The Biosynthesis of Showdomycin: Studies with Stable Isotopes and the Determination of Principal Precursors

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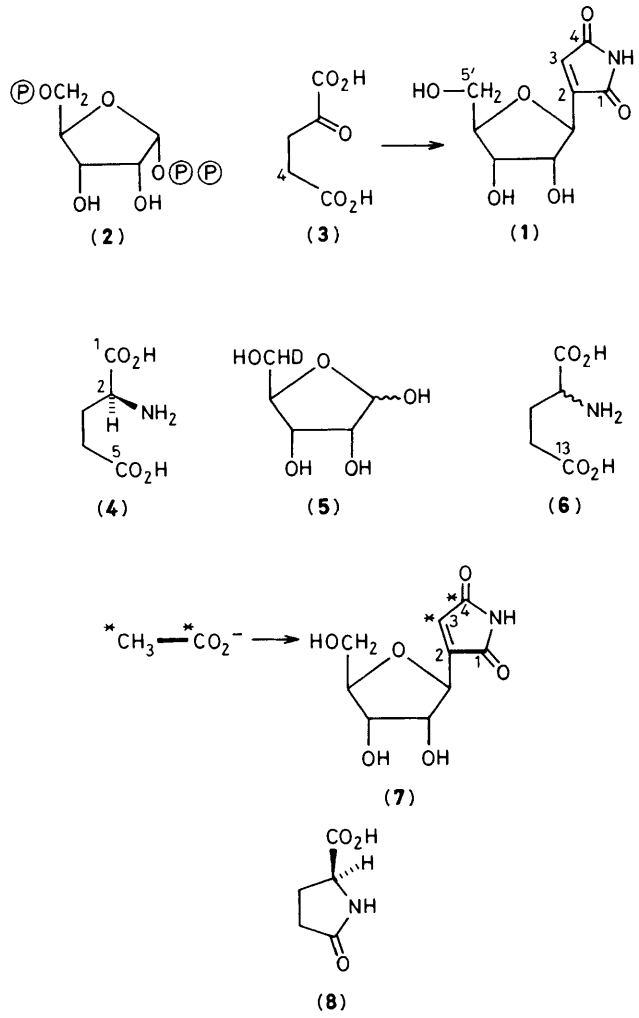
Studies with stable isotopes show that D-ribose and L-glutamic acid are direct biosynthetic precursors of showdomycin (**1**), with carbons 2—5 and the nitrogen of L-glutamate giving rise to the maleimide ring: L-pyroglutamate may be an intermediate.

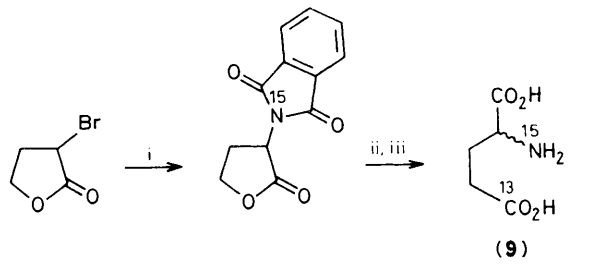
Showdomycin (**1**), one of the *C*-nucleoside group of antibiotics,[1] is a metabolite of *Streptomyces showdoensis* with antibacterial and anti-tumour activity.[1] Elstner and Suhadolnik have reported evidence,[2] using radiotracers, that the biosynthesis of showdomycin involves as major precursors D-ribose, [presumably as phosphoribosylpyrophosphate (**2**)], and an unsymmetrical metabolite on or closely related to the Krebs (tricarboxylic acid) cycle, such as α-oxoglutarate (**3**), glutamate, or succinyl CoA, with the linkage occurring from the carbon atom corresponding to C-4 of α-oxoglutarate. These conclusions were supported by later experiments with singly labelled ^{13}C-acetates.[3] We now report experiments



using stable isotopes (^{13}C, ^{2}H, and ^{15}N) which clearly establish D-ribose and L-glutamic acid (**4**) as direct precursors, with carbons 2—5 and the nitrogen atom of L-glutamate providing the maleimide ring.

When [5-$^{2}H_1$]-D-ribose,[4] shown as the furanose form (**5**) (550 mg), admixed with [1-^{14}C]-material, was administered to growing cultures of *S. showdoensis*, the resultant showdomycin (**1**) incorporated radioactivity well (2.5% specific incorporation). The ^{1}H n.m.r. spectrum of showdomycin (**1**) (200 MHz, D_2O) shows the protons of the 5′-position as the AB part of an ABX system, centred at δ 3.75, and clearly resolved from other signals. The ^{2}H n.m.r. spectrum (55.3 MHz, H_2O) of the biosynthetic showdomycin showed a single rather broad absorbtion centred at δ 3.74, thus indicating the intact and specific incorporation of D-ribose.

Concerning the origin of the maleimide ring, we firstly demonstrated that [U-^{14}C]-L-glutamate [as (**4**)] was efficiently incorporated (7.9% specific incorporation) into showdomycin (**1**), whilst [1-^{14}C]-L-glutamate was incorporated only very poorly (0.13% specific incorporation); earlier similar results[2] had been obtained with racemic materials. [5-^{13}C]-DL-Glutamic acid (**6**) was synthesised by reaction of 2-benzoylaminobutyrolactone with ^{13}C-cyanide and subsequent acid hydrolysis.[5] When this material (890 mg) was administered to *S. showdoensis*, the $^{13}C\{^{1}H\}$ n.m.r. spectrum (50.32 MHz, D_2O) of the showdomycin produced showed a pronounced enhancement (3% enrichment above natural abundance) of the upfield of the two carbonyl signals (δ 175.15 and 175.77),



Scheme 1. i, Potassium [^{15}N]phthalimide, *N*,*N*-dimethylformamide (DMF), reflux, 12 h, 78%; ii, K^{13}CN, DMF, reflux, 48 h, 42%; iii, 6 M HCl, reflux, 81%.

other signals not being measurably enriched. That the upfield signal was assignable to C-1 was clear from the fully proton-coupled spectrum of unlabelled showdomycin; the upfield signal displayed a large (12.3 Hz) three-bond transoid coupling to H-3,[6] together with a smaller coupling (2.5 Hz) to H-1′, whilst the lower field signal showed only relatively small couplings (5.3 and 0.9 Hz).

We felt it necessary to establish clearly whether one or both stereoisomers of glutamate could act as precursors. When [5-^{14}C]-L- and [5-^{14}C]-D-glutamic acids (synthesised as for ^{13}C material above, and resolved *via* the action of hog kidney acylase on the *N*-acetyl derivative) were fed in parallel (320 mg of each) to *S. showdoensis*, incorporation of the L-isomer was good (1.85% specific incorporation)† whilst the D-isomer was poorly incorporated (0.29%). Our results thus indicate clearly that carbons 2—5 of L-glutamate are specific precursors of carbons 4—1 of showdomycin.

This conclusion was supported by experiments with sodium [$^{13}C_2$]acetate. When this precursor (100 mg, 90% ^{13}C each position, admixed with 400 mg unlabelled material and [U-^{14}C]acetate) was fed, the showdomycin isolated (13.2% specific incorporation of ^{14}C) was labelled with ^{13}C as indicated in (**7**). The singlet signals for C-3 and C-4 (δ 130.9 and 174.8) were each enriched approximately equally (1.6 and 1.5% ^{13}C above natural abundance respectively), whilst the signals for C-1 and C-2 (δ 174.2 and 148.8) displayed doublets (*J* 55.4 Hz) of approximately equal intensity (2.1 and 1.8% ^{13}C respectively). The central singlets for C-1 and C-2 showed only slight enhancement (*ca.* 0.3 and 0.2% ^{13}C respectively), ascribable to the presence of singly-labelled precursors in the material fed. This result is in agreement with prediction based on metabolism of acetate *via* the Krebs (tricarboxylic acid) cycle,[7] and incorporation into showdomycin in the proposed manner.

To investigate the origin of the nitrogen atom in showdomycin, [5-^{13}C,^{15}N]-D,L-glutamic acid (**9**) was synthesised as outlined in Scheme 1. This compound (90% ^{13}C, 99% ^{15}N, 408 mg) admixed with [U-^{14}C]-L-glutamic acid, gave showdomycin (**1**) with 9.8% specific incorporation of radioactivity. The ^{13}C n.m.r. spectrum of this material (90.56 MHz, D_2O) showed the signal for C-1 as an enriched singlet, flanked by a weak doublet (*J* 14.99 Hz, 0.7 Hz upfield isotope shift). No other signals showed enrichment or satellites. Total ^{13}C incorporation was 1.1%,† and from the relative intensities of main peak and satellite, it was estimated that *ca.* 35% of the doubly labelled glutamate molecules that had been incorporated into showdomycin had retained both labelled nitrogen

† One would predict that incorporation of [5-^{13}C or -^{14}C] glutamate should be significantly less than that of uniformly labelled material, since metabolism of the specifically labelled material *via* transamination and the Krebs cycle leads to loss of label.

and carbon. This extensive exchange of nitrogen is presumed to be the result of transaminase activity; nonetheless, the appearance of ^{13}C–^{15}N coupling for the C-1 signal clearly indicates that the nitrogen of L-glutamate is retained in the biosynthesis of showdomycin.

Structural analogy suggests that L-pyroglutamate (**8**) could be a possible intermediate in showdomycin biosynthesis. When [U-^{14}C]-L-pyroglutamate (prepared by cyclisation of the amino acid)[8] was tested as a precursor, a 5.8% specific incorporation was observed, and a similar result (2.9% incorporation) was observed with specifically labelled material, [5-^{14}C]-D,L-pyroglutamic acid. In the light of these results we prepared [5-^{13}C,^{15}N]-D,L-pyroglutamic acid [as (**8**)]. When this precursor (305 mg, 90% ^{13}C, 99% ^{15}N) was administered to *S. showdoensis* the resultant showdomycin (**1**) gave a ^{13}C n.m.r. spectrum (90.56 MHz, D_2O) showing C-1 as an enriched singlet flanked by a clear doublet (J 14.95 Hz), the remainder of the spectrum being unchanged. Total ^{13}C incorporation was 3.4%, and it could be calculated that retention of nitrogen relative to ^{13}C was 28%. This partial exchange of nitrogen can best be rationalised by conversion of pyroglutamate (**8**) into glutamate (**4**), followed by transamination. The ready interconversion of L-glutamate and L-pyroglutamate and the enzymes catalysing these processes are well established in animal tissues, and although the situation is less well defined, similar processes are thought to occur in micro-organisms.[9] Thus, although L-pyroglutamate (**8**) can clearly act as a specific precursor of showdomycin (**1**) its involvement as an obligatory intermediate is not yet established.

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