Pergamon

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The Biosynthesis of Sparsomycin. Further Investigations of the Biosynthesis of the Uracil Acrylic Acid Moiety

Ronald J. Parry,* Jeffrey C. Hoyt, and Yan Li

Department of Chemistry, Rice University, P. O. Box 1892, Houston, TX 77251

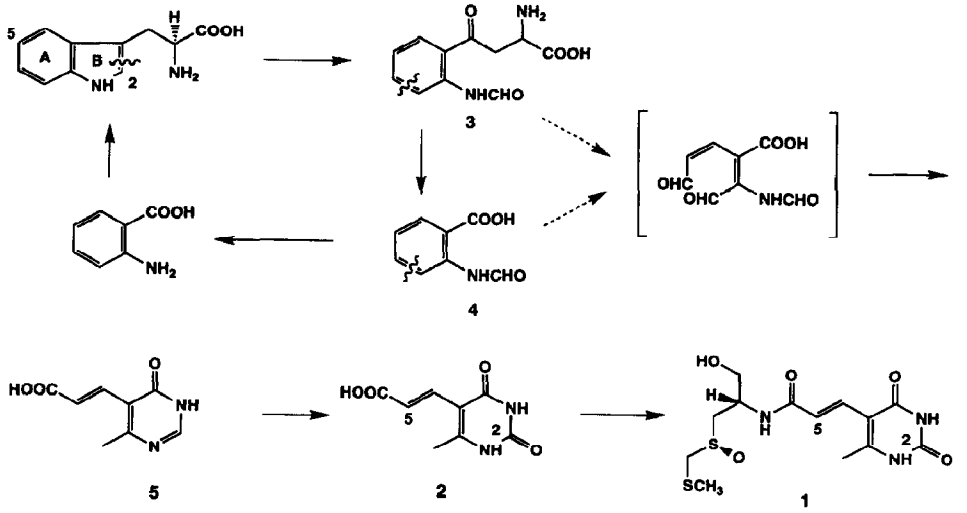
Abstract: Precursor incorporation experiments have ruled out the involvement of N'-formylkynurenine in the biosynthesis of the uracil acrylic acid moiety of sparsomycin. An enzyme has been partially purified from the sparsomycin-producer that catalyzes the synthesis of the uracil acrylic acid from a pyrimidine acrylic acid previously shown to be a sparsomycin precursor. The enzymatic synthesis of the uracil acrylic acid requires NAD^+, a result which indicates that the mechanism of the uracil acrylic acid synthase is similar to that of inosine-5'-monophosphate dehydrogenase. Analog studies demonstrate that the enzyme lacks rigid substrate specificity.

Sparsomycin (**1**) is a novel antibiotic isolated from the fermentation broth of *Streptomyces sparsogenes* var. *sparsogenes* [1] and from *Streptomyces cuspidosporus* .[2] The structure of sparsomycin was assigned in 1970,[3] and several total syntheses of the antibiotic have been reported.[4-6] Sparsomycin exhibits antibiotic activity against a variety of gram-negative and gram-positive bacteria, and it shows potent antitumor activity against KB human epidermoid carcinoma cells in tissue culture.[7] The biological activity of sparsomycin is the result of its ability to inhibit the peptide bond forming step of protein biosynthesis by interacting with the large ribosomal subunit.[8] The biosynthesis of sparsomycin is a problem of unusual interest due to its unique structure which includes a uracil acrylic acid moiety (**2**) and a monooxo-dithioacetal group.

Previous investigations of sparsomycin biosynthesis demonstrated that the uracil acrylic acid moiety **2** is derived from L-tryptophan by a pathway that involves loss of the amino acid side chain and the oxidative cleavage of both of the aromatic rings of the amino acid.[9] Since tryptophan is known to undergo oxidative cleavage of ring B to yield N'-formylkynurenine (**3**), it was initially hypothesized (Scheme I) that tryptophan could be converted into **2** via N'-formylkynurenine and N-formylanthranilic acid (**4**). Oxidative cleavage of the aromatic ring of the latter compound would then yield an intermediate that could be converted into **2** via the pyrimidine acrylic acid (**5**). Evidence for the intermediacy of **5** was obtained from precursor incorporation experiments. However, precursor incorporation experiments with doubly-labeled forms of N-formylanthranilic acid demonstrated unequivocally that this compound was incorporated into **2** by deformylation to anthranilic acid, which was then converted back to tryptophan.[9] Nevertheless, these experiments did not rule out the possible involvement of the kynurenine pathway in sparsomycin biosynthesis, since cleavage of ring A of the indole nucleus could occur at the stage of N'-formylkynurenine (Scheme I). We would now like to report the results of experiments which evaluate the possible role of N'-formylkynurenine in sparsomycin biosynthesis as well as experiments that provide additional support for the intermediacy of the pyrimidine acrylic acid **5** in the biosynthesis of the uracil acrylic acid moiety **2**. The possible role of N'-formylkynurenine in sparsomycin biosynthesis was evaluated in two ways. First, (5-$^2H_1$)-DL-tryptophan[9] was converted into (5-$^2H_1$)-DL-N'-formylkynurenine by ozonolysis.[10] Administration of the deuterated N'-formylkynurenine to *S. sparsogenes* in the usual way then

yielded a sample of sparsomycin that was analyzed by ^2H NMR spectrometry. The analysis revealed that ca.10% deuterium enrichment was present at the expected position (C-5) of the sparsomycin skeleton. While this result

Scheme I



appears promising, the behavior previously observed with N-formyl anthranilic acid suggested that a second experiment should be carried out. Therefore, a sample of (*formyl*-^{13}C)-DL-N'-formylkynurenine was prepared by ozonolysis of (2-^{13}C)-DL-tryptophan[9] and administered to *S. sparsogenes*. The sparsomycin formed in this experiment showed no trace of ^{13}C enrichment. The results of these two experiments indicate that the deuterated form of N'-formylkynurenine is incorporated into sparsomycin after deformylation and conversion to anthranilic acid. In combination with the results of previous experiments,[9] they appear to rule out the involvement of the kynurenine pathway in sparsomycin biosynthesis, and favor a pathway in which ring A of the indole nucleus is cleaved prior to ring B.

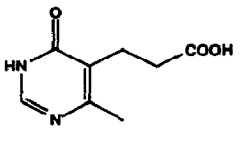
Additional evidence for the intermediacy of the pyrimidine acrylic acid **5** in the biosynthesis of **2** has been obtained from cell-free studies. Cell-free extracts were prepared by sonication of 3-day old cultures of *S. sparsogenes* in phosphate buffer, nucleic acids were precipitated with polymin P, and the proteins were then subjected to chromatography on DEAE cellulose. When the protein fraction eluting with 1 M salt was incubated with the pyrimidine acrylic acid **5** and NAD$^+$, the formation of the uracil acrylic acid **2** could be detected by HPLC. The formation of **2** was dependent upon the addition of both **5** and NAD$^+$ to the incubation mixture, and the identity of the product as **2** was confirmed by NMR and mass spectral analysis of material isolated by preparative HPLC. NADP$^+$ could not be substituted for NAD$^+$. The substrate specificity of the uracil acrylic acid synthase was investigated using protein that had been further purified by high-resolution anion exchange chromatography

and chromatofocusing. The activity of the substrate analogs was determined by monitoring the rate of conversion of NAD^+ to NADH (λ_m = 340 nm). The results of these studies, which are summarized in Table I, indicate that the enzyme lacks rigid substrate specificity and can utilize both the pyrimidine propionic acid **6** and hypoxanthine (**7**) as substrates. However, neither inosine (**8**) nor inosine-5'-monophosphate (IMP) (**9**) are active as substrates. This selectivity may reflect the inability of inosine and IMP to enter the active site of the enzyme for steric reasons. The fact that the pyrimidine carboxylic acid **5** is the best substrate of those examined suggests that the enzyme is very likely to be associated with the sparsomycin biosynthetic pathway, and it provides additional evidence that the pyrimidine acrylic acid **5** is a true intermediate in sparsomycin biosynthesis. The NAD^+ dependence of the conversion of the pyrimidine acrylic acid **5** into the uracil acrylic acid **2** demonstrates that the formation of **2** is mechanistically related to the conversion of IMP into xanthosine-5'-monophosphate which is catalyzed by IMP dehydrogenase. IMP dehydrogenase has been purified and characterized from a variety of sources including bacteria,[11-16] mammals,[17] and the parasitic protozoan *Tritrichomonas foetus*.[18] Additional purification of uracil acrylic acid synthase will be required before a thorough comparison of this enzyme with IMP dehydrogenase can be attempted.

Table 1: Relative Activity of Substrate Analogs with Uracil Acrylic Acid Synthase[a]

Substrate	Relative Activity
5	1.00
6	0.30
6	0.45
8	0.0
9	0.0

[a]Incubations were carried out at 32° C in 50 mM phosphate buffer, pH 6.8, with 4.8 mM NAD^+ and 1 mM substrate.



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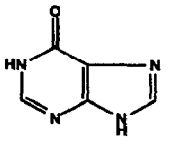

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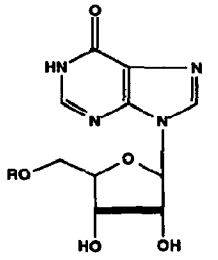

8, R = H
9, R = P

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