Biosynthesis of the Antitumor Antibiotic Anthramycin by *Streptomyces refuineus*

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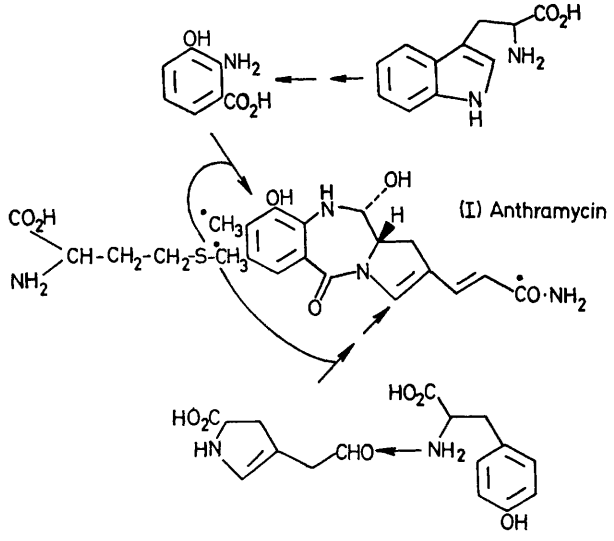
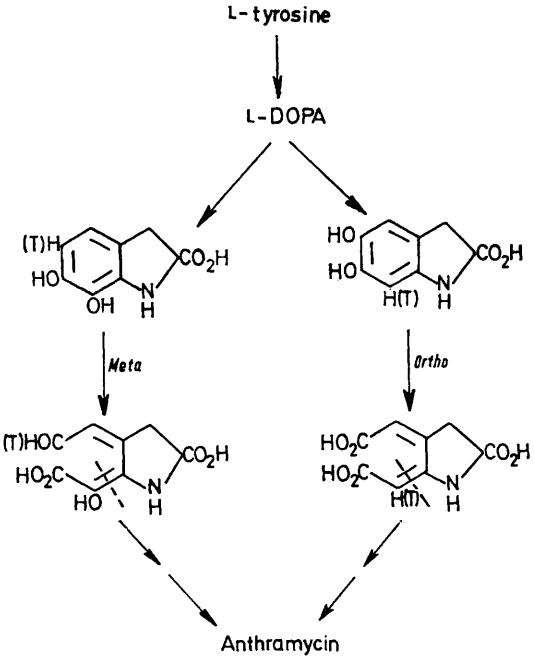
Summary The biogenetic building blocks for anthramycin have been established as tryptophan, tyrosine, and two one-carbon units *via* methionine.

ANTHRAMYCIN (I) is an antibiotic produced by *Streptomyces refuineus* var *thermotolerans* (NRRL 3143).[1] The biogenetic origin of the non 4-methyl-3-hydroxyanthranilic acid part of anthramycin was of prime interest in this investigation. The results reported here establish that anthramycin is derived from tryptophan, tyrosine, and two one-carbon units *via* methionine. We consider it likely that the biogenetic building blocks are as shown in Scheme 1.



SCHEME 1. Suggested biosynthetic building blocks for anthramycin.

Streptomyces refuineus was grown in 50 ml baffled shake cultures at 47° in a medium containing peptonized milk (2%), dried yeast (0·3%), and cornstarch (1%) in distilled water for 12 h. Radioactive precursors were then added and after incubating for a further 3 h the cultures were extracted with butanol saturated with water. After removing the butanol by distillation with water under reduced pressure at 35° the residue was dissolved in methanol and crystallized to constant specific activity. The incorporation data indicate L-tryptophan-(7a-^{14}C)(13·4%), L-methionine-(CH_3-^{14}C)(20·6%), L-tyrosine-(U-^{14}C)(9·9%),



SCHEME 2. Alternative pathways for the conversion of tyrosine-(3-5^{3}H)/(1-^{14}C) into the "acrylamide proline" group of anthramycin involving either *ortho*- or *meta*-cleavage.

and L-DOPA-(1-^{14}C)(14·8%) were all efficiently incorporated into anthramycin, while DL-tryptophan-(alanine-3-^{14}C), L-phenylalanine-(U-^{14}C), D-glucose-(1-^{14}C), D-glucose-(6-^{14}C), acetate-(1-^{14}C), L-proline-(U-^{14}C), and δ-aminolevulinic acid-(4-^{14}C) were all incorporated less than 0·1% into anthramycin.

To obtain further evidence for the specific precursor role of tryptophan-(7a-^{14}C), L-methionine-(CH_3-^{14}C), and L-tyrosine-(U-^{14}C), samples of anthramycin labelled from these amino-acids were degraded to give 4-methyl-3-hydroxyanthranilic acid (MHAA). The data from these degradations show that whereas all the radioactivity from tryptophan was found in MHAA, only 42% of that from methionine, and none of that from the tyrosine was found in this compound. Subsequent degradation of the MHAA labelled from methionine showed that all the radioactivity in MHAA was located in the methyl group. The origin of the MHAA from tryptophan and methionine is analogous to that in actinomycin biosynthesis[2] and probably involves a diversion of the well known metabolic pathway leading to the formation of 3-hydroxyanthranilic acid from L-tryptophan.[3]

To learn more about the conversion of tyrosine into the "acrylamide proline" part of anthramycin a series of experiments with tyrosine labelled with both tritium and carbon-14 were carried out. Upon feeding L-tyrosine-[(3–5)^{3}H/(1-^{14}C)] $\left(\frac{^3H}{^{14}C} = \frac{3{\cdot}73}{1}\right)$ almost exactly one half of the tritium was lost during its conversion to anthramycin $\left(\frac{^3H}{^{14}C} = \frac{1{\cdot}81}{1}\right)$. This suggests that the aromatic-ring cleavage of tyrosine involves a *meta*-cleavage pathway rather than *ortho*-cleavage since the latter would lead to complete loss of tritium from these positions (Scheme 2).†

A feeding experiment with L-tyrosine-[(3–5)^{3}H/(U-^{14}C)] $\left(\frac{^3H}{^{14}C} = \frac{8{\cdot}96}{1}\right)$ showed a 64% retention of tritium during its conversion into anthramycin $\left(\frac{^3H}{^{14}C} = \frac{5{\cdot}73}{1}\right)$. This is in accord with transfer of seven of the nine carbons of tyrosine to the "acrylamide proline" part of anthramycin. The origin of the eighth carbon atom of this group must be a one-carbon unit from methionine since only 42% of the label from a feeding experiment with L-methionine-(CH_3-^{14}C) was found in the MHAA. By analogy with the biosynthesis of the propyl proline group of lincomycin from tyrosine[4] the amide carbon of the acrylamide side chain should be that which is derived from methionine. If this were true then a feeding experiment with doubly labelled methionine-(CH_3-$^{14}C^3H_3$) should show transfer of a one-carbon unit to the acrylamide proline group with complete loss of tritium. The data from this experiment (Table) substantiate our postulation but do not supply unequivocal proof of this. These data also are indicative of a transfer of an intact methyl group to the 3-hydroxyanthranilic acid group. We are presently undertaking a study using C-13 n.m.r. to verify these preliminary results.

TABLE. Feeding experiment with doubly labelled methionine.

	Relative specific radioactivities of anthramycin and its degradation products	$\frac{^3H}{^{14}C}$ ratio	Tritium retention (%)	Number of hydrogens retained on transferred C-1 unit
[CH_3-^{14}C,$^3H_3$]Methionine	—	2·92	100	—
Anthramycin	100	1·41	48	—
Acetic acid*	44	2·90	99	2·98
Calculated values for "acrylamide proline" group	56	0·24	8·3	0·25

* Derived from Kuhn Roth oxidation of anthramycin.

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† The conclusion is valid if no NIH shift has taken place in the conversion of tyrosine into dopa. The absence of such a shift during this transformation has, in fact been shown in other systems. (J. W. Daly, D. M. Jerina, and B. Witkop, *Experientia*, 1972, **28**, 1128.)

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