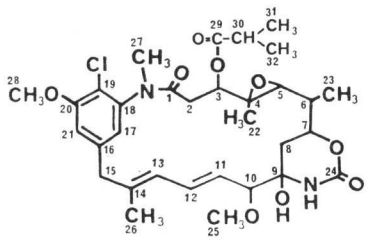
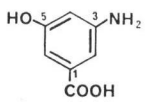
BIOSYNTHETIC ORIGIN OF AMINOBENZENOID NUCLEUS (C_7N-UNIT) OF ANSAMITOCIN, A GROUP OF NOVEL MAYTANSINOID ANTIBIOTICS

Sir:

Ansamitocin P-3(**1**)[1], a new maytansinoid antitumor antibiotic produced by *Nocardia* sp. No. C-15003, has an aminobenzenoid nucleus (C_7N-unit) in its molecule.



(**1**)



(**2**)

Recently, GHISALBA *et al.*[2,3] showed that the aminobenzenoid nucleus of rifamycin produced by *Nocardia mediterranei* was derived from an intermediate between 3-deoxy-D-arabino-heptulosonic acid 7-phosphate and shikimate in the shikimate pathway, perhaps 5-dehydroquinate or 5-dehydroshikimate. KIBBY *et al.*[4,5] concluded that the primary precursor of the aminobenzenoid nucleus of actamycin and mitomycin was 3-amino-5-hydroxybenzoic acid (AHBA) (**2**), by feeding experiments using ^{14}C- and ^{13}C-labeled AHBA. Furthermore, GHISALBA *et al.*[6,7] came to the same conclusion from studies on the biosynthesis of rifamycin using block mutants capable of producing rifamycin only in the presence of AHBA. RINEHART *et al.*[8] assumed that the biosynthetic origin of the aminobenzenoid nucleus of pactamycin would be *m*-aminobenzoic acid derived from dehydroquinate or dehydroshikimate according to the results of feeding experiments using [U-^{13}C]glucose and *m*-amino-[ring-U-^{14}C]benzoic acid.

We also have been interested in the origin of the nucleus of the maytansinoid antibiotics. First, the effect of benzoic acid derivatives on ansamitocin biosynthesis was examined. *Nocardia* sp. No. C-15003 was incubated in 1% glucose, 1% glycerol, 1% peptone and 0.5% yeast extract, pH 7.0 medium at 28°C for 42 hours on a rotary shaker. Cells were harvested, washed by centrifugation and resuspended into sterile distilled water. Biosynthesis of ansamitocin was performed at 28°C for 72 hours on a rotary shaker with a washed cell system consisting of 5 ml of 0.1 M tris-HCl buffer (pH 8.5), 5 ml of 0.05 M $MgCl_2$, 5 ml of 0.05 M sodium isobutyrate, 10 ml of 0.25 M lactose and 25 ml of the washed cell suspension (250~300 mg, dry cell weight).

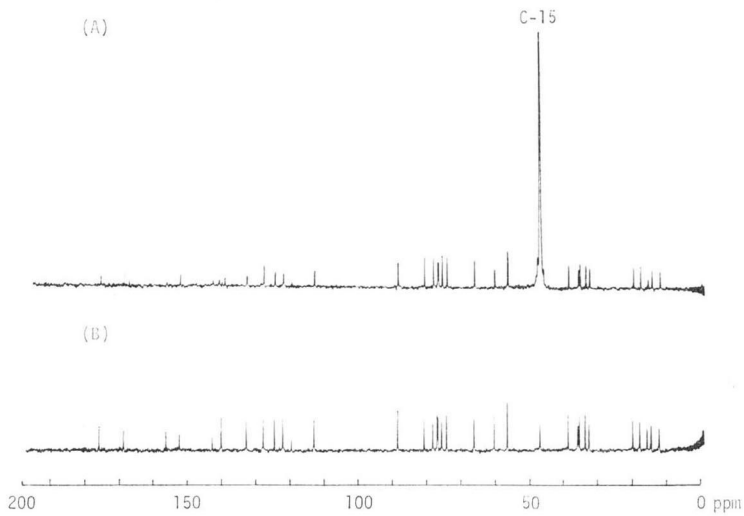
Table 1. Effect of benzoic acid derivatives on ansamitocin production.

Additive	Concentration (mM)	Relative productivity
3-Aminobenzoic acid	2	28
3-Hydroxybenzoic acid	2	13
3,5-Diaminobenzoic acid	2	21
3,5-Dihydroxybenzoic acid	2	29
3-Amino-5-hydroxybenzoic acid	2	130
None (control)	—	100

As shown in Table 1, the addition of AHBA into the incubation mixture stimulates ansamitocin production, but 3-amino-, 3-hydroxy-, 3,5-diamino- and 3,5-dihydroxybenzoates strongly inhibit production. This suggests that the aminobenzenoid nucleus of ansamitocin originates from AHBA and the other benzoates tested inhibit the *de novo* synthesis of AHBA or the incorporation of AHBA into the nucleus. Then, we tried to confirm this by the feeding experiment using [^{13}C] AHBA.

[Carboxy-^{13}C]AHBA(hydrochloride) (86 atom %), synthesized by the method of HERLT *et al.*[9], was pulse-fed to the washed cell system at zero time (40 μg/ml), 24 hours (20 μg/ml) and 48 hours (20 μg/ml) from the beginning of the incubation. After incubation for 72 hours, 43 μg/ml ansamitocin P-3 accumulated and was extracted with ethyl acetate from one liter of the incubation mixture. The solvent layer was concentrated and subjected to silica gel column chromatography (Silica gel 60, Merck)[10]. Ansamitocin was eluted by chloroform - methanol (100: 1, v/v). After concentration of the eluate, 28 mg of colorless crystals of ansamitocin P-3 were obtained from ethyl acetate. Twenty-five milligrams of ansamitocin P-3 obtained were dissolved in deuterated chloroform ($CDCl_3$) and analyzed at 25.2 MHz with a Fourier transform ^{13}C NMR spectrometer (Varian XL-100-12) by proton-

Fig. 1. ^{13}C NMR spectra of ansamitocin.
A) Labeled with [carboxy-^{13}C]AHBA.
B) Natural abundance.



noise-decoupled tcehnique. Operation conditions were as follows: Spectral width, 200 ppm; data point, 8192; accumulation, 74,000. Chemical shifts were expressed in ppm relative to tetramethylsilane. As shown in Fig. 1, the ^{13}C NMR spectrum of ansamitocin obtained by the feeding experiment shows that the C-15 (δ 47.0) position of ansamitocin is specifically enriched and the relative enrichment factor at C-15 is 77.6 to that of natural abundance. This finding indicates that AHBA is directly incorporated into the aminobenzenoid nucleus of ansamitocin as a primary precursor and that almost all the nucleus of ansamitocin produced is derived from AHBA added exogenously to the incubation mixture.

We conclude that the most reasonable precursor of the aminobenzenoid nucleus (C_7N-unit) of maytansinoid antibiotics is 3-amino-5-hydroxybenzoic acid.

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