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BIOTIN BIOSYNTHESIS. INCORPORATION OF 5(RS)-^{3}H-DETHIOBIOTIN INTO BIOTIN

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Abstract: 5(RS)-^{3}H-(±)-Dethiobiotin has been synthesized and its incorporation into biotin by Aspergillus niger has been investigated. The incorporation was found to proceed without tritium loss.

A number of important natural products containing sulfur are biosynthesized by the apparent introduction of sulfur at a saturated carbon atom. The unusual nature of this reaction has stimulated considerable interest. An example that has been well studied is the biosynthesis of the vitamin (+)-biotin (2). It has been known for some time[1] that the biosynthesis of 2 proceeds via (+)-dethiobiotin (1) in a number of microorganisms. Recent work with doubly-labeled forms of dethiobiotin has shown that sulfur is introduced at C-1 and C-4 of 1 without apparent involvement of C-2 and C-3.[2] In addition, it has been found that sulfur is introduced at C-4 of dethiobiotin with overall retention of configuration.[3] The introduction of sulfur at C-2 of penicillin (3) has also been found to proceed with retention of configuration[4] while the introduction of sulfur at C-6 of lipoic acid (4) takes place with inversion of configuration.[5] In the case of penicillin and lipoic acid, all of the sites in the precursors that are adjacent to the site of sulfur introduction have been shown to be uninvolved in the functionalization process.[4-6] In the case of biotin, however, the potential involvement of C-5 in the introduction of sulfur at C-4 of dethiobiotin has not been evaluated. An investigation of the role of C-5 is necessary to allow interpretation of the stereochemical results at C-4. We now report the results of such an investigation.

5(RS)-^{3}H-(±)-Dethiobiotin was synthesized from the known[6] 1(RS)-^{3}H-alcohol 5. The tritiated alcohol 5 (sp. act. 54 mCi/mmole) was converted to the corresponding mesylate 6 (99%) by treatment with methanesulfonyl chloride and triethylamine.[7] Nitrile 7 (sp. act. 32 mCi/mmole) was then prepared (77%) from 6 by reaction with aqueous potassium cyanide in the presence of tri-n-butylamine.[8,9] Hydrolysis of nitrile 7 under basic conditions (40% NaOH in 1:1 EtOH-H_2O, 20 hr reflux) followed by lithium aluminum hydride reduction of the resulting acid yielded the 2(RS)-^{3}H-alcohol 8 (sp. act. 28 mCi/mmole, 56% from 7).[10] The labeled alcohol 8 was then transformed into 5(RS)-^{3}H-(±)-dethiobiotin in 16 steps using previously reported methods.[2a]

The resulting 5(RS)-^{3}H-(±)-dethiobiotin (sp. act. 1 μCi/mg) was mixed with 10-^{14}C-(±)-1 and the resulting doubly-labeled precursor administered to Aspergillus niger (ATCC 1004). After 5 days, radioactive biotin sulfone was isolated using published methods[2a] and purified by recrystallization to constant specific radioactivity and constant tritium to carbon-14 ratio. The results of this experiment are summarized in the Table. The data clearly indicates that sulfur is introduced at C-4 of dethiobiotin without apparent involvement of C-5.

Table: Incorporation of Dethiobiotin into Biotin

Precursor	Precursor $^{3}H/^{14}C$	$^{3}H/^{14}C$ for Biotin Sulfone	% ^{3}H Retention	% Incorp.
5(RS)-^{3}H-10-^{14}C-1	5.72	5.33	93	0.45

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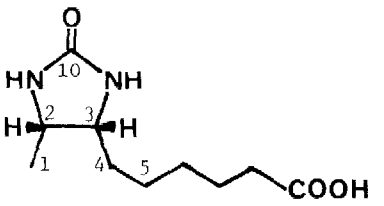

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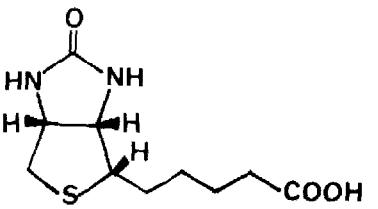

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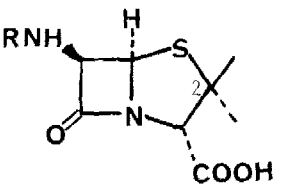

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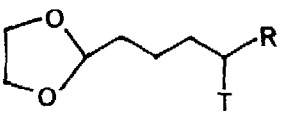
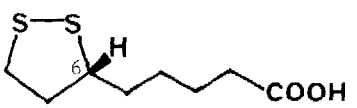


5 R = OH
6 R = OMs
7 R = CN
8 R = CH_2OH



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9. Some loss of tritium accompanies formation of the nitrile. This is presumably due to base catalyzed exchange of the nitrile under the reaction conditions.

10. Hydrolysis of nitrile 7 to the corresponding acid proceeds with ca. 13% tritium loss.

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