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The Different Effects of an Epoxy and a Methylene Group on Enzymic Reductions of a Vicinal Oxo Group

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In the case of two 3-hydroxysteroid dehydrogenases, 1α,2α-epoxy-17β-hydroxy-5α-androstan-3-one was shown to be a substrate, whereas 17β-hydroxy-1α,2α-methylene-5α-androstan-3-one scarcely served as a substrate and was an inhibitor. This is unlikely to be a simple steric effect, but its electronic basis is not clear.

The enzymes cortisone reductase (EC 1.1.1.53) and 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50) catalyse the transfer of hydrogen from NADH to various 3-oxosteroids, giving corresponding 3-ols (*1*, *2*). This communication describes the different behaviour of the potential substrates 1α,2α-epoxy-17β-hydroxy-5α-androstan-3-one and 17β-hydroxy-1α,2α-methylene-5α-androstan-3-one.

Reactions were carried out at 25°C and pH 7.0, using 150 μM NADH in 0.1 M sodium phosphate buffer and dimethyl sulfoxide (1.5% v/v in kinetic experiments; 10% in preparative experiments). The progress of the reaction (oxidation of NADH) was followed spectrophotometrically at 340 nm as described elsewhere (*3*), the amount of enzyme used being adjusted so that 20% reaction was completed in 2–4 min, except in the case of the 1α,2α-methylene compound. This compound gave slow reaction rates and less than 10% total reaction even with very large amounts of enzyme (e.g., 93 $\mu g \cdot ml^{-1}$ of cortisone reductase, or a similarly large amount of 3α-hydroxysteroid dehydrogenase). Initial rates of reaction were determined from the slopes of the progress curves, which were approximately linear for the first 20% of reaction, apart from the short period immediately after addition of substrate and mixing. The cortisone reductase preparation was crystalline, and gave one band on polyacrylamide disc electrophoresis. The 3α-hydroxysteroid dehydrogenase was a freeze-dried preparation, purified by precipitation with streptomycin sulfate, ammonium sulfate, and chromatography on Sephadex G-100 (*6*). No "contaminating activity" appeared to be present in either case, though cortisone reductase has 20β- activity as well as 3-activity (*1*, *3*, *7*).

The 17β-hydroxy-1α,2α-methylene-5α-androstan-3-one was scarcely a substrate for either enzyme (Table 1). It was, however, a moderately effective inhibitor of reduction of 17β-hydroxy-1α-methyl-5α-androstan-3-one. The type of inhibition was not investigated, but if simple competitive inhibition was assumed, K_i values of about 26 μM (3α-hydroxysteroid dehydrogenase) and about 70 μM (cortisone reductase) were obtained.

Failure of the 1α,2α-methylene compound to serve as a good substrate could not easily be attributed to simple obstruction by the 1α,2α-methylene group because both

TABLE 1

RATE AND EXTENT OF REDUCTION OF SUBSTITUTED 17β-HYDROXY-5α-ANDROSTAN-3-ONES CATALYSED BY TWO DIFFERENT ENZYME PREPARATIONS[a]

Substituent	3α-Hydroxysteroid dehydrogenase		Cortisone reductase	
	Relative rate (20 μM)	Percent reaction (20 μM)	Relative rate (50 μM)	Percent reaction (20 μM)
None	1.00[b]	95	1.00[b]	86
1α,2α-Epoxy	1.53	92	0.39	84
1α,2α-Methylene	0.003	<10	0.002	<10
1α-Methyl	0.20	80	0.80	82
2α-Methyl	0.23	97	0.72	84

[a] Conditions: 25°C, pH 7.0, 150 μM NADH.
[b] Rate for unsubstituted compound put equal to 1.00 for each enzyme.

the 1α-methyl and 2α-methyl compounds were substrates for both enzymes (Table 1). Moreover, although the conformation of the 1α,2α-methylene compound [in which the A-ring is probably in a half-boat form (*4*)] probably differs from that of 17β-hydroxy-5α-androstan-3-one itself and the 1α- and 2α-methyl derivatives, it is not likely to differ much from the 1α,2α-epoxy compound, which was quite a good substrate for both enzymes (Tables 1 and 2). Hydrolysis of the epoxide before its reduction was not likely to be the explanation of its functioning as a substrate because 1α,17β-dihydroxy-5α-androstan-3-one was a very poor substrate (*5*), and the 1,2-diol would be expected to be even worse. It was conceivable, however, that hydride transfer occurred to C-2 instead of C-3, reducing the 1α,2α-epoxide instead of the 3-oxo group. That the reaction of the epoxy compound did, in fact, follow the expected course (reduction of the 3-oxo group) was indicated by the following findings.

The reduction product obtained from the epoxy compound using cortisone reductase was examined by thin-layer chromatography (silica gel layers; solvent hexane–ethyl acetate 1:2 v/v; stain phosphomolybdic acid or dinitrophenyl hydrazine). The starting material (both a reference specimen and material extracted from a control incubation mixture that contained no enzyme) had R_F 0.56, and was stained by phosphomolybdic acid and by dinitrophenylhydrazine. The reduction product had R_F 0.25, and was stained by phosphomolybdic acid but scarcely by dinitrophenylhydrazine, indicating the absence of an oxo group. The reduction product purified by preparative thin-layer chromatography showed no absorption in the carbonyl region of the infrared, whereas the starting material showed an absorption peak close to 1720 cm^{-1} (KBr). The reduction product was a substrate for cortisone reductase under oxidising conditions (2 *mM* NAD^+ at pH 7.0), and the product of this reaction had the same R_F value (0.47) as 1α,2α-epoxy-17β-hydroxy-5α-androstan-3-one, and quite distinct from the reduced material (R_F 0.14), when examined by thin-layer chromatography (layer, silica gel; solvent, hexane–ethyl acetate 1:1 v/v). The product obtained by reduction of 1α,2α-epoxy-17β-hydroxy-5α-androstan-3-one using 3α-hydroxysteroid dehydrogenase had the same R_F value as the product obtained using cortisone reductase. Thus, the reduction

TABLE 2

MICHAELIS CONSTANT AND MAXIMUM VELOCITY FOR ENZYMIC REDUCTION OF 17β-HYDROXY-5α-ANDROSTAN-3-ONE AND THE 1α,2α-EPOXIDE[a]

Enzyme and substrate	Correlation coefficient $1/v$ upon 1/[S]	Nonweighted K_M (μM)	Weighted[b] $K_M \pm 2$ SE	Nonweighted V	Weighted[b] $V \pm 2$ SE	Nonweighted K_M/V	Weighted[b] K_M/V
3α-Hydroxysteroid dehydrogenase (substrates 2–20 μM)							
Parent compound	0.94	3.64	3.06 ± 0.74	1.18[c]	1.15 ± 0.1[c]	3.1[c]	2.7[c]
1α,2α-Epoxide	0.99	15.1	14.2 ± 3.8	2.74[c]	2.8 ± 0.4[c]	5.5[c]	5.1[c]
Cortisone reductase (substrates 30–100 μM)							
Parent compound	0.94	162	112 ± 38	18.3[d]	13.9 ± 3.4[d]	8.9[d]	8.0[d]
1α,2α-Epoxide	0.99	364	319 ± 84	14.1[d]	12.6 ± 2.8[d]	25.8[d]	25.3[d]

[a] Conditions: 25°C, pH 7.0, 150 μM NADH.

[b] Procedure of Wilkinson, G. N., *Biochem. J.* **80**, 324 (1961).

[c] Enzyme concentration not known. V expressed in arbitrary units taking mean observed rate for parent compound at 20 μM as 1.00.

[d] V in μmoles min^{-1} mg^{-1}.

of 1α,2α-epoxy-17β-hydroxy-5α-androstan-3-one by both these enzymes appeared to leave the epoxy group intact, and to reduce the 3-oxo group.

Both enzymes utilised 17β-hydroxy-5α-androstan-3-one more efficiently than the epoxide (Table 2). (A lower value for K_M/V represents more efficient substrate utilisation.) Also, the K_M value for the epoxide was higher than for the parent compound in the case of both enzymes. However, in the case of cortisone reductase the V value was about the same for the epoxide and the parent compound, whereas in the case of the 3α-hydroxysteroid dehydrogenase the V value of the epoxide was roughly three times that of the parent compound. The influence of the epoxy group on the functional behaviour, therefore, differed somewhat between the enzymes, no doubt because the active sites differ. It is, therefore, the more striking that in both cases the 1α,2α-epoxy but not the 1α,2α-methylene compound served as quite a good substrate. Some conjugation of electrons from the 1α,2α-methylene group with the 3-oxo group might make C-3 less susceptible to nucleophilic attack; the 1-ene, for example, was a very poor substrate (*5*). The 1α,2α-epoxy group might increase slightly the electrophilic character of C-3. In this context it is interesting to compare the C=O stretching frequencies. The 17β-hydroxy-5α-androstan-3-one, the 1α,2α-epoxy derivative, and the 2α-methyl derivative, which were all substrates, showed absorption peaks (KBr) in the region 1715–1725 cm^{-1}. The 1α,2α-methylene derivative and 1-ene, which were scarcely substrates, absorbed close to 1670 cm^{-1}. This association should be regarded at present as indicating merely a starting point for further studies. The 1α,2α-methylene compound, for example, did not show any strong absorption peak in the u.v. above 210 nm (compare the 1-ene, λ_{max} 230 nm, ϵ_{Max} 10,000), but resembled the 1α,2α-epoxy compound in showing a weaker absorption in the 207–209 nm region, and differed in this respect from the unsubstituted compound (which showed less than 0.5% of the absorption at these wavelengths). It is suggested that the major difference in substrate behaviour of the 1α,2α-epoxy and 1α,2α-methylene compounds depends upon some specific effect the nature of which is not yet apparent.

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