ring I as shown in Fig. 2.



Aspyrone

Vulgamycin

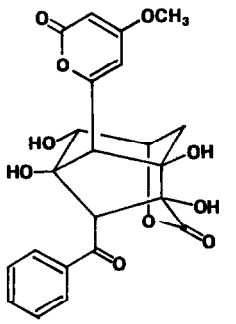
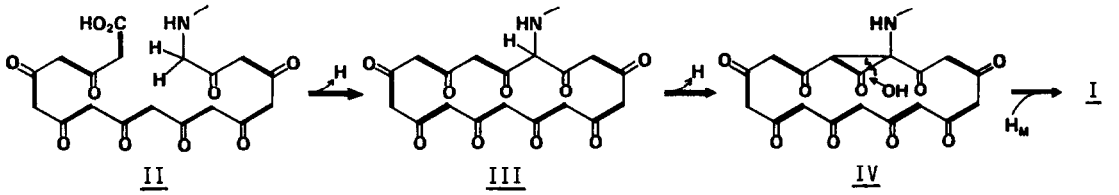


Fig. 2 Alternative Pathway



II III IV

Clear difference in the biosynthesis of lankacidins from those of aspyrone and vulgamycin was that Favorskii-type rearrangements in the latter were suggested on the basis of C-C bond cleavage of one of the acetate units observed by the ^{13}C-NMR analyses on the metabolites produced from ^{13}C- doubly labeled acetate,[2,3,4,5] wheras all acetate units were incorporated intact in the former.[1]

To clarify this intriguing problem of the lankacidin ring formation, feeding experiments of deuterated glycine were undertaken, since the fate of hydrogens seemed to be crucial to test the above-mentioned alternative pathway.

In a preliminary experiment, [$^2H_5$]-glycine (98 Atom% enriched, MSD Canada) was administered to the fermentation of a lankacidin producing organism Streptomyces sp.,[1] and the produced lankacidins A and C were simultaneously extracted with CH_2Cl_2. Acetylation of the mixture of lankacidins A and C with acetic anhydride and pyridine gave lankacidin C diacetate as a sole product, which was further purified by preparative TLC. The diacetate was then analyzed by MS and ^2H-NMR spectroscopy, which showed no incorporation of deuterium except for weak labeling of the methyl groups on the macrocyclic ring.

To confirm this observation, a mixed substrate composed of [1-^{13}C]-glycine (91 Atom% enriched, Prochem) and [$^2H_5$]-glycine (98 Atom% enriched, MSD Canada) in a ratio of 2 : 1 was fed

similarly to the lankacidin fermentation and the labeled lankacidin C diacetate was prepared as described above. The diacetate was again analyzed by the ^{13}C- and ^{2}H-NMR spectra,[6] which are shown in the following Fig. 3 and Fig. 4.

Fig. 3

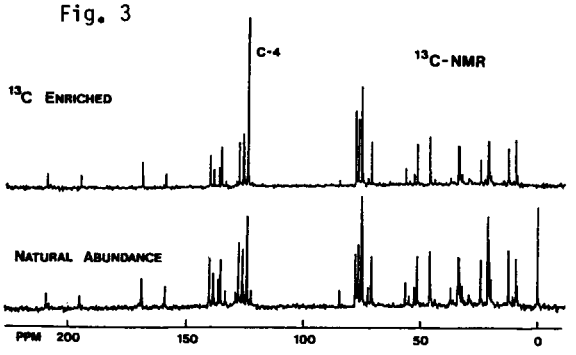
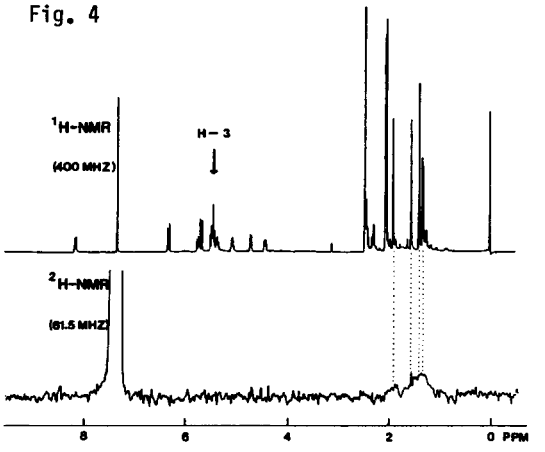


Fig. 4



Incorporation of the exogenous glycine was clearly demonstrated by the enhanced signal of the C-4 carbon observed at 124.6 ppm in the ^{13}C-NMR spectrum,[1] however, no deuterium incorporation was observed at all at the C-3 methine group, which would resonate arround 5.4 ppm based on the ^{1}H-NMR assignment,[7] while slight labeling was apparent into the methyl groups as can be seen in the ^{2}H-NMR spectrum. Labeling of the methyl groups is not surprising because C-2 of glycine can be converted into methyl group of methionine through 5,10-CH_2-tetrahydrofolic acid prodoced by the action of glycine decarboxylase.

As to the result of no incorporation of deuterium from glycine into H-3, it seems less likely that the deuterium is lost by chemical exchange at the stage of either a linear polyketide II or a 17-membered product such as I because of the reactivity of the corresponding position. The deuterium loss can be rationalized instead by assuming involvment of a Favorskii-type rearrangement in the biosynthesis, since the Favorskii mechanism requires removal of the corresponding hydrogen from a plausible 18-membered cyclic polyketide intermediate (III $\rightarrow$ IV $\rightarrow$ I) *vide supra*. Deuterium of glycine might otherwise be lost at the stage of an 18-membered intermediate III, since the corresponding methine hydrogen could be rather activated by the flanking two carbonyl groups.

In any case, the present results strongly imply intermediacy of an 18-membered carbocyclic polyketide precursor, which is afterwards transformed into the unique 17-membered ring of lankacidin antibiotics probably through a Favorskii-type rearrangement. It seems also worth to note that this is the first example suggesting this type of biological ring contraction of polyketide by means of deuterium labeling.

Acknowledgement

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References and Notes

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6) ^{13}C-NMR spectra were recorded on a JEOL FX-100 spectrometer using $CDCl_3$ as solvent and TMS as internal standard. ^{2}H-NMR spectrum was taken with a JEOL FX-400 spectrometer operated unlocked at 61.5 MHz with complete proton decoupling. The chemical shift was standardized by a natural abundance signal of $CHCl_3$ solvent.
7) The H-3 resonance was unambiguously assigned by spin decoupling experiments. Pertinent proton signals were a doublet at 8.09 ppm (J= 8.0 Hz, CONH), a double-doublet at 5.43 ppm (J= 8.0 & 9.1 Hz, H-3) and a doublet at 4.70 ppm (J= 9.1 Hz, H-4).

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