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Biotransformation of some ring A and B epoxy steroids by the fungus *Mucor plumbeus*

KHALID O. AL-FOOTY

Department of Chemistry, Faculty of Science, King Abdul Aziz University, Jeddah - 21589, P.O. Box 80203 (Saudi Arabia)

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ABSTRACT

The preparation of some ring A and B epoxy steroids derived from testosterone, pregnenolone and epiandrosterone using m-chloroperbenzoic acid is described. The biotransformation of the epoxy products by the fungus *Mucor plumbeus* are performed. The introduction of the epoxy group into the steroids moiety has affected the biotransformation results and gave only hydrolyzed products.

Key words: *Mucor plumbeus*, Microbiological hydroxylation, expoxy steroids, m-chloroperbenzoic acid.

INTRODUCTION

The ability of enzymes to perform reactions, under mild conditions, with regio-and stereospecificity offers a valuable potential for the production of chiral molecules in such diverse applications as synthesis of natural products, medicinal chemistry and synthesis of drug metabolites[1].

The factors that govern the microbiological hydroxylation of steroids involve a combination of effects based on the site, stereochemistry and nature of the existing functional groups in the substrate[2]. Predictive models for the microbiological hydroxylation of steroids envisage three active centers on the hydroxylase, two of which binds the substrate and one delivers the oxygen[3-4] (Fig. 1). The presence of the two binding oxygen groups (hydroxyl of carbonyl) in the steroid molecule increase the rate of reactivity of biotransformation and gives only monohydroxylated products[5].

Hydroxylation commonly takes place at least four or five atoms distant from the binding groups. This paper deals with synthesis of epoxy steroids to replace one of the hydroxyl or carbonyl group to study the effect of this new epoxy group on the biotransformation results.

RESULTS AND DISCUSSION

In order to examine the effect of introduction of epoxy group into steroids moiety on the biotransformation results three epoxy steroids in ring A and B were prepared and biotransformed using the fungus *Mucor plumbeus*.

Epiandrosterone was converted to 3β-(toluene-p-sulfonyloxy)-5α-androstan-17-one by

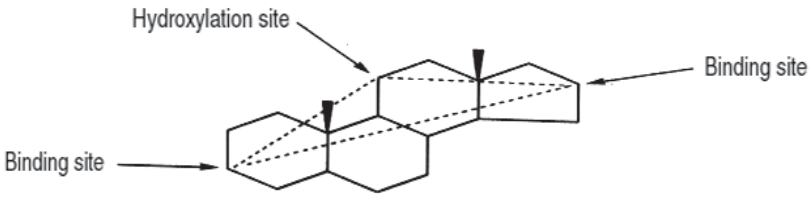
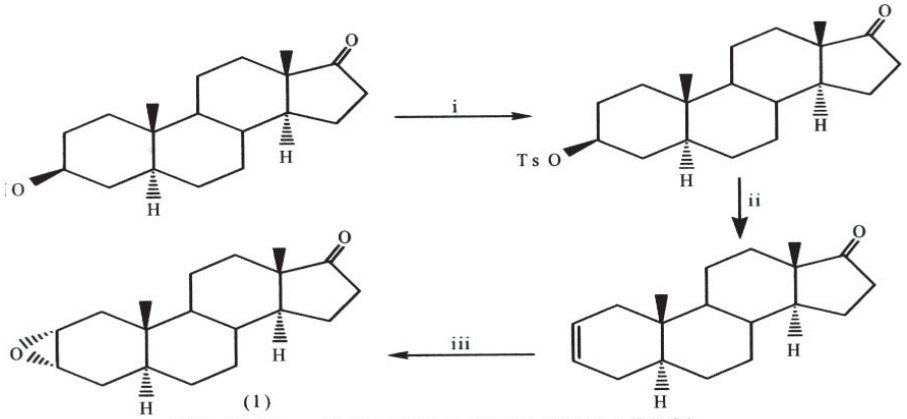


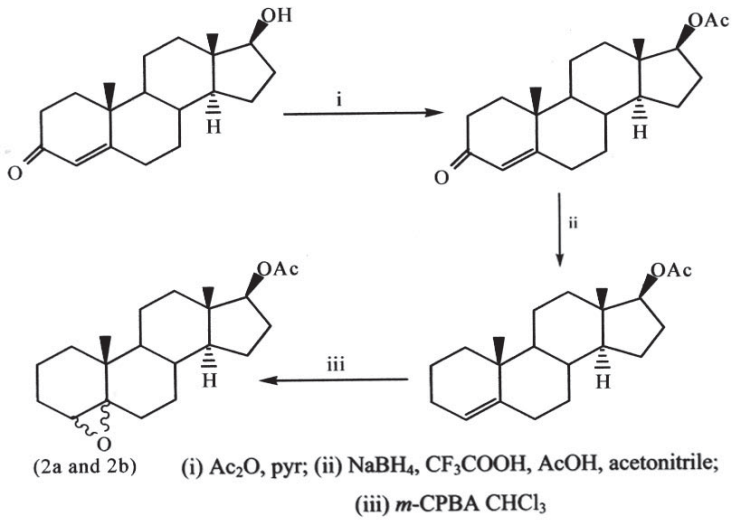
Fig. 1: The Jones model of enzyme e-substrate interaction

using toluene-p-sulfonyl chloride in pyridine. The elimination of tosyl group by collidine gave 5α-androstan-2-en-17-one which were identified by comparing their spectroscopic data with the literature. 5α-androstan-2-en-17-one was treated with m-chloroperbenzoic acid in chloroform to afford the known compound 2α,3α-epoxy-5α-androst-17-one (1). The product was identified by comparing its spectroscopic data with the literature[6]. Scheme 1.



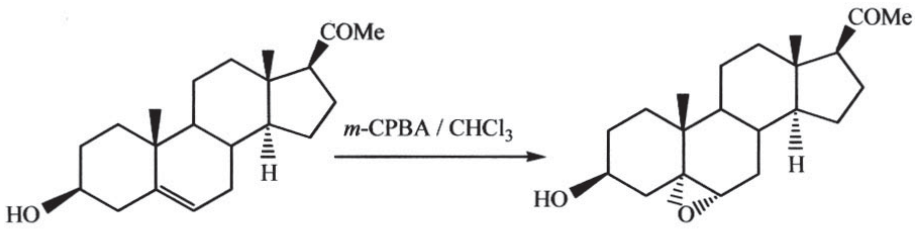
Scheme 1

Testosterone was acetylated to give testosterone acetate. This product was treated with sodium borohydride and trifluoroacetic acid in acetic acid and acetonitrile to give 17β-acetoxy-androst-4-ene. 17β-Acetoxy-androst-4ene in chloroform was treated with m-chloroperbenzoic acid to afford an inseparable mixture of the α and β-epoxides (60:40) (2a & 2b). The products were identified by comparing its spectroscopic data with the literature[7]. Scheme 2.



Scheme 2

Pregnenolone was treated with m-chloroperbenzoic acid in chloroform to give the known 3β-hydroxy-5α,6α-epoxypregnan-20-one(3) (Scheme 3). The product was identified by comparing its spectroscopic data with the literature[8].



Scheme 3 (3)

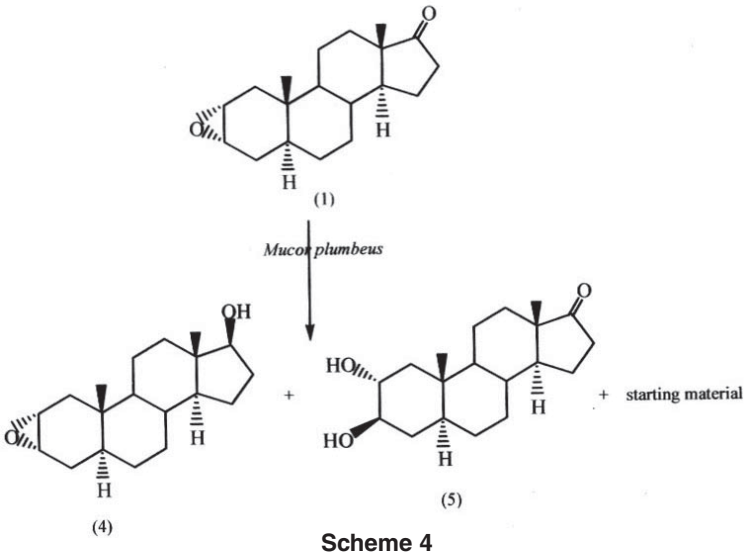
Biotranformations

The metabolism of the epoxy steroids by *Mucor plumbeus*

Incubation of 2α,3α-epoxy-5α-androstan-17-one (1)

Incubation of 2α,3α-epoxy-5α-androstan-17-one (1) with *Mucor plumbeus* for 7 days give two metabolite products and 14% of the starting material was recovered following column chromatography on silica.

The first metabolite was eluted with 5% ethyl acetate in light petroleum and was identified as the known 2α,3α-epoxy-5α-androstan-17β-ol(4) (17%). The ^{1}H NMR spectrum of the product (4) showed new signal at δ3.56 ppm (1H, t, J=8.5 Hz, 17α-H) which was assigned to the 17α proton. The ^{13}C NMR spectrum of the product (4) showed the absence of carbonyl signal at δ221 (C-17) and the presence of new signal at δ67.2 (C-17) which was consistent with the presence of the 17β-hydroxy. The second metabolite was eluted with 30% ethyl acetate in light petroleum and was identified as the known hydrolysed product. 2α,3α-dihydroxy-5α-androstan-17-one (5) (23%) (Scheme 4). The product was identified by comparing its spectroscopic data with the literature[9].



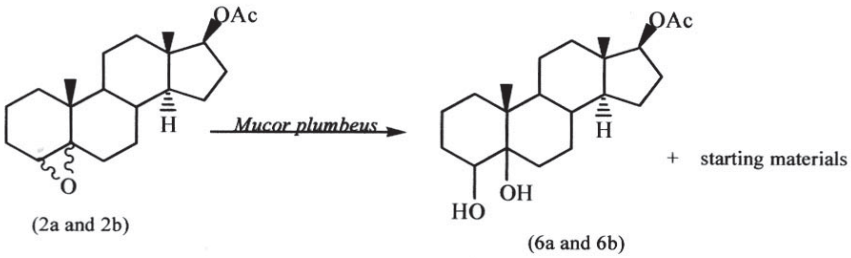
Scheme 4

Incubation of the 17β-acetoxy-4,5-epoxyandrostane (2a, 2b)

Under similar condition incubation of the inseparable mixture 17β-acetoxy-4,5 epoxyandrostane (2a, 2b) gave one product and 22% of the starting materials.

The only metabolite was eluted with 40% ethyl acetate in light petroleum and was identified as inseparable mixture 2:1 4α-5β-dihydroxy-17β-acetoxyandrostane (6a) and 4β-5α-dihydroxy-17β-acetoxyandrostane (6b) (28%). The ^{1}H NMR spectrum of the product showed the lacked of the

signals at δ2.92ppm (1H, d, J=3.6 Hz, 4β-H) and 2.90 ppm (1H, d, J=4.3 Hz, 4α-H) which were assigned to the 4β and 4α protons of the inseparable epoxide (2a and 2b). The ^{1}H NMR spectrum of the product also contained two new signals at δ3.42 ppm (1H, d, J=8.9 Hz, 4β-H) and at δ3.59 ppm (1H, q, J=3.4Hz, 4α-H) which was assigned to the 4β and 4α protons of the products (6a&6b. The stereochemistry of the products was assigned by analogy with the ^{1}H NMR spectra of similar 4α-5β and 4β,5α dihydroxy steroid[10]. ^{1}H NMR spectrum of (6a and 6b) proved that the ratio of products based on the relative integrals was (6a):(6b) (3:2), respectively.



(2a and 2b) (6a and 6b)

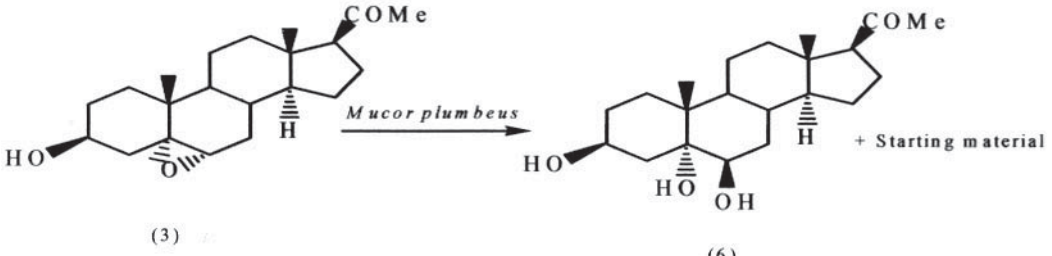
Scheme 5

Incubation of 3β-hydroxy-5α,6α-epoxypregnan-20-one (3)

Under similar condition incubation of 3β-Hydroxy-5α,6α-epoxyapregnan-20-one (3) gave one biotransformation product and 30% of the starting material was recovered following column chromatography on silica.

The only metabolite eluted with 50% ethyl acetate in light petroleum and was identified as 3β-5α,6β,-trihydroxypregnan-20-one (3) (7) (27%). The ^{1}H NMR spectrum of the product showed the absence of the signal at δ2.84ppm (1H, d, J=4.4Hz 6βH) which was assigned to the 6β proton attached to the 5α,6α epoxy group. The ^{1}H NMR spectrum of this product (30) contained a new signal at δ 3.52 ppm (aH, d, J-7.88 Hz, 6αH) which was not present in the spectrum of the starting material. The 3α-H responance at δ3.82 ppm (1H, tt, J=10.8, 5.8Hz) of the starting material moved to δ4.2 ppm (1H, tt, J=10.8 Hz) (Δδ0.4) in accordance with a transannular 1:3-diaxial intraction with a hydroxyl group. This was consistent with the presence of the 5α-hydroxy group. The ^{13}C NMR spectrum of the product two new signals at δ75.8ppm (C-5) and at δ76.1ppm (C-6) in agreement of the signals at δ59.11ppm (C-6) and δ65.78 ppm (C-5) of the starting material. This was with the presence of the 5α- and 6β-dihydroxy groups.

The stereochemistry of the product was assigned by analogy with the ^{1}H NMR spectra of similar 5,6-dihydroxy[8].



(3) (6)

Scheme 6

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

references at 400MHz on a Brucker DPX 400 spectrometer while ^{13}C NMR spectra were recorded in deuteriochloroform at 75MHz with a Brucker DPX 400 spectrometer. Mass spectra were recorded on a VG Autospec. Micro-analysis were carried out using Perking Elmar.

The following three substrates were prepared by the literature methods reported previously[6-8].

2α,3α-epoxy-5α-androstan-17-one (1): m.p. 153-155°C (lit.[6], 155-157°C).

17β-acetoxy-4,5-epoxyandrostane (2a, 2b)

3β-hydroxy-5α,6α-epoxypregnan-20-one (3): m.p. 168-170°C (lit.,[8] m.p. 170-173°C)

Biotransformation experiment

General fermentation details

The fungus *Micro plumbeus* (IMI 116688) was grown on shake culture in 250ml 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. 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 subtrate (0.5) in ethanol (30ml) was distributed over 50 flasks. The fermentation was then continued for further 7days. The broth was filterd 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 chromatogrphed on silica and eluted with an increasing gradient of ethyl acetate in light petroleum.

Table 1: ^{13}C NMR data determined in $CDCl_3$ at 75MHz of the compounds 4,5 and 7

Carbon no	4	5	7
C-1	38.2	44.1	32.5
C-2	50.5	73.1	33.2
C-3	51.8	75.8	67.2
C-4	28.8	34.3	42.6
C-5	33.0	44.8	75.6
C-6	28.3	27.6	75.8
C-7	28.9	29.4	34.4
C-8	33.5	33.7	30.8
C-9	57.9	55.8	46.1
C-10	33.8	36.9	39.5
C-11	20.2	20.1	20.7
C-12	31.5	31.5	32.3
C-13	47.7	47.8	48.4
C-14	51.6	52.1	51.4
C-15	21,5	21.3	22.0
C-16	35.8	35.8	36.1
C-17	80.6	221.2	63.7
C-18	13.9	13.6	13.7
C-19	12.8	12.4	17.2
17β-$COCH_3$	31.6	-	-
17β-$COCH_3$	205.4	-	-

Incubation of 2α,3α-epoxy-5α-androstan-17-one (1) gave 2α,3α-epoxy-5a-androstan-17-o1 (4) (52mg, 17%); m.p. 163-166°C (lit.,[6] 161-163°C); FTIR 3349 (OH),

^1H NMR ($CDCl_3$, 400 MHz) δ0.72 (3H, s, 18-H), 0.82 (3H, s, 19-H), 3.04 (1H, dd, J=4.8, Hz, 2β-H), 3.09 (1H, dd, J=4.8, 1.6 Hz, 3β-H), 3.56 (1H, t, J=8.5 Hz, 17α-H).

Further elution with 30% ethyl acetate in light petroleum gave

2α,3β-dihydroxy-5α-androstan-17-one (5) (64mg, 21%), m.p. 172-173°C (lit.[6], 168-171°C), FTIR 3423 (OH), 1729 (C=O), ^1H NMR ($CDCl_3$, 400MHz) δ0.72 (3H, s, 18-H), 0.82 (3H, s, 19-H), 4.01 (1H, m, 3α-H), 4.29 (1H, q, J=3Hz, 2β-H).

Incubation of 17β-acetoxy-4,5-epoxyandrostane (2a,2b), inseparable mixture 2:1 of 4α,5β-dihydroxy-17β-acetoxyandrostane(6a) and 4β,5α-dihydroxy-17β-acetoxyandrostane (6b) as an oil (84 mg, 28%), (Found, 350.231. $C_{21}H_{34}O_4$ calculated, 350.246); FTIR 3396 (OH), ^1H NMR (CD_3OD, 400 MHz) (of the major product 4α,5β-dihydroxy-17β-acetoxyandrostane (6a) δ0.71 (3H, s, 18-H), 1.21 (3H, s, 19-H), 2.0 (3H, s, 17β-$OCOCH_3$), 3.59 (1H, q, J=3.4Hz, 4β-H), 4.4 (1H, J=8.5 Hz, 17α-H), ^1H NMR (CD_3OD, 400 MHz) (of the major product 4β,5α-dihydroxy-17β-acetoxyandrostane (6b) δ0.71 (3H, s, 18-H), 1.21 (3H, s, 19-H), 2.0 (3H, s, 17β-$OCOCH_3$), 3.42 (1H, d, J=8.9Hz, 4α-H), 4.35 (1H, t, 8.5 Hz, 17α-H), ^1H-NMR spectrum of (6a and 6b) proved that the ratio of products based on the relative integrals was (6a) : (6b) (3:2), respectively.

Incubation of 3β-hydroxy-5α, 6α-epoxyandrostan-17-one (3) gave 3β,7α-dihydroxy-5α, 6α-epoxyandrostan-17-one (7) (80mg, 27%); as white neddles m.p. 241-246°C (Found C, 70.8, H, 8.32 $C_{19}H_{28}O_4$ requires C, 71.22, H, 8.81%). FTIR 3390 (OH) and 1747 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz) δ0.81 (3H, s, 18-H), 1.12 (3H, s, 19-H), 3.3 (1H, d, J=4.6, Hz, 6β-H), 3.85 (1H, tt, J=10.8, 5.8 Hz, 3α-H), 4.05 (1H, brs, 7βH).

CONCLUSION

The introduction of the epoxy group into steroid moiety has severally affected the biotransformation results. These epoxy steroids (1)(2) and (3) seem to behave like the mono-oxogenated steroids in giving poor yields and no hydroxylation products comparing with the 3,17-dioxogenated steroids. The epoxy steroids do not seem to bind will with the enzyme and they were hydrolyzed during these biotransformation and one reduction product was isolated. Investigations was done to show that these hydrolysed products were not just hydrolysis in the slightly acidic medium and that fungus was involved. We repeat the biotransformation experiment of the epoxy steroid (1),(2) and (3) without adding the fungus and no hydrolyzed products was obtained. These results confirm that the fungus was involved in this hydrolysis reaction.

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