3-Amino-5-hydroxybenzoic Acid as a Key Intermediate in Ansamycin and Maytansinoid Biosynthesis

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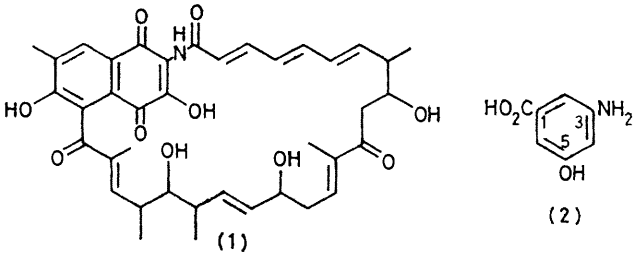
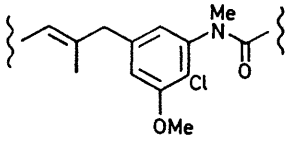
Summary The specific incorporation of 3-amino-5-hydroxybenzoic acid (**2**) into actamycin (**1**) by a *Streptomycete* culture establishes this amino-acid as a key intermediate in ansamycin and maytansinoid biosynthesis

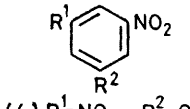
ANSAMYCIN antibiotics of microbial origin include antibacterial agents and potent inhibitors of reverse transcriptase, while the maytansinoids from higher plants and a species of *Nocardia* have antimitotic, antitumour, and antileukaemic activity [1] The two groups are structurally and biogenetically related For several microbial ansamycins, including rifamycins S [2–5] and W,[6] streptovaricin D,[7] geldanamycin,[8] actamycin (**1**),[9] and herbimycin,[10] it has been shown that the ansa chain is polyketide in origin, as is part of the nucleus in those cases where it is naphthalenoid It was suggested, in 1973,[2] that the remaining segment of the naphthalenoid ansamycin nuclei arises from the same unknown C_7N_1 precursor as the benzenoid nuclei of geldanamycin and the maytansinoids Despite considerable effort, the precise nature of this species has not been established We present here evidence that this key precursor is the unusual amino-acid 3-amino-5-hydroxybenzoic acid (**2**)

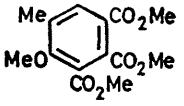
Previous labelling experiments with rifamycin S[4,5] and geldanamycin[8] have indicated that the C_7N_1 progenitor which initiates the polyketide chain is derived from glucose, probably *via* the shikimate pathway [11] Since shikimic acid itself, however, was not effectively converted into rifamycin S[4] or geldanamycin,[8] the biosynthesis of this C_7N_1 intermediate was considered to diverge prior to the level of shikimate, perhaps from 3-dehydroquinate or 3-dehydroshikimate [4] Transamination of the carbonyl functions of these two C_7 keto-acids could then generate the unusual 1,3-relationship of carbon and amino-substituents required in the carbocyclic intermediate [4] Mutant studies with *Nocardia mediterranei*, a rifamycin B producer, showed that the C_7N_1 species was derived from an intermediate of the shikimate pathway between 3-deoxy-D-arabinoheptulosonic acid 7-phosphate and shikimate [12]


(1)

(2)


(3)


(4) $R^1=NO_2$; $R^2=OMe$
(5) $R^1=NH_2$; $R^2=OMe$
(6) $R^1=I$; $R^2=OMe$
(7) $R^1=CN$; $R^2=OMe$
(8) $R^1=CO_2H$, $R^2=OH$


(9)

Analysis of the structures[1,9,10,13,14] of the known ansamycins and maytansinoids permits definition of the C_7N_1 precursor The one-carbon substituent is presumably at the oxidation level of the carboxy-group, since the unit is required to initiate the formation of a polyketide chain The C_7 amino-acid must also carry an oxygen function at the 5-position of the ring This oxygen function may subsequently be methylated or otherwise etherified as in the maytansinoids and some rifamycins, respectively, acetylated as in the streptovaricins, or commonly oxidised, with involvement of the 2-position, to the *p*-quinone (or *p*-quinol) level The apparent exceptions are tolypomycin Y, where the oxygen, in *p*-quinonoid form, is masked by imine formation with the amino-sugar tolyposamine, and the halomycins, where a similar quinonimine is reduced to an aminophenol In the maytansinoids, which have the partial structure (**3**), the aromatic 3-amino-5-hydroxy-acid is further modified only by *O*-methylation and introduction of chlorine into the 4-position In other ansamycins, this 4-position either retains hydrogen, or carries chlorine, hydroxy-, methyl-, or methylthio-functions introduced by secondary processes In these microbial products the 6-position of the precursor remains unsubstituted or is oxidised, as in the benzoquinonoid herbimycin and geldanamycin, respectively, or is involved in ring closure to the polyketide chain in the naphthalenoid ansamycins Actamycin (**1**)[9] exemplifies several of these modifications The functionality and reactivity required for this range of enzymic processes define the primary C_7N_1 precursor as 3-amino-5-hydroxybenzoic acid (**2**), a logical by-product of the shikimic acid pathway [11]

[*carboxy*-^{14}C]-3-Amino-5-hydroxybenzoic acid (**2**) was synthesised from 1-methoxy-3,5-dinitrobenzene (**4**) † Partial reduction gave 3-methoxy-5-nitroaniline (**5**) which was further converted into the iodo-compound (**6**) Isotope was then introduced with cuprous [^{14}C]cyanide and the resulting nitrile (**7**) hydrolysed to the nitro-acid (**8**) before reduction to the required amino-acid (**2**)

This amino-acid (**2**), as its hydrochloride (15 mg, 7 60 μCi), was pulse fed to a culture of *Streptomyces* sp E/784 (500 ml fermentation medium, 5 mg after 28, 36, and 49 h growth)

† Full details will be published elsewhere

The actamycin (**1**),[9] obtained on harvest (72 h growth), was purified to constant radioactivity (50 mg, 0·60 μCi). A triglyceride fraction isolated from the same fermentation carried no radioactivity, indicating that the label from the precursor was not being randomised into the polyketides, which include, in particular, the ansa chain of actamycin (**1**). The specificity of incorporation was confirmed by oxidation (O_3, H_2O_2–HCO_2H) of 3,6-di-*O*-methylactamycin, subsequent methylation affording the triester (**9**) which carried all the actamycin radioactivity. The [^{14}C]-labelled acid (**2**) was thus specifically utilised for actamycin synthesis with high isotope incorporation (7·9%) and low dilution (1 : 11·6) with endogenous substrate. As with other ansamycins,[4,8] [2,3,4,5-^{14}C]shikimic acid was not incorporated into actamycin.

These results establish 3-amino-5-hydroxybenzoic acid (**2**) as the key C_7N_1 nuclear precursor which initiates ansa chain formation in the ansamycins and maytansinoids. This amino-acid (**2**) occurs naturally in the *Streptomycete* antibiotic ferrimycin A_1.[15] It was suggested[16] that the then unknown C_7N_1 unit of the ansamycins shared a common biogenetic origin with similar units in other antibiotics, including the mitomycins, validamycin, and kinamycin. 3-Amino-5-hydroxybenzoic acid (**2**), or a closely related species, may also participate in the biosynthesis of manumycin[17] and asukamycin.[18] Studies in these areas are in progress.

We are indebted to Dr. L. Coronelli, Gruppo Lepetit S.P.A., Milan, for a culture of *Streptomyces* sp. E/784 and thank Mrs. M. G. Anderson, Mrs. J. Rothschild, and Mr. P. J. Green for technical assistance.

(*Received*, 12*th December* 1979; *Com.* 1293.)

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