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BIOSYNTHETIC INCORPORATION OF GLUCOSE [U-$^{13}C_6$] INTO THE C_7N_2 UNIT OF ANTIBIOTIC A23187

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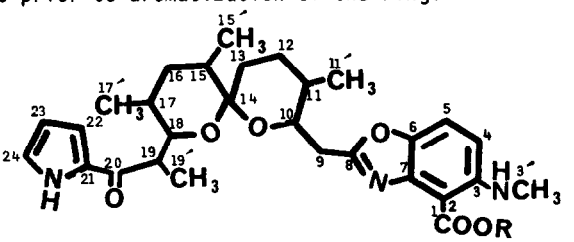
Abstract. The biosynthetic incorporation of Glucose [U-$^{13}C_6$] into the C_7N_2 unit of A23187 indicated that this functionality is formed by a divergence of the shikimate pathway. The possibility that 2,6-diaminobenzoic acid is a free intermediate in the biosynthesis of this unit was eliminated.

Antibiotic A23187(I) is a divalent cation ionophore produced in submerged culture by S. chartreusis (NRRL3882).[1] Our recent work[2] has demonstrated that the C_7N_2 unit of A23187 is labeled by glucose [1-^{13}C] and [6-^{13}C] at the nitrogen bearing carbons, in agreement with a shikimate-type pathway. Unlike the C_7N_1 unit of a number of other interesting and important antibiotics[3], shikimic acid [U-^{14}C] is apparently incorporated into the C_7N_2 unit of A23187.[2] This fact and the lack of incorporation of anthranilic acid ($^{14}COOH$) and tryptophan-[7a-^{14}C] suggest that the pathway to this unit represents a divergence of shikimate pathway after shikimic acid but prior to aromatization of the ring.



I, R = H
II, R = CH_3

Glucose [U-$^{13}C_6$] was used as a precursor for the C_7N_2 unit to test more rigorously the role of the shikimate pathway in the biosynthesis. Such a pathway would involve condensation of an intact three carbon unit (phosphoenol pyruvate) and an intact four carbon unit (erythrose-4-phosphate). These units should be coupled to adjacent carbons in the same unit but not to those in the other unit if sufficient unlabeled glucose is also added along with the labeled material. Three possible pathways can be suggested for the formation of this C_7N_2 unit via such a pathway. Pathway A would require that the three carbon unit label C-1, C-2, and C-7 and the four carbon unit label C-3 through C-6. Pathway B would result if the orientation of condensation product were the opposite and the three carbon unit would label C-1 through C-3 and the four carbon unit label C-4 through C-7. Pathway C would result if a symmetrical intermediate like 2,6-diaminobenzoic acid was involved in the biosynthesis. This would give rise to a more complicated coupling pattern since C-2 would be coupled to both C-7 and C-3.

In order to differentiate between these pathways, glucose [U-$^{13}C_6$] was diluted with unlabeled glucose (0.25 gm labeled to 0.75 gm unlabeled) and fed to 10 production cultures (50 ml) of S. chartreusis in divided amounts at 72, 96 and 120 hours of cell growth. The antibiotic was isolated as before[2], treated with diazomethane and A23187 methylester (II) purified by column chromatography to yield 77 mg of pure compound.

The couplings found in the NMR spectrum (Table) of the labeled antibiotic are straightforward and consistent with the involvement of pathway A in the biosynthesis.[5] The resonance for carbon 2 is spanned by a multiplet (J_{CC}=74 and 76 Hz) and is coupled to C-1 (doublet, J_{CC}=76 Hz) and C-7 (J_{CC}=74 Hz). The resonance at C-3 is spanned by a doublet (J_{CC}=62 Hz) and is coupled to C-4 (multiplet, J_{CC}=62 and 63 Hz) which is also coupled to C-5 (multiplet, J_{CC}=63 and 66 Hz). Carbon 5 is also coupled to C-6 (doublet, J_{CC}=66 Hz). These results are only in accord with the involvement of Pathway A.

The results reported here are consistent with the involvement of a shikimate-type pathway in the formation of the C_7N_2 unit of A23187 and also eliminate the possibility that 2,6-diaminobenzoic acid is a free intermediate in the biosynthesis. This is also suggested by the finding that additions of unlabeled 2,6-diaminobenzoic acid to production cultures of S. chartreusis did not reduce the incorporation of shikimic acid [U-^{14}C] into A23187.[6] The possibility that 3-hydroxyl-2,6-diaminobenzoic acid is a true intermediate in the biosynthesis is currently being investigated.[7].

TABLE. ^{13}C-^{13}C coupling of carbons of the C_7N_2 unit of A23187 methylester labeled from [U-$^{13}C_6$] glucose

Carbon	Chemical Shift, ppm[a]	J_{CC}, Hz
1	168	76
2	100.5	76, 74
3	150.8	62
4	108.4	62, 63
5	116.9	63, 66
6	142.5	66
7	142.3	74

[a]Spectrum recorded on a JEOL FX-270 multinuclear spectrometer in $CDCl_3$.[4]

References and Notes

1. R. M. Gale, C. E. Higgins, and M. M. Hoehm, U. S. Patent No. 3,960,667. June 1, 1976.
2. M. J. Zmijewski, Jr., R. Wong, J. W. Paschal and D. E. Dorman, manuscript in preparation.
3. K. L. Rinhart, Jr., D. D. Weller, and C. J. Pearce. J. Nat. Prod. 43.1 (1980), K. L. Rinhart, Jr., M. Potgieter, D. L. Delaware, and H. Seto. J. Amer. Chem. Soc. 103 2099 (1981) and references therein.
4. The carbon resonances for C-2, 6 and 7 are slightly shifted in the spectrum of the methylester when compared to the free acid. The carbon 13 resonance for C-2 occurs at 100.5 ppm in the methylester and is better resolved from C-14 than in the free acid. This is why the methylester was used instead of the free acid.
5. The only other coupled carbon resonances in the C-13 NMR spectrum of this enriched antibiotic were the carbon signals at 166.1 ppm (doublet, J_{CC}=59.6 Hz) and 32.3 ppm (doublet, J_{CC}=59.6 Hz) due to carbons 8 and 9 respectively. These two carbons are derived from the acetate starter unit for the spiroketal ring.
6. The 2,6 diamino benzoic acid used was prepared from 2,6-dinitrobenzaldehyde.
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