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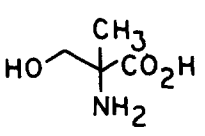
STREPTONIGRIN BIOSYNTHESIS. 2. THE ISOLATION OF β-METHYLTRYPTOPHAN AND ITS INTERMEDIACY IN THE STREPTONIGRIN PATHWAY[1]

Steven J. Gould* and Dennis S. Darling

School of Pharmacy, Section of Medicinal Chemistry and Pharmacognosy,
University of Connecticut, Storrs, Connecticut 06268

Although biological methylation is a common reaction in biosynthetic pathways, C-methylated amino acids are quite rate. α-Methylserine 1 occurs in the antibiotic amicetin[2]; β-methylproline 2 and β-methylphenylalanine 3 occur in bottyromycin[3]; and β-methyltryptophan 4 occurs in telomycin[4]. Indole isopropionic acid[5] 5 produced by a Claviceps, is probably a degradation product of β-methyltryptophan[6].



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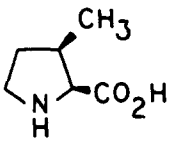

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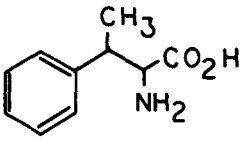

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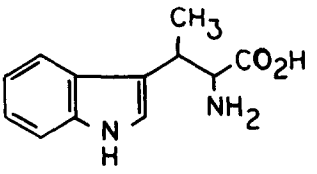

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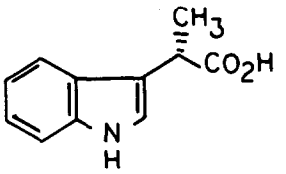

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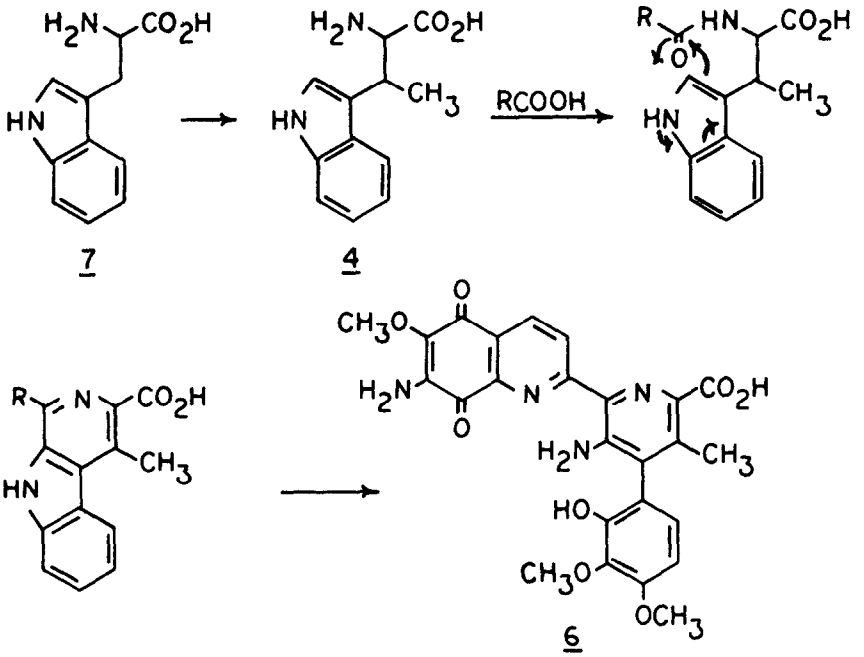
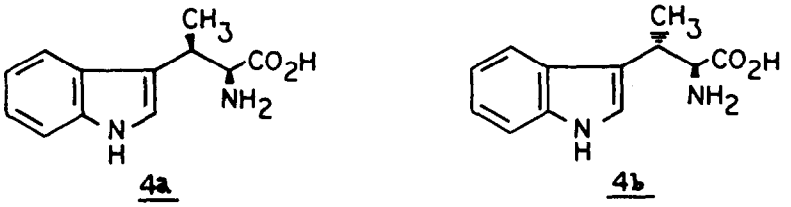
We now report the first isolation of β-methyltryptophan, 4, as a free natural product, and its incorporation into the anticancer antibiotic streptonigrin, 6, produced by Streptomyces flocculus ATCC 13257[7]. Our previous work on streptonigrin biosynthesis[1a] had indicated that phenylpicolinic acid moiety was derived from tryptophan, 7, and that C-methylation was occuring at an early stage in the biosynthetic pathway. The proposed pathway involving 4 is shown in Scheme 1.

In order to test the intermediacy of 4 in streptonigrin biosynthesis, it was necessary to demonstrate 1) that 4 is produced under conditions yielding streptonigrin and 2) that 4 is incorporated into streptonigrin.

Scheme 1



Authentic samples of the 2RS,3SR Isomer "A", 4a, and the 2RS,3RS Isomer "B", 4b, were synthesized according to the procedure of Snyder and Matteson[8], separation being effected by fractional crystallization of the acetamides. Isomeric purity was clearly evident from the



NMR spectra of the acetamides, as well as those of the β-methyltryptophans. The signal from the amide N-H of the Isomer A acetamide appeared at δ8.10, whereas that from the Isomer B acetamide appeared at δ8.83. After hydrolysis to the amino acids, the signals due to the α and β hydrogens appeared at δ3.45 and 4.05 and at δ4.30 and 4.90, for 4a and 4b respectively.

S. flocculus was cultured (50 ml) under standard conditions[1a]. At the first appearance

of streptonigrin production, 9.7 x 10^6 dpm of $^{14}CH_3$-L-methionine was added to the broth. Six hours later the broth was divided in two, one-half receiving a small quantity (10 mg) of Isomer A (4a) and the other receiving a small quantity (15 mg) of Isomer B (4b). After cell disruption by sonication to release any endogenous labeled 4 and centrifugation to remove solids, each supernatant was purified by ethyl acetate extractions at pH 2 and 10, followed by neutralization to pH 7 and chromatography on Dowex 50W-X4 (H+) ion exchange resin. The Dowex column was eluted with water, 2% NH_4OH, and 10% NH_4OH. The late 2% NH_4OH fractions contained radioactivity and β-methyltryptophan -- but no methionine -- as demonstrated by liquid scintillation counting and by radioscanning of analytical paper chromatograms (BuOH : HOAc : H_2O = 65 : 13 : 22). These fractions were combined, concentrated, and further purified by preparative paper chromatography (Whatman 3MM, same solvent system). The band containing the β-methyltryptophan was cut out and eluted with water. Dilution of half of the eluate with an additional 50 mg of the relevant isomer of 4 and recrystallization of this material to constant specific radioactivity (3.76 x 10^5 dpm/mmole) clearly showed that 4 had been produced by S. flocculus. The natural enantiomer is one of the racemates of Isomer A (4a). The precursor methionine had been incorporated to the extent of 2%.

The remaining half of the Isomer A eluate (6 x 10^4 dpm Isomer A) was fed to a new 100 ml fermentation of S. flocculus. Work-up[1a] 15 hours later afforded 1.72 mg of labeled streptonigrin. This was diluted with 25 mg of authentic streptonigrin and recrystallized to constant specific radioactivity (4.53 x 10^4 dpm/mmole). Thus, β-methyltryptophan was incorporated to the extent of 4%.

We have, therefore, demonstrated the intermediacy of β-methyltryptophan in the biosynthesis of streptonigrin. Efforts are now underway to determine the absolute stereochemistry of the natural enantiomer.

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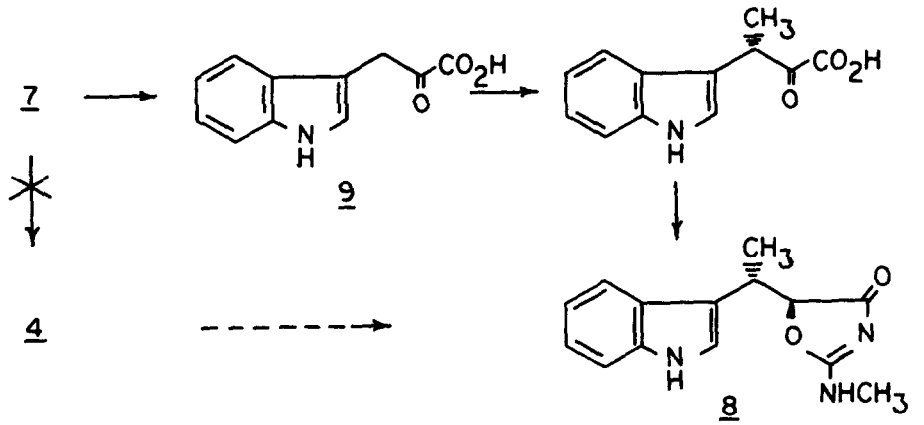

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