Stereochemistry of the Enzymic 3-Hydroxylation of 1,3-Dihydro-2*H*-1,4-benzodiazepin-2-ones

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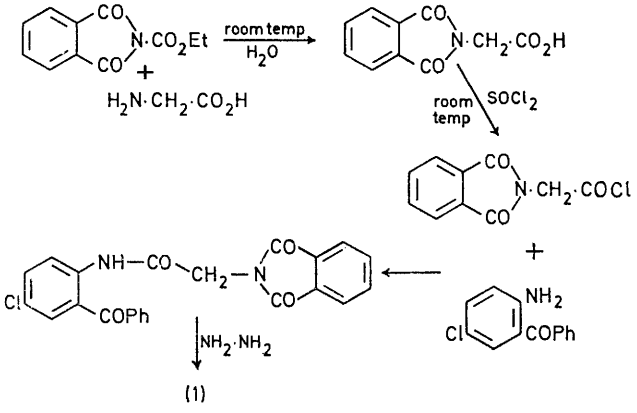
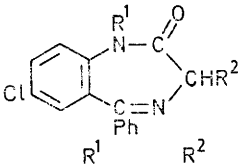
Summary The enzymic hydroxylation at C-3 of demethyldiazepam (**1**) and diazepam (**2**) proceeds through stereospecific removal of the *pro-S* hydrogen atom.

MONO-OXYGENASES[1] of the hepatic microsome fraction have an unusually broad substrate specificity;[2,3] in particular they are known to hydroxylate 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**2**) (diazepam; DZ) and its demethyl analogue (**1**) (DMDZ) at the *pro*-chiral centre C-3,[4] yielding oxazepam (**3**) (OX) and *N*-methyloxazepam (**4**) (MOX), respectively. We now present evidence of the stereochemical course of this reaction.

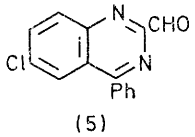


SCHEME

The synthesis of (**1**) labelled at C-3 from labelled glycine by modification of known methods[5] is outlined in the Scheme. Methylation of DMDZ (**1**) with dimethyl sulphate in ethanolic sodium hydroxide at room temperature gave DZ (**2**) (50%); mass spectral determinations and radioactivity measurements showed that more than 90% of the label was retained during the synthesis of (**1**) and (**2**). (2*R*)-[2-$^2H_1$]- and (2*R*)-[2-$^3H_1$]-glycine were biosynthesised from glycine and deuterium or tritium oxide in the presence of L-alanine aminotransferase;[6] (2*S*)-[2-$^3H_1$]glycine was obtained by the same enzymic exchange with [2-$^3H_2$]glycine and H_2O.



	R^1	R^2
(1)	H	H
(2)	Me	H
(3)	H	OH
(4)	Me	OH
(6)	H	Me
(7)	H	Pr^i
(8)	H	$[CH_2]_2 \cdot CO_2Me$
(9)	H	$O \cdot CO \cdot [CH_2]_2 \cdot CO_2H$
(10)	Me	$O \cdot CO \cdot [CH_2]_2 \cdot CO_2Me$
(11)	H	OMe
(12)	H	$O \cdot C_6H_9O_6$



(5)

In order to ascertain that racemization does not occur during the synthesis of the benzodiazepine ring, c.d. data for (3*R*)-[3-$^2H_1$]DMDZ were obtained ($\Delta\epsilon_{333}$ + 0·1; $\Delta\epsilon_{280}$ − 0·61; $\Delta\epsilon_{258}$ − 1·56). The shape of the curve is similar to that of the chiral benzodiazepines with the 3*R*-configuration (see Table 1); although this result does not prove optical purity, it provides strong support for the presence of a large amount of the 3*R*-enantiomer in the synthetic DMDZ.†

The biological hydroxylation of labelled (**1**) and (**2**) was performed with a hepatic microsome preparation of mice;‡

TABLE 1

Physical constants of optically active 3-substituted benzodiazepines

	M.p. (°C)	$[\alpha]_D^{20}$ (°)[a]	c.d. $\Delta\epsilon$ at 315, 280, 258, 237, and 222 nm	C-3 config.
(+)-(**6**)[b]	199—200	+314	−7·2; +8·7; +31·4; −5·1; +44·4	*S*
(+)-(**7**)	198—199	+244	−7·2; +8·5; +28·4; −5·8; +42·1	*S*
(+)-(**8**)	148—149	+204	−7·5; +7·9; +30·2; −7·4; +42·6	*S*
(−)-(**7**)	198—199	−244	+7·1; −8·4; −28·4; +5·9; −42·0	*R*
(+)-(**9**)	156	+170	−7·3; + 14·5; +21·8; −10·8; +48·9	*S*
(+)-(**10**)	(Glass)	+159		*S*
(+)-(**11**)	226—227	+293	−8·5; + 16·1; +31·5	*S*
(**12**)			−2·1; +4·3; +3·9; −2·7; +12·9	*S*

[a] Optical rotations were measured in dioxan (*c* 1). [b] Ger. Pat. 2,212,526 (*Chem. Abs.*, 1973, **78**, 16,248).

† In any case, glycine obtained by enzymic exchange has an optical purity not higher than 90%.

‡ To increase the oxidative conversion of (**1**) into (**3**), hepatic microsome preparations from phenobarbital-induced mice were used for experiments on substrates which were not stereospecifically labelled; however when stereospecifically labelled compounds were employed the mice used were not induced, to avoid a lower stereospecificity in the hydroxylation (see ref. 2*a*, p. 243).

TABLE 2

Incorporation of labelled benzodiazepines into oxazepam (OX) *and methyloxazepam* (MOX)

		OX	MOX	% Retention
[3-^{14}C,3-$^{3}H_2$]DMDZ	9·3 (^{3}H:^{14}C)	5·02 (^{3}H:^{14}C)		54
[3-^{14}C,3-$^{3}H_2$]DZ	9·7		5·3 (^{3}H:^{14}C)	54
(3*R*)-[3-^{14}C,3-$^{3}H_1$]DMDZ	6·72	6·3		94
(3*S*)-[3-^{14}C,3-$^{3}H_1$]DMDZ	13·0	3·9		30
(3*R*)-[3-^{14}C,3-$^{3}H_1$]DZ	10·5		8·4	80
(3*S*)-[3-^{14}C,3-$^{3}H_1$]DZ	13·0		4·1	31
[3-$^{2}H_2$]DMDZ	74% $^{2}H_2$	84% $^{2}H_1$		48
	24% $^{2}H_1$	16% $^{2}H_0$		
(3*R*)-[3-$^{2}H_1$]DMDZ	76% $^{2}H_1$	71% $^{2}H_1$		90
	3% $^{2}H_2$	20% $^{2}H_0$		
	21% $^{2}H_0$			

the results are summarized in Table 2. The rate of conversion of (**1**) into (**3**) is significantly lowered by the presence of deuterium at C-3.[7] Thermal conversion[8] of labelled (**3**) into the aldehyde (**5**) left the label unchanged whereas oxidation of (**5**) to the corresponding acid resulted in complete loss of the label.

In different runs, with various ^{3}H:^{14}C ratios in the DMDZ, OX (**3**) was obtained with a tritium retention of 80—97% from the *R*-isomer and a loss of 60—70% from the *S*-isomer. The (3*S*)-isomer always gave the less satisfactory results; this is not surprising since enzymic exchange between [2-$^{3}H_2$]glycine and water never proceeds in quantitative yield.

Reaction of 2-amino-5-chlorobenzophenone with the chloride hydrochlorides of chiral amino-acids affords 3-alkyl-5-phenyl-2*H*-1,4-benzodiazepin-2-ones with known configuration at C-3.[9] We have already reported[10] that the racemate of oxazepam hemisuccinate (**9**) can be resolved into the two enantiomers; comparison of their c.d. data with those of optically active alkyl derivatives clearly shows that the dextrorotatory enantiomer has the 3*S*-configuration.¶ Treatment of (3*S*)-(**9**) with an anhydrous alcohol and 2 equiv. of hydrogen chloride gave 3-alkoxybenzodiazepin-2-ones [*e.g.* (**11**)] with complete retention of configuration. Hydrolysis of the same compound (3*S*)-(**9**) in anhydrous ethanol with at least 3 equiv. of sodium ethoxide at room temperature caused precipitation of the optically active sodium salt of (**3**);§ if the reaction was run in water or if the isolated salt was treated with water, rapid hydrolysis to racemic (**3**) occurred, probably as a consequence of equilibration *via* an open aldehydic form. Analogous results were obtained with compound (**10**). On the other hand, from the urine of oxazepam-treated rabbits, oxazepam β-glucuronide (**12**) can be isolated,[12] the c.d. data of which suggest an *S*-configuration at C-3 of the aglycone.

The biological hydroxylation of (**1**) and (**2**) occurs with a high degree of stereospecificity. Owing to the configurational lability of OX and MOX, the results described do not allow us to specify whether the reaction proceeds with retention or inversion of configuration. However, since the reaction involves implicit consumption of molecular oxygen[13] and is considerably slowed by the presence of deuterium at C-3,[7] comparison with other hydroxylations catalysed by the same enzymes strongly supports the hypothesis of retention of configuration.[14] Furthermore we have never found evidence of the intermediacy of an *N*-oxide and we have proved that the enzymic preparation does not convert the *N*-oxide of DMDZ into OX, suggesting the probable occurrence of direct hydroxylation at C-3.[15]

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¶ While this work was in progress, a paper[11] appeared on the separation of the diastereoisomeric esters of oxazepam with (−)-camphanic acid. From our results it is evident that "das vorderen diastereoisomere" is the 3*R*-isomer and not the 3*S*- as suggested in that paper.

§ The secondary amide is acidic enough to form alkaline salts; (3*S*)-(**3**) sodium salt shows $[\alpha]_D^{20}$ + 534° (*c* 0·5 in $Me_2N{\cdot}CHO$).

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