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BIOTRANSFORMATION OF TESTOSTERONE BY FUNGI AND THE EXPERIMENTAL METHODS: A MINI-REVIEW

Prathyash Ushus M. J[1]*, Siva Kumar K[1]*, Muddukrishnaiah Kotakonda[2], Sunnapu Prasad[3], Raslamol K[4], Sangeetha S[5], Mahesh Kumar MVS[1], Satyanarayana Swamy Ch[1], Koteswari Peddi[1], Bala Murali[1], Priyanka Mishra[1].

[1]Department of Biotechnology, GITAM Institute of Science, Visakhapatnam 530045.India.
[2]Department of Pharmaceutics, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram District, Kerala 673 637, India.
[3]Centre for Biotechnology and PhytoPharmacognosy Research, Koundampalayam, Coimbatore, Tamil Nadu, India.
[4]Department of Pharmaceutics, Nirmala College of Health Science, Meloor, Chalakkudi, India.
[5]Department of Pharmaceutical Chemistry, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram, Kerala, India.

Corresponding Authors*: skandula@gitam.edu, pmanchar@gitam.in

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Abstract

Introduction: Steroids are significant biomolecules, such as cell membrane components and hormones, because of their biological activity. Steroid drugs show a wide range of biological significance, such as anti-inflammatory, immunosuppressant, contraceptive, and anticancer properties. There are reports of certain derivatives with anticancer or antileishmanial properties. Aim: Biotransformation of testosterone by fungus and the experimental methods: A Mini-Review. Methods: An extensive search was performed in different databases. Scopus, Embase, Cochrane Library, and PubMed. The keywords used in the search were fungal biotransformation, steroidal fungal biotransformation, and fungal biotransformation. All studies published in English and those on fungal biotransformation were included in the analysis. Results: Biological transformation of steroids is a better alternative to chemical synthesis because biocatalysts are more stereo- and regio-specific. The biotransformation of steroids is an important area of research in microbiology. Although bacteria, algae, and yeast can transform steroids, many fungi from diverse taxa transform steroids of various classes. The obtained products are important for pharmaceutical and therapeutic applications. Conclusion: This review summarizes the fungal fermentation of androstane, elaborating on testosterone biotransformation, which takes place in 17 different genera, and the experimental methods followed by various researchers in the extraction, analysis, and structural identification of products.

Keywords: Fungal biotransformation of steroids, steroid biotransformation, testosterone biotransformation by fungi, steroid derivatives, androstane biotransformation.

Introduction

Steroids are biological compounds that function as significant cell membrane components and signalling molecules (such as hormones) (Moss, 1989). Cholesterol is the precursor for steroidogenesis *in vivo*, which leads to the synthesis of distinct steroids, including pregnanes, androstanes, estranes, and corticosteroids, by microsomal and non-microsomal enzymes (Ortsäter et al., 2012).

Steroids or their derivatives are also used as drugs with a broad range of therapeutic applications and represent the highest-marketed category of pharmaceuticals after antibiotics, with an annual production of more than one million tons (Fernández-Cabezón et al., 2018). Steroid drugs have a wide range of biological activities. They have anti-inflammatory (Ko et al., 2000), antidepressants, immunosuppressant, contraceptive (Agoston et al., 2006; Fragkaki et al., 2009; Tuba et al., 2000), and cancer treatment effects (Nicolás Díaz-Chico et al., 2007; Siemes et al., 2010). The derivatives of these intermediates are more biologically significant than the starting materials (Cano-Flores et al., 2020). Steroid derivatives exhibit cytotoxicity against cancer cell lines (Choudhary et al., 2017) and leishmanicidal activity (Baydoun et al., 2014). Because of the above properties of steroids and their derivatives, they are of tremendous importance. Trade names of some of the steroid-based drugs available commercially, their generic names and importance are as follows: Cortef (hydrocortisol); Prednisone, Celestone (betamethasone), Orapred (Prednisolone Sodium Phosphate), Florinef (fludrocortisone) used for the allergic disorders, skin diseases, immune system disorders, arthritis, sclerosis and Addison disease.

Biotransformation is the biological modification of a compound’s structure, giving rise to a new blend. Steroid transformations can be achieved either biologically or chemically. Biological organisms, such as yeast (Pajič et al., 1999), fungi (Kollerov et al., 2020; Nassiri-Koopaei & Faramarzi, 2015), bacteria (Donova, 2007), and microalgae (Faramarzi et al., 2008), transform steroids enzymatically.

Steroid transformation by biocatalysts synthesises highly stereospecific and regiospecific products, which are more complicated or impossible by chemical synthesis (Cano-Flores et al., 2020). Biotransformation has an advantage over chemical synthesis because it activates non-activated carbon centres, making it more significant than the chemical synthesis method (Leslie & Jon, 2001). They occupy a small size (surface-to-volume ratio), have a high multiplication rate, and are tolerant to extreme conditions (Hegazy et al., 2015).

Microbial biotransformation reactions of steroids

Among the microbial species, fungi have a pivotal role in catalysing a wide range of different reactions with steroids. Moreover, many fungal transformations belonging to several genera have been reported in the past, especially in the last two decades (Donova et al., 2005).

Steroid biotransformations undergo reactions like hydroxylation, dehydroxylation, O-methylation, O-demethylation, glycosylation, deglycosylation, dehydrogenation, hydrogenation, and C ring cleavage, the benzo-γ-pyrone system, cyclisation, and carbonyl reduction (Andres et al., 2009; Schrewe et al., 2013; Tong & Dong, 2009).

Substrates used so far for fungal biotransformation

Androstanes and their derivatives are the substrates commonly used in biotransformation studies. Transformations of androstanes examined from fungi and their end products are in Table 1. Besides, few fungal species can produce transformed steroid products from testosterone. The majority of them belonged to the phylum Ascomycota, Zygomycota, and Basidiomycota. They were either isolated and purified from soil or obtained from culture collection centres. Testosterone transformations were reported in 17 different genera: *Absidia*, *Aspergillus*, *Beauveria*, *Botryosphaerica*, *Botrytis*, *Cladosporium*, *Colletotrichum*, *Curvuluria*, *Fusarium*, *Mucor*, *Pencillium*, *Phanerochaete*, *Rhizopus*, *Pleurotus*, *Thamnostylum*, *Ulocladium* and *Whatzelinia* (Table2) (Al-Aboudi et al., 2008b; Brzezowska et al., 1996; Farooq & Tahara, 2000; Ghasemi et al., 2014; Hu et al., 1995; Huszcza et al., 2005a; Huszcza & Dmochowska-Gladysz, 2003; Kolek &Swizdor,1998; Lamm et al., 2007; Mahato&Mukherjee,1984; Paneketal.,2020; Peartetal.,2013; Rahman et al.,1998a; K. E. Smith et al., 1990; Wilson et al., 1999; Yamashita et al., 1976; Yildirim et al., 2010, 2018, 2019; Yildirim & Kuru, 2016). The structures of the bio-transformed products of androstanes including testosterone are represented in Fig. 1.

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Table 1: List of Androstanes, bio-transforming fungi and their products

S. No	Androstane substrate	Fungal species	Products	Reference
1.	Androst-4-ene-3,17-dione (AD)	*Absidiagriseolla var. igachii*	7α-hydroxy-AD[1] 6β-hydroxy-AD[2] 7β-hydroxy-AD [3] 14α-hydroxy-AD [4]	(Heidary & Habibi, 2016)
2.		*Circinella muscae*	7β-hydroxy-AD [3] 6β-hydroxy-AD [2] 14α-hydroxy-AD [4]	
3.		*Trichoderma virens*	Testosterone [5]	
4.	Dehydroepiandrosterone (DHEA)	*Aspergillus niger*	androst-4-ene-3,17-dione [6] 17β-hydroxy androst-4-ene-3,16- dione [7] 16β,17β-dihydroxyandrost-4-en-3-one [8] 16β-hydroxy androst-4-ene-3,17-dione [9]	(Yamashita et al., 1976)
5.		*Aspergillus sydowii*	3β,7β-Dihydroxyandrost-5-en-17-one [10] 3β,7α-Dihydroxyandrost-5-en-17-one [11] 6β-Hydroxyandrost-4-en-3,17-dione [12]	(Yildirim & Kuru, 2016)
6.		*Beauveria bassiana*	5-androsten-3,11α,17-triol [13] 7α-hydroxy dehydroepiandrosterone [14] Androstenediol [15]	(Huszcza et al., 2005)
7.		*Beauveria caledonica*	11α- hydroxyandrost-3,7,17-trione [16] 3β,7α-dihydroxyandrost-5-en-17-one [17] 3β,11α-dihydroxyandrost-5-en-7,17-dione [18] 3β- hydroxy androstan -7,17-dione [19] 3β,7β-dihydroxyandrost-5-en-17-one [10] 3β-hydroxyandrost-5-en-7,17-dione [21]	(Kozłowska, Urbaniak, et al., 2018)
8.		*Isariafumosorosea*	3β,7α-dihydroxyandrost-5-ene-17-one [11] 3β,7β-dihydroxyandrost-5-ene-17-one [10] 3β-hydroxy androst-5-ene-7,17-dione [21] 3β,7β-dihydroxy-17a-oxa-d-homo-androst-5-ene-17-one [25] 3β,7α-dihydroxy-17a-oxa-d-homo-androst-5-ene-17-one [26]	(Ewa Kozłowska et al., 2017)

9.		*Mucor recemosus*	7α-hydroxy-dehydroepiandrosterone [14] 7β-hydroxy-dehydroepiandrosterone [29]	(Li et al., 2005)
10.		*Spicaria fumosorosea*	7α-OH-DHEA [30] 7β-OH-DHEA [31] 3β,7β-dihydroxy-17a-oxa-D-homoandrost-5-en-17-one [32]	(Lobastova et al., 2015)
11.		*Aspergillus versicolor*	androst-4-ene-3,17-dione (AD) [6] diene-3,17-dione (6βOH-ADD) [34] 6β-hydroxyandrost-4-ene-3,17-dione (6βOH-AD) [35]	(Kozłowska et al., 2017)
12.		*Fusarium acuminatum*	3β,7α-dihydroxyandrost-5-ene17-one (7α-OH-DHEA) [36]	
13.		*Mucor hiemalis*	3β,7α-dihydroxyandrost-5-ene-17-one (7αOH-DHEA) [36] androst-5-ene-3β,7α,17α-triol [37] 3β-hydroxyandrost-5-ene-7,17-dione (7Oxo-DHEA) [38]	
14.		*Pencillium chrysogenum*	17α-oxa-D-homo-androst-4-ene-17-one [39] androst-4-ene-3,17-dione (AD) [6]	
15.		*Pencillium commune*	17α-oxa-D-homoandrost-4-ene-17-one [39] 3β-hydroxy-17α-oxa-D-homo-androst-5-ene17-one (3βOH-lactone) [41]	
16.	Androstenediol (3β,17β-dihydroxyandrost-5-ene)	*Aspergillus niger*	17β-hydroxyandrost-4-en-3-one [42] 17β-hydroxyandrost-4-ene-3,16-dione [7]	(Peart et al., 2012)
17.	Oxymetholone	*Aspergillus niger*	17βhydroxy-2α-(hydroxymethyl)-17α-methyl-5α-androstan-3-one [44] 2α-(hydroxymethyl)-17α methyl-5α-androstan-3β,17β-diol [45]	(Khan et al., 2012)
18.	17α-methyltestosterone	*Absidia coerulea*	17α-methyl-6-dehydrotestosterone [46] 7α-hydroxy-17 α –methyltestosterone [47]	(Brzezowska et al., 1996)
19.		*Beauveria bassiana*	11α-hydroxy-17α-methyltestosterone [50]	(Huszcza et al., 2005)
20.		*Isaria fumosorosea*	15β-hydroxy-17α-methyltestosterone [51] 6β-hydroxy-17α-methyltestosterone [52] 6β,12β-dihydroxy-17α-methyltestosterone [53]	(Ewa Kozłowska et al.,

				2017)
21.	1-dehydrotestosterone (Boldenone)	*Absidia coerulea*	14α-hydroxy-l-dehydrotestosterone [48] 1,4,14-androstatriene-3-one [49]	(Brzezowska et al., 1996)
22.		*Beauveria bassiana*	11α-hydroxy-1-dehydrotestosterone [54] 11α-hydroxyandrost-1,4-diene-3,17-dione [55] 11α-hydroxytestosterone [56] 11α-hydroxyandrost-4-ene-3,17-dione [57]	(Huszcza et al., 2005)
23.	Drostanolone enanthate	*Cephalosporium aphidicola*	2-methylandrosta-14α-hydroxy-1,4-diene-3,17-dione [58] 2-methylandrosta-11α-hydroxy-1, 4-diene-3,17-dione [59] 2α-methyl-7α-hydroxy-5α-androstan-3,17-dione [60] 2α-methyl-3α,17β-dihydroxy-5α-androstane [61] 2-methylandrosta-1, 4-diene-3,17-dione [62] 2α-methyl-3α,14α,17β-trihydroxy-5α-androstane [63]	(Choudhary et al., 2017)
24.		*Fusarium lini*	2α-methyl-7α-hydroxy-5α-androstan-3,17-dione [60] 2α-methyl-3α,17β-dihydroxy-5α-androstane [61] 2-methylandrosta-1, 4-diene-3,17-dione [62] 2-methyl-5α-androsta-7α-hydroxy-1-ene-3,17-dione [64] 2α-methyl-5α-androsta-17β-hydroxy-3-one [65]	
25.	Testosterone enanthate	*Circinella muscae*	8β,14α-dihydroxytestosterone [66] 6β-hydroxytestosterone [67] 9α-hydroxytestosterone [68]	(Zoghi et al., 2019)
26.	Methasterone	*Cunninghamella blakesleeana*	7α,17β-Dihydroxy-2α,17α-dimethyl-5α-androstane-3-one [69] 7α,16β,17β-Trihydroxy-2α,17α-dimethyl-5α-androstane-3-one [70] 5α,12β,17β-Trihydroxy-2α,17α-dimethylandrostane-3-one [71] 7α,12β,17β-Trihydroxy-2α,17α-dimethyl-5α-androstane-3-one [72] 7α,9α,17β-Trihydroxy-2α,17α-dimethyl-5α-androstane-3-one [73]	(Ahmad et al., 2017)
27.		*Fusarium lini*	6β,17β-Dihydroxy-2,17α-dimethylandrosta-1,4,14-triene-3-one [74]	

			15α,17β-Dihydroxy-2,17α-dimethylandrosta-1,4-diene-3-one [75] 6β,17β-Dihydroxy-2,17α-dimethylandrosta-1,4-diene-3-one [76] 14α,15α-Dihydroxy-2,17-dimethylandrosta-1,4,16-triene-3-one [77] 17β-Hydroxy-2,17α-dimethylandrosta-1,4-diene-3,6-dione [78] 17β-Hydroxy-2,17α-dimethylandrosta-1,4-diene-3-one [79]	
28.		*Macrophomina phaseolina*	17β-Hydroxy-17α-(hydroxymethyl)-2α-methyl-5α-androstane-3,6-dione [80]	
29.	Oxandrolone	*Cunninghamella blakesleeana*	12β,17β-dihydroxy-17α-methyl-2-oxa-5α-androstan-3-one [81]	(C. Smith et al., 2015)
30.		*Macrophomina phaseolina*	11β,17β-dihydroxy-17α-(hydroxymethyl)-2-oxa-5α-androstan-3-one [82] 5α,11β,17β-trihydroxy-17α-methyl-2-oxa-androstan-3-one [83] 17β-hydroxy-17α-methyl-2-oxa-5α-androstan-3,11-dione [84] 11β,17β-dihydroxy-17α-methyl-2-oxa-5α-androstan-3-one [85]	
31.	Adrenosterone	*Isaria farinosa*	6β-hydroxyandrost-4-ene-3,11,17-trione [27]	(Kozłowska, Hoc, et al., 2018)
32.		*Isaria fumosorosea*	6β-hydroxyandrost-4-ene-3,11,17-trione (6β-OH-Adr) [27]	(Ewa Kozłowska et al., 2017)
33.	19-Hydroxyandrostenedione	*Pencillium vinaceum*	19-Hydroxyandrostenedione [86] 17β,19-dihydroxyandrost-4-en-3-one [87]	(Panek et al., 2020)
34.	17β-hydroxyandrost-1,4,6-triene-3-one	*Isaria farinosa*	15α,17β-dihydroxy-6β,7β-epoxyandrost-1,4-diene-3-one [88] 15α,17β-dihydroxy-6β,7β-epoxyandrost-4-ene-3-one [89] 14α,17β-dihydroxy-6β,7β-epoxyandrost-1,4-diene-3-one	(Kozłowska, Hoc, et al., 2018)

			[90]	
35.	5α-androstan-3-one,	*Cephalosporium aphidicola*	17β-hydroxyandrostan-3- one [91]	(Hanson & Nasir, 1993)
36.	5α-androstan-17-one,	*Cephalosporium aphidicola*	3α,17β-dihydroxyandrostan-6-one[92] 17β-hydroxyandrost-4-en-3,6-dione [93] 3β,5α-dihydroxyandrostan-6,17-dione[94] 3β,5α, l7β-trihydroxyandrostan-6-one [95]	
37.	5α-androstan-3,6-dione,	*Cephalosporium aphidicola*	17β-hydroxyandrost-4-en-3,6-dione [93] 3α,17β-dihydroxyandrostan-6-one [92] 3β,5α-dihydroxyandrostan-6,17-dione [94] 3β,5α,17β-trihydroxyandrostan-6-one [95]	
38.	5α-androstan-3,17-dione	*Cephalosporium aphidicola*	17β-acetoxy-5α-androstan-3-one [96] 17β-hydroxy-5α-androstan-3-one [91]	
39.	Androsta-1,4-diene-3,17-dione (ADD)	*Aspergillus brasiliensis*	17β-hydroxyandrost-1,4-dien-3-one [97] 11α-hydroxyandrost-1,4-diene-3,17-dione [98] 12β-hydroxyandrost-1,4-diene-3,17-dione [99]	(Hosseina badi et al., 2014)

Table 2: Biotransformation of Testosterone by fungi

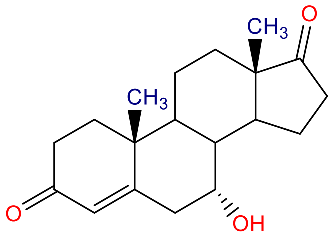
S No	Fungal species	Biotransformation product	Reference
1	*Aspergillus niger*	androst-4-ene-3,17-dione [6] 17β-hydroxyandrost-4-ene-3,16-dione [43] 16β-hydroxyandrost-4-ene-3,17-dione [9] 16β,17β-dihydroxyandrost-4-en-3-one [8] 16β,17α-dihydroxyandrost-4-en-3-one [33]	(Yamashita et al., 1976)
2	*Aspergillus fumigatus*	15 β-hydroxytestosterone [40]	(Mahato & Mukherjee, 1984)

3	*Botryosphaerica obrusa*	6β-Hydroxyandrostenedione [100] 6β-Hydroxytestosterone [67] 7α-Hydroxytestosterone [101] 7β-Hydroxytestosterone [102] 11β-Hydroxytestosterone [103] 12β-Hydroxytestosterone [104] 15α –Hydroxyandrostenedione [105] l5α -Hydroxytestosterone [106] 6β,7β-Dihydroxyandrostenedione [107]	(K. E. Smith et al., 1990)
4	*Mucor griseocyanus*	14α-hydroxytestosterone [108] 14α-hydroxyandrost-4-ene-3,17-dione [109]	(Hu et al., 1995)
5	*Thamnostylum piriforme*	6β-hydroxyandrost4-ene-3,17-dione [110] 9α-hydroxytestosterone [68] 14α-hydroxytestosterone [108]	
6	*Absidia coerulea*	14α-hydroxytestosterone [108] 14α-hydroxyandrostenedione [112]	(Brzezowska et al., 1996)
7	*Fusarium culmorum*	6β –hydroxyandrostenedione [100] 6β –hydroxytestosterone [67] 15α -hydroxyandrostenedione [105] 15α –hydroxytestosterone [106]	(Kolek & Swizdor, 1998)
8	*Curvuluria lunata*	17- dehydrotestosterone [6]	(Rahman et al., 1998)
9	*Pleurotus oestreatus*	15α-hydroxytestosterone [106]	
10	*Colletotrichum musae*	Androst-4-ene-3,17-dione [6]	(Wilson et al., 1999)
11	*Fusarium oxysporum var cubense*	Androst-4-ene-3,17-dione [6] 15α-Acetoxyandrost-4-ene-3,17-dione [113] 6β,17β-Diacetoxyandrost-4-en-3-one [114] 15α,17β-Diacetoxyandrost-4-en-3-one [115]	
12	*Botrytis cinerea*	7β,17β -dihydroxyandrostan-3-one [20]	(Farooq & Tahara, 2000)

13	*Absidia glauca*	7α-hydroxyandrostenedione [22] 6β,11α-dihydroxy androstenedione [23]	(Huszcza & Dmochowska-Gladysz, 2003)
14	*Beauveria bassiana*	11α-hydroxytestosterone [56] 5α-androstan-11α,17-diol-3-one [28] 11α-hydroxyandrost-4-ene-3,17-dione [111] 5α-androstan-11α-ol-3,17-dione [43]	(Huszcza et al., 2005)
15	*Phanerochaete chrysosporium*	6β,17β-Dihydroxyandrost-4-en-3-one [116]	(Lamm et al., 2007)
16	*Mucor plumbeus*	6β,17β-Dihydroxyandrost-4-en-3-one [116] 14α,17β-Dihydroxyandrost-4-en-3-one [117]	
17	*Whetzelinia sclerotiorum*	Androst-4-ene-3,17-dione [6] 6β,17β -Dihydroxyandrost-4-en-3-one [116] 2β,17β -Dihydroxyandrost-4-en-3-one [118] 2β,16β,17β -Trihydroxyandrost-4-en-3-one [119]	
18	*Fusarium lini*	Androst-1,4-dien-3,17-dione [120] 17β-Hydroxyandrost-1,4-dien-3-one [121] 11α-Hydroxyandrost-1,4-dien-3,17-dione [122] 11α,17β-Dihydroxyandrost-4-en-3-one [56] 11α,17β-Dihydroxyandrost-1,4-dien-3-one [123]	(Al-Aboudi et al., 2008)
19	*Rhizopus stolonifer*	Androst-4-en-3,17-dione [6] Testolactone [124] 17β-Hydroxy-5α-androstan-1,6-dione [125] 11α-Hydroxyandrost-4-en-3,17-dione [126] 11α-Hydroxytestolactone [127]	
20	*Pencillium digitatum*	5α-androstane-3,17-dione [128] 3α-hydroxy-5α-androstan-17-one [129] 3β-hydroxy-5α-androstan-17-one [130] androst-4-ene-3,17-dione [6]	(Yildirim et al., 2010)

21	*Aspergillus niger*	17β-hydroxyandrost-4-ene-3,16-dione [7] 16β-hydroxyandrost-4-ene-3,17-dione [9] 16β,17β-dihydroxyandrost-4-en-3-one [8] 16β,17α-dihydroxyandrost-4-en-3-one [33] 6β,17β -dihydroxyandrost-4-en-3-one [131]	(Peart et al., 2013)
22	*Rhizopus oryzae*	6β-hydroxyandrost-4-ene-3,17-dione [110] 6β,17β-dihydroxyandrost-4-en-3-one [131] 1β,17β-dihydroxyandrost-4-en-3-one [132] 7α,17β-dihydroxyandrost-4-en-3-one [133] 11α,17β-dihydroxyandrost-4-en-3-one [134] 6β,11α,17β-trihydroxyandrost-4-en-3-one [135]	
23	*Mucor plumbeus*	14-hydroxyandrost-4-ene-3,17-dione [109] 6β,17β -dihydroxyandrost-4-en-3-one [131] 14,17-dihydroxyandrost-4-en-3-one [136] 6,14-dihydroxyandrost-4-ene-3,17-dione [137] 15,17-dihydroxyandrost-4-en-3-one [138] 6,14,17-trihydroxyandrost-4-en-3-one [139] 6β -hydroxyandrost-4-ene-3,17-dione [110]	
24	*Absidia chrysogenu*	androst-4-ene-3,17-dione [6] 14α-hydroxy testosterone [108]	(Ghasemi et al., 2014)
25	*Fusarium fujikuroi*	androst-4-ene-3,17-dione [6]	(Ghasemi et al., 2014)
26	*Aspergillus sydowii*	6β, 17β-Dihydroxyandrost-4-en-3-one [116] 15α,17β-Dihydroxyandrost-4-en-3-one [138] 14α,17β-Dihydroxyandrost-4-en-3-one [117]	(Yildirim & Kuru, 2016)
27	*Ulocladium chartarum*	12β,17β-Dihydroxyandrost-4-ene-3-one [140] 5α-Androstane-3,6,17-trione [141] 17β-Hydroxy-5α-androstane-3,6-dione [142] Androst-4-ene-3,17-dione [6] 7β-Hydroxyandrost-4-ene-3,17-dione [143] 7β,17β-Dihydroxyandrost-4-ene-3-one [144] 14α,17β-Dihydroxyandrost-4-ene-3-one [145]	(Yildirim et al., 2010)

28	*Cladosporium sphaerospermum*	6β,16β,17β-trihydroxyandrost-4-en-3-one [146] 6β,12β,17β-trihydroxyandrost-4-en-3-one [147]	(Yildirim et al., 2019)
29	*Pencillium vinaceum*	Testololactone [124]	(Panek et al., 2020)



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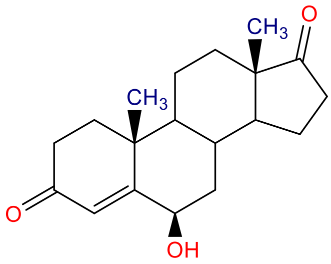

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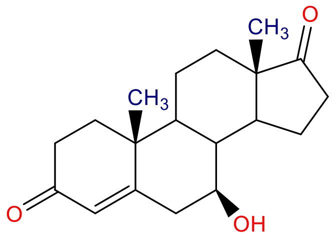

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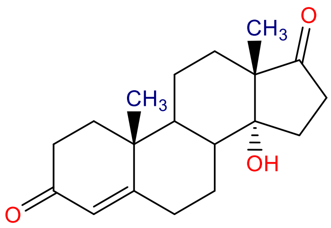

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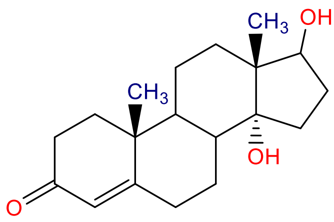

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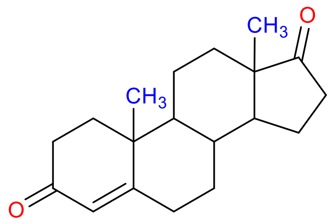

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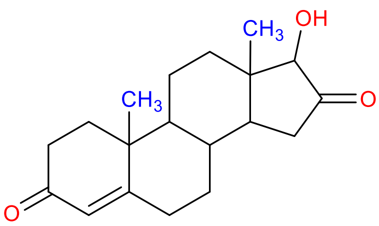

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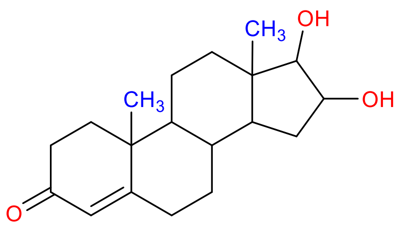

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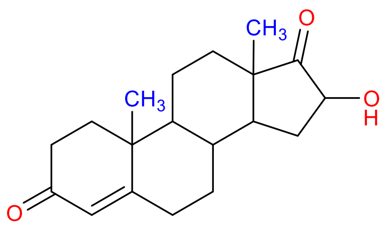

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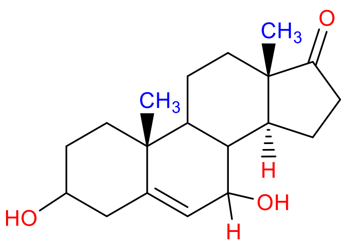

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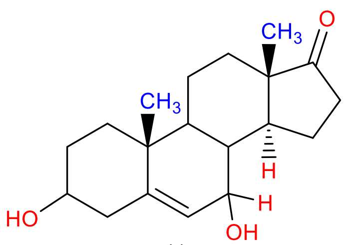

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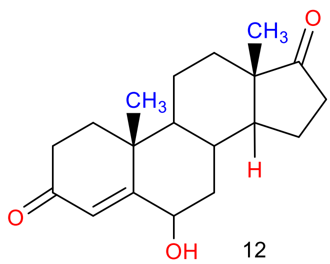

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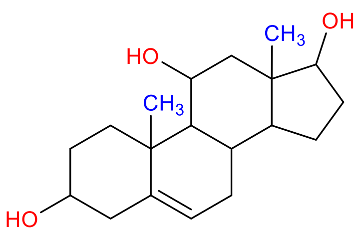

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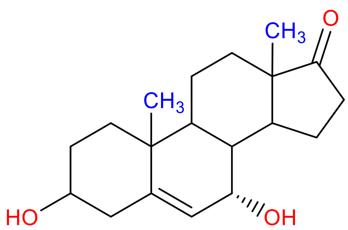

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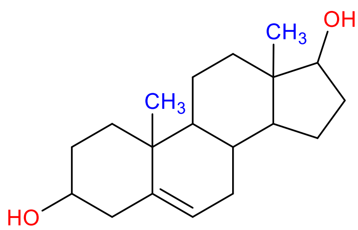

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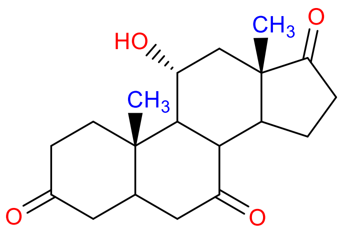
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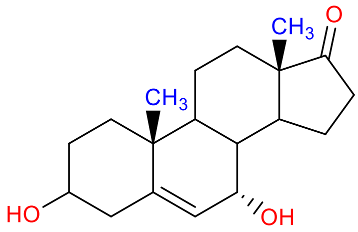
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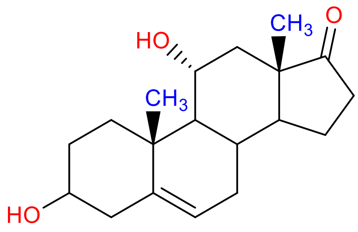
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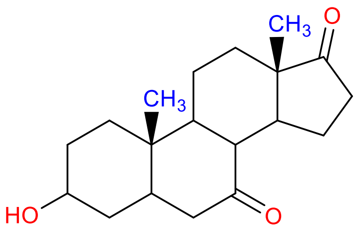
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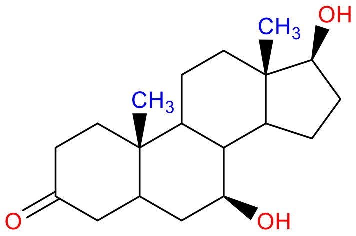
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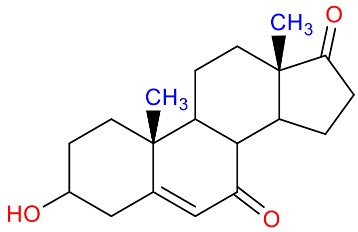
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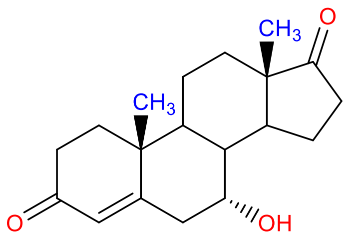
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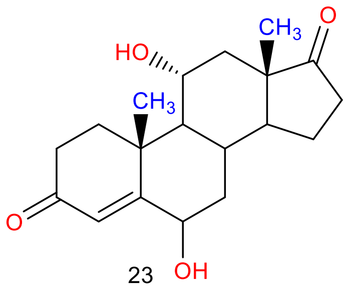
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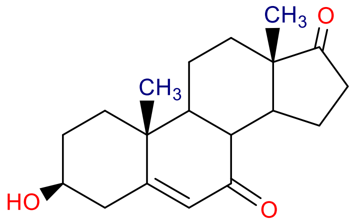
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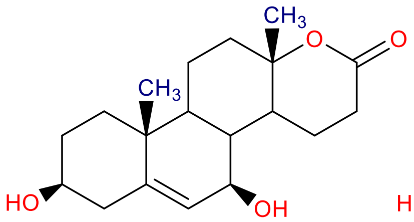
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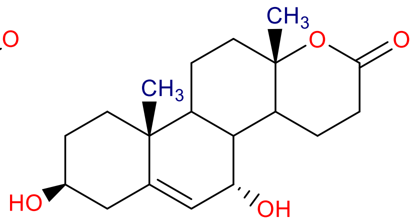
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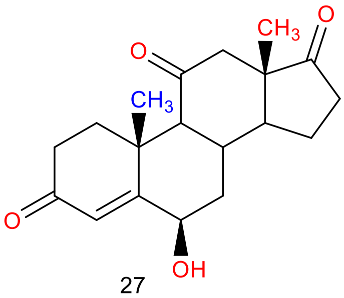
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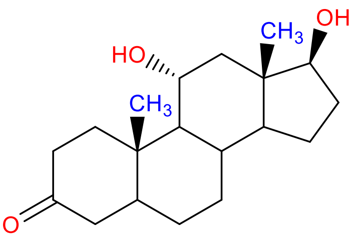
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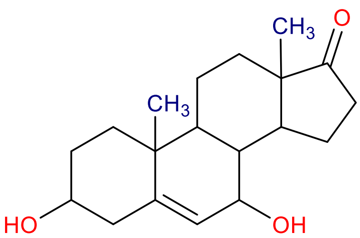
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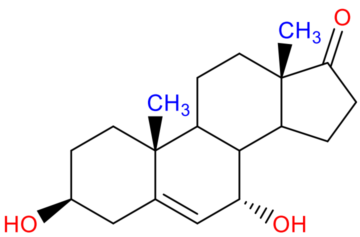
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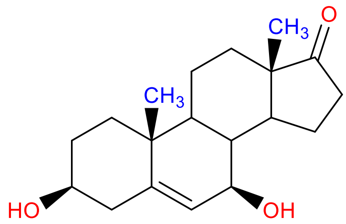

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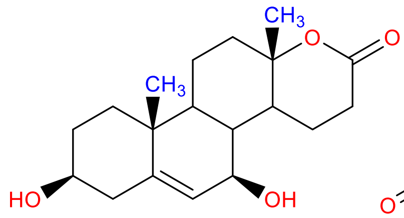

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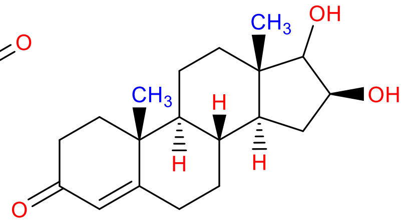

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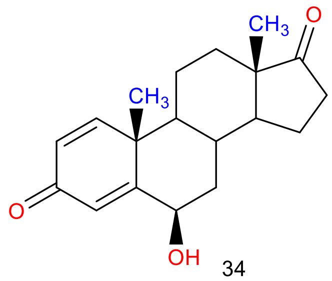

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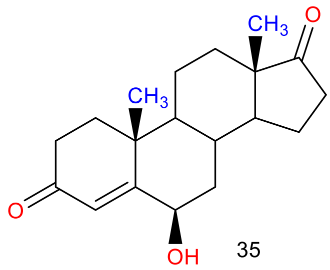

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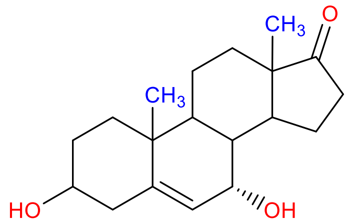

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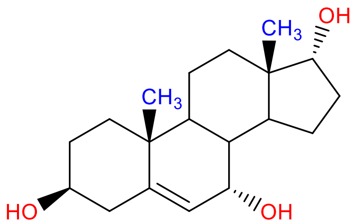

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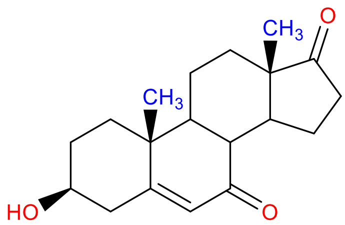

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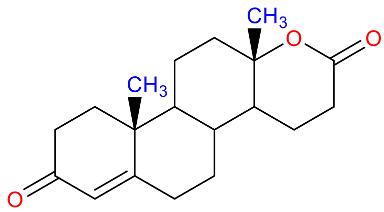

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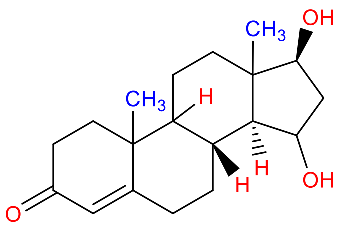

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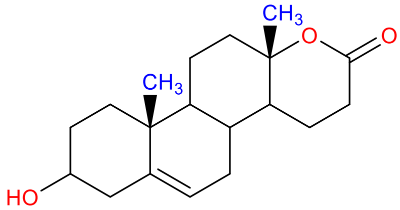

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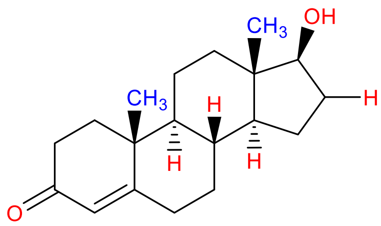

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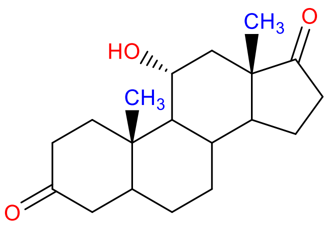

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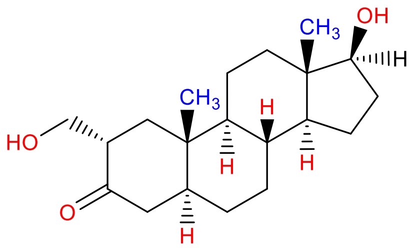

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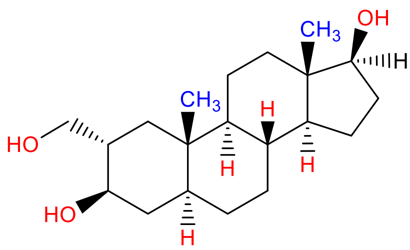

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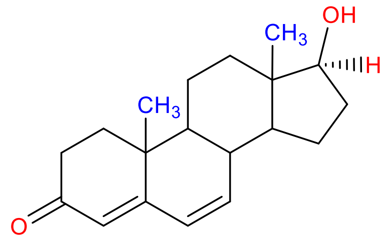

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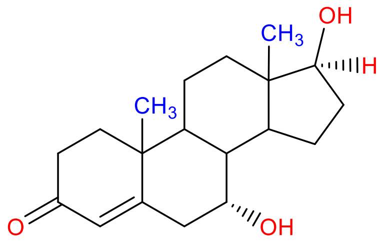

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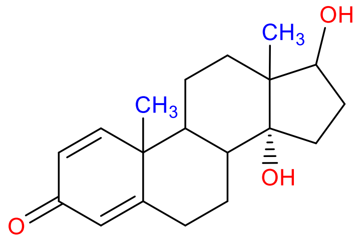

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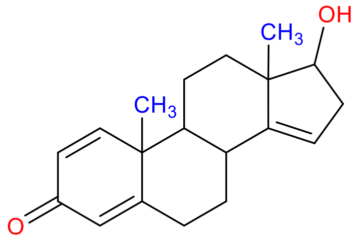

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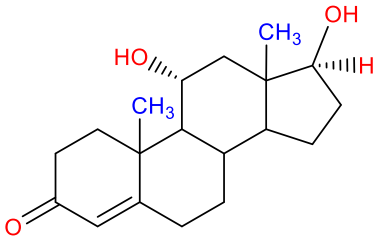

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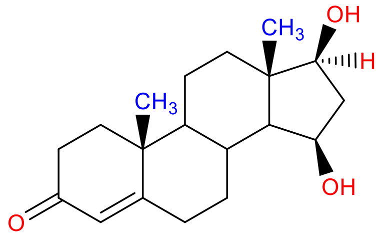

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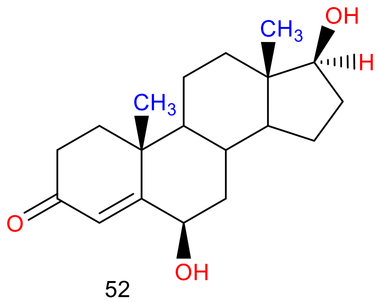

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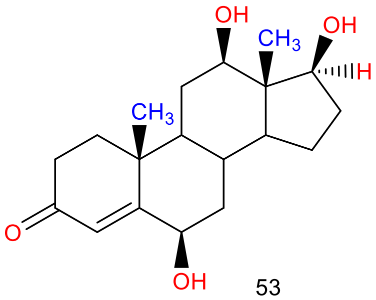

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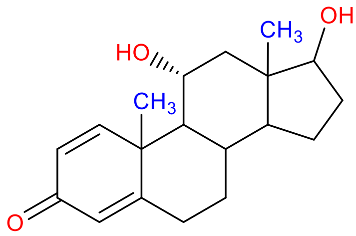

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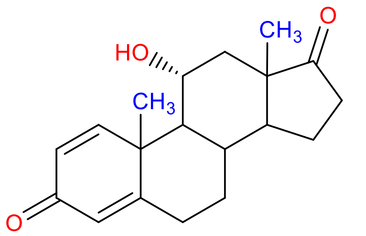

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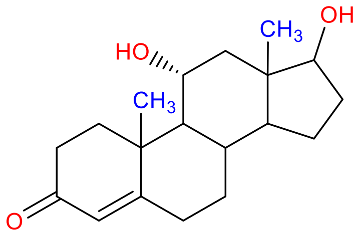

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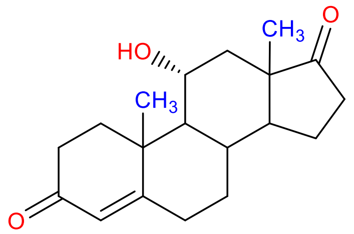

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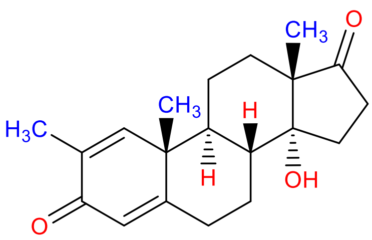

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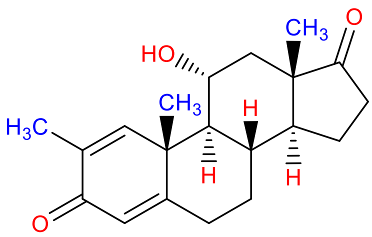

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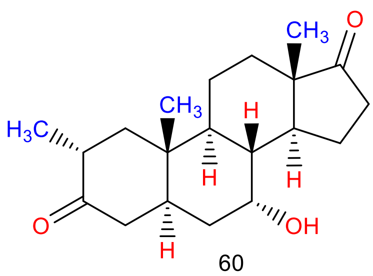

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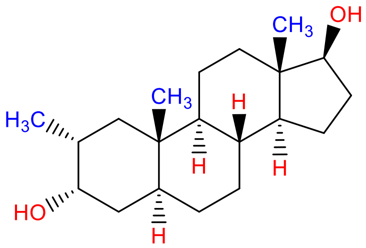
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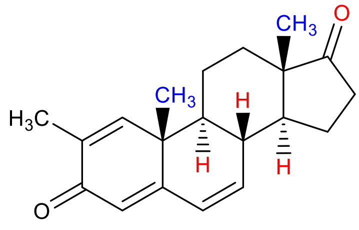
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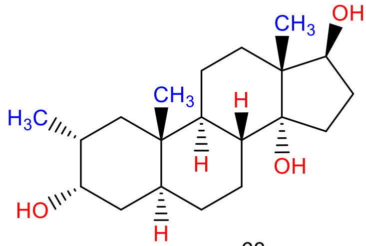
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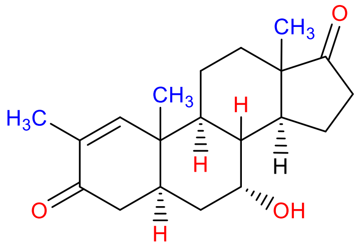
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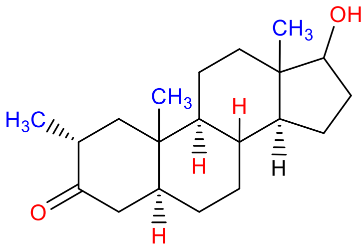
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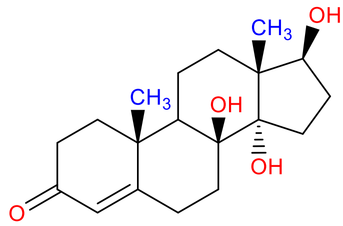
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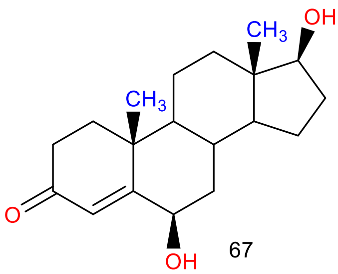
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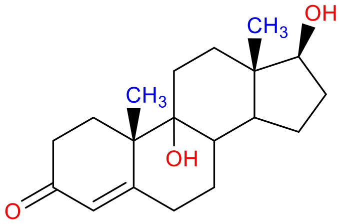
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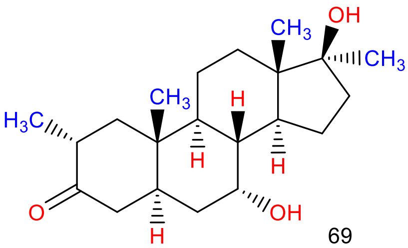
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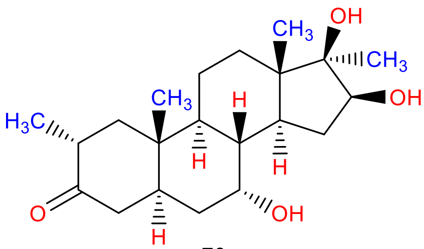
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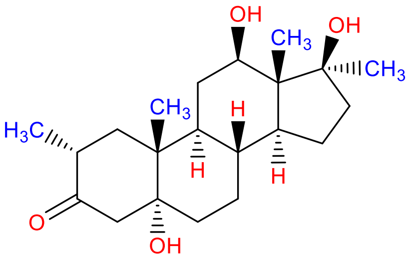
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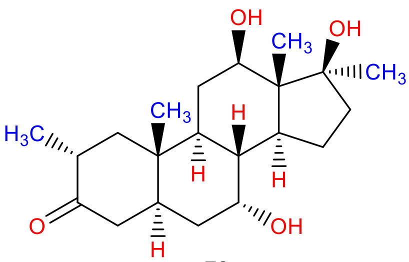
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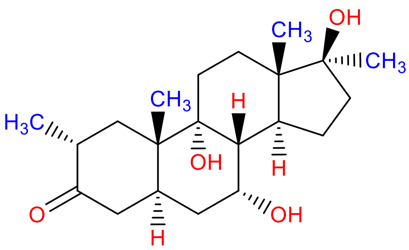
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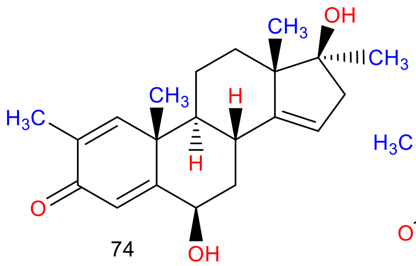

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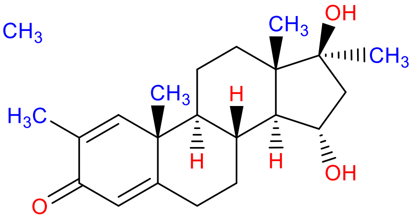

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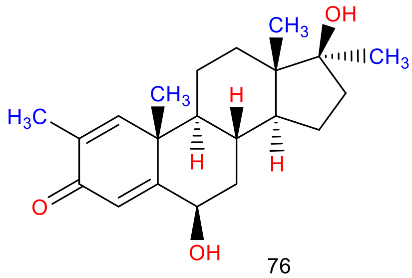

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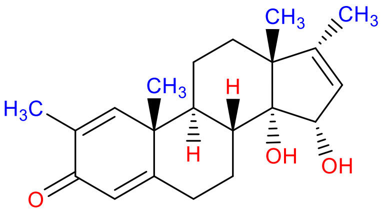

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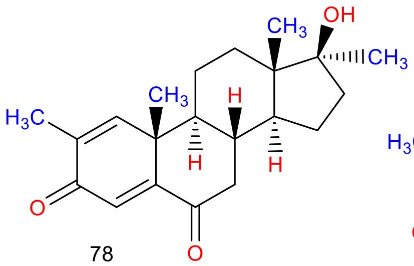

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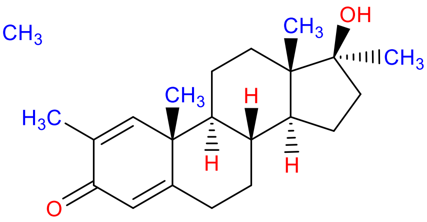

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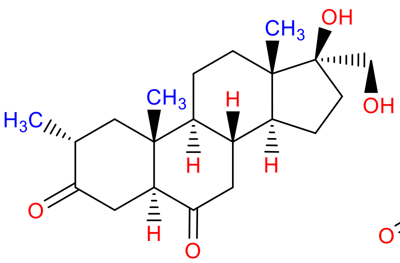

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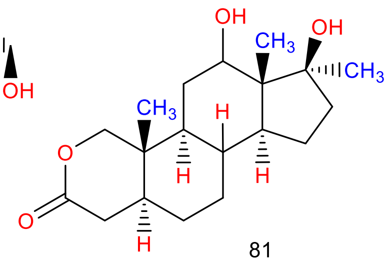

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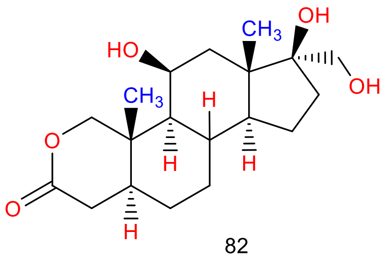

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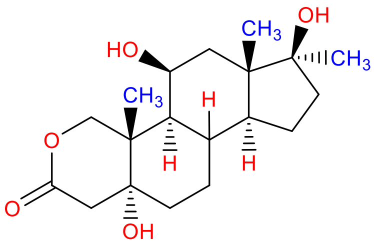

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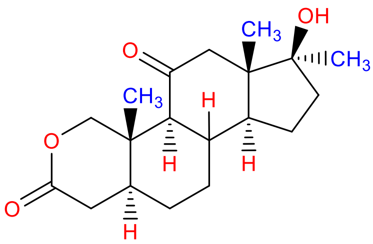

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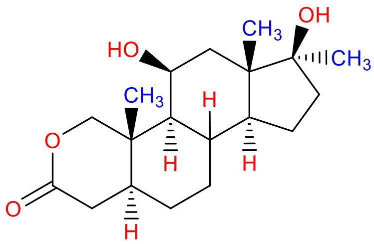

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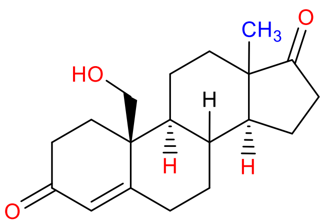

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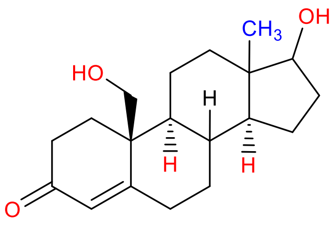

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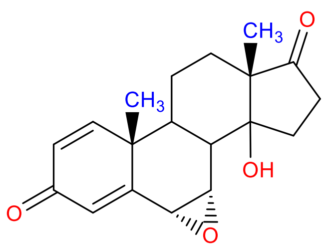

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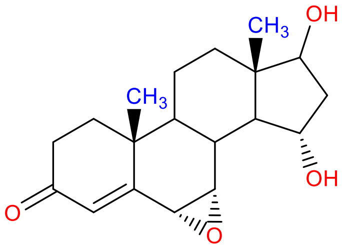
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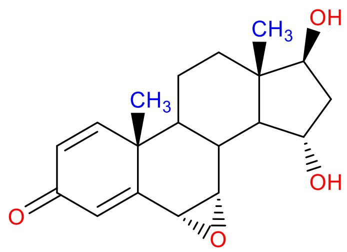
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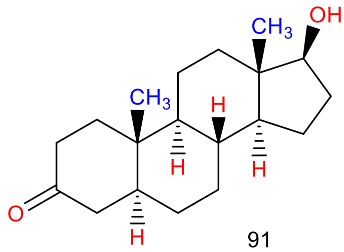
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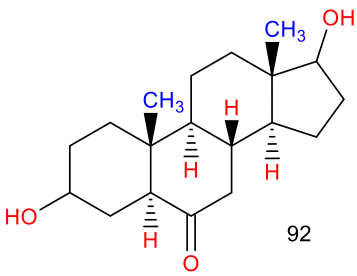
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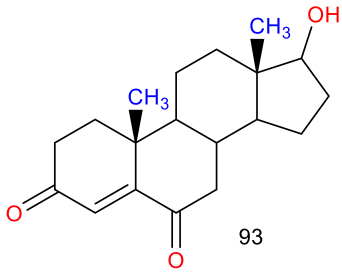
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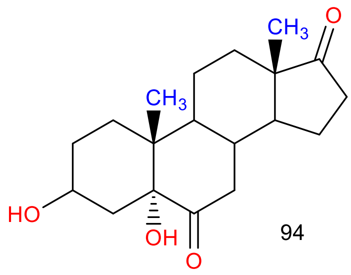
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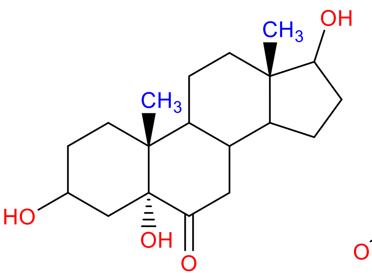
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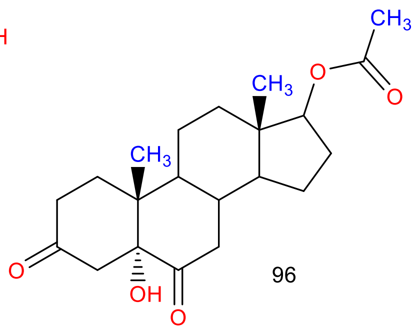
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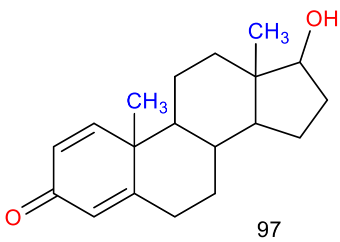
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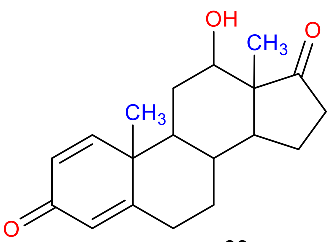
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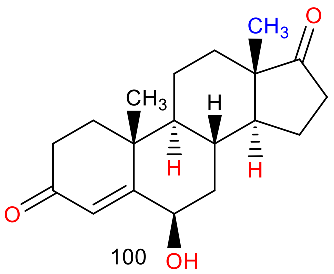
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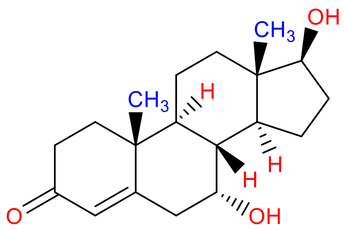
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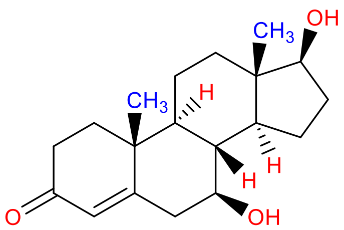
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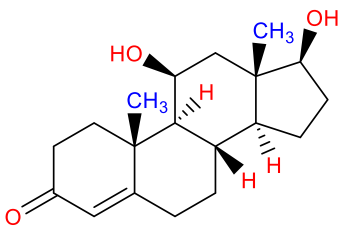
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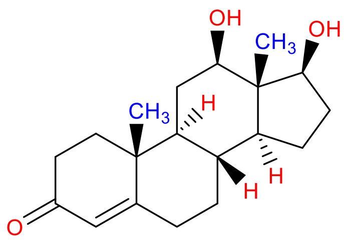
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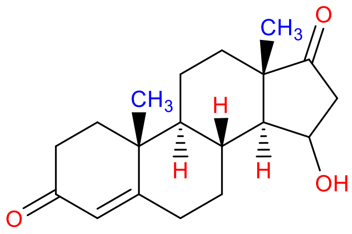

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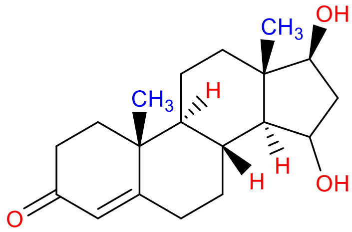

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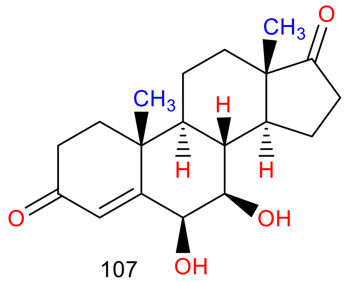

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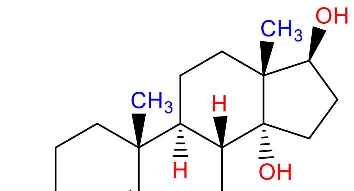

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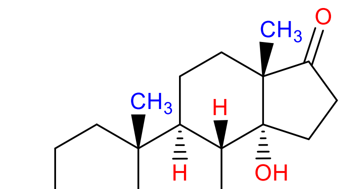

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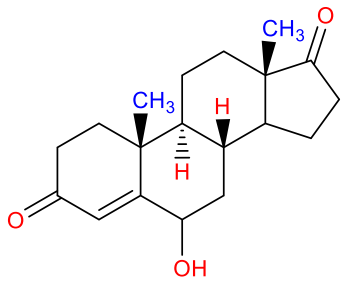

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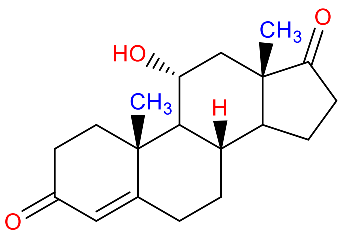

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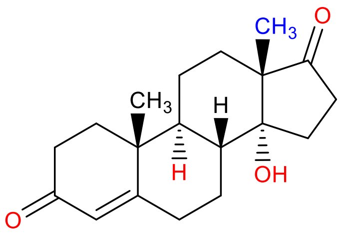

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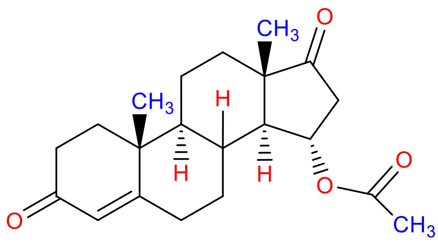

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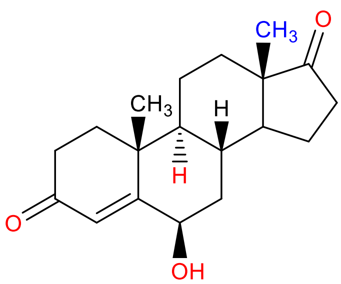

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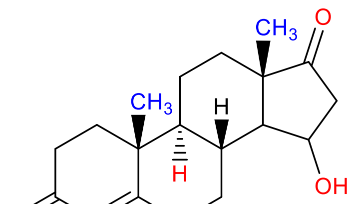

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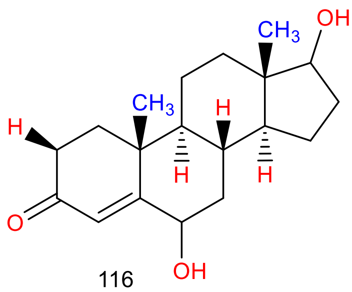

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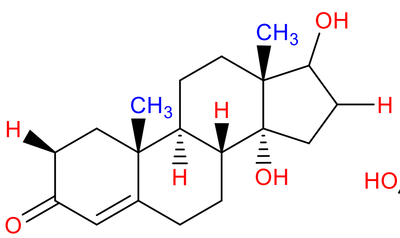

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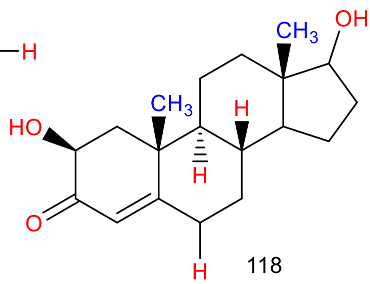

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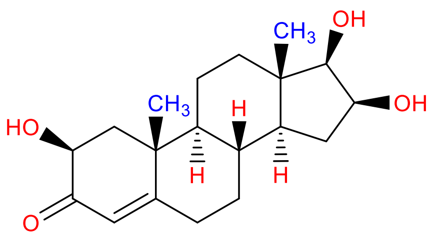

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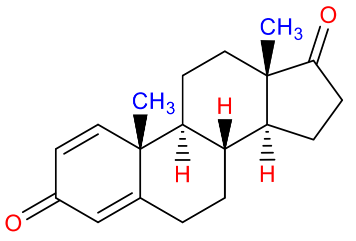

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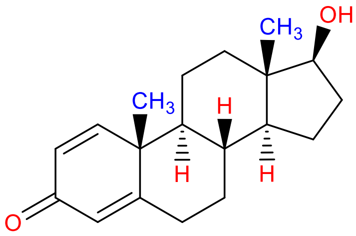

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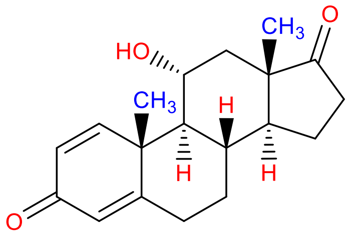

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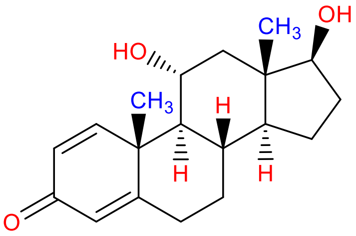

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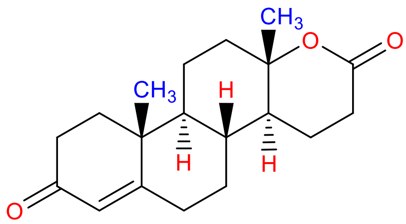

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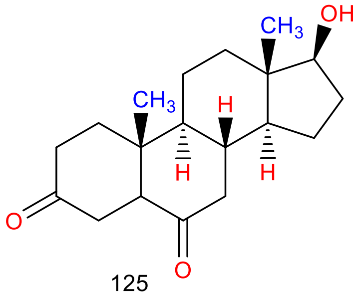

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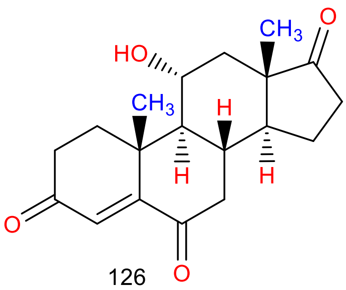

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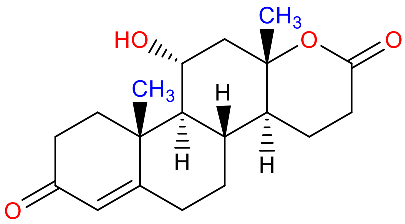

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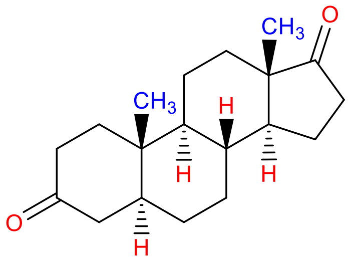

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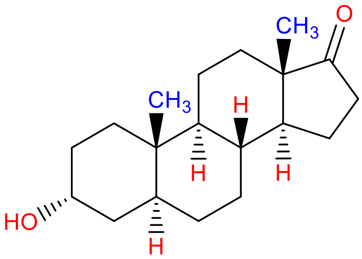

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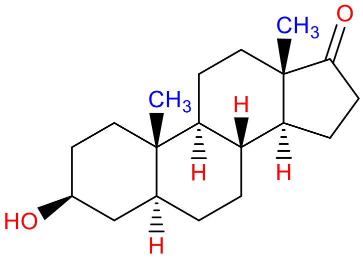

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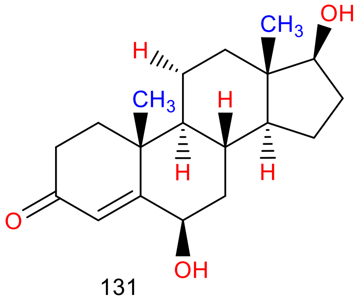

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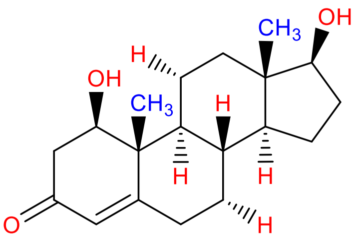

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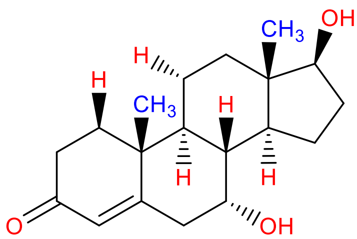

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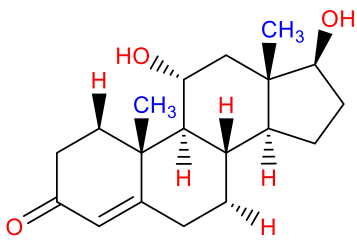

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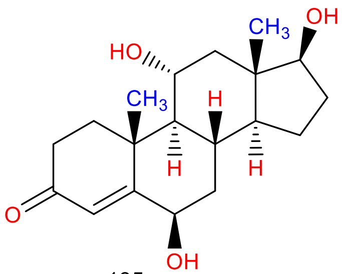

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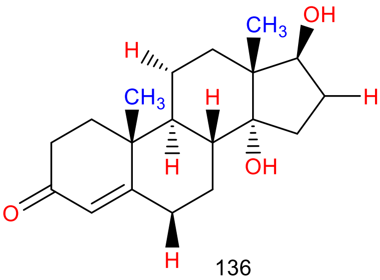

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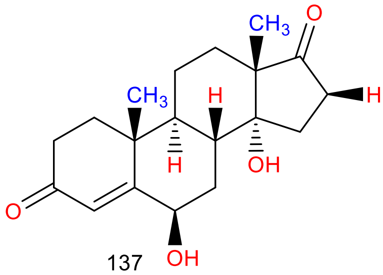

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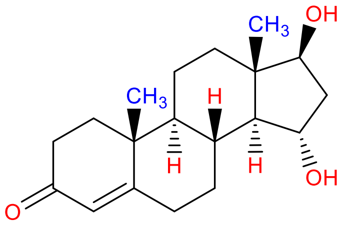

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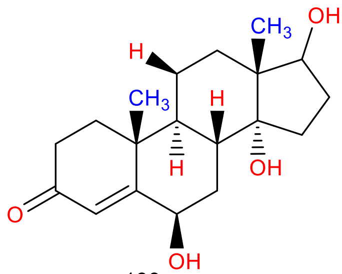

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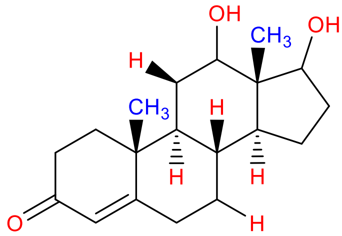

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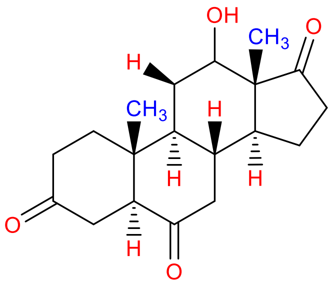

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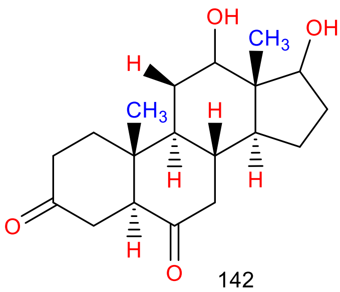

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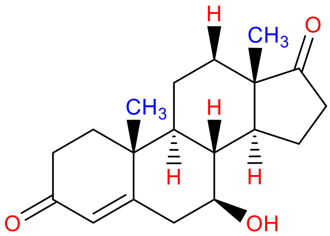

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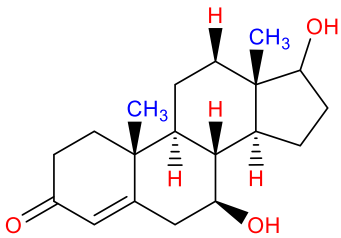

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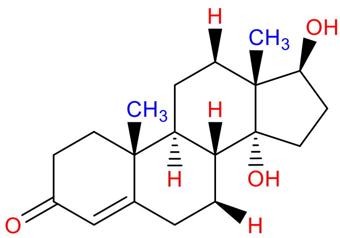

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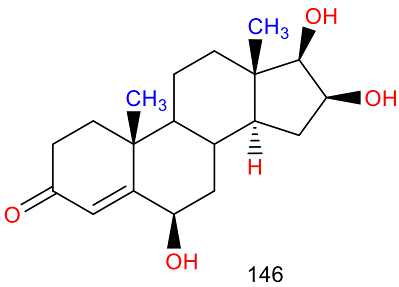

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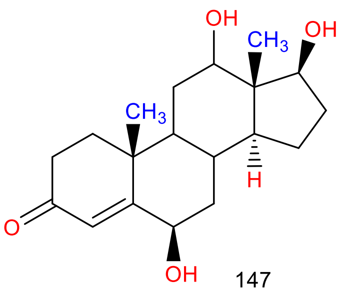

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Figure 1: The figure represents the structures of the biotransformed products of androstanes and testosterone represented in Tables 1 & 2.

Biotransformation of Testosterone

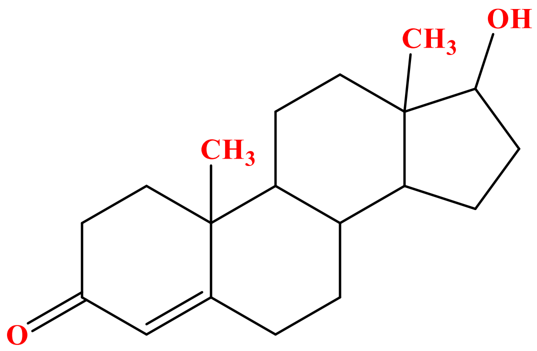

Figure 2. Structure of testosterone

Several chemical shifts occur during the fungal biotransformation reactions. In general, hydroxylations occur at 2β, 6α, 6β, 7α, 7β, 11α, 14α, 15α, 15β and 16β positions and other reactions such as oxidation followed by hydroxylation, dehydrogenation, Baeyer-Villiger lactonisation of ring-D and hydrogenation at C4 (Lamm et al., 2007). take place during testosterone (Fig. 2) biotransformation.

Fermentation

Fungal culture grows well in mediums with high carbohydrate and nitrogen sources. Commonly, 3% of glucose as a source of carbohydrate and 1% of peptone or amino bac as a nitrogen source were used. Major and minor elements such as $MgSO_4$, $NaNO_3$, K_2HPO_4, $FeSO_4$, and $ZnSO_4$ were also used to grow certain fungi such as *Acremonium chrysogenu* PTCC 5271, *Botrytis*, *R. stolonifera* and *Fusarium lini* (Al-Aboudi et al., 2008; Farooq and Tahara, 2000; Ghasemi et al., 2014a).

Addition of substrate (testosterone)

For fungal cultures grown for 72 hours, a clear and dissolved form of the substrate in absolute ethanol (Ghasemi et al., 2014), acetone (Al-Aboudi et al., 2008b), or dimethylformamide (DMF) (Yildirim & Kuru, 2016) was added to the culture flasks. Fermentation takes approximately 3–14 days. Researchers have used a single-phase pulse-feed technique for incubation (Lamm et al., 2007). The quantity of substrate used in the fermentation process varies from culture to culture. Many investigators added substrate to the culture medium ranging from 0.3 (Brzezowska et al., 1996) to 2.4 mM concentrations (Al-Aboudietal., 2008).

The products in the culture broth were analysed using qualitative methods periodically at 24, 48, 72, 96 h, and 8th day (Panek et al., 2020).

Extraction of products

Extraction of products after harvesting is a laborious and time-consuming process. Filtration or centrifugation separates the mycelium and the culture broth. The extraction of the broth with chloroform or methanol or ethyl acetate in 20 ml v/v of solvent for 150ml of liquid medium (Ghasemi et al., 2014; Brzezowska et al., 1996; Huszcza et al., 2005a; Huszcza & Dmochowska-Gladysz, 2003; Al-Aboudi et al., 2008). Anhydrous sodium sulfate (Farooq & Tahara, 2000) or magnesium sulfate (Panek et al., 2020) was added to dry the acquired filtrate. Then, the solvent was evaporated under reduced temperature and pressure, resulting in brown gum (Farooq & Tahara, 2000).

Analysis of products

The purification was done by Column Chromatography eluted with a gradient of hexane/acetone (2:3 v/v) (Panek et al., 2020). The preparative thin-layer chromatography (TLC) method was used in the purification step to elute the mixture as a single compound. A mixture of solvents used in TLC analysis as the mobile phase was hexane–acetone (2:1or1:1 v/v) (Huszcza et al., 2005a), Cyclohexane-Ethyl Acetate-Isopropanol (45:45:10 v/v), or Cyclohexane-Ethyl Acetate-Acetone (45:45:10 v/v) (Brzezowska et al., 1996). Sulfuric acid-ethanol (1:1 v/v) was sprayed, followed by heating to develop the sample mixtures. High-Performance Liquid Chromatography was used to quantify the products.

Structural identification of products

High-Resolution Mass Spectrometry (HRMS) analysis determines the molecular mass and formula of the obtained purified product (Lamm et al., 2007). Advanced spectroscopic techniques, such as electron ionisation mass spectrometry (EI-MS) and one- or two-dimensional NMR techniques coupled with Heteronuclear Multiple Bond Coherence (HMBC) and Heteronuclear Multiple Quantum Coherence (HMQC) (Ghasemi et al., 2014; Zoghi et al., 2019), elucidated the structures and determined the melting points. Deeper knowledge of the steric atoms can be obtained from 2D NMR techniques that provide information on the conformation of the protons of the molecules, such as Rotating frame Overhauser effect spectroscopy (ROESY) and nuclear Overhauser effect spectroscopy (NOESY).

Conclusion and Future Perspectives

Although the biological transformation of steroids poses many challenges, advancements in the research and evolution of the methods have made it easier to do research in this area. The cost of the substrates, insolubility of the substrates, toxicity of steroids, and solvents used to dissolve the substrates are the significant challenges commonly faced during fermentation.

Steroid biotransformation is a promising research area. The efficiency of steroid transformation in biological organisms varies from species to species and organisms under different stress conditions. Certain microbial species may yield a beneficial product higher than other species on the same substrate. Biotransformation by microbes belonging to different taxa using a range of steroid substrates must be studied to understand the applications of their products. We can determine various biological activities, such as immunosuppressive, anti-rheumatic, contraceptive, diuretic, anti-inflammatory, sedative, cancer, and other properties.

Although the biotransformation procedure involves challenging steps, we can overcome them by further research. It is vital to determine the biological activity of a product to solve clinical, livestock, and other problems.

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