Pergamon

Tetrahedron Letters, Vol. 36, No. 3, pp. 477-480, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(94)02291-7

Biotransformation of Alosetron: Mechanism of Hydantoin Formation

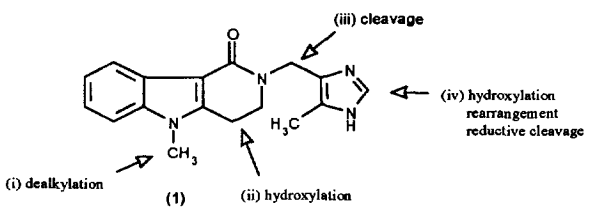
Andrew R. Knaggs,[a]* Karl M. Cable,[b] Richard J. P. Cannell,[a] Philip J. Sidebottom,[c] Guy N. Wells [b] and Derek R. Sutherland [b]

[a]Natural Products Chemistry, [b]Isotope Chemistry ,[c]Structural Chemistry,
Glaxo Research & Development Ltd, Greenford, Middlesex UB6 0HE, UK

Abstract : The formation of the hydantoin biotransformation product (3) of Alosetron (1) has been shown to proceed *via* migration of the tricyclic lactam moiety and occurs with complete retention of both hydrogens in the C-7' methylene group.

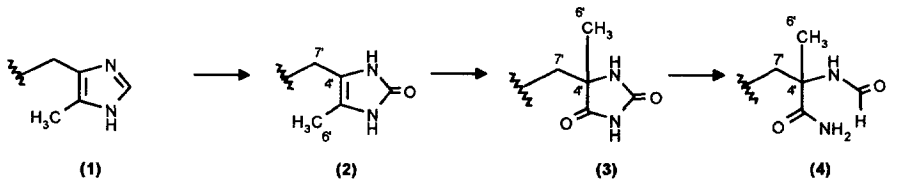
Microbial biotransformations are increasingly being used in the pharmaceutical industry and academia, and often provide mild and high-yielding alternatives to chemical syntheses, chiral resolutions, and functional group modifications.[1] Interest has focussed on the use of microbial biotransformations to aid drug development, in particular for the metabolic evaluation of drug candidates. Comparative studies of drug metabolism in mammals and microorganisms have demonstrated similar biotransformation pathways, especially those leading to aromatic hydroxylation.[2] These findings have led to use of microbial systems to mimic mammalian metabolism, and for the production of putative mammalian metabolites. Microbial biotransformation has the advantage of ease of scale-up, and can give rise to novel products possessing enhanced pharmacological activity. Use of this approach can lead to rapid and efficient structural characterisation and biological evaluation of mammalian drug metabolites. This paper describes some of our microbial metabolism studies on the $5HT_3$ receptor antagonist Alosetron (1), and presents the results of investigations into the mechanism of formation of hydantoin metabolite (3).

Alosetron was incubated separately with 25 microorganisms in shaken liquid culture for 7 days. Samples of each culture broth were taken, together with those from the corresponding control cultures (incubated without drug substrate), extracted and analysed by gradient reverse-phase HPLC. Comparison of the chromatograms obtained from control culture extracts with those for cultures incubated with Alosetron indicated that one of the most efficient biotransforming microorganisms was *Streptomyces griseus* (ATCC 13272). Consequently, this organism was refermented with Alosetron (75mg) on a larger scale (3 x 50ml), and the resulting microbial metabolites of Alosetron extracted and purified. A total of 10 biotransformation products were identified and characterised by NMR and mass spectroscopic analysis. Metabolism of the drug substrate had taken place at 4 sites: (i) N-demethylation of the indole moiety, (ii) hydroxylation at C-4 of the lactam ring, (iii) dealkylation of the lactam nitrogen and (iv) extensive metabolism (both oxidative and reductive) of the imidazole portion of Alosetron (Scheme 1).



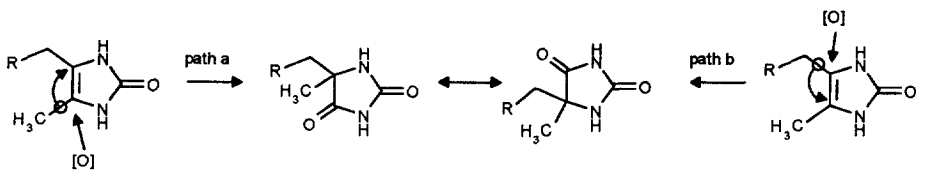
Scheme 1: Sites of Metabolism of Alosetron (1)

The observed dealkylations and hydroxylation of the tricyclic lactam substructure were unsurprising for a molecule of this type, but an interesting sequence of biotransformations was apparent from the metabolism of the imidazole moiety.[3] The three major biotransformation products of Alosetron, modified in the imidazole ring, are shown in scheme 2. Consideration of these structures suggested a potential metabolic pathway leading from Alosetron (**1**), *via* (**2**) and (**3**), to the ring-opened metabolite (**4**). The first step in the biotransformation sequence probably proceeds through a cytochrome P-450 monooxygenase mediated hydroxylation at C-2 of the imidazole ring, followed by tautomerisation to yield imidazolone (**2**). In the next step further oxidation of (**2**) must occur, accompanied by a skeletal rearrangement to produce hydantoin (**3**); reductive cleavage of (**3**) will then give rise to the ring-opened amide (**4**).



Scheme 2: Proposed Biotransformation Pathway for Imidazole Residue

The rearrangement which occurs during the formation of the hydantoin metabolite (**3**) is to our knowledge a unique biotransformation. Two possible rearrangement pathways exist (Scheme 3): (i) oxidation with migration of the methyl group (path a) or (ii) oxidation followed by shift of the methylene carbon attached to the tricyclic lactam appendage (path b).

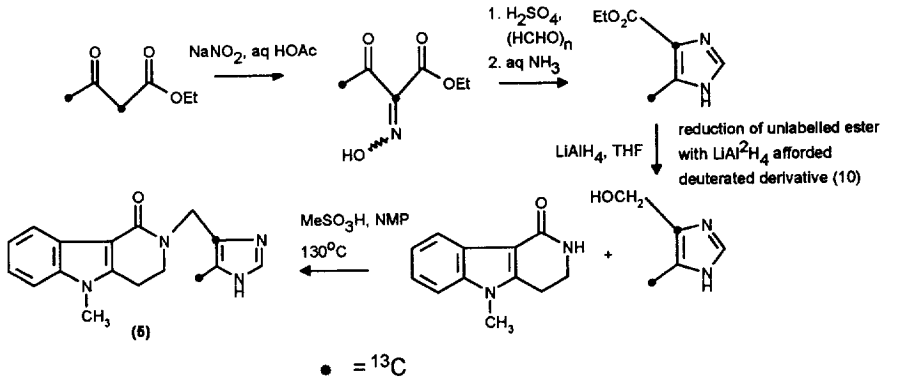


Scheme 3: Rearrangement pathways to hydantoin (**3**)

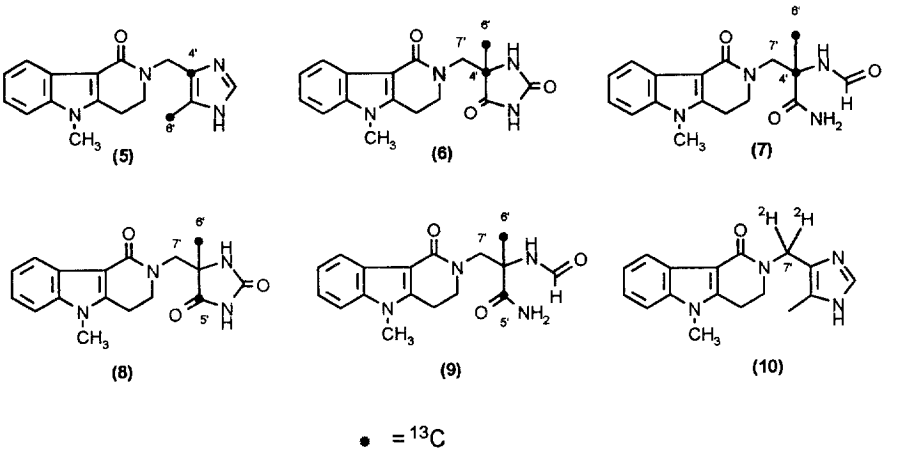
In order to differentiate between these two pathways [4'-^{13}C, 6'-^{13}C]Alosetron (**5**) (Scheme 5) (15 atom % ^{13}C at each position) was prepared[4] (Scheme 4), and incubated with cultures of *Streptomyces griseus*. Labelled versions of hydantoin (**3**) and amide (**4**) were isolated and analysed by ^{13}C NMR and mass spectroscopy. Operation of path a would give rise to labelled species (**6**) and (**7**) and hence would be indicated by the presence of J_1 ^{13}C-^{13}C homonuclear coupling associated with the ^{13}C NMR signals for C-6' and C-4', whilst path b would result in labelled species (**8**) and (**9**) possessing isolated ^{13}C labels at C-6' and C-5'. The ^{13}C NMR spectra obtained of hydantoin (**3**) and amide (**4**) displayed enhanced signals for C-6' and C-5', no J_1 ^{13}C-^{13}C doublets were observed. Furthermore, mass spectroscopic analysis of the isolated metabolites (**3**) and (**4**) showed the presence of only unlabelled compound and the corresponding $^{13}C_2$ species, no singly labelled isotopomers were detected. These findings

support a rearrangement pathway that leads from metabolite (**2**) *via* oxidation and a 1,2-shift of the tricyclic lactam moiety to the hydantoin (**3**) (path b, Scheme 3).

The mechanism of the rearrangement of imidazolone (**2**) was investigated further by incubating [7'-$^2H_2$]Alosetron[4] (**10**) with cultures of *Streptomyces griseus*. Retention of both deuterons would imply that a direct 1,2-shift was occurring, whereas loss of one or more of the deuterium atoms in (**10**) would indicate a more complex mechanistic process, such as the involvement of a cyclopropane intermediate.[5] MS analysis of the hydantoin and ring-opened amide metabolites following extraction and purification from the *S. griseus* culture broth showed that complete retention of both deuterium atoms had occurred in each case.

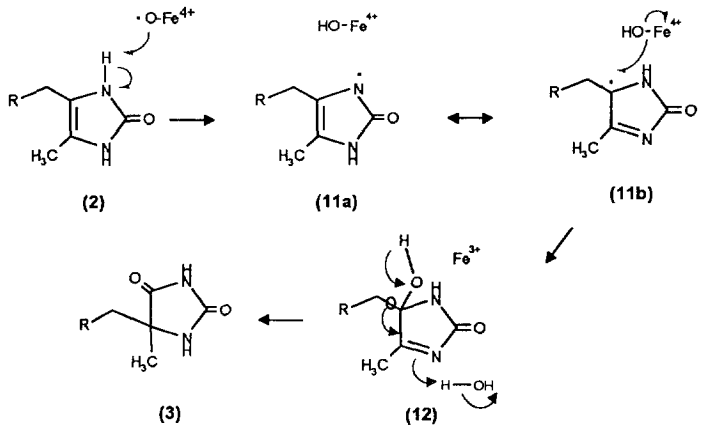


Scheme 4: Synthesis of Labelled Versions of Alosetron



Scheme 5: Possible Rearrangement Products

This result gave further support to a rearrangement pathway involving a direct 1,2-shift of the tricyclic lactam moiety. The migration of the lactam residue is almost certainly linked to the introduction of the second oxygen atom which again is most likely mediated by a cytochrome P-450 monooxygenase. Several mechanisms can be put forward for this hydroxylation-migration process. One hypothetical mechanism is shown in Scheme 6.[6] Here an iron (IV) oxo species generated in the catalytic site of the P-450 monooxygenase (or other iron oxidase) abstracts a hydrogen atom from the N-1 amide of (**2**) forming the radical intermediate (**11a**). Recombination of the resulting iron (IV) hydroxy species with the canonical form (**11b**) of the radical intermediate gives hydroxy-imine (**12**). Migration of the tricyclic lactam appendage in (**12**) driven by carbonyl formation and concomitant imine addition then produces the hydantoin metabolite (**3**). The rearrangement of hydroxy-imine (**12**) could be envisaged as occurring either within the catalytic pocket of the enzyme or could alternatively proceed non-enzymically in the cytosol following release from the monooxygenase.



Scheme 6: Hypothetical Mechanism for Formation of Hydantoin (**3**)

Acknowledgements : We gratefully acknowledge Mr N Taylor and Dr P. S. Marshall for MS analysis.

References:

1. Roberts, S. M.; Biotransformations: Preparative Organic Chemistry, 1989, Academic Press.
2. Smith, R. V.; and Rosazza, J. P.; *Arch. Biochem. Biophys.*, 1974, **161**, 551.
3. A similar metabolic profile with a related compound has been observed in mammalian and *in vitro* systems, Walsh, J. S.; unpublished results.
4. Wells, G. N.; Cable, K. M.; and Sutherland, D. R, Proceedings of Synthesis and Applications of Isotopically Labelled Compounds, Strasbourg, July 1994, Wiley, in press.
5. Whiting, D.A.; and Crombie, L.; *Tetrahedron Letters* 1992, **33**, 3663.
6. Akhtar, M.; and Wright, J. N.; *Natural Product Reports* 1991, 527.

(*Received in UK* 2 *November* 1994; *revised* 16 *November* 1994; *accepted* 18 *November* 1994)