Pure & Appl. Chem., Vol. 61, No. 3, pp. 485–488, 1989.
Printed in Great Britain.

Studies on the biosynthesis of antibiotics

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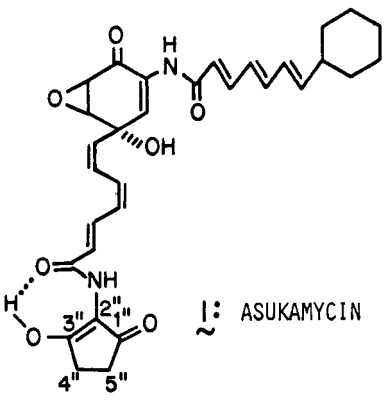
Abstract - Studies with stable isotope-labeled precursors followed by NMR analysis of the resulting antibiotics have revealed the biosynthetic origin and mode of formation of the cyclohexanecarboxylic acid moiety in ansatrienin, the mC_7N unit in ansatrienin and asukamycin, the 2-amino-3-hydroxycyclopent-2-enone moiety of asukamycin and reductiomycin, and the dihydrofuranylacrylic acid moiety of reductiomycin.

This report deals with the biosynthesis of three antibiotics, asukamycin (**1**) from *Streptomyces nodosus* var. *asukaensis* (ref. 1), ansatrienin (mycotrienin) (**2**, Figure 2) from *S. collinus* and *S. rishiriensis* (ref. 2,3) and reductiomycin (**3**, Figure 4) from *S. xanthochromogenus* (ref. 4). These compounds contain several biosynthetically unique structural units, namely a cyclohexane ring (**1** and **2**), a mC_7N unit (**1** and **2**) and a 2-amino-3-hydroxycyclopent-2-enone moiety (C_5N unit) (**1** and **3**). In **1** and **2** these are either connected by or attached to a framework of olefinic or saturated carbon chains which are derived biosynthetically from acetate/propionate units via the polyketide pathway.



1: ASUKAMYCIN

The cyclohexanecarboxylic acid moiety of **2** was found to arise intact from the seven carbon atoms of shikimic acid (**4**, Figure 2) via 1-cyclohexenecarboxylic acid (ref. 5). To obtain more information on the mode of conversion of **4**, a precursor of aromatic compounds, into a fully reduced hydroaromatic ring, we synthesized [2-^{13}C]-**4** and examined its incorporation into **2**. The ^{13}C-NMR spectrum of the resulting sample of **2** showed only one enriched carbon, C-2 or C-6 of the cyclohexane ring. By degradation to the *S*-mandelate ester of cyclohexylcarbinol and NMR comparison with independently synthesized samples of the *R*- and *S*-mandelate esters of [*1R, 2R*)-[2-$^2H_1$]cyclohexylcarbinol it was established that the label resided exclusively at C-6, i.e., the processing of [2-^{13}C]-**4** was stereospecific to give **2** with *S* configuration at C-1 of the cyclohexane ring (ref. 6). Similarly, [2-$^2H_1$]-**4** labeled **2** exclusively in the *pro*-6*R* (axial) position of the cyclohexane ring (ref. 7). A similar conclusion had been drawn, from a feeding experiment with [6-$^2H_2$]glucose, by Furukawa *et al.* (ref. 8) on the biosynthesis of the cyclohexane ring in ω-cyclohexylundecanoic acid. However, their interpretation that this represents a *Re, Re* reduction of the double bond in **4** is contradicted by our observation that a small amount of the 1-cyclohexenecarboxylic acid analog accompanying the sample of **2** from [2-^{13}C]-**4** carried the ^{13}C label at C-6 (CH_2) rather than at C-2 (CH), i.e., the double bond migrates in the ring. In addition, 6-deuterated **4** gave no deuterium incorporation into **2** (ref. 7). Based on these results we propose the pathway shown in Figure 1 for the formation of the cylcohexanecarboxylic acid moiety of **2**. Attempts to establish the mode of formation of the cyclohexane ring of **1** in the same way met with failure because of very poor incorporation of **4**, presumably due to permeability problems. However, the coupling patterns in the cyclohexane ring and adjacent carbon of **1** biosynthesized from [U-$^{13}C_3$]glycerol leave no doubt about its shikimate origin (ref. 9).

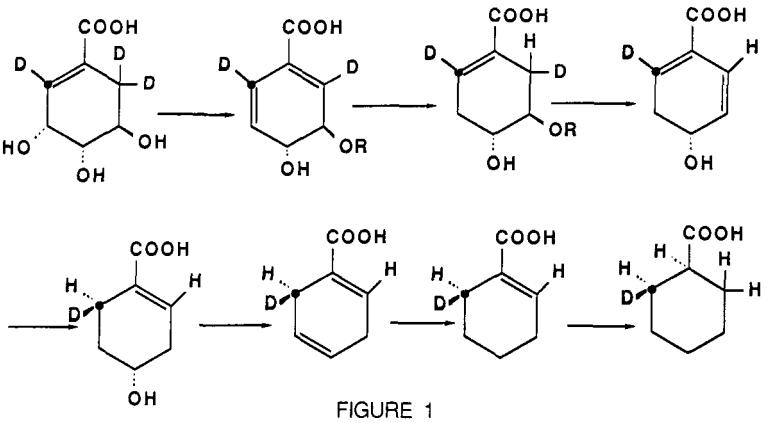


FIGURE 1

A number of antibiotics, mainly ansamycins and mitomycins, contain mC_7N units, 6-membered carbocyclic rings carrying a carbon and a nitrogen substituent in a 1,3 (meta) orientation. Their origin has been traced to the shikimate pathway, but **4** itself is not incorporated into these mC_7N units. This could mean that either **4** is not taken up into the producing organism or the formation of the mC_7N unit branches off earlier in the shikimate pathway. 3-Amino-5-hydroxybenzoic acid (AHBA) has been established as a specific precursor of these mC_7N units (ref. 10, 11). Consistent with this we observed very efficient incorporation of [7-^{13}C]AHBA into **2**, labeling exclusively C-17 of the antibiotic (Figure 2). Since the feeding of [2-^{13}C]-**4** described earlier had labeled the cyclohexane ring of **2** but not the mC_7N unit, we can conclude that, at least in this case, non-incorporation of **4** is not due to permeability problems. Hence, the formation of AHBA must branch off earlier in the shikimate

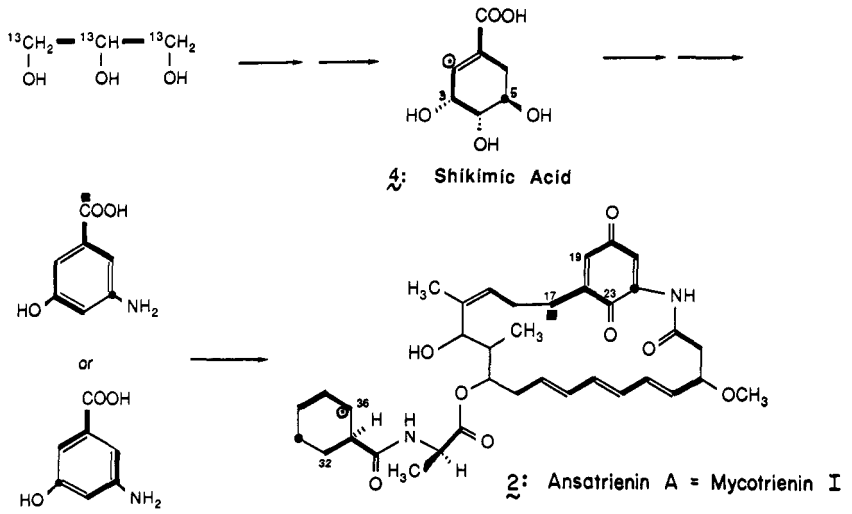


FIGURE 2

pathway. Analysis of the ^{13}C-^{13}C coupling pattern of a sample of **2** biosynthesized from [U-$^{13}C_3$]glycerol revealed that the nitrogen of the mC_7N unit is attached to the carbon corresponding to C-5, not C-3, of **4** (Figure 2) (ref. 12), consistent with the observations of Hornemann *et al.* on mitomycin (ref. 13) and Rinehart *et al.* on geldanamycin (ref. 14) biosynthesis. Based on these results and the finding of Jiao *et al.* (ref. 15) that the amide nitrogen of glutamine is the best source of the nitrogen of rifamycin, we propose the pathway shown in Figure 3 for the formation of AHBA, the precursor of the mC_7N unit.

Surprisingly, a feeding experiment with [7-^{13}C]AHBA gave no incorporation into **1** at all (ref. 9). Further examination revealed a completely different pathway for the formation of the mC_7N unit in **1** involving succinic acid (carbon atoms 6, 5, 4 and 7) and glycerol (carbons 1, 2, 3) as precursor. Details of this new pathway need to be established.

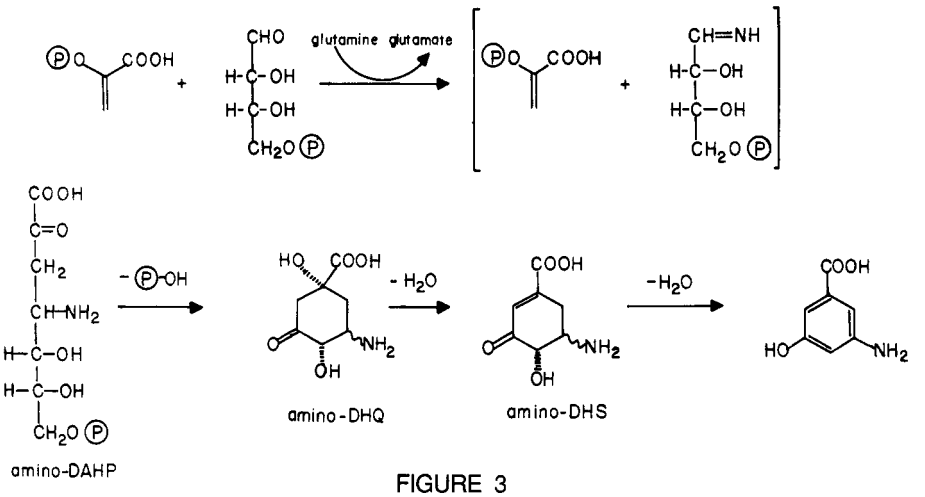


FIGURE 3

Inspection of the C_5N unit in **1** and **3** shows that it contains all the carbon, hydrogen, oxygen and nitrogen atoms of 5-aminolevulinic acid (ALA) less one molecule of water. In a series of feeding experiments with glycine and ALA it was established that the C_5N unit in both **1** and **3** does indeed arise by an intramolecular cyclization of ALA (ref. 16, 17). The most conclusive evidence comes from a feeding experiment with [4,5-$^{13}C_2$]ALA that gave **3** in which one half of the labeled molecules showed coupling between C-1 and C-2 and the other half between C-2 and C-3 of the C_5N unit (Figure 4) (ref. 17). It is proposed that the cyclization of ALA is mediated by an enzyme using pyridoxal phosphate as a cofactor.

The remaining 9 carbon atoms of **3** where first thought to arise from tyosine. However, it was quickly found that the acetoxy group comes from acetate, leaving only seven carbon atoms to account for. Coupling analysis of **3** biosynthesized from [U-$^{13}C_3$]glycerol pointed to a pathway involving ring cleavage of a symmetrical precursor generated via the shikimate pathway (ref. 17). Following this lead it was found that p-hydroxy-[7-^{13}C]benzoic acid and p-hydroxy-[7-^{13}C]benzaldehyde efficiently and specifically labeled **3** (55-64% and 35% enrichment, respectively at C-5') (ref. 17, 18). Of the four ring hydrogens of p-hydroxy-[2,3,5,6-$^{2}H_4$]benzoic acid three are completely retained in the conversion and only the one appearing at C-2'' of **3** undergoes partial exchange with solvent protons, possibly during a double bond *cis-trans* isomerization (ref. 18). The pathway of **3** biosynthesis can thus be formulated as shown in Figure 4.

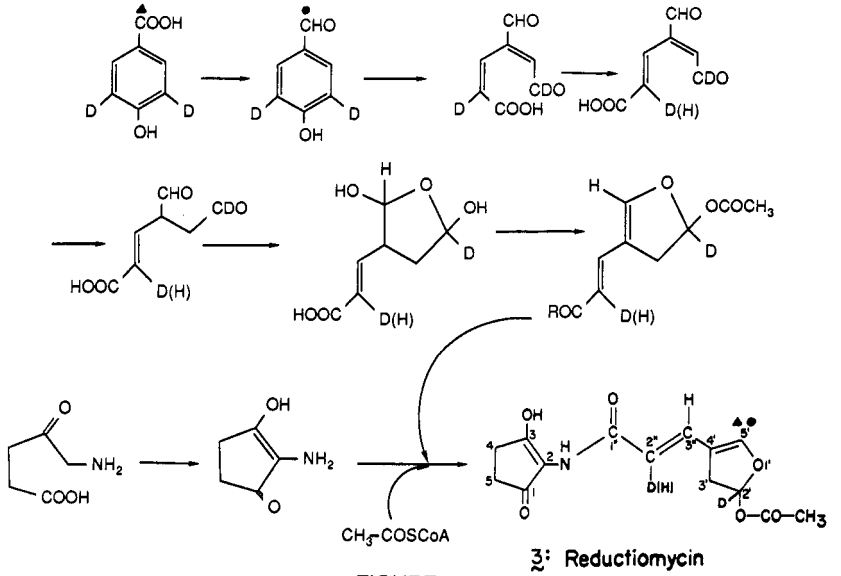


FIGURE 4

Acknowledgements This work was supported by NIH research grant AI20264. We gratefully acknowledge the services of the Los Alamos Stable Isotope Resource (supported by NIH grant RR02231 and the Department of Energy).

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