The Biosynthesis of Showdomycin: Stereochemical Aspects of Maleimide Ring Formation

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The biosynthesis of the maleimide ring of showdomycin (**1**) from L-glutamic acid proceeds with retention of the 3-*pro*-*S*-hydrogen at the vinylic position of showdomycin, whilst the 3-*pro*-*R*-hydrogen is lost.

We have recently demonstrated unambiguously that the *C*-nucleoside antibiotic showdomycin (**1**) is formed biologically from D-ribose [presumably as phosphoribosylpyrophosphate (**2**)] and L-glutamic acid (**3**), with loss of C-1 of glutamate.[1] Here we report our findings concerning the origin of the vinylic hydrogen of showdomycin (**1**).

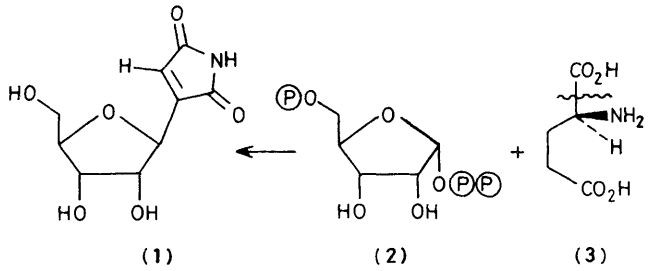
When [3-$^2H_2$]-DL-glutamic acid (**4**) (600 mg)[2] was administered to growing liquid cultures of *Streptomyces showdoensis*, the resultant showdomycin (**1**) showed in its deuterium n.m.r. spectrum (55.28 MHz, H_2O) a broad singlet at δ 6.79; the ^1H n.m.r. spectrum of showdomycin (200 MHz, D_2O) shows the vinylic proton as a narrow doublet at δ 6.72 (lit.[3] δ 6.82), well resolved from all other signals. Hydrogen from C-3 of glutamate is thus retained during the formation of the maleimide ring.

In the light of this result, it was clearly of interest to investigate the biosynthesis using L-glutamic acid samples stereospecifically labelled at C-3. The synthesis of such materials has recently been reported by two of us[4] and samples of (2*S*,3*S*)-[3-^2H]glutamic acid (**5**) and (2*S*,3*R*)-[2,3-$^2H_2$]-glutamic acid (**6**) were prepared by a minor modification of this route.

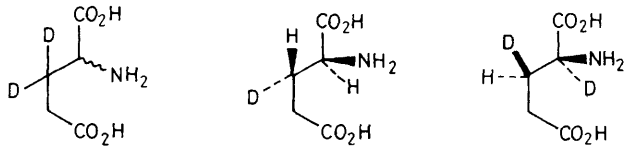
When (2*S*,3*S*)-[3-$^2H_1$]glutamic acid (**5**) (302 mg), admixed with [U-^{14}C]-L-glutamic acid, was administered to *S. showdoensis*, the ^2H n.m.r. spectrum of the resultant showdomycin (6.6% specific incorporation of radioactivity) again showed a broad singlet at δ 6.79; integration of the signal and that due to the methyl groups of t-butyl alcohol (^2H at natural abundance) added as an internal standard indicated a specific incorporation of deuterium of *ca.* 2.4%.† On the other hand, when (2*S*,3*R*)-[2,3-$^2H_2$]glutamic acid (**6**) (280 mg) was fed, again mixed with [U-^{14}C]-labelled material, there was no detectable incorporation of deuterium into the resultant showdomycin (**1**), although specific incorporation of radioactivity was again good (5.2%).

These results indicate that the formation of the maleimide ring of showdomycin (**1**) from L-glutamate (**3**) involves loss of the 3-*pro*-*R*-hydrogen of glutamate, whilst the 3-*pro*-*S*

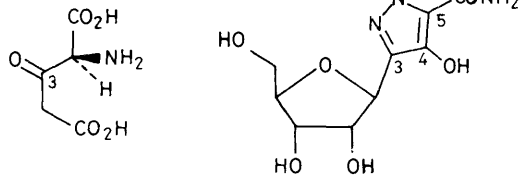
† One would expect deuterium incorporation to be less than that of ^{14}C, since *uniformly* labelled glutamic acid can give rise to incorporation of radioactivity after transamination and metabolism *via* the Krebs cycle. Such metabolism leads to loss of deuterium. See also refs. 1 and 5.



(1) (2) (3)



(4) (5) (6)



(7) (8)

hydrogen is retained. The fact that one of the hydrogens is retained also has implications for the mechanism of linkage of the ribose and glutamate units. An attractive way of providing the required nucleophilicity at C-4 of glutamate would involve a 3-oxo intermediate [(**7**) or a related compound], and (**7**) has been postulated as an intermediate in the biosynthesis of muscarine, ibotenic acid, and related metabolites.[6] It would also be appealing as an intermediate in the biosynthesis of pyrazofurin (**8**), carbons 3—6 of which are thought to correspond to carbons 4—1 of glutamic acid.[5] Our findings indicate that such a theory is untenable in the case of showdomycin.

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