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Review

A REVIEW ON BIOTRANSFORMATIONAL STUDIES OF DANAZOL

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ABSTRACT

Biotransformation is the basis of life. Various chemical modifications are occurred by drugs in the body to give metabolites as new molecules having their own features, generally different from those of drug. Danazol (**1**) is used more effectively for the treatment of endometriosis and benign fibrocystic mastitis. As a result of biotransformation of danazol (**1**) variety of metabolites, **2-17** were identified by using different sources including a monkey, human (male and female) volunteers and horse, and fermentation with *Cephalosporium aphidicola, Aspergillus niger, Fusarium lini, Fusarium solani, Gibberella fujikuorii* and *Bacillus cerus*. This present review will discuss metabolites **2-17**, obtained from danazol (**1**) up to 2012.

Keywords: Biotransformation, Danazol, *Cephalosporium aphidicola, Aspergillus niger, Fusarium lini, Fusarium solani, Gibberella fujikuorii, Bacillus cerus*

INTRODUCTION

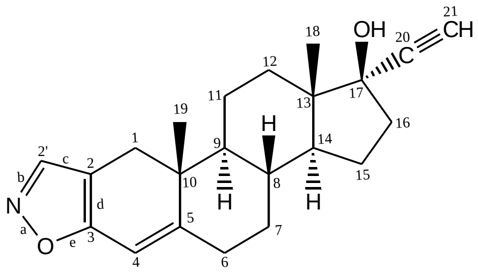
Most of the drugs in the body are converted into one or many metabolites. The metabolite can be more or less toxic and more or less active; and can have different, similar, or even antagonist properties compared to those of the drug. According to pharmacokinetic studies, each metabolite of a drug must be a new molecule having its own features (half-life, elimination, the volume of distribution, etc.) generally different from those of drug.

Biotransformation are chemical reactions that are catalyzed by microorganisms (in terms of growing or resting cells) or isolated enzymes. Biotransformation is more efficient and economical routes to target compounds. It offers many benefits over traditional synthetic chemistry including moderate reaction conditions, solvent waste, less side products and regio- and stereo-selective transformations. It has the ability to produce molecules that are difficult to prepare using traditional organic synthesis. This technology is being advanced in the manufacture of specialty chemicals, molecules of high biological activity, especially flavors and fragrances (Cheetham, 1993). Because the biotransformation processes are environmentally friendly, is attracting more and more attention. On industrial scale, biotransformation reactions including isomerization, epoxydation, hydroxylation, double bond formation, reduction, oxidation, alkylation, glycosylation, acetylation and hydrolysis using micro-organisms are now routinely performed.

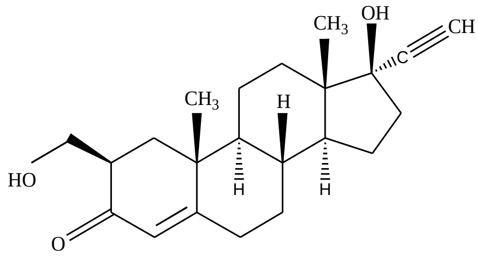
Danazol or 17*β*-hydroxy-17*α*-pregna-2,4-dien -20-yno[2,3-*d*] isoxazole (**1**) is a steroidal compound having isoxazole ring. It is effectively used in the treatment of adenomyosis, cramps, digestive disorders, cyclical mastalgia, premenstrual syndrome, endometriosis, pelvic pain, precocious puberty, dyspareunia and benign fibrocystic mastitis (Rosi et al., 1977). It possesses neither progestational nor estrogenic activities, as well as an orally effective pituitary gonadotropin inhibitory agent (Rosi et al., 1977). It was approved as the first drug for the treatment of endometriosis specifically by the US Food and Drug Administration (FDA) in the early 1970s (Dmowski et al., 1971). Heavy menstrual bleeding in premenopausal women is a noble cause of ill health. Danazol (**1**) proved highly successful for treatment of heavy menstrual bleeding as compared to other treatments (Beaumont et al., 2007). Migraine in women generally is a hormonal event and danazol's (**1**) action in preventing these hormonal changes is a natural deterrent. Danazol (**1**) appears to be an effective in the control of women's cyclic migraine by inhibiting estrogen fluctuation about 51% of women. It is the first practical approach to preventing previously untreatable women's hormonal headaches (Lichten et al., 1991).

TRANSFORMED PRODUCTS FROM BIOTRANSFORMATION OF DANAZOL (1):

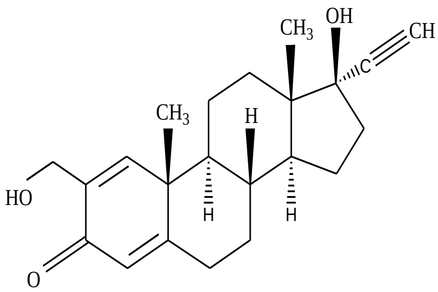
Various transformed products **2-17** were obtained by separate ways of biotransformation of danazol (**1**) up to 2012 (Fig. 1). This review may be of general interest and helpful in comparative studies among transformed products **2-17**, obtained by diverse ways. The detail of these compounds **2-17** is also mentioned in Table 1.



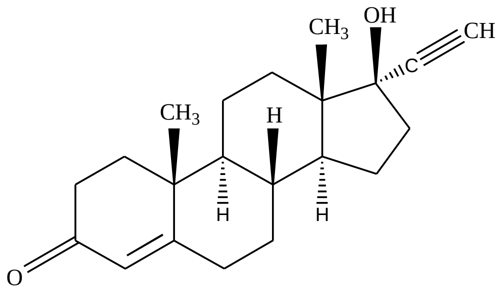
Danazol (**1**)



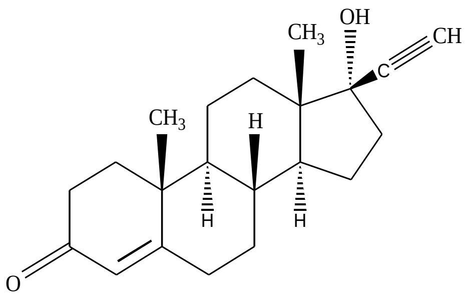
2β-Hydroxymethylethisterone (**2**)



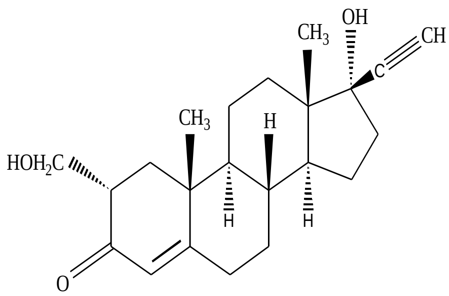
Δ¹-2-Hydroxymethylethisterone (**3**)



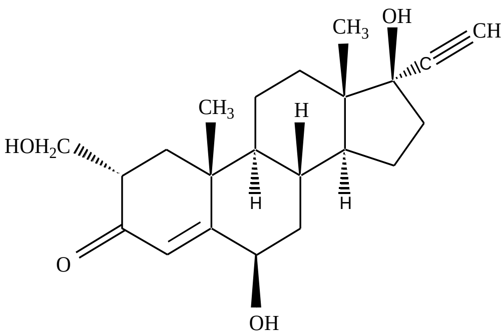
Ethisterone (**4**)



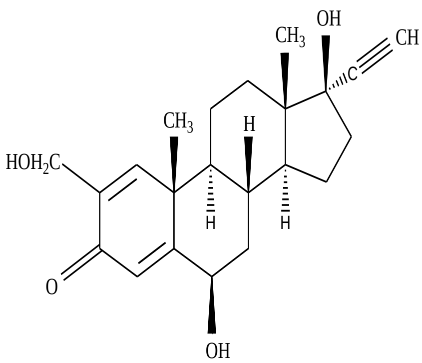
17α-Hydroxypregn-4-en-20-yn-3-one (**5**)



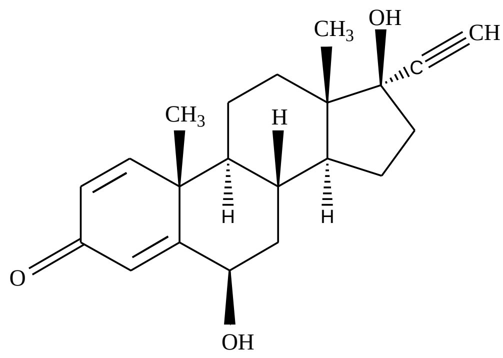
17α-Hydroxy-2α-(hydroxymethyl)pregn-4-en-20-yn-3-one (**6**)



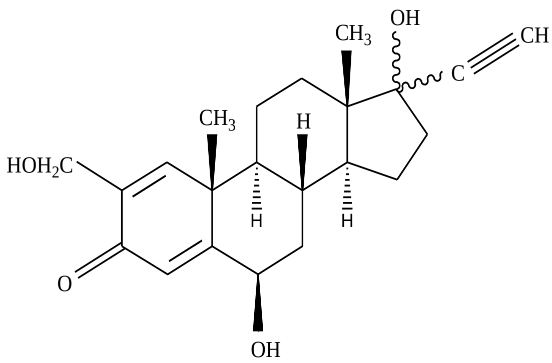
6β,17β-Dihydroxy-2α-(hydroxymethyl)
pregn-4-en-20-yn-3-one (**7**)



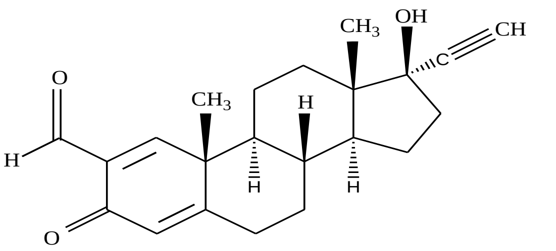
6β,17β-Dihydroxy-2-(hydroxymethyl)
pregna-1,4-dien-20-yn-3-one (**8**)



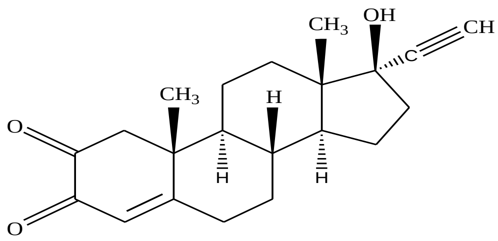
6β,17β-Dihydroxy-17α-pregna-1,4-dien-20-yn-3-one (**9**)



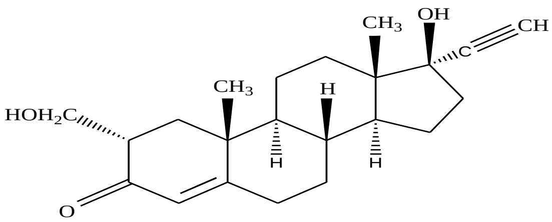
17ω-Hydroxy-2-(hydroxymethyl)-17ω-pregna-1,4-dien-20-yn-3-one (**10**)



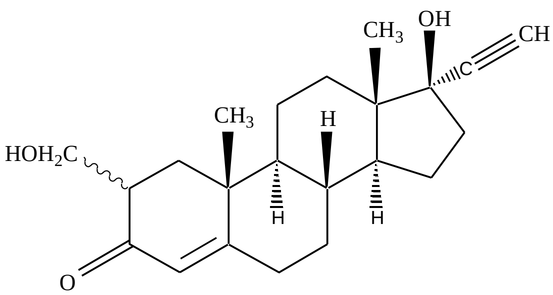
17β-Hydroxy-3-oxo-17α-pregna-1,4-dien-20-yn-2-carboxaldehyde (**11**)



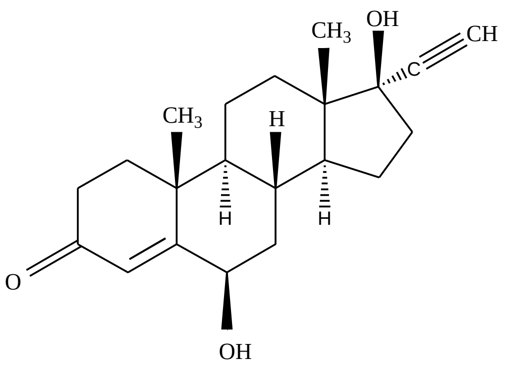
17β-Hydroxy-17α-pregna-4-en-20-yn-2,3-dione (**12**)



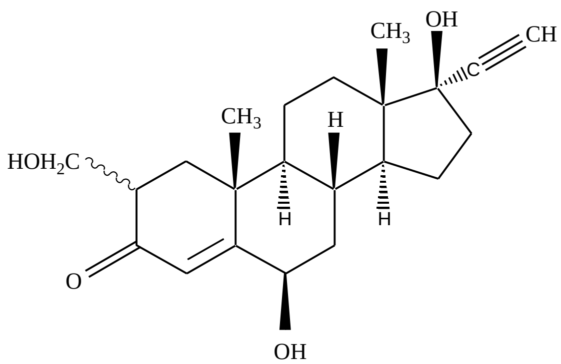
17β-Hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**13**)



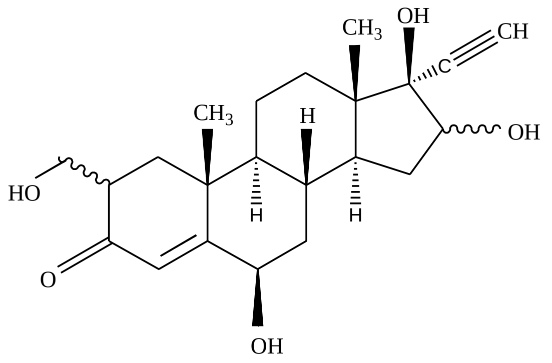
2ξ-Hydroxymethylethisterone (**14**)



6β-Hydroxyethisterone (**15**)



6β-Hydroxy-2ξ-hydroxymethylethisterone (**16**)



6β,16ξ-Dihydroxy-2ξ-hydroxymethylethisterone (**17**)

Fig. 1. Structures of danazol (**1**) and its biotransformed products **2-17**

Table 1: Biotransformed products **2-17** of danazol (**1**).

S. No.	Biotransformed products	Biotransformation pathways	References
1	2β-Hydroxymethylethisterone (**2**)	from monkey urine and fecal extract	Dmowski et al., 1971
2	Δ¹-2-Hydroxymethylethisterone (**3**)	from monkey urine and fecal extract, urine of female (human), *Fusarium lini*, *A.niger* & *Cephlosporium aphidicola,* urine of two healthy male volunteers	Beaumont et al., 2007; Dmowski et al., 1971; Lichten et al., 1991; Porto et al., 2006; Rosi et al., 1977
3	Ethisterone (**4**)	from monkey urine and fecal extract, urine of two horses, urine of two healthy male volunteers	Kim et al., 2001; Porto et al., 2006
4	17α-Hydroxypregn-4-en-20-yn-3-one (**5**)	from urine of female (human)	Beaumont et al., 2007
5	17α-Hydroxy-2α-(hydroxymethyl) pregn-4-en-20-yn-3-one (**6**)	from urine of female (human)	Beaumont et al., 2007
6	6β,17β-Dihydroxy-2α-(hydroxy-methyl)pregn-4-en-20-yn-3-one (**7**)	from urine of female (human)	Beaumont et al., 2007
7	6β,17β-Dihydroxy-2-(hydroxymethyl)pregna-1,4-dien-20-yn-3-one (**8**)	from urine of female (human), urine of two horses, urine of two healthy male volunteers	Beaumont et al., 2007; Kim et al., 2001; Porto et al., 2006
8	6β,17β-Dihydroxy-17α-pregna-1,4-dien-20-yn-3-one (**9**)	from urine of two horses	Kim et al., 2001
9	17ω-Hydroxy-2-(hydroxymethyl)-17ω-pregna-1,4-dien-20-yn-3-one (**10**)	from urine of two horses	Kim et al., 2001
10	17β-Hydroxy-3-oxo-17α-pregna-1,4-dien-20-yn-2-carboxaldehyde (**11**)	from urine of two horses	Kim et al., 2001
11	17β-Hydroxy-17α-pregna-4-en-20-yn-2,3-dione (**12**)	from urine of two horses	Kim et al., 2001
12	17β-Hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**13**)	from *Fusarium lini*, *Aspergillus niger*, *Cephlosporium aphidicola* & *Bacillus cerus*	Lichten et al., 1991; Rosi et al., 1977
13	2ξ-Hydroxymethylethisterone (**14**)	from urine of two healthy male volunteers	Porto et al., 2006
14	6β-Hydroxyethisterone (**15**)	from urine of two healthy male volunteers	Porto et al., 2006
15	6β-Hydroxy-2ξ-hydroxymethyl ethisterone (**16**)	from urine of two healthy male volunteers	Porto et al., 2006
16	6β,16ξ-Dihydroxy-2ξ-hydroxymethylethisterone (**17**)	from urine of two healthy male volunteers	Porto et al., 2006

Biotransformation of danazol (1) in monkey: Davison and co-workers in 1976 investigated the metabolism of danazol (**1**) into the human volunteers, monkey and rat. Danazol (**1**) was rapidly metabolized by good absorption into 60 end products in monkey fecal extracts. The major identified fecal and urinary end-products were 2βhydroxymethylethisterone (**2**), Δ¹-2-hydroxymethylethisterone (**3**) and ethisterone (**4**).

Biotransformation of danazol (1) in human (female): Rosi and co-workers in 1977 were studied metabolism of danazol (**1**) into human. A female had taken danazol (**1**) orally for seven days at a dose 800 mg/day. They were identified and isolated Δ¹-2-hydroxymethylethisterone (**3**), 17α-hydroxypregn-4-en-20-yn-3-one (**5**) and 17α-hydroxy-2α-(hydroxymethyl) pregn-4-en-20-yn-3-one (**6**) from urine of female. The other two metabolites isolated and identified as 6β,17β-dihydroxy-2α-(hydroxymethyl) pregn-4-en-20-yn-3-one (**7**) and 6β,17β-dihydroxy-2-(hydroxymethyl) pregna-1,4-dien-20-yn-3-one (**8**). This study indicated that these transformed metabolites showed less pituitary inhibiting activity than danazol (**1**) (Rosi et al., 1977).

Biotransformation of danazol (1) in horse: Kim and co-workers in 2001 described the androgenic effects of danazol (**1**). For this purpose, it was orally administered to two horses and identified urinary metabolites as well as studied the urinary excretion pattern of major metabolites testosterone and ethisterone. The major metabolites of danazol (**1**) were ethisterone (**4**), 6β,17β-dihydroxy-2-(hydroxymethyl) pregna-1,4-dien-20-yn-3-one (**8**), 6β,17β-dihydroxy-17α-pregna-1,4-dien-20-yn-3-one (**9**), 17ω-hydroxy-2-(hydroxymethyl)-17ω-pregna-1,4-dien-20-yn-3-one (**10**), 17β-hydroxy-3-oxo-17α-pregna-1,4-dien-20-yn-2-carboxaldehyde (**11**) and 17β-hydroxy-17α-pregna-4-en-20-yn-2,3-dione (**12**) (Kim et al., 2001). Several minor peaks were also present but their intensities were minimal.

Biotransformation of danazol (1) by *Fusarium lini*: Choudhary and co-workers in 2002 were examined the transformation of the isoxazole ring in danazol (**1**) by microbial transformation. The fungus *Fusarium lini* cleaved the isoxazole ring of danazol (**1**) by reduction on fermentation and yielded Δ¹-2-hydroxymethylethisterone (**3**) and 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**13**).

Biotransformation of danazol (1) by *Aspergillus niger*: Choudhary and co-workers in 2002 reported transformed metabolites of danazol (**1**) by microbial transformation. This is a new approach to obtain these metabolites Δ¹-2-hydroxymethylethisterone (**3**) and 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**13**) by using *Aspergillus niger*.

Biotransformation of danazol (1) by *Cephalosporium aphidicola*: Choudhary and co-workers in 2002 obtained metabolites Δ¹-2-hydroxymethylethisterone (**3**) and 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**13**) also through the fungus *Cephalosporium aphidicola* on fermentation with danazol (**1**).

Biotransformation of danazol (1) by *Bacillus cerus*: Choudhary and co-workers in 2002 were also used bacteria *Bacillus cerus* for the microbial transformation of danazol (**1**), yielded only 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**13**).

Biotransformation of danazol (1) in healthy human (male) volunteer: Porto and co-workers in 2006, danazol (**1**) was orally subjected to two healthy male volunteers. Samples of urine were collected after one week. They identified different metabolites Δ¹-2-hydroxymethylethisterone (**3**), ethisterone (**4**), 6β,17β-dihydroxy-2-(hydroxymethyl) pregna-1,4-dien-20-yn-3-one (**8**), 2ξ-hydroxymethylethisterone (**14**), 6β-hydroxyethisterone (**15**), 6β-hydroxy-2ξ-hydroxymethyl ethisterone (**16**) and 6β, 16ξ-dihydroxy-2ξ-hydroxymethylethisterone (**17**).

Biotransformation of danazol (1) by *Fusarium solani*: Azizuddin and Choudhary in 2010 reported transformed metabolites of danazol (**1**) by microbial transformation. This is a new approach to obtain these metabolites Δ¹-2-hydroxymethylethisterone (**3**) and 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**13**) by using *Fusarium solani*. Danazol (**1**) was found to be the most potent prolyl endopeptidase inhibitor (C_{50} = 57.4 ± 0.002) among all the compounds, while its metabolites **3** (IC_{50} = 827.7 ± 0.04) and **13** (IC_{50} = 379.3 ± 0.00081) showed significant inhibiting activity but lesser activity than danazol (**1**). Z-Pro-prolinal is used as standard (IC_{50} = 880 ± 0.001) (Azizuddin and Choudhary, 2010).

Biotransformation of danazol (1) by *Gibberella fujikuorii*: Azizuddin and Choudhary in 2010 reported transformed metabolites of danazol (**1**) by microbial transformation. This is also a new approach to obtain metabolite 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**13**) by using *Gibberella fujikuorii*.

CONCLUSION

This review aimed to highlight the biotransformed metabolites **2-17** of danazol (**1**) from many ways. Regarding this detailed survey, it is assumed that it will assist in comparative studies among transformed products obtained by separate ways of biotransformation.

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