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Synthesis and Biotransformation of Some Halogenated Steroids by the Fungus *Mucor plumbeus*

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Abstract. Preparation of some chloro, bromo and iodo steroids derived from testosterone and epiandrosterone is described. The biotransformation of halogenated products by fungus *mucor plumbeus* are preformed. Introduction of the halogen atom into the steroids moiety severely affected the biotransformation results.

Keyword: *Mucor plumbeus*, biotransformation, steroids, 3-dibromomethylene-5α-androstan-17-one.

Introduction

The application of enzymes as reagents is one of the fastest growing areas of research[1] . The value of this application – biotransformation – was first noticed when it helped to overcome a major problem in the synthesis of the cortical steroids by using the fungus *Rhizopus arrhizus*[2], Fig. 1.

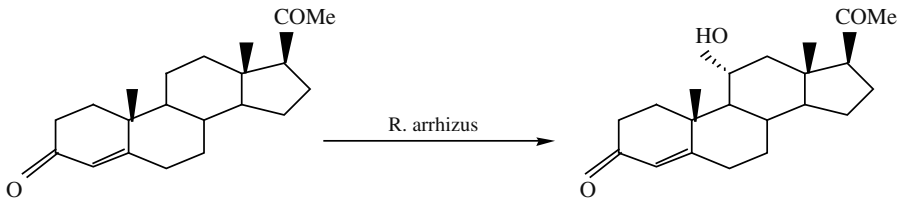


Fig. 1.

The biotransformation of the dioxosteroids (hydroxyl or carbonyl) gave monohydroxylated products[3]. This has been widely observed by variety of fungi[4]. The presence of the two binding oxygen groups

(hydroxyl or carbonyl) in the steroid molecule increases the rate of reactivity of biotransformation[4]. The increased substrate polarity will increase water solubility which will aid permeation into cell. The Jones model of hydroxylation[5] (Fig. 2) 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.

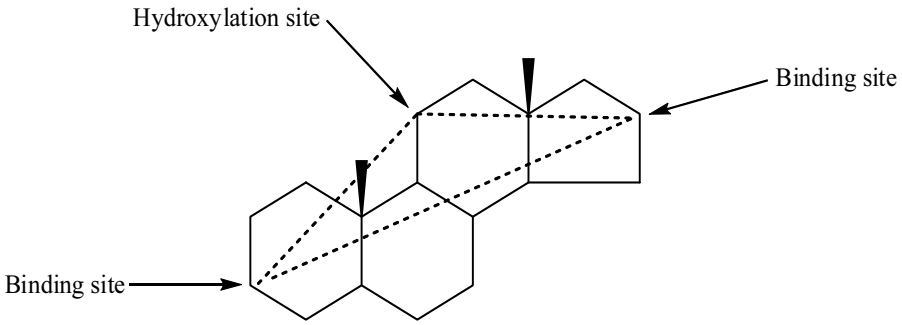


Fig. 2. The Jones model of enzyme-substrate interaction.

Thus in the case of dioxygenated substrate the third catalytic center will hydroxylate at the nearest carbon atom. Hydroxylation commonly takes place at least four or five atoms distant. This paper deals with synthesis of halogenated steroids to replace one of the oxygen containing group to study the effect of this new halogen atom on the biotransformation results.

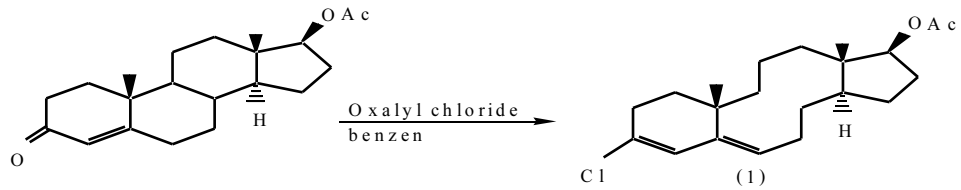
Results and Discussion

In order to examine the effect of introduction of halogen atom to steroids moiety on the biotransformation results, five steroids containing halogen atom were prepared and biotransformed.

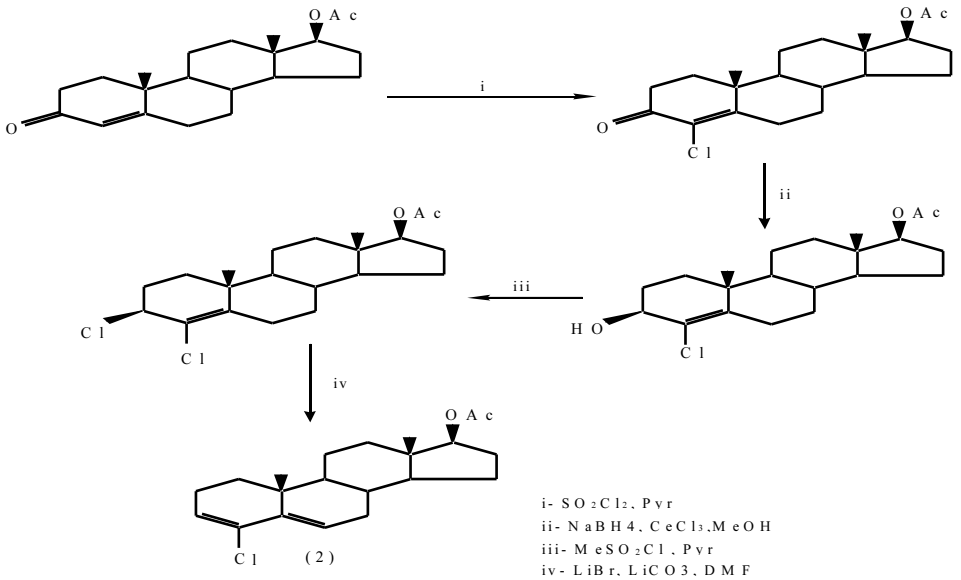
Testosterone acetate was treated with oxalyl chloride in benzen. The product was 17β-acetoxy-3-chloroandrosta-3,5-diene (1) (Scheme 1) which was identifed by comparing its spectroscopic data with the literature values [6].

Testosterone acetate was treated with sulfuryl chloride in pyridine. The product which separated as17β-acetoxy-4-chloroandrost-4-en-3-one was reduced by sodium borohydride in methanol in the presence of

cerium trichloride. Treatment of the product 17β-acetoxy-4-chloro-3β-hydroxyandrost-4-ene with methanesulfonyl chloride in pyridine gave 17 β-acetoxy-3,4-dichloroandrost-4-ene. This compound was refluxed with lithium bromide and lithium carbonate in dimethylformamide. The product was 17β-acetoxy-4-chloroandrost-3,5-diene (2) (Scheme 2). These compounds were identified by comparison of their spectroscopic data with the literature values[7].



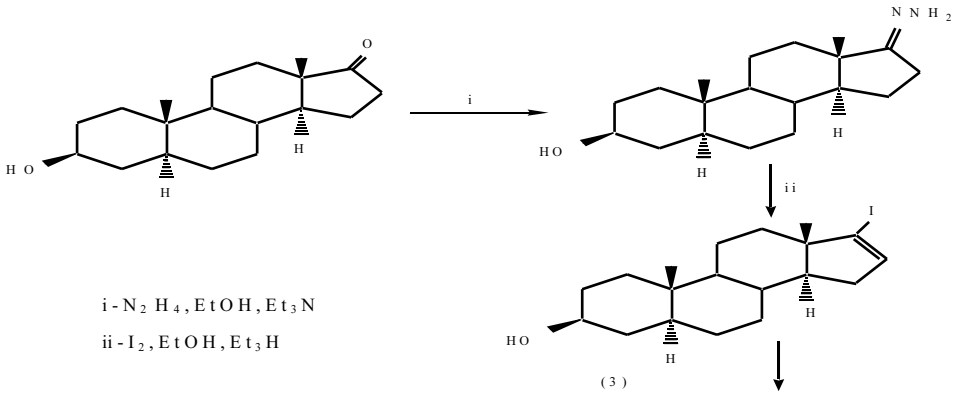
Scheme 1



Scheme 2

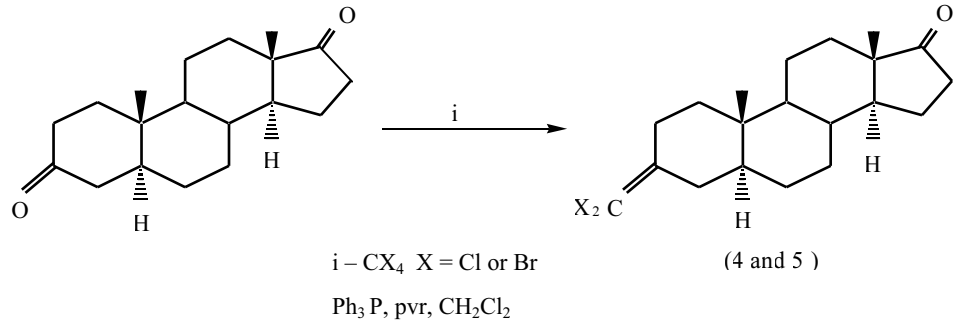
3β-Hydroxy-5α-androstan-17-one was refluxed with hydrazine hydrate and triethylamine in ethanol. The product was 3β-hydroxy-5α-androstan-17-hydrazone which was identified by its spectroscopic data. The hydrazone in THF and triethylamine was treated with iodine to

afford the known compound 17-iodo-5α-androst-16-en-3β-ol (3)[7] (Scheme 3).



Scheme 3

5α-Androstan-3,17-dione was treated with triphenylphosphine carbon tetrachloride mixture in pyridine as a Wittig type reaction, to give a product which was purified by column chromatography. The product was identified from the IR and ^{13}C NMR spectra. The IR spectrum showed the double bond absorption at 1663 cm^{-1}. The ^{13}C NMR signals at δ 111.38 and δ 137.7 were assigned to the $C{=}CCl_2$ group. The mass spectrum showed also the presence of two chlorine atom. All these suggested that the compound was 3-dichloromethylene-5α-androstan-17-one(5) (Scheme 4).

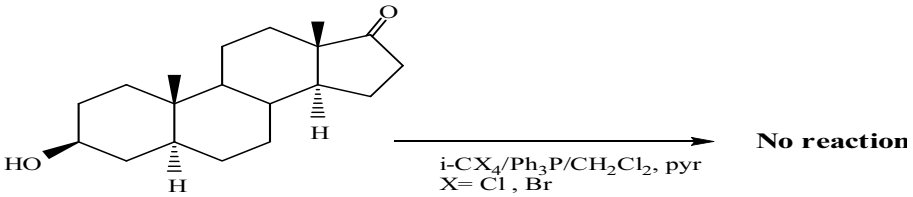


Scheme 4

In similar procedure 3-dibromomethylene-5α-androstan-17-one (4) was prepared. The product was identified by its IR and ^{13}C NMR spectra. In the IR spectrum the double bond absorption appeared at 1680 cm^{-1}.

The ^{13}C NMR showed signals at δ 125.65 and 147.5 were assigned to the $C=CBr_2$ group. The mass spectrum showed the presence of two bromine atoms. These suggested that the product was 3-dibromomethylene-5α-androstan-17-one (4) (Scheme 4).

Synthesis of dihalomethylene derivatives from a ketone at C-17 failed due to stiric hinderance. (Scheme 5).



(5)

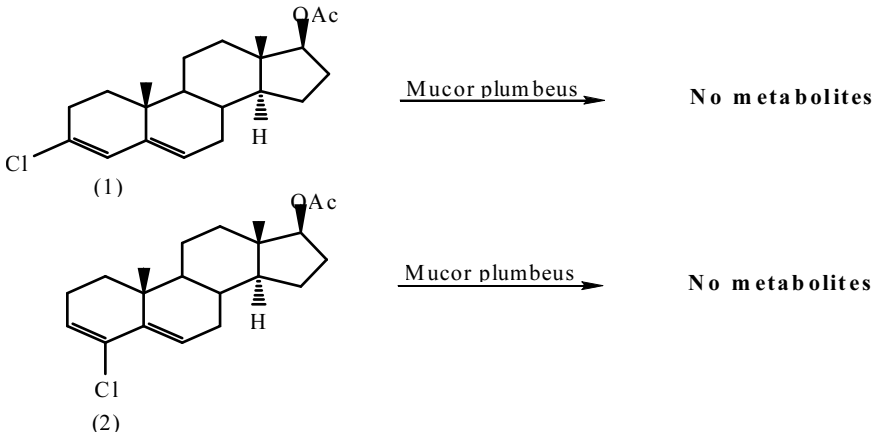
Scheme 5

Biotransformations Results

The metabolism of the halogenated steroids by *Mucor plumbeus.*

Incubation of 17β-Acetoxy-3-chloroandrost-3,5-diene (1)

The incubation of 17β-Acetoxy-3-chloroandrost-3,5-diene (1) with *Mucor plumbeus* for 7 days gave no biotransformation products and 46% starting material (Scheme 6)



(1)

(2)

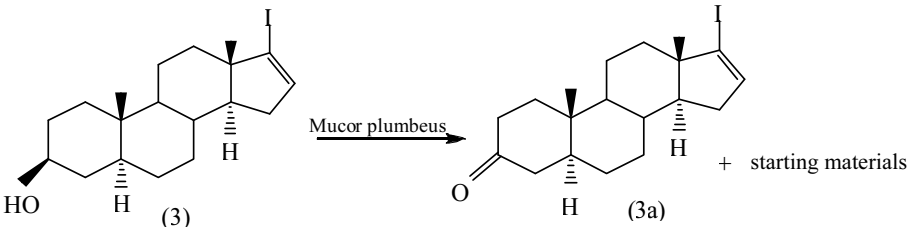
Scheme 6

Incubation of 17β-Acetoxy-4-chloroandrost-3,5-diene (2)

In the same way17β-Acetoxy-4-chloroandrost-3,5-diene (2) gave no biotransformation products and 52% starting material (Scheme 6).

Incubation of 17-Iodo-5α-androst-16-en-3β-ol (3)

Under the same condition 17-Iodo-5α-androsta-16-en-3β-ol (3) gave one biotransformation product and 25% starting materials. The crude product was purified by column chromotography, eluteion with 5% ethyl acetate in light petroleum afforded the oxidized product 17-Iodo-5α-androsta-16-en-3-one (3a) (22%) which was easily identified by its ^{1}H NMR spectrum (Scheme 7).



Scheme 7

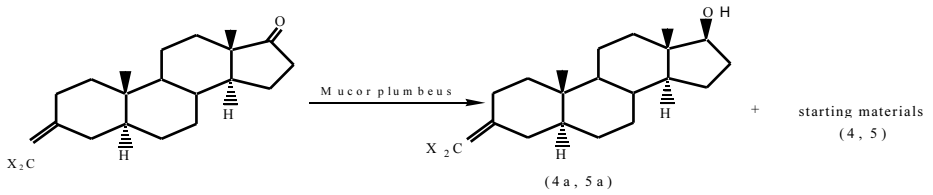
Incubation of 3-Dibromomethylene-5α-androstan-17-one (4)

The incubation of 3-Dibromomethylene-5α-androstan (4) with *Mucor plumbeus* for 7 days gave one biotransformation products and 55% of starting material. The product was eluted with 10% ethyl acetate in light petroleum to reach a pure substance which was identified as the reduction product 3-Dibromomethylene-5α-androstan-17β-ol (4a)(17%) (Scheme 8) which was easily identified by its ^{1}H NMR spectrum. The ^{1}H NMR spectrum of the reduced product showed new signal at δ 3.70 ppm (1H, t, J= 8.5 Hz, 17α-H), which is characteristic for the 17α- H. The ^{13}C NMR spectrum of the product (4a) contained a new signal at δ 67.4 for 17 C−OH in place of the signal at δ 220.9 ppm (17C=O) in the starting material.

Incubation of 3-Dichloromethylene-5α-androstan-17-one (5)

In similar way this compound gave one biotransformation products and 45% starting materials. The product was eluted with 10% ethyl acetate in light petroleum to reach a pure product which was identified as

3-dichloromethylene-5α-androstan-17β-ol (5a) (15%) which was easily identified by its ^{1}H NMR spectrum as shown above.



Scheme 8

Conclusion

The halogenated steroid compounds seem to be very toxic, and they were preventing the fungus from growing, and gave very poor biotransformation results. They gave just oxidation and reduction products, and no hydroxylation products at all. The absence of hydroxylation products suggest that the halogenated steroids do not bind well with the enzyme.

Experimental

Melting points were determined by Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded using KBr disks on a Nicolet Magna 520 Fourier transform spectrometer. ^{1}H NMR spectra were determined in deuteriochloroform with TMS as an internal standard reference at 400 MHz on a Brucker Avance DPX 400 spectrometer, while ^{13}C NMR spectra were recorded in deuteriochloroform at 100 MHz with a Brucker Avance DPX 400 spectrometer. Mass spectra were recorded on a VG Autospec. Micro-analysis were carried out using Perkin Elmar.

The following compounds were prepared as previously reported[11-13]:

17β-Acetoxy-3-chloroandrost-3,5-diene (1) mp 149-150ºC (lit.[8], 151.5-152.5ºC).

17β-Acetoxy-4-chloroandrost-3,5-diene (2) mp 147-148ºC (lit.[9], 149-151ºC).

17-Iodo-5α-androsta-16-en-3β-ol (3) m.p.(143-146°C) (Lit.[10], 148-150°C).

Preparation of 3-Dibromomethylene-5α-androstan-17-one (4)

Triphenylphosphine (700mg) was added to well stirred solution of carbon tetrabromide (500mg) in dry dichloromethane (120cm^3) to give an orange solution. 5α-Androstan-3,17-dione (750mg 9.4 mmol) was added and the mixture was stirred for 3 hrs. The reaction was controlled till no starting material is detected. The solution was washed with water and the organic layer was separated and dried over anhydrous sodium sulfate. The solvent was removed in vacuo to give a yellow solid which was chromatographed on silica. Elution with 2% ethyl acetate: light petroleum gave:

3-dibromomethylene-5α-androstan-17-one (4) (531 mg 76%).

m.p.161-164ºC (Found C, 54.13; H, 6.7. $C_{20}H_{28}OBr_2$ requires C, 54.07; H, 6.35%).

FTIR 1680 (C=C).

^{1}H NMR ($CDCl_3$, 400 MHz) 0.75 (3H, s, 18-H), 0.82 (3H, s, 19-H).

Preparation of 3-Dichloromethylene-5α-androstan-17-one (5)

3-dichloromethylene-5α-androstane-17-one (5) was prepared in 83% yield in the same above procedure from 5α-androstan-3,17-dione .

m.p.149-151ºC (Found C, 68.27; H, 7.87. $C_{20}H_{28}OCl_2$ requires C, 67.60; H, 7.94%).

FTIR 1663 (C=C).

^{1}H NMR ($CDCl_3$, 400 MHz) 0.75 (3H, s, 18-H), 0.82 (3H, s, 19-H).

Biotransformation Experiment

General Fermentation Details

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

^{13}C NMR data determined in $CDCl_3$ at 100 MHz of new compounds 4, 4a, 5 and 5a.

	4	5	4a	5a
C-1	38.35	39.88	38.35	39.88
C-2	30.21	27.59	30.21	27.59
C-3	127.65	111.38	127.65	111.38
C-4	36.35	37.26	36.35	37.26
C-5	53.35	51.01	53.35	51.01
C-6	29.11	28.87	29.11	28.87
C-7	30.91	31.73	30.91	31.73
C-8	35.93	36.12	35.93	36.12
C-9	56.59	54.35	56.59	54.35
C-10	41.09	40.85	41.09	40.85
C-11	23.39	21.03	23.39	21.03
C-12	38.96	38.64	38.96	38.64
C-13	49.01	50.27	49.01	50.27
C-14	46.82	46.58	46.82	46.58
C-15	26.21	23.91	26.21	23.91
C-16	32.03	34.28	32.03	34.28
C-17	221	220.3	81.62	81.72
C-18	12.27	11.94	12.27	11.94
C-19	13.31	12.54	13.31	12.54
$3C=CX_2$	147.01	137.72	147.65	136.87

Trace element solution contained (per litre) zinc sulfate (1.6g), ferrous sulfate (1g), cobalt nitrate (1g), ammonium molybdate (1g), copper sulfate (0.1g) 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 7 days. The broth was filtered and 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.

3-Dibromomethylene-5α-androstan-17-one(4))(0.5g) gave

3-Dibromomethylene-5α-androstan-17β-ol(4a)(17%) which was crystallized from ethyl acetate as white needles

m.p.181-184ºC (Found C, 53.83; H, 6.78. C20H30OBr2 requires C, 54.1; H, 6.65%).

FTIR 3512 (OH).

^{1}H NMR ($CDCl_3$, 400 MHz) 0.73 (3H, s, 18-H), 0.84 (3H, s, 19-H) 3.66 (1H, t, J=8.5Hz, 17α-H).

3-Dichloromethylene-5α-androstan-17-one(5)

(0.5g) gave 3-Dichloromethylene-5α-androstan-17β-ol(5a) (15%) which was crystallized from acetone as white plates.

m.p.177-178°C.

(Found C, 67.22; H, 8.46. $C_{20}H_{30}OBr_2$ requires C, 67.16; H, 8.52%).

FTIR 3525 (OH).

^{1}H NMR ($CDCl_3$, 400 MHz) 0.70 (3H, s, 18-H), 0.77 (3H, s, 19-H) 3.58 (1H,t, J= 8.5 Hz, 17α-H)

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الاصطناع والتحولات الحيوية لبعض هالوجينات الاسترويدات باستخدام الفطر موكور بلامبيوس

خالد عمر الفوتي

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جدة – المملكة العربيه السعودية

المستخلص. تم في هذا البحث تحضير خمس من الاسترويدات الهالوجينية (كلورو، برومو وايودو) المشتقة من التيستسترون والايبي اندروسترون. تم عمل تجربة التحول الحيوي لهذه الاسترويدات الهالوجنية باستخدام الفطر موكور بلامبيوس. لوحظ أن هذه الاستبدالات الهالوجينية لها تاثير كبير على نتائج تجربة التحول الحيوي.