*JKAU: Sci., **Vol. 27. No. 2** pp: 1-5 (1436 A.H./2015 A.D.)*
DOI:10.4197/Sci.27-2.1

Synthesis and biotransformation of some steroidal imines by the fungus *Mucor plumbeus*

Khalid Omar Al-footy

Chemistry Department, Faculty of Science, King Abdul Aziz University, Jeddah, Saudi Arabia
E-mail : kalfooti@kau.edu.sa

Abstract. Preparation of four different imino steroids derived from androsterone was described. The biotransformation of the imino steroids by fungus mucor plumbeus was preformed. The new imino groups affected severely the biotransformation results.
Keyword Index: mucor plumbeus, biotransformation, steroidal imines

I. Introduction

Humankind has used biotransformation procsses for thousands of years. For example, the bioconversion of ethanol to acetic acid (vinegar) by Acetobacter was most likely developed concomitantly with ethanol production from fermentable sugars by our ancestors in several places in the world [1]. Enzymatic reactions (Biotransformation) are often unique in their potential to produce fine chemicals in high optical purity. The biotransformation of the dioxosteroids (hydroxyl or carbonyl) gave monohydroxylated products [2]. Presence of the two binding oxygenated groups (hydroxy or carbonyl) in the steroid increases the reactivity of biotransformation experiments[2]. The increased substrate polarity will increase water solubility, which will aid permeation into cell. The Jones model of hydroxylation[3] (Fig.1) indicates that the two hydrophilic oxygen containing groups in the steroid will attach to the enzyme binding site, in an orientation that will ensure the maximum possible hydrophobic interaction between the substrate and the enzyme.

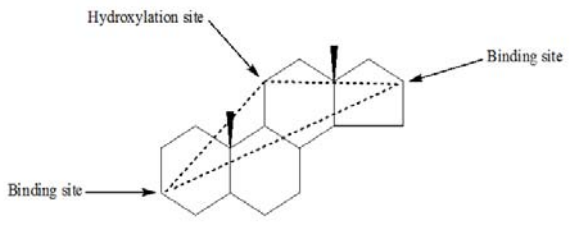


Fig.1. The Jones model of enzyme-substrate interaction.

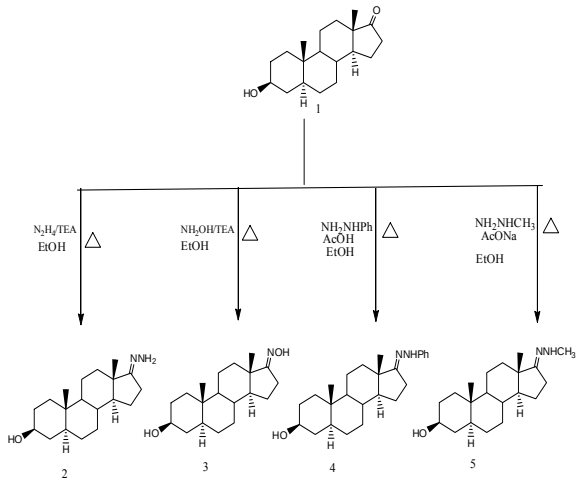
Thus, in the case of dioxygenated substrate, the third catalytic center will hydroxylate at the nearest carbon atom. Hydroxylation usually happens at least four or five atoms distant. This paper deals with preparation of imino steroids to replace one of the oxygen-containing group at C-

17 in order to study the effect of new imino groups on the biotransformation experiments.

II. Results and Discussions

Four steroids containing imino groups were prepared and biotransformed in order to examine the effect of the new imino groups on the biotransformation results.

3β-Hydroxy-5α-androstan-17-one (1) was refluxed with hydrazine hydrate and triethylamine as a base in ethanol to yield 3β-hydroxy-5α-androstan-17-hydrazone (2) in 91% yield (scheme 1) which was identified by comparing its spectroscopic data with the literature values [4]. Compound (1) was treated with phenyl hydrazine and acetic acid in ethanol to yield 3β-hydroxy-5α-androstan-17-phenyl hydrazone (3) in 92 % yield. (scheme 1) [4]. 3β-Hydroxy-5α-androstan-17-one(1) was treated with hydroxylamine and triethylamine in ethanol to give 3β-hydroxy-5α-androstan-17-oxime (4) in 76% yield. (Scheme 1) [5]. 3β-hydroxy-5α-androstan-17-methyl hydrazone (5) was achieved in 84.22 % yield by the reaction of compound (1) with one equivalent only of methyl hydrazine and Sodium acetate as a mild base in ethanol (scheme 1) which was identified by comparing its spectroscopic data with the literature values [5].



Scheme 1

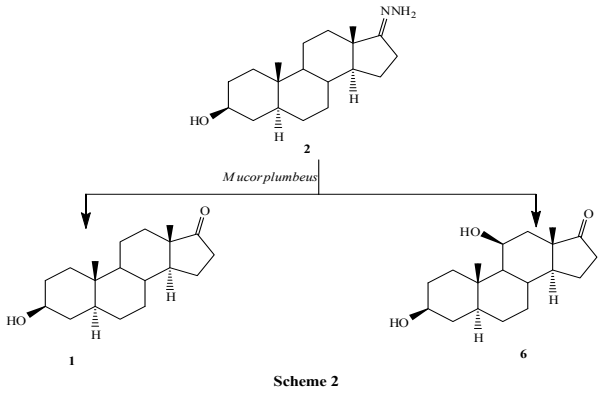
Biotransformation Results

The metabolism of the imino steroids by *Mucor plumbeus*

1-Incubation of 3β-hydroxy-5α-androstan-17-hydrazone (2)

The incubation of 3β-hydroxy-5α-androstan-17-hydrazone (2) with *Mucor plumbeus* for 7 days gave two biotransformation products (Scheme 2). The first metabolite was identified as the hydrolyzed product 3β-hydroxy-5α- androstan-17-one (1)25% which was identified by comparing its spectroscopic data with that of authentic sample.

The second metabolite was identified as 3β, 11β-dihydroxy-5α-androstan-17-one (6)15%. The IR spectrum of the product (6) showed a band at 1727 cm^{-1} that was consistent with the presence of carbonyl group at C−17. The ^1H NMR spectrum of (6) did not reveal the signal at δ_H 4.65 ppm (2H, brs, NH_2) of (2). The ^1H NMR spectrum of the product (12) also showed a signal at δ_H 4.08 ppm (1H, brs, 11α–H) which was assigned to the 11α-proton. The ^{13}C NMR spectrum of the product (6) contained a new signal at δ_C 68.5 for C−OH in place of the signal at δ_C 23.41 ppm (C-11) in the starting material. The site and the stereochemistry of hydroxyl group were confirmed by comparing its ^1H NMR spectra with the ^1H NMR of an authentic sample, which had been prepared by incubation of this compound with another fungus [6].

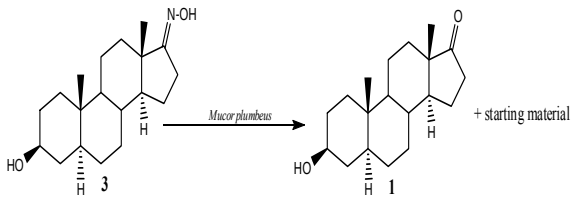


Scheme 2

2-Incubation of 3β-hydroxy-5α-androstan-17-oxime (3).

3β-Hydroxy-5α-androstan-17- oxime (3) was incubated with *Mucor plumbeus* for 7 days to afford 10% starting material and one hydrolyzed

product after preparative tlc (Scheme 4). The only metabolite was identified as 3β-hydroxy-5α-androstan-17- one (1) 8%. Which was identified by comparison its spectroscopic data with that of authentic sample.

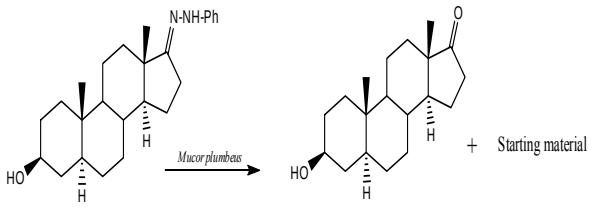


Scheme 3

3-Incubation of 3β-hydroxy-5α-androstan-17-phenyl hydrazone (4).

3β-Hydroxy-5α-androstan-17-phenyl hydrazone (3) was incubated with *Mucor plumbeus* for 7 days to afford 15% starting material and one hydrolyzed product after preparative tlc (Scheme 4).

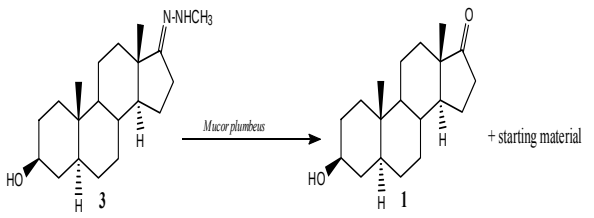
The only metabolite identified was 3β-hydroxy-5α-androstan-17- one (1) 20%; which was identified by comparing its spectroscopic data with that of authentic an sample.



4 Scheme 4 1

4-Incubation of 3β-hydroxy-5α-androstan-17-methyl hydrazone (5).

3β-Hydroxy-5α-androstan-17- methyl hydrazone (5) was incubated with *Mucor plumbeus* for 7 days to afford 10% starting material and one hydrolyzed product after preparative tlc (Scheme 5). The only metabolite identified was the hydrolyzed product 3β-hydroxy-5α-androstan-17- one (1)19%. This compound was identified by comparing its spectroscopic data with that of authentic sample.



Scheme 5

III. Conclusion

The 17-imino steroids gave very poor biotransformation results. They gave hydrolyzed products in the form of just one hydroxylation product. The absence of hydroxylation products suggested that the 17-imino steroids do not bind well with the enzyme. The imino groups were hydrolysed easily during the biotransformation experiments and more investigations were done to show that fungus was involved in these hydrolysis reactions. We repeated the biotransformation experiment on some of the steroidal imines in the medium without the fungus and no hydrolyzed products were obtained. These results prove that the fungus is directly involved in the hydrolysis process.

IV. Experimental

Melting points were determined with Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded for the compounds in a KBr disks on a Nicolet Magna 520 Fourier transform spectrophotometer. ^{1}H NMR spectra were reconded in deuteriochloroform on DPX-400 and a Bruker AC 400 MHz spectrometers using Me_4Si as the δ scale. ^{13}C NMR were recorded in deuteriochloroform at 75 MHz on Bruker DPX-400 spectrometer.

Mass spectra were recorded on a VG Autospec. Column chromatography was performed as described [8]. Merck 9385 silica (mesh 230-400) was used for column chromatography. Column chromatography and all reactions were followed by thin layer chromatography on Merck 5735 silica developed in solvent systems consisting of ethyl acetate in light petroleum. Light petroleum refers to the fraction, b.p.60-80 °C. The spots

were revealed by spraying with a mixture of sulfuric acid and methanol (1:1) on the plates, which were then left in an oven at 150 °C for 30 seconds to a few minutes. Extracts were dried over anhydrous sodium sulfate and solvents for both chromatography and recrystallisation were dried and purified as described in *Purification of Laboratory Chemicals*, Pergamon Press, 1980. All reactions were performed in air and at room temperature unless otherwise stated.

The following compounds were prepared as previously reported [7], [8], [9],[10]:

3β-hydroxy-5α-androstan-17-hydrazone (2) mp (180-182 °C) (Lit [7], 183-187 °C);

3β-hydroxy-5α-androstan-17-oxime (3) mp (176-179 °C) (Lit. [8], 175-177 °C).

3β-hydroxy-5α-androstan-17-phenylhydrazone (4) mp (149-152 °C) (Lit. [9], 148-150 °C).

3β-hydroxy-5α-androstan-17-methylhydrazone (5) mp (140-144°C) (Lit. [10], 141-143°C).

V. Biotransformation Experiment

General Fermentation Details

The fungus *Mucor plumbeus* (IMI 116688) was grown on shake culture in 250 ml conical flasks using 100ml medium comprising (per litre), Glucose (30g), magnesium sulfate (1g), potassium dihidrophosphate (1g), Ammonium tartrate (2g), calcium chloride (0.25g), ferrous ammonium sulfate (1)g, Yeast (1g), and sodium chloride (1g), Trace element solution 2 ml were diluted with distilled water to 1L, and neutralized to pH 7 by adding NaOH.

The trace element solution contained (per litre), ferrous sulfate (1g) zinc sulfate (1.6g), ammonium molybdate (1g) , copper sulfate (0.1g) cobalt nitrate (1g), and magnesium sulfate (0.1g). The fungus was grown for 2 days before the substrate (0.5g) in ethanol (30ml) was distributed over 50 flasks. The fermentation was then continued for further 7days. The broth was filterd, the mycelium was washed, and the water layer extracted with ethyl acetate. The extracts were washed with water and dried. The solvent was evaporated and the residue was chromatogramphed on silica and eluted with an increasing gradient of ethyl acetate in light petroleum ether.

3β-hydroxy-5α-androstan-17-hydrazone (2) (0.5g) gave two products as in Scheme 2.

Starting material and 3β, 11β-dihydroxy-5α-androstan-17-one (6) were recrystallized as white prisms

m.p. 194-195°C (Lit.[11], 190-192°C), FTIR 3414 cm^{-1} (OH), 1727 cm^{-1} (C=O); ^1H NMR($CDCl_3$, 400 MHz) δ 0.81(3H, s, 18-H), 0.83 (3H, s, 19-H), 3.56 (1H, tt, *J=11.3, 5.2 Hz, 3α-H*), 4.08 (1H, brs, 11α–H).

3β-hydroxy-5α-androstan-17-oxime (3) gave starting materials and one hydrolyzed product as in Scheme 3.

3β-hydroxy-5α-androstan-17-phenylhydrazone (4) gave starting materials and one hydrolyzed product as in Scheme 4.

3β-hydroxy-5α-androstan-17-methylhydrazone (5) gave starting materials and one hydrolyzed product as in Scheme 5.

VI. References

[1]**Alarcón J., guila S., Cornejo F., Alderete J.,** J. Mol.Catal. B: Enzym., 48 (2007)

[2]**D. H. Peterson and H. C. Murray,** *J. Am. Chem. Soc*,. 74, 1871, 1952.

[3]**K. Faber,** *Biotransformation in Organic Chemistry*, Spring-Verlag, 1992, p.178.

[4]**E. R. H. Jones,** *Pure. Appl. Chem*., 1973, 33, 39

[5]**Hanson, J. R.,** *An Introduction to Biotransformation in Organic Chemistry*, W. H. Freeman, 1998.

[6]**Jones, E. R. H.,** *Pure. Appl. Chem*., 33, 39 , 1973.

[7]**Mahato, S. B., Banerjee, S. and Podder, S.,** *Phytochemistry*, .28, 7, 1998.

[8]**Hanson J.R, and Yildirim K**., *J.Chem.Research (s)*, 698, 1999.

[9]**Gurst, J. E. and Djerassi,** C., J. Am. Chem. Soc., 86, 5544, 1964.

[10]**Hanson *etal*.,** J. R. , *J.Chem.Research (s)*, 118, 1998.

[11]**Andrew S. Lamma., Avril R.M. Chena., William Reynolds., Paul B. Reesea**., *steroids*, 72 (2007) 713.

تحضير بعض مشتقات الاستيرويد الايمينية وعمل التحول الحيوي لها بواسطة الفطر موكر بلامبيوس

خالد عمر الفوتي

قسم الكيمياء – كلية العلوم – جامعة الملك عبدالعزيز–جدة

المملكة العربية السعودية

Email : kalfooti@kau.edu.sa

المستخلص: تم تحضير أربعة من الاسترويدات الايمنية في الموقع 17 من الانروستيرون وعمل التحول الحيوي لها بواسطة الفطر موكر بالامبيوس لمعرفة مدى تاثير مجموعة الايمين الجديدة على عملية التحول الحيوي, ووجد ان مجموعة الايمين اثرت بشكل كبير على عملية التحول الحيوي مقارنة بالاسترويدات التي تحمل مجموعتين اكسجينية في الموقعين 3 و17 .

الكلمات المفتاحية: *موكوربلامبيوس–التحول الحيوي– الاسترويدات الايمنية*