The Mechanism of C-21 Dehydroxylation of Tetrahydrodeoxycorticosterone by *Eubacterium lentum*

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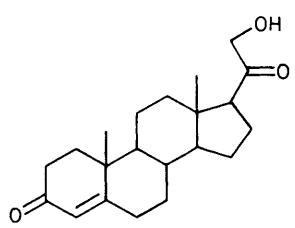
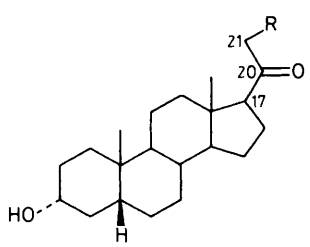
The C-21 hydroxy group of C-17 and C-21 deuterium labelled tetrahydrodeoxycorticosterone is removed by the anaerobic bacteria *Eubacterium lentum* with retention of deuterium label at C-17α and loss of one of the two deuterium atoms originally present at C-21.

The enzymic removal of hydroxy groups from corticosteroids by intestinal micro-organisms represents a major route for the *in vivo* metabolic deactivation of this class of hormone.[1] The removal of a hydroxy group from C-21 of 20-oxo corticosteroids, *e.g.* (**1**), is a common example of this class of reaction.[2] The related dehydroxylations of bile acids at C-7α[1] and of 16α-hydroxyprogesterone[3] by intestinal micro-organisms have also been reported; in these cases, the reaction has been shown to proceed by a dehydration–reduction mechanism.[1,3,4]

Apart from preliminary work on relevant enzymology,[5,6] mechanistic work on the C-21 dehydroxylation reaction has hitherto been confined to a report that C-21 dehydroxylation of ^{3}H labelled deoxycorticosterone [21-hydroxypregn-4-ene-3,20-dione, (**1**)] by human faecal flora proceeded with substantial but unreproducible loss of label from an undetermined site in the molecule.[7]



(**1**)



(**2**) **a**; R = OH

b; R = H

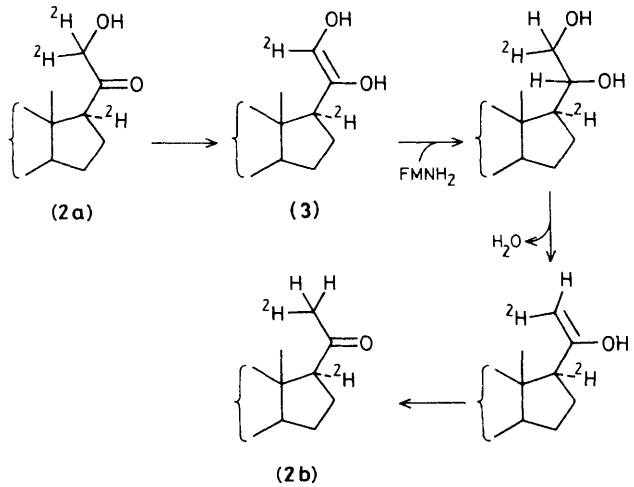
In view of the medical significance of the metabolic deactivation of corticosteroids,[8] we have studied the conversion of tetrahydrodeoxycorticosterone (**2a**) into the corresponding 21-deoxy steroid (**2b**) by the intestinal bacterium *Eubacterium lentum* ATCC 25559. This organism, when grown in mixed culture with *Escherichia coli* exactly as described,[9] performed the conversions of C-17 and -21 deuterium labelled substrate summarized in Table 1. Control experiments have shown that *E. coli* alone did not metabolise (**2a**) under these conditions.[5]

Labelled substrate, prepared from unlabelled material by base-catalysed exchange,[10] was analysed by ^{1}H and ^{2}H n.m.r. spectroscopy. Product (**2b**), of both synthetic and enzymic origins, was additionally analysed by electron-impact mass spectrometry. Comparison of the values for absolute deuterium content (from ^{1}H n.m.r. and mass spectral data), relative deuterium content (from ^{2}H n.m.r. data), and deuterium location (from ^{1}H and ^{2}H n.m.r. data) demonstrates that the conversion of (**2a**) into (**2b**) by *E. lentum* takes place without significant loss of label from C-17α, but with loss of one of the two hydrogens originally present at C-21. The

Table 1. Metabolism of labelled (**2a**) by *E. lentum*.

Substrate	Conditions	Product	^{2}H content, %		^{2}H n.m.r. : δ, p.p.m.	
			C-17	C-21	C-17	C-21
(**2a**)	Synthetic sample		44[a]	95	2.43	4.12
(**2b**)	Synthetic sample		>95	80	2.51	2.07
(**2a**)	Phosphate buffer, pH 6.3	(**2a**) recovered	44	95		
(**2a**)	*E. lentum*, 4 days	(**2a**) recovered	45	>95		
(**2b**)	*E. lentum*, 7 days	(**2b**)	>95	80		
(**2a**)	*E. lentum*, 4 days	(**2b**)	38	52		
(**2a**)	*E. lentum*, 7 days	(**2b**)	41	47		

[a] Values are accurate to ±2%



Scheme 1. Proposed route for C-21 dehydroxylation by *E. lentum*.

relevant control experiments which confirm that the integrity of label in both (**2a**) and (**2b**) is maintained during the incubation are also outlined in Table 1.

A mixed labelling incubation was carried out in which substrate consisted of unlabelled and ^{2}H labelled (**2a**) in equal quantities. Analysis of the product deuterium content, as described above, with the assumption of the loss of one deuterium atom from C-21, gave a value for the apparent kinetic isotope effect, k_H/k_D, at C-21 for C-21 dehydroxylation of (**2a**) of 2.6 ± 0.1.[11]

The data presented, in conjunction with the known requirements of C-21 dehydroxylation for a C-20 oxo substrate[9] and a reduced flavin cofactor,[5,6] and the reported inhibition of the enzyme by metal chelating agents,[12] suggest the route for this reaction proposed in Scheme 1. Binding of the substrate as the enediol (**3**) (a process which would be subject to a primary kinetic isotope effect of appropriate magnitude)[13] probably occurs to an as yet unidentified metal ion of the enzyme. Subsequent reduction, dehydration, and enol–keto tautomerism can give the product (**2b**).

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References

1 I. A. Macdonald, V. D. Bokkenheuser, J. Winter, A. M. McLernon, and E. H. Mosbach, *J. Lipid Res.*, 1983, **24**, 675.
2 J. Winter, V. D. Bokkenheuser, and L. Ponticorvo, *J. Biol. Chem.*, 1979, **254**, 2626.
3 T. L. Glass, J. Winter, V. D. Bokkenheuser, and P. B. Hylemon, *J. Lipid Res.*, 1982, **23**, 352.
4 B. Samuelsson, *J. Biol. Chem.*, 1960, **235**, 361.
5 V. D. Bokkenheuser, J. Winter, P. Dehazya, and W. G. Kelly, *Appl. Environ. Microbiol.*, 1977, **34**, 511.
6 S. D. Feighner, V. D. Bokkenheuser, J. Winter, and P. B. Hylemon. *Biochim. Biophys. Acta*, 1979, **574**, 174.
7 W. G. Kelly, O. de Leon, J. Winter, and V. D. Bokkenheuser, *J. Steroid Biochem.*, 1977, **8**, 73.
8 V. D. Bokkenheuser, J. Winter, J. W. Honour, and C. H. L. Shackleton, *J. Steroid Biochem.*, 1979, **11**, 1145.
9 J. Winter and V. D. Bokkenheuser, *J. Steroid Biochem.*, 1978, **9**, 379.
10 D. M. Johnson, *J. Org. Chem.*, 1982, **47**, 198.
11 H. L. Holland and G. J. Taylor, *Can. J. Chem.*, 1981, **59**, 2809.
12 S. D. Feighner and P. B. Hylemon, *J. Lipid Res.*, 1980, **21**, 585.
13 H. L. Holland and B. J. Auret, *Can. J. Chem.*, 1975, **53**, 845.