ISOLATION OF 4-AMINOANTHRANILIC ACID: A NEW SHIKIMATE PATHWAY PRODUCT FROM *STREPTOMYCES FLOCCULUS*

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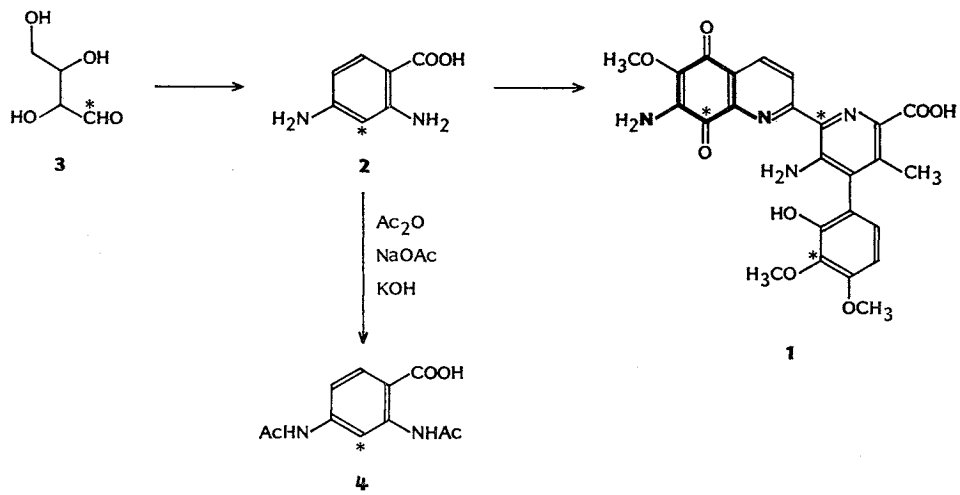
Streptonigrin (**1**), a potent anticancer antibiotic, was isolated from *Streptomyces flocculus* in 1959[1]. We have reported evidence from biosynthetic experiments fully consistent with derivation of the A-ring from the shikimic acid pathway[2,3] and recently reported the specific incorporation of 4-aminoanthranilic acid (**2**) into this portion of the molecule[4]. This was the first indication that **2** may be a natural product. We now report that **2** is, indeed, produced by *S. flocculus*.

Two 50-ml fermentation broths, each in 250-ml Erlenmeyer flasks, were inoculated with 2 ml of a 48-hour seed culture in the usual manner[5]. After 12 hours, 8×10^8 dpm of D-[1-^{14}C]erythrose (8.0 μCi/mmol) (**3**) was synthesized[6,7] and was added to each flask. One flask was allowed to incubate for 92 hours as a control, and workup yielded 1.7 mg of **1**. The other fermentation was terminated 2 hours after addition of **3**. Authentic **2** was synthesized from 4-nitroanthranilic acid by catalytic reduction in methanol in the presence of Pd/C - H_2 and concentrated HCl, and a portion (102.2 mg, 0.454 mmol) was added as carrier to the broth immediately prior to workup in order to trap any **2** produced *de novo*, which would be radioactive. The pH was adjusted to 9.0 with 1 N KOH to dissolve the carrier material, and the mixture was then sonicated†† in an ice bath for 5 minutes. After the solids were removed by centrifugation, the pellet was washed with water and re-centrifuged. The combined supernatants were filtered through a bed of Celite, and the filtrate adjusted to pH 5 with 6 N HCl. The resulting precipitate was removed and the supernatant lyophilized. Repeated trituration with methanol extracted the desired material. The combined methanolic extracts were concentrated *in vacuo*, an additional quantity of **2** (50.4 mg, 0.22 mmol) was added, and the mixture dissolved in 20 ml of water.

Acetylation of the crude material was effected with a mixture of KOH (63.8 mg), NaOAc (0.29 g) and Ac_2O (1 ml). After stirring overnight at room temperature, the bis-acetamide **4**†††,[8] had precipitated as a pale brown solid. The mixture was adjusted to pH 2~3 with concentrated HCl and then extracted with ethyl acetate. The combined extracts were concen-



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†† Heat Systems, Inc., Model W-225R, 50% duty cycle, output control setting 6.

††† An authentic sample had been prepared from unlabeled **2**; physical data matched those in the literature, ref 8. It was also fully characterized by IR and ^{1}H NMR spectroscopies.

trated to near dryness and residual liquid removed as an azeotrope with dichloromethane, and the pale brown powder thus obtained (26.8 mg) was repeatedly recrystallized from methanol - water. The fifth through eighth recrystallizations had specific molar radioactivities† of 2.59, 2.78, 2.33 and 2.58×10^4 dpm/mmol. Since the specific activity of the erythrose fed had been 1.18×10^7 dpm/mmol, a 0.22%-incorporation into **2** had been obtained.

In recent years a number of new aromatic amino acids have been shown to be effective biosynthetic precursors to a variety of microbial metabolites[9~18]. In order to prove that such a compound — previously unknown in nature — is a true intermediate, it is necessary to demonstrate that it is produced by the relevant organism. This has now been done for 4-aminoanthranilic acid.

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† Scintillation counting was done with a Beckman Model LS7800 counter using automatic quench correction by external standardization to provide dpm values.