Stereochemistry of Incorporation of Serine into the Oxazole Ring of Virginiamycin M_1

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Incorporation of serine into the oxazole ring of virginiamycin M_1 proceeds *via* the (*S*)-isomer of serine with loss of the 3-(*pro-S*) hydrogen, as deduced from feeding studies with various stereospecifically labelled precursors.

The antibiotic virginiamycin M_1, which is also known *inter alia* as ostreogrycin A, is an unsaturated peptolide antibiotic produced by *Streptomyces virginiae*. It has the structure (**1**), as deduced from chemical evidence[1,2] and *X*-ray crystallography.[3] It contains the unusual oxazole ring, which is found in only a few other natural products such as berninamycin[4] and the recently isolated marine natural products ulapualide A and B[5] and calyculin A.[6] Previous work by the Virginia group has shown that the oxazole ring arises biosynthetically from an acylserine intermediate,[7] and in this communication we report that the formation of the oxazole ring from serine is stereoselective, both with respect to the serine stereochemistry at C-2, and also with respect to the loss of a hydrogen from C-3.

Conditions for the production of virginiamycin M_1 from *S. virginiae* strain PDT 30, for the incorporation of labelled serines, and for the isolation of labelled antibiotic were as previously described.[7] The isolated antibiotic was hydrogenated over platinum and hydrolysed to yield racemic alanine from the oxazole portion,[1] and this was converted into its N-benzoyl derivative and isolated by h.p.l.c.,[8] followed by dilution with unlabelled material and recrystallization to constant specific activity. (2*S*,3*R*)-[3-^{3}H]Serine and (2*S*,3*S*)-[3-^{3}H]serine were prepared from D-[1-^{3}H]mannose and D-[1-^{3}H]glucose respectively, as previously described.[9]

The results of four incorporation experiments are given in Table 1. The first experiment serves as a control to demonstrate that the methods used do not result in wash-out of tritium, while the second indicates that (2*S*)-serine is incorporated preferentially compared with racemic serine.† In the third and fourth experiments the two chirally ^{3}H-labelled serines were incorporated, and the results of these experiments show clearly that the 3-(*pro-R*) hydrogen is retained, while the 3-(*pro-S*) hydrogen is lost on formation of the oxazole ring. The fact that retention of tritium is not 100% and 0% respectively can be explained by the known stereochemical randomization accompanying the competing but reversible side-reaction of the transfer of the hydroxymethyl group of serine to tetrahydrofolate by serine transhydroxymethylase.[10] In addition, a secondary tritium isotope effect may also be a factor; such an isotope effect would be of the order of 1.2—1.3 for a reaction involving the conversion of an sp^3 into an sp^2 hybridized carbon.[11]

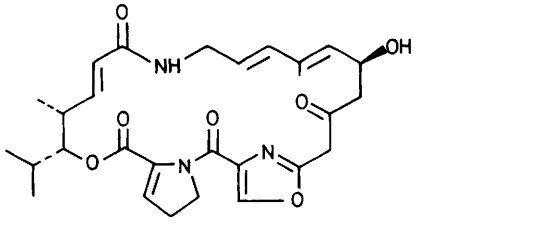
Formation of the oxazole ring from an acylserine intermediate could in principle proceed by several pathways. Although it is not known whether the oxygen atom of the ring arises from serine or from the acyl group, analogy with the formation of the thiazole ring from cysteine suggests that it is derived from serine. With this reasonable assumption, the major pathways for formation of the oxazole ring are shown in Scheme 1. In pathways a and c, oxidation of serine to the aldehyde is followed by enolization and cyclization to yield an

† The fact that the %^{3}H label retained in experiment 2 is not exactly twice that in experiment 1 may be due to the (2*S*)-[U-^{14}C]serine not being completely chirally homogeneous. Alternatively, the result could indicate some utilization of (2*R*)-serine.

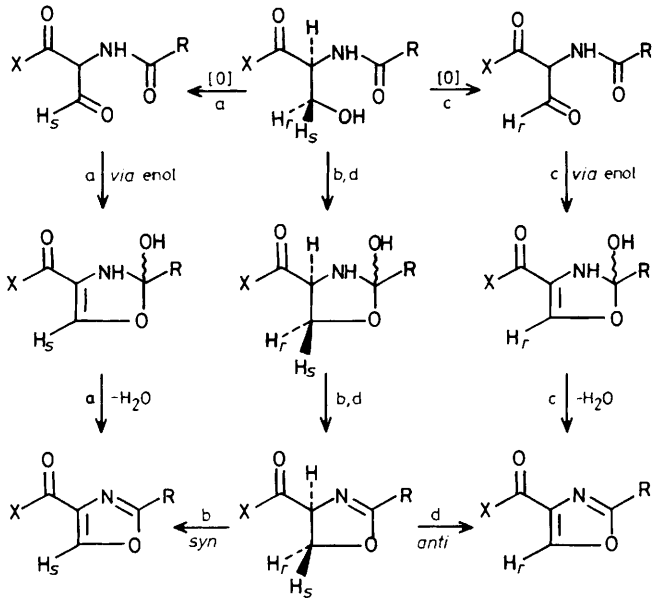
Table 1. Incorporation of labelled serine samples into the oxazole portion of virginiamycin M_1.

Experiment	Precursor	$^3H/^{14}C$ ratio of precursor	$^3H/^{14}C$ ratio of product[a]	% ^3H retained
1	(2*S*)-[G-^{3}H]Serine (2*S*)-[U-^{14}C]Serine	6.02	2.95	49
2	(2*S*)-[G-^{3}H]Serine (2*RS*)-[carboxy-^{14}C]Serine	4.81	3.46	72
3	(2*S*,3*R*)-[3-^{3}H]Serine (2*S*)-[U-^{14}C]Serine	2.42	1.60	66
4	(2*S*,3*S*)-[3-^{3}H]Serine (2*S*)-[U-^{14}C]Serine	3.32	0.38	11

[a] Product *N*-benzoylalanine, formed from virginiamycin M_1 as described in the text, recrystallized to constant radioactivity ratio.



(1)



Scheme 1

adduct which can dehydrate to the oxazole. In pathways b and d cyclization and dehydration occur first, and are followed by dehydrogenation to yield the oxazole. The stereochemical outcome of each of these pathways is indicated in Scheme 1, with a key conclusion that pathways b and d would yield a product from (2*S*)-serine with retention of the 3-(pro-*S*) hydrogen if the dehydrogenation step occurs with an overall *syn* stereochemistry. Since dehydrogenation reactions normally occur with a *syn* stereochemistry, as shown by studies on the biosynthesis of cryptoechinuline,[12] mycelianamide,[13] and elaiomycin,[14] and the side-chain dehydrogenation of *N*-benzyloxycarbonyl-L-tryptophan,[15] formation of the oxazole ring with retention of the 3-(*pro-R*) hydrogen suggests that ring formation does *not* involve a dehydrogenation process, and may thus be taken as evidence favouring pathway c.

The stereochemistry of the proposed oxidation of serine to the aldehyde in pathway c is unknown. In the case of oxidation by alcohol dehydrogenase, ethanol binding occurs so that the 1-(*pro-R*) hydrogen is transferred,[16] but the conversion of (2*R*,3*R*)-[2,3-$^3H_2$]cysteine into benzylpenicillin, which may proceeded *via* a thioaldehyde intermediate, proceeds with retention of the tritium labels.[17] If our interpretation of the pathway is correct, it would seem that oxidation of serine to its aldehyde also proceeds with retention of the 3-(*pro-R*) hydrogen.

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