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Studies on the Functional Specificity of Cortisone Reductase

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Crystalline preparations of cortisone reductase (EC 1.1.1.53) have certain 3- and 20-hydroxy steroid-NAD⁺ oxidoreductase activities that may possibly depend on one active centre with complex steroid-binding properties (Gibb & Jeffery, 1971). Studies are now described in which a series of test substrates was used to elucidate certain aspects of the specificity of these reactions. It was shown that there is no absolute requirement for the hydrogen added to or removed from C-3 to be α , β , axial or equatorial, or for the chirality at C-3 to be *R* or *S*. In the 5 α -androstan-3-one compounds studied, the hydrogen added to or removed from C-3 was β and equatorial, but C-3 had *R* or *S* chirality. The 5 β -androstan-3-one compounds studied were very poor substrates, and among these compounds preference was found for utilization of 3 β -hydrogen, which was axial and C-3 *R*, rather than 3 α -hydrogen, which was equatorial and C-3 *S*, though the latter was observed. These poor substrates were utilized so inefficiently that it was not possible to establish kinetically the relationship between the slight 3 β -hydroxy steroid dehydrogenase activity and either the 3 α - or the 20-hydroxy steroid dehydrogenase activity.

The non-steroid adamantanone was a substrate, undergoing a reaction clearly analogous to the 3-hydroxy steroid dehydrogenase activity towards

steroids, but achiral, and equally related to 3 α , 3 β , 3-axial and 3-equatorial activity. This activity was not kinetically independent of the 20-hydroxy steroid dehydrogenase activity (Gibb & Jeffery, 1972).

The hydrogen added to or removed from C-20 was *R* or *S*, but was consistently 20 α in the compounds so far investigated.

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Studies on the Active Centre for the 3-Hydroxy Steroid-Nicotinamide-Adenine Dinucleotide Oxidoreductase Activity of Cortisone Reductase

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Kinetic studies were carried out with 17 β -hydroxy-5 α -androstan-3-one (I), a series of related steroids differing in structure only in ring A, and the analogous non-steroid adamantanone. The 1 α -methyl and 2 α -methyl derivatives of compound (I) had apparent *K_m* and apparent *V* values similar to those of compound (I). The 1 β -methyl and 4 α -methyl derivatives of compound (I) were not substrates, but were moderately effective inhibitors. The 1 α -hydroxy derivative of compound (I) was a very poor substrate with high apparent *K_m* and low apparent *V*. These findings would allow the view that the binding site is hydrophobic in the region around the A-ring; that it is possibly of somewhat more restricted dimensions (or lower flexibility) in the direction across the A-ring (1 β –4 α), and somewhat bigger than a steroid (or highly flexible) in the region out of the plane of the A-ring (i.e. from above the β -side to below the α -side). This would lead to an estimate of roughly 0.60×0.52nm for the minimum dimensions of the site where the A-ring binds. Such a view could accommodate in a general way the further findings that 17 β -hydroxy-5 β -androstan-3-one and the non-steroidal but analogous adamantanone were poor substrates; and even that 17 β -hydroxy-1 α ,2 α -methylene-5 α -androstan-3-one and the corresponding 1 β ,2 β -methylene compound were moderately effective inhibitors, though it is less clear why these two compounds were not substrates. Certain other