Biosynthesis of the Shikimate-derived Starter Unit of the Immunosuppressant Ascomycin: Stereochemistry of the 1,4-Conjugate Elimination

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The potent immunosuppressants ascomycin, FK506 and rapamycin are polyketide secondary metabolites that are biosynthesized from an usual shikimate-derived (1'S,3R,4R)-3,4-dihydroxycyclohexanecarboxylic acid (DHCHC) starter unit1-4. The biosynthetic pathway to this DHCHC moiety of ascomycin has been investigated in Streptomyces hygroscopicus var. ascomyceticus by a series of incorporation experiments with isotopically labeled shikimic acid and various putative pathway intermediates1. The results obtained were consistent with a process that initiates with either a syn or an anti 1,4-conjugate elimination of a C6 hydrogen and the C3 hydroxyl group of shikimic acid to produce (3R,4S)-3,4-dihydroxy-1,5-cyclohexadienecarboxylic acid (1) (Fig. 1). A syn reduction of the A^double bond of 1 gives of (1'S,4R,5R)-4,5-dihydroxycyclohex-2-ene carboxylic acid (2), which is subsequently converted to (4R,5SR)-4,5-dihydroxycyclohex-1-ene carboxylic acid (3) by an isomerization of the remaining double bond from the A^2 to the A^1 position. In the final step of the process 3 is converted to DHCHC by an anti addition of hydrogen to the re faces of C1 and C2. An alternative pathway in which the first double bond is reduced by an anti addition of hydrogen (1→2), while consistent with the experimental results, seems unlikely as it would require a subsequent unprecedented antarafacial rearrangement to generate 31.

The only stereochemical detail of DHCHC biosynthesis that remains undetermined is the steric course of the initial 1,4-elimination step. Previous incorporation studies with (-)-(6R)-[6-2H]shikimic acid had generated ascomycin which exhibited a single 2H signal at 0.9 ppm, consistent with deuterium occupying the C30R (axial) position11. This signal clearly demonstrated that the pathway to DHCHC involves loss of one hydrogen (no signal was observed for deuterium occupying the C30S position), but it does not distinguish which of the heterotropic C6 hydrogens is removed. We have now circumvented this problem by developing a synthetic strategy for preparing stereospecifically deuterated (−)-(6R)-[6-2H]shikimic acid from mannose5. In separate experiments each stereospecifically labeled shikimic acid (30 mg) was added to 5×25 ml 24 hours cultures of S. hygroscopicus var. ascomyceticus to a final concentration of 1.4 him. After an additional 5 days of fermentation the cells were combined with those obtained from a 2×25 ml six day fermentation, and the ascomycin was isolated, purified and characterized by 1H and 2H NMR as described previously1. Inspection of the 46.07 MHz 2H NMR spectra of ascomycin (71 mg) from the feeding experiment with (−)-(6R)-[6-2H]shikimic acid exhibited no discernible signal even after prolonged accumulation. In contrast, the 2H NMR spectrum of ascomycin (56 mg) from the (−)-(6S)-[6-2H]shikimic acid experiment was indistinguishable from that obtained previously using (−)-(6(R,S)-[6-2H]shikimic acid, with a single 2H signal at 0.9 ppm.

These results clearly indicate that the initial 1,4-elimination step proceeds in an anti fashion with loss of the pro-6R hydrogen of shikimate. The same anti elimination has been shown to occur in the conversion of shikimate to I during the biosynthesis of cyclohexanecarboxylic acid (CHC), a process which occurs in Streptomyces collinus and Allicyclobaccillus acidocaldarius (Fig. 1)5. The CHC and DHCHC pathways apparently diverge after this first step. Analysis of the rapamycin polyketide synthase provides strong evidence that this enzyme utilizes DHCHC as a free acid suggesting that the syn reduction of the A^double bond of 1 is first converted to a coenzyme A thioester and that the A^double bond of this compound is then reduced in an anti fashion (Fig. 1)7-9. It has been proposed that these stereochemical differences reflect differing progenitors for the enoyl reductases in these two pathways10. By the same token the dehydratases responsible for converting shikimate to I in the same stereochemical fashion in these two pathways may be related. It has previously been noted that chorismate synthase also catalyzes an anti 1,4-elimination reaction using 5-enolpyruvylshikimate.© 2023 Springer Science+Business Media, LLC
3-phosphate in a later step in the shikimate pathway\(^5\).
The evolutionary relationship of all of these dehydratases, however, will only be determined once the corresponding genes have been cloned. Some progress has been made in this direction; an enoyl CoA reductase involved in the CHC pathway has already been cloned and sequenced\(^7\), and analysis of the surrounding genes has provided evidence of a CHC biosynthetic gene cluster (unpublished data).

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References


