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

STEVEN J. GOULD and W. RANDAL ERICKSON
Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, U.S.A.

(Received for publication December 25, 1987)

Streptonigrin (1), a potent anticancer antibiotic, was isolated from Streptomyces flocculus in 1959. We have reported evidence from biosynthetic experiments fully consistent with derivation of the A-ring from the shikimic acid pathway and recently reported the specific incorporation of 4-aminoanthranilic acid (2) into this portion of the molecule. 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. After 12 hours, $8 \times 10^6$ dpm of d-[1-14C]erythrose ($8.0 \mu$Ci/mmol) (3) was synthesized 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 - H2 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 9.0 with 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 Ac2O (1 ml). After stirring overnight at room temperature, the bis-acetamide 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-
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$^1$ of 2.59, 2.78, 2.33 and 2.58 \times 10^4 \text{ dpm/mmol}. Since the specific activity of the erythrose fed had been 1.18 \times 10^7 \text{ 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$^9$-$^{13}$. 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.

Acknowledgment

Strains of *Streptomyces flocculus* were originally obtained from Dr. John Dezeeuw of Pfizer International Inc., Groton, CT, U.S.A. This work was supported by Public Health Service Grant GM 31715 to S.J.G.

References


