Tetrahedron Letters,Vol.24,No.49,pp 5445-5448,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

THE BIOSYNTHESIS OF STREPTONIGRIN FROM [1-^{13}C]-D-ERYTHROSE[1]

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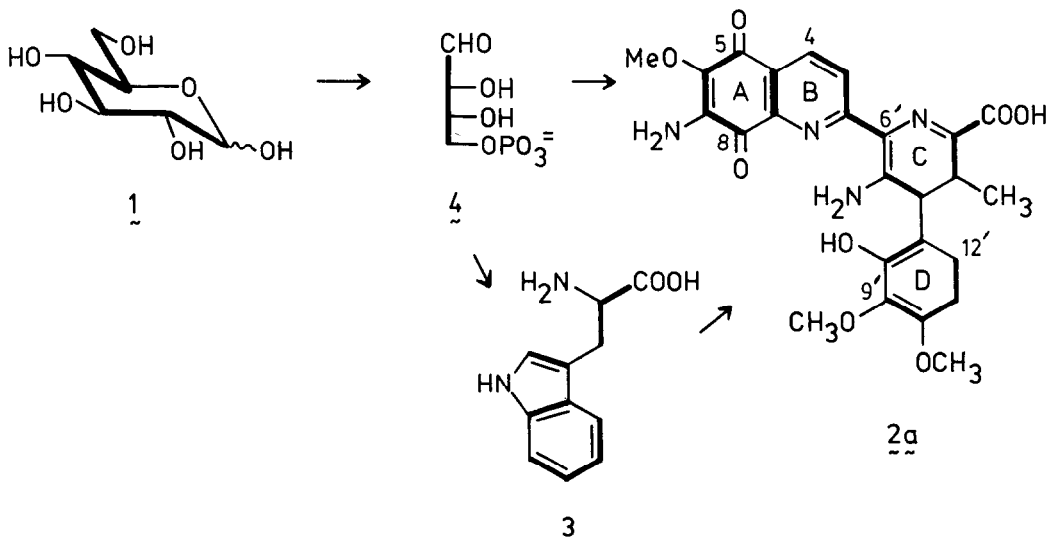
Abstract: Twelve of the twenty-five carbon atoms of streptonigrin are derived from three intact erythrose units.

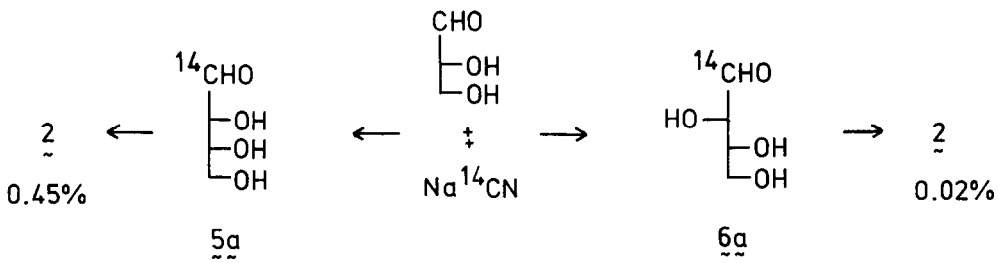
We reported[4] that feeding [U-$^{13}C_6$]-D-glucose (1) to growing cultures of Streptomyces flocculus ATCC 13257 yielded streptonigrin specifically labeled at all carbons. An analysis of the ^{13}C-^{13}C spin coupling patterns making use of ^{13}C-^{13}C homonuclear decoupling experiments revealed the size and location of each of the primary precursors, as shown in structure 2a.



The labeling pattern of the C-D rings was fully consistent with their known[5] derivation from β-methyl tryptophan (3), while the C_4+C_2 pattern of the A-ring was consistent with its derivation from a shikimate-type pathway. In total, three C_4 biogenetic units were clearly involved in streptonigrin biosynthesis. We now present evidence that all three C_4 units are derived from D-erythrose-4-phosphate (4).

[4-^{14}C]-D-Erythrose was prepared from [6-^{14}C]-D-glucose by modification of the lead tetraacetate oxidation reported by Bailey.[6] A portion of this (1.40×10^7 dpm) was divided between two 250 ml fermentations,[7] twelve hours after their innoculation with a seed culture. Standard work-up and purification yielded 13.5 mg of streptonigrin, and recrystallization to constant specific activity indicated a 1.8% incorporation ^{14}C.

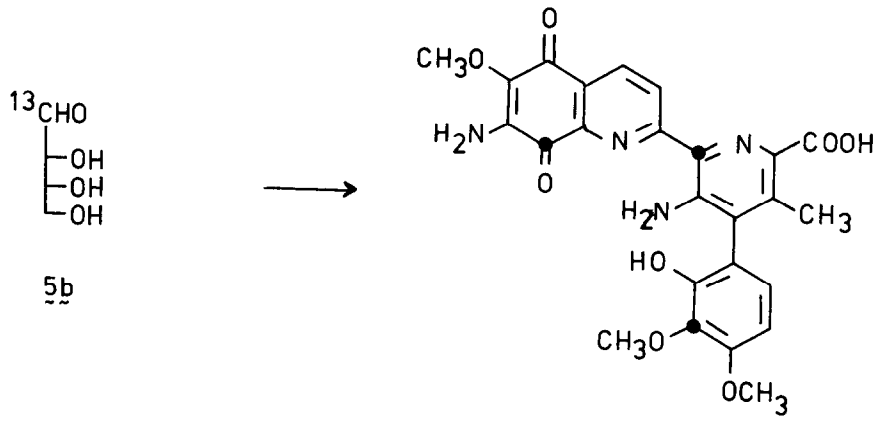
[1-^{14}C]-D-Erythrose (5a) and [1-^{14}C]-D-threose (6a) were then synthesized[8,9] from D-glyceraldehyde (1.9 mmole) and Na^{14}CN (0.32 mCi diluted with 1.9 mmole carrier cyanide), and separated by Dowex chromatography. A seed culture of S. flocculus was used to innoculate two 250 ml production broths; to one was added 11.15 μCi of erythrose and to the other was added 10.22 μCi of threose. Initial work-up of each afforded 0.68% incorporation of ^{14}C (7.0 mg of streptonigrin) and 0.02% incorporation of ^{14}C (6.0 mg of streptonigrin) from the erythrose and threose feedings, respectively. After addition of carrier streptonigrin and recrystallization, the incorporation of [1-^{14}C]-D-erythrose was 0.45%.



[1-^{13}C]-D-Erythrose (5b) was next synthesized in an analogous manner. A portion of this (27.7 mg, 90% enriched) and 3.31 μCi of [1-^{14}C]-D-erythrose (4.0 mg) were divided between four 250 ml production fermentations; combined, these ultimately afforded 23.5 mg of pure streptonigrin 2b (0.56% incorporation of ^{14}C). The 67.88 MHz ^{13}C NMR spectrum of this sample was compared with that of authentic, unlabeled streptonigrin, normalizing the peak integrals with a selection of resonances that should not have been enriched.[4] Only three resonances showed significant enrichment: C-9' (1.0%), C-6' (1.1%), and C-8 (0.6%). This is in good agreement with the 0.8% enrichment calculated on the basis of three sites labeled. It is noteworthy that there was no enrichment at C-12', C-4, or C-5.

These results clearly show that D-erythrose, presumably as erythrose-4-phosphate, is the specific precursor to all three C_4 biogenetic units. This also supports our view that the A-ring is derived from a shikimate-type pathway.

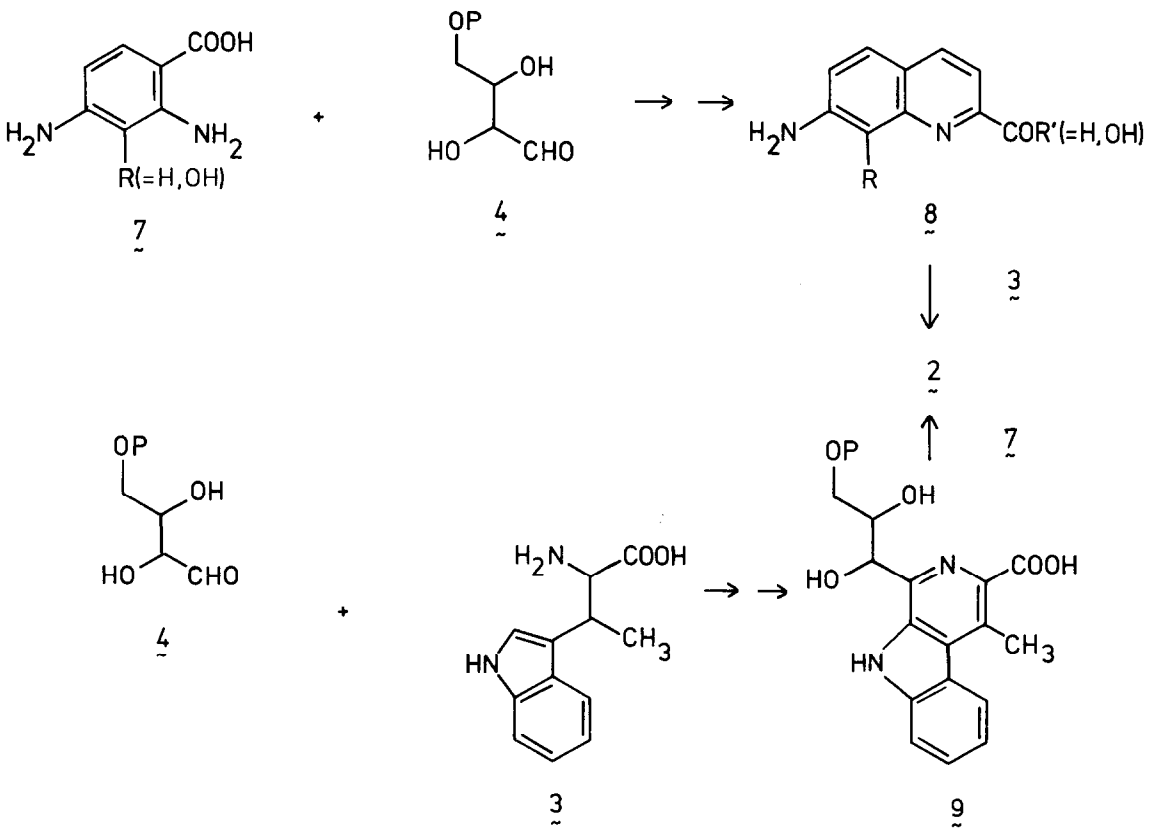
The middle C_4 unit, C-6' to C-4, can be viewed as the lynch-pin in the molecule. It may first couple with an amino anthranilic acid 7 to yield a



5b

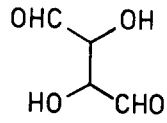
2b (● ≡ ^{13}C)

quinoline carboxylic acid or aldehyde 8 that condenses with 7, or it may first condense with 3 to yield a ß-carboline 9 that condenses with 7. These pos-



7 4 8 3 2 7 4 3 9

sibilities are currently under investigation. It should be noted that 4 is not at the correct oxidation state to directly yield a quinoline. While oxidation could occur at a number of points in the pathway, one possibility - oxidation to erythro-2,3-dihydroxysuccinaldehyde (10) - is clearly not involved since such a symmetrical intermediate would lead to labeling at C-4, as well.



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Acknowledgements. We are grateful to Dr. John Douros of Drug Research and Development, Chemotherapy, NCI, for a generous gift of streptonigrin, and to Dr. John DeZeeuw of Pfizer and Co., Inc., Groton, Conn., for strains of S. flocculus. This work was supported by Public Health Service Grant CA 27339 to S.J.G. ^{13}C NMR spectra were obtained on a Bruker HX 270 spectrometer at the Northeast Regional NSF-NMR Facility, Yale University, partially supported by the National Science Foundation, Grant CHE 79-16210.

1. This is Part 6 in the series "The Biosynthesis of Streptonigrin".
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(Received in USA 14 September 1983)