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BIOSYNTHESIS OF OCTOSYL ACID A: INCORPORATION OF C-13 LABELED GLUCOSE[1]

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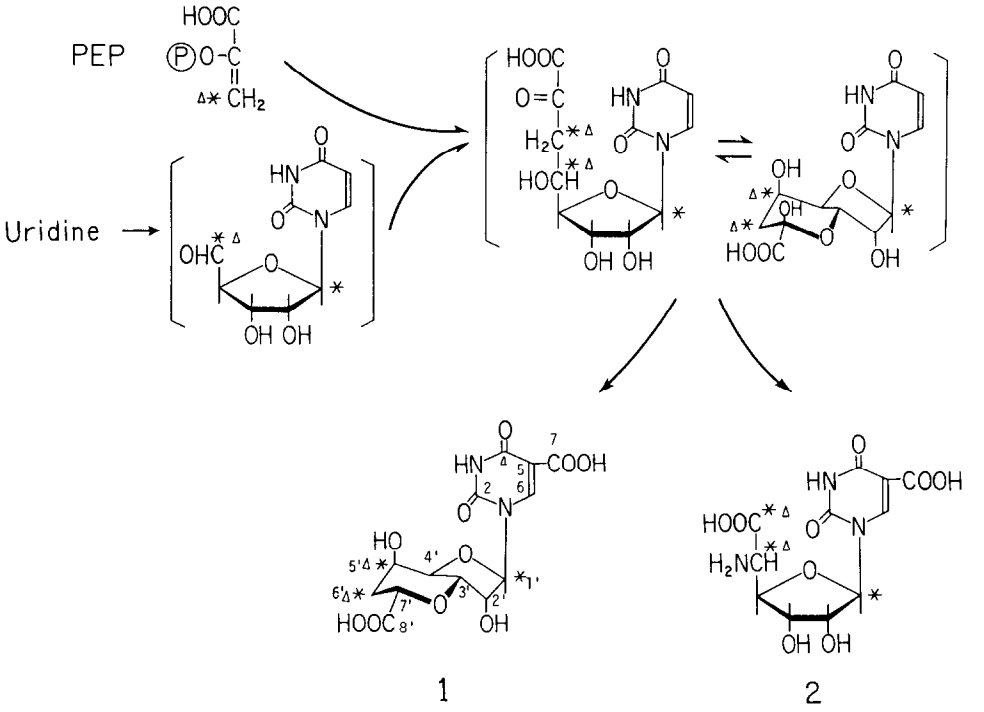
Summary: *D-[1 and 6-^{13}C]Glucose feeding experiments followed by ^{13}C-NMR analysis supported that octosyl acid A is a shunt metabolite of the polyoxin biosynthesis, being formed by the condensation of uridine and phosphoenolpyruvate.*

We have recently shown[5] that the nucleoside skeleton of the antibiotic polyoxin[6] is biosynthesized by the condensation of uridine and phosphoenolpyruvate. This conclusion was based on the tracer experiment using whole cells of Streptomyces cacaoi var. asoensis using [1-^{13}C]glucose as well as [U-^{14}C]-uridine, [3-^{14}C]glycerate, and [3-^{14}C]pyruvate. Unfortunately, the biosynthesis of octosyl acids,[7] anhydro-octose uronic acid nucleosides produced by the same organism was not clarified because under the specified fermentation conditions using glycerol as carbon source, octosyl acids were not sufficiently produced. By utilizing a blocked mutant[8] which accumulates octosyl acid A (1) and polyoxin C acid (2), we have been able to study the incorporation of [1-^{13}C]glucose into both 1 and 2. ^{13}C-NMR analysis showed that the labeling pattern of both compounds is exactly the same. The incorporation of [6-^{13}C]-glucose was also studied to support further the biosynthetic pathway shown in Scheme I.

D-[1-^{13}C]- and D-[6-^{13}C]Glucose[9] (each 400 mg, 90 atom %) were administered[10] respectively in two portions to a shaking culture of a mutant strain of S. cacaoi 110 and 120 hrs after inoculation (40 mL of the medium in a 500 mL flask: Medium composition: 10% soluble starch, 1% glucose, 1% soybean flour, 1.5% yeast, 2.5% wheat embryo, and 0.3% $NaNO_3$). After an additional 41 hrs incubation, 1 was isolated from the culture filtrate by the procedure described before[7] (yield: ca 10 mg). The results of ^{13}C-NMR analysis are shown in Table 1. Significant enrichment of the sugar carbons was observed on C-1', C-5', and C-6' from the [1-^{13}C]glucose experiment. In contrast, [6-^{13}C]glucose enriched only C-5' and C-6'. The distribution of the label is the same to that of the polyoxin nucleoside[11] and supports our earlier proposal that octosyl acid is a shunt metabolite of the biosynthesis of the polyoxins.[5] Known sugar metabolism shows the formation of [1,5-^{13}C]ribose from [1-^{13}C]glucose, and [5-^{13}C]ribose from [6-^{13}C]glucose via the pentose phosphate cycle. Also the

Scheme I. Proposed biosynthetic pathway for octosyl acid A (1) and polyoxin C acid (2): * label from D-[1-^{13}C]glucose, Δ from D-[6-^{13}C]glucose. Incorporation stage of the one-carbon unit into C-7 is not known.



glycolytic pathway proves the formation of [3-^{13}C]phosphoenolpyruvate from both [1- and 6-^{13}C]glucose. Enrichment of the pyrimidine carbons can be explained by the known metabolism: C-4 ∼ C-6 originate from aspartate and C-2 from carbamoyl phophate. C-7 was proved to come from C-3 of serine.[12] We have already shown that uridine and phosphoenolpyruvate are the most probable direct precursors for the polyoxin nucleoside.[5] As shown in Scheme I, uridine (or UMP) may first be oxidized to the 5'-aldehyde, which undergoes aldol condensation with phosphoenolpyruvate followed by reduction at the C-7 ketal would result in the formation of 1.

Antifungal antibiotics, ezomycins[13] possess the same sugar skeleton and may be biosynthesized by a similar pathway. The present study may also suggest that the C_{11}-sugars of nucleoside antibiotics, tunicamycin[14] and hikizimycin (anthelmycin)[15] could also be biosynthesized by a similar type of condensation between nucleoside and sugar carbonyl. This type of condensation might also be valid for the biosynthesis of amino acid derivatives of nucleosides such as sinefungin[16] and mildiomycin.[17]

Table 1. ^{13}C NMR[a] Chemical Shift Assignment[b] and Enrichment of Carbons of Octosyl Acid A Labeled by [1 and 6-^{13}C]Glucose.

Carbon atom	Chemical shift[c]	Relative enrichment[d]	
		[1-^{13}C]Glucose	[6-^{13}C]Glucose
2	148.6	1.49	2.42
4	164.0	1.40	2.28
5	101.4	1.67	2.92
6	147.7	1.57	3.00
7	162.9	1.24	1.97
1'	91.8	2.18	0.95
2'	71.2	1.04	0.95
3'	70.4	0.92	0.95
4'	77.2	1.04	0.99
5'	63.0	1.91	3.36
6'	34.8	1.92	3.11
7'	72.1	1.00	1.00
8'	171.4	1.01	1.01

a ^{13}C NMR spectra were taken on a JEOL FX-100 spectrometer in dimethylsulfoxide-1,2-$^{12}C,d_6$. b C-2,5,6,1',and 6' were assigned straightforwardly by chemical shift and splitting pattern in a gated decoupling spectrum. Three carbonyl signals were asigned as follows. Signal at 171.4 ppm was assigned as C-8' by long-range selective decoupling with H-6'. Coupling constants of C-7 (4.3 Hz) and C-4 (9.1 Hz) with H-6 indicate that signal at 162.9 ppm is C-7 and 164.0 ppm is C-4, since trans coupling is larger than cis [U. Vogeli and W. von Philipsborn, Org. Magn. Reson., 7, 617 (1975)]. C-2',3',4',5', and 7' were assigned by selective decoupling with H-3' (δ 3.80), H-4' (δ 4.00), H-2' (δ 4.28), H-7' (δ 4.41) and H-5' (δ 4.94). C-2',3', and 7' were further confirmed by selective population inversion method ($J_{C_2,-H_2}$ = 160 Hz, $J_{C_3,-H_3}$ = 143 Hz, $J_{C_7,-H_7}$ = 146 Hz) [K. G. R. Pachler and P. L. Wessels, J. Magn. Reson., 12, 337 (1973)]. c Chemical shifts were expressed in ppm relative to internal tetramethylsilane. d Relative enrichment was obtained by comparison of the integral curves of enriched and unenriched samples under identical conditions.

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References and Notes

(1) This paper constitutes a part of the series entitled "Biosynthesis of the polyoxins, nucleoside peptide antibiotics". For the preceding paper, see ref 5.

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(5) K. Isono, T. Sato, K. Hirasawa, S. Funayama, and S. Suzuki, J. Am. Chem. Soc., 100, 3937 (1978).

(6) K. Isono, K. Asahi, and S. Suzuki, J. Am. Chem. Soc., 91, 7490 (1969).

(7) K. Isono, P. F. Crain, J. A. McCloskey, J. Am. Chem. Soc., 97, 943 (1975).

(8) Will be published elsewhere.

(9) D-[1-^{13}C]- and D-[6-^{13}C]glucose were synthesized by some modification of the methods described in (a) H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel, and T. T. Galkowsky, J. Res. Nat. Bur. Stand., 48, 163 (1952); (b) R. Schaffer and H. S. Isbell, ibid., 56, 191 (1956).

(10) L-Aspartate (5×10^{-3} M) was added to the culture 15 min before addition of glucose.

(11) C-1',5', and 6' of polyoxin C were enriched by D-[1-^{13}C]glucose as reported in the previous paper (ref 5). In the present experiment, polyoxin C acid (2) was isolated. Labeling pattern was the same to octosyl acid A (1) in both [1 and 6-^{13}C]glucose experiments (data not shown).

(12) (a) K. Isono and R. J. Suhadolnik, Arch. Biochem. Biophys., 173, 141 (1976). (b) S. Funayama and K. Isono, Agric. Biol. Chem., 40, 1039 (1976).

(13) K. Sakata, A. Sakurai, and S. Tamura, Tetrahedron Lett., 1974, 4327; ibid., 1975, 3191.

(14) (a) T. Ito, Y. Kodama, K. Kawamura, K. Suzuki, A. Takatsuki, and G. Tamura, Agric. Biol. Chem., 41, 2303 (1977). (b) A. Takatsuki, K. Kawamura, M. Okina, Y. Kodama, T. Ito, and G. Tamura, ibid., 41, 2307 (1977).

(15) (a) S. Ennifar, B. C. Das, S. M. Nash, and R. Nagarajan, JCS Chem. Commn., 1977, 41. (b) M. Vuihorgne, S. Ennifar, B. C. Das, J. W. Paschal, R. Nagarajan, E. W. Hagaman, and E. Wenkert, J. Org. Chem., 42, 3289 (1977).

(16) D. R. Berry and B. J. Abbott, J. Antibiotics, 31, 185 (1978).

(17) S. Harada, E. Mizuta, and T. Kishi, J. Am. Chem. Soc., 100, 4895 (1978).

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