Hydroxysteroid dehydrogenases catalyze the interconversion of hydroxyl and carboxyl groups of steroids. Those from *Pseudomonas testosteroni* are of two types:

1. 3α-Hydroxysteroid Dehydrogenase (1.1.1.50), (α enzyme):

2. 3β- and 17β-Hydroxysteroid Dehydrogenase:

The enzymes have useful application in the assay of steroids (Hurlock and Talalay 1958; Stempfel and Sidbury 1964; Moore 1972). They have been used for the determination of bile acids in blood (Iwata and Yamasaki 1964; Palmer 1969; Leslie 1969; Murphy et al. 1970; Schwarzet et al. 1974).

It should be noted that Aladdin supplies two preparations: one from the regular *Pseudomonas testosteroni* (ATCC 11966) culture which produces both the α and β enzymes, and a second from a mutant strain which produces almost only the α enzyme. By using both, the β-hydroxysteroid can be determined by difference.

Teller and Bongiavanni (1963) reported only slight activity of the mutant strain on epiaandrosterone indicating but a trace of the β enzyme. Roe and Kaplan (1969) have investigated the specificities of the mutant and wild type strains, and this reference should be consulted for specific details.

**Characteristics of Hydroxysteroid Dehydrogenase from Pseudomonas testosteroni:**

**3α-Hydroxysteroid Dehydrogenase:**

- **Molecular weight:** 47,000 (Squire et al. 1964; Skålhegg 1974).
- **Composition:** Amino acid composition has been reported by Squire et al. (1964).
- **Isoelectric point:** pH 6.1 (Squire et al. 1964).
- **Specificity:** α enzyme oxidizes only 3α-hydroxysteroids of the C19, C21, C24 series (Talalay 1963).
Inhibitors: Heavy metals and sulfhydryl-binding reducing agents (Talalay 1963).

Stability: The Aladdin preparation of a enzyme is stable for 6-12 months when stored at -20°C.

3β(or 17β)-Hydroxysteroid Dehydrogenase:

Molecular weight: 100,000 (Squire et al. 1964).

Composition: Amino acid composition has been reported by Squire et al. (1964).

Isoelectric point: pH 6.5 (Squire et al. 1964).

Specificity: Catalyzes the oxidation of 3β-hydroxysteroids of the C19 and C21 series, 17β-hydroxysteroids of the C18, C19, and C21 series, as well as certain 16β-hydroxysteroids (Talalay 1963).

Inhibitors: Heavy metals and reducing agents. The oxidation of testosterone is inhibited by 3,17α-estradiol and other 1,3,5-estratriene derivatives (Talalay 1963).

Stability: The Aladdin preparation of mixed α and β enzyme is stable for 6-12 months when stored at -20°C.