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Nitrile hydratase from *Rhodococcus erythropolis*: Metabolization of steroidal compounds with a nitrile group

Günter Kaufmann[a,*], Horst Dautzenberg[b,1], Harry Henkel[a], Gerd Müller,[a] Thomas Schäfer,[b,2] Bernd Undeutsch[a], Michael Oettel[a]

[a]*Division of Research and Development, Jenapharm GmbH & Co. KG, D-07745 Jena, Germany*
[b]*Research Group Polyelectrolyte Complexes, University Potsdam, D-14513 Teltow, Germany*

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Abstract

The progestin dienogest (17α-cyanomethyl-17β-hydroxy-estra-4,9-dien-3-one) was metabolized by the nitrile hydratase-containing microorganism *Rhodococcus erythropolis*. An enzymatic hydrolysis of the nitrile group at the 17α-side chain was intended to obtain novel derivatives and to test them for progesterone receptor affinity. In contrast to the rapid enzymatic hydrolysis of nonsteroidal nitriles, the nitrile group of dienogest was cleaved very slowly. The dominant reaction was an aromatization of ring A. After prolonged fermentation, the 17α-acetamido derivatives of estradiol and of 9(11)-dehydroestradiol were formed. Three of the metabolites were also prepared synthetically. They were tested for hormonal activity by assessing their binding to progesterone and estrogen receptors in vitro. Neither the aromatized 17α-acetamido derivatives nor the dienogest derivative 17α-acetamido-17β-hydroxy-estra-4,9-dien-3-one, which was prepared synthetically only, exhibited affinity for the progesterone receptor.

Keywords: Progestin; Microbial transformation; Steroid aromatization; Nitrile hydratase; Progesterone receptor

1. Introduction

Dienogest (17α-cyanomethyl-17β-hydroxy-estra-4,9-dien-3-one) (DNG) is a progestin derived from nortestosterone but characterized by a cyanomethyl group in position 17α instead of the common ethinyl substituent. It is used as a progestin in hormonal contraception and is under development for further indications, such as endometriosis and hormone replacement therapy. DNG shows a comparatively strong progestational transformation effect on the endometrium. In estrogen-primed female rabbits (Clauberg/McPhail assay), it is about five times more active than levonorgestrel after oral administration [1]. Using the so-called Kaufmann assay, the transformation dose in estrogen-primed postmenopausal women was estimated. The total dose of 6.3 mg of DNG administered over 14 days resulted in a complete secretory transformation of the endometrium. In this assay, the oral progestational activity of DNG was 2 and 7 times that of chlormadinone acetate and medroxyprogesterone acetate, respectively [2]. On the other hand, DNG exhibits only moderate binding affinity for the progesterone receptor, about 10% of that of progesterone [3,4]. This discrepancy has induced speculations that dienogest may be a prodrug, a metabolite of which may be responsible for its high biologic efficacy. Such is the case with desogestrel, for example. In this regard, a number of metabolites and chemically derived compounds were tested but found to be less active than dienogest [4]. Until now, no derivatives with a metabolized 17α-cyanomethyl side chain have been included in such studies. Thus, it was interesting to find possible means of obtaining such derivatives, e.g. by enzymatic hydrolysis of the nitrile function, and to test them for progesterone receptor affinity.

The broad variety of microbial reactions available opens the possibility of performing difficult reactions and preparing the corresponding products. Microorganisms containing nitrile hydratase can transform a variety of aliphatic and aromatic nitriles to the corresponding amides [5,6] and

* Corresponding author. Jenapharm GmbH & Co KG, Otto-Schott-Str. 15, D-07745 Jena, Germany.

[1] Dr. Horst Dautzenberg, head of the Research Group Polyelectrolyte Complexes, University Potsdam, died in August 1998 while this paper was in preparation.

[2] Present address: Morus-Verlag GmbH, Götzstr. 65, D-12099 Berlin, Germany.

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further to the corresponding acids through the use of amidase. Alternatively, microorganisms containing nitrilase can hydrolyze nitriles directly to acids and ammonia [7,8]. Here, a microbial transformation of DNG to the acetamido compound was tried with *Rhodococcus erythropolis*. Because the fermentation was only partially successful, the potential metabolites were also prepared synthetically and tested for their hormonal activity in vitro.

2. Experimental

2.1. Steroids

Dienogest (STS 557) is a product of Jenapharm. The aromatic steroids STS 433 (later: J 540), STS 528, and J 1201 were synthesized by Ponsold and coworkers [9–11] and by Jenapharm, respectively.

2.2. Synthesis of the 17α-acetamide compounds

2.2.1. J 1113 **(I)**

Sodium hydroxide solution (6 M, 12 ml) and tetrabutylammonium chloride (0.9 g, 2.3 mmol) were added to a solution of 17α-cyanomethyl-17β-hydroxy-estra-4,9-dien-3-one (2.7 g, 8.7 mmol) in methylene chloride (40 ml). Hydrogen peroxide (30%, 15 ml) was added dropwise with vigorous stirring at room temperature to the two-phase system. The two phases were separated after 3 h of stirring at room temperature. The organic phase was washed with sodium hydrogensulphite solution and water, dried over Na_2SO_4, and evaporated. Crystallization from ethanol/water gave 17α-acetamido-17β-hydroxy-estra-4,9-dien-3-one **(I)**, m.p. 233–240°C (87% yield).

2.2.2. J 1358 (formula see **IIIa**)

17α-Acetamido-17β-hydroxy-estra-4,9-dien-3-one (0.5 g, 1.5 mmol) was suspended in methylene chloride (20 ml). 3-Chlorperbenzoic acid (420 mg, 2.4 mmol) was added to the suspension at room temperature. Sodium hydrogensulphite solution was added to the reaction mixture after 4 h and stirred again. The organic phase was washed with diluted sodium hydroxide solution and water, dried, and evaporated. The residue obtained was dissolved in methanol (10 ml). After addition of sodium hydroxide solution (2 M, 6 ml), the reaction mixture was stirred for 2 h at room temperature. The 17α-acetamido-estra-1,3,5(10),9(11)-tetraene-3,17β-diol was precipitated by addition of HCl (6 M, 2.5 ml). The compound was purified by column chromatography and by crystallization from *tert*-butylmethyl ether/methylene chloride. Melting point (m.p.) 282–287°C (75% yield).

2.3. Microorganism and fermentations

Rhodococcus erythropolis FZB 53 was grown at 30°C on medium containing 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 3 g yeast extract, 5 g alanine, 0.03 g $CoSO_4$, and 0.5 g acetonitrile as an inductor. The cells were stored at 5°C in 67 mM phosphate buffer, pH 7.8 (Soerensen). Enzyme activity was checked using the substrate 3-cyanopyridine. No remarkable loss of activity was found during storage for up to two months.

The steroid fermentations were performed with cell suspensions in phosphate buffer containing 20% dimethylsulfoxide (DMSO) or 2% acetone by shaking at 25°C. These solvent concentrations had been shown to only minimally influence the enzymatic hydrolysis of the reference substance 3-cyanopyridine to nicotinamide. Steroid concentrations of 0.06–0.5 mM and cell concentrations of 1.7–7.4 mg/ml (calculated as dry weight) were used.

In order to monitor the fermentations directly, samples were taken at suitable intervals, mixed with the same volume of methanol, centrifuged, and 20 μl of the resulting solution were subjected to HPLC without further purification. HPLC conditions: column C_8 (Nowa-Pak, 4 μm, 4.6 × 250 mm), 37°C, eluent methanol/water (70:30 v/v); UV detection at 275 nm. T_R of DNG: 3.8 min.

For preparative isolation of the metabolites, fermentations were extracted with ethyl acetate and subjected to preparative thin-layer chromatography (TLC).

2.4. Analytics

TLC was carried out on silica gel GF_{254} (Merck) prewashed with chloroform/methanol (1:1 v/v) or on TLC aluminium sheets of silica gel 60 F_{254} (Silufol, Merck) using the solvent systems given in Table 1.

High-performance liquid chromatography (HPLC) characterization of isolated DNG metabolites was done with a Shimadzu CBM-10A equipped with an auto injector (SIL-10A), a column oven (CTO-10A), and an UV diode-array detector (SPD-M10A VP), using a hypersil ODS column, 5 μm (250 × 4.6 mm) at 35°C. For further conditions, see Table 1.

NMR Spectra were recorded on a VARIAN Gemini 300 at 300 MHz and 75.4 MHz. DMSO-d_6 was used as solvent. Chemical shifts were reported as δ-values in ppm downfield from the internal standard tetramethylsilane; J were given in Hz.

High resolution mass spectra were recorded on a Joel JMS-D 100.

2.5. Receptor affinity

Binding affinities for the progesterone receptor (PR) and estrogen receptor (ER) were measured by competitive binding of the compounds of interest together with the tracers [6,7-^{3}H]ORG 2058 (5 nM, for PR) or [6,7-^{3}H]Estradiol (3 nM, for ER) to receptors in rabbit uterus cytosol (rabbits for PR primed with estradiol, immature rabbits for ER). Buffer: Tris/HCl, 20 mM, pH 7.4, containing 1 mM EDTA, 2 mM DTT, and 250 mM sucrose. Incubations were carried out for

Table 1
Data of DNG metabolites

Metabolite (see formula, Fig. 1)	**IIa**	**IIb**	**IIIa**	**IIIb**
TLC				
Rf in system A[a]	0.42	0.42	0.s21	0.21
Rf in system B[a]	0.56	0.56	0.22	0.22
HPLC: t_R (method[b])	9.5 min (C)	10.1 min (C)	14.0 min (D)	14.6 min (D)
λ_{max} (nm)	280 nm	262; 299 nm	280 nm	262; 299 nm
HRMS				
M^+ Found	311.1882	309.1714	329.1991	327.1856
Calculated	311.1885	309.1729	329.1991	327.1834
Formula	$C_{20}H_{25}NO_2$	$C_{20}H_{23}NO_2$	$C_{20}H_{27}NO_3$	$C_{20}H_{25}NO_3$
^{1}H-NMR	8.98 (s, 3-OH), 7.03 (d, 8.7, H-1), 6.51 (dd, 8.1, 2.2, H-2), 6.43 (d, 2.2, H-4), 4.94 (s, 17-OH), 0.80 (s, H-18)	9.26 (s, 3-OH), 7.42 (d, 8.7, H-1), 6.53 (dd, 8.7, 2.2, H-2), 6.44 (d, 2.2, H-4), 6.05 (m, H-11), 5.06 (s, 17-OH), 0.80 (s, H-18)	8.99 (s, 3-OH), 7.04 (d, 8.4, H-1), 6.50 (dd, 8.4, 2.3, H-2), 6.43 (d, 2.3, H-4), 5.63 (s, 17-OH), 0.81 (s, H-18)	9.26 (s, 3-OH), 7.42 (d, 8.7, H-1), 6.53 (dd, 8.7, 2.3, H-2), 6.44 (d, 2.3, H-4), 6.07 (m, H-11), 5.67 (s, 17-OH), 0.81 (s, H-18)
^{13}C-NMR	154.8 (3), 137.0 (5), 130.1 (10), 125.9 (1), 119.9 (-CN), 114.8 (4), 112.6 (2), 80.5 (17), 48.6 (14), 46.1 (13), 42.9 (9), 39.3 (8), 14.1 (18)	157.3 (3), 138.7 (5), 136.5 (10), 127.4 (9), 126.2 (1), 120.3 (-CN), 117.2 (11), 115.9 (4), 114.7 (2), 82.2 (17), 14.9 (18)	175.7 (-$CONH_2$), 154.8 (3), 137.0 (5), 130.2 (10), 125.9 (1), 114.8 (4), 112.6 (2), 81.2 (17), 48.6 (14), 46.0 (13), 43.3 (9), 22.8 (15), 13.9 (18)	175.6 (-$CONH_2$), 155.9 (3), 136.9 (5), 134.2 (10), 125.2 (9), 124.8 (1), 116.5 (4), 114.7 (2), 113.6 (11), 80.9 (17), 46.3 (14), 44.4 (13), 39.5 (8), 23.4 (15), 14.0 (18)

[a] TLC system A: chloroform/methanol (92:8 v/v); TLC B: benzene/ethyl acetate/ethanol/acetic acid (50:42:8:0.1 v/v).
[b] HPLC method C: eluent acetonitrile/water (40:60 v/v), 1 ml/min. Method D: eluent methanol/sodium phosphate buffer (25 mM, pH 7.5) (55:45 v/v); 0.5 ml/min.

18 h at 0–4°C. Separation of free and bound steroid was achieved by charcoal/dextran (1%/0.1%) treatment.

The molar IC_{50}s were evaluated from a series of concentrations, and the relative molar binding affinities (RBA) were calculated as the quotient of the IC_{50} of the reference compound and that of each test substance ($\times$100%).

2. Results

Although *Rhodococcus erythropolis* cells were able to enzymatically hydrolyze the reference compound 3-cyanopyridine to nicotinamide within minutes, the steroid substrate was metabolized relatively slowly. No metabolite with the intact estra-4,9-diene system was found in a number of incubations with different concentrations of dienogest and cells. Instead of the intended hydrolysis of the nitrile group, an aromatization of ring A was noticed. After prolonged fermentation of DNG with *Rhodococcus erythropolis*, a second, more polar type of metabolite, was detected. Both of these products were isolated by preparative TLC from the culture extract of a 27-day fermentation, together with a residue of about 5% DNG.

The less polar product II corresponded to the known estra-1,3,5(10),9(11)-tetraene compound with a 17α-CH_2CN group [11]. Although it was crystallized, the ^{1}H-NMR spectra clearly showed that it consisted of a mixture of the estra-1,3,5(10)-triene and the estra-1,3,5(10),9(11)-tetraene compounds **IIa** and **IIb**. There were also two metabolites in product **III**, **IIIa**, and **IIIb**, which were shown by ^{13}C-NMR to have lost the cyano group and to contain a carbonyl group instead. Mass spectra showed the M^+ peaks of the estratriene and estratetraene derivatives. Resolution of both substance pairs was achieved by HPLC. The structures were proved by UV spectra and by comparison with the synthetic steroids STS 433 (later code J 540), J 1201, and J 1358. The data of the four metabolites are given in Table 1; for formulas, see Fig. 1.

A trace of an even more polar product was found also in prolonged fermentations. The expected carboxylic acid derivative with an aromatized ring A was extracted from ether solution into aqueous sodium carbonate or sodium bicarbonate. However, the amount was insufficient for characterization.

The velocity of the metabolization of the nitrile group of DNG to the acetamido compounds was estimated from samples taken at suitable intervals from different fermentations. Furthermore, the aromatic compound STS 433 (identical with **IIa**) was compared to DNG with respect to metabolization velocity. Both compounds, when compared to the reference 3-cyanopyridine, showed that the steroidal 17α-CH_2CN group was metabolized very slowly, differing

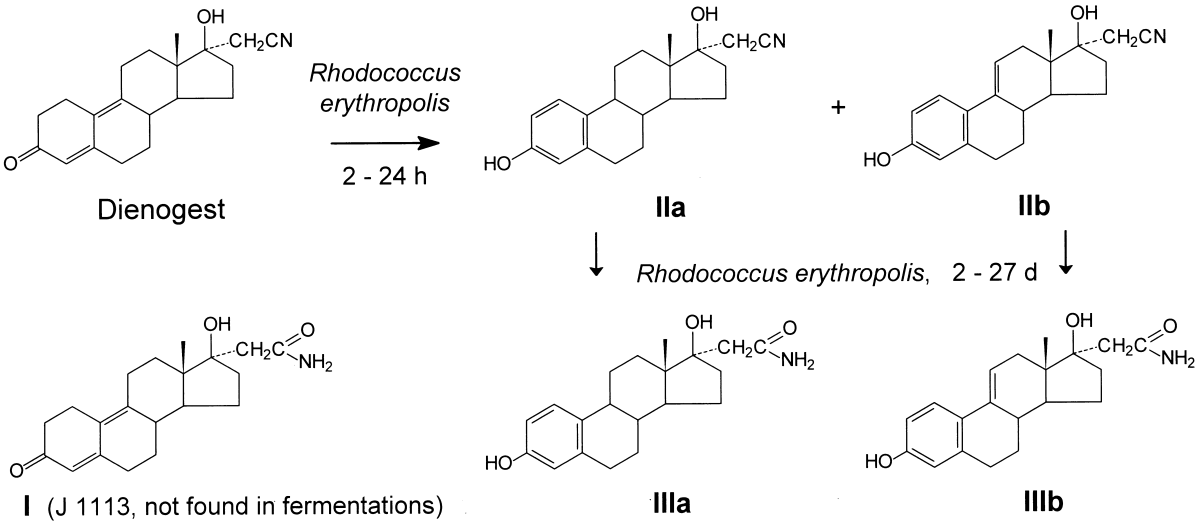

Fig. 1. Metabolites of dienogest.

in reactivity from cyanopyridine by several orders of magnitude (see Table 2).

In search for possible hormonal activities of the metabolites, receptor binding affinities for the progesterone and the estrogen receptors were determined using the corresponding synthetic compounds. The results are listed in Table 3. Estrogen receptor affinity was found for the ring A aromatized derivatives of DNG, corresponding to **IIa** and **IIb**. Further, some progesterone receptor affinity was found for those derivatives that were characterized by the unmetabolized 17α-CH_2CN group. The 17α-acetamido compounds **IIIa, IIIb,** and **I** were nearly or completely inactive at the receptor level. Furthermore, compound **I** exhibited no progestin activity when tested for pregnancy maintenance s.c. in mice (Elger W, 1998, unpublished observations).

3. Discussion

Normally, the strain *Rhodococcus erythropolis* FZB 53 can rapidly hydrolyze a variety of aliphatic or aromatic nitrile compounds [5], and the intermediate amides can also be isolated. Other species of *Rhodococcus* have been used for the hydrolysis of nitriles to drugs of pharmaceutical interest, like pyrazinoic acid [7] and (S)-naproxen [8], which was obtained by stereoselective nitrile hydrolysis.

When the steroidal nitrile compound dienogest was used as substrate, the A-ring aromatization was found to be the dominant reaction instead of the nitrile hydratation reaction. Although unwanted, this result correlates with aromatization reactions of other 19-nor componds. The species *Rhodococcus rhodochrous* was able to transform 3-keto-desogestrel (13-ethyl-17-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3-one) to the corresponding A-ring aromatized compound [12]. DNG itself was metabolized to aromatized metabolites by *Mycobacterium smegmatis* [13]. Interestingly, the aromatized metabolite **IIb** was also formed by mammalian and human metabolism [14,15]. The estrogenic effect of this metabolite was one order of magnitude lower than that of estriol s.c. in mice, that of **IIa** even lower [4]. Nevertheless, the antiprogestational activity of DNG in some animal experiments may be mimicked by the aromatized metabolite [16].

The desired microbial transformation of the nitrile group of DNG, in contrast to that of nonsteroidal nitriles, e.g. cyanopyridine, was seen only in prolonged fermentations, where ring A of the metabolites had already been aromatized (see Fig. 1). Thus, the 17α-acetamido analog of dienogest was only synthetically prepared (J 1113) and included in the receptor binding tests, see below. The 17α-cyanomethyl group of DNG seems to be relatively stable against the microbial nitrile hydratase, an enzyme of low substrate specificity. The stability of the 17α-cyanomethyl group was also seen in the metabolization of DNG in humans. Of the variety of metabolites produced, only one was found that had lost the nitrogen of

Table 2
Comparison of metabolization rates in fermentations with *Rhodococcus rhodochrous* at 25°C

Substrate	Substrate Concentr. (mmol/l)	Biomass Conc.[a] (mg/ml)	Time for 50% metabolization by	
			Aromatization	Nitrile Hydratase
DNG	0.1	7.4	1.5 h	48 h
DNG	0.5	1.7	8 h	264 h
STS 433 (= **IIa**)	0.06	7.4		24 h
Cyanopyridine	480	2.2		24 min
Cyanopyridine	1260	7.4		15 min

[a]Biomass as dry weight.

Table 3
Receptor binding affinities of dienogest derivatives

Steroid (Code)	Structure (see Fig. 1)	Relative molar binding affinity (RBA) [%] Mean ± SD (*n*)	
		Progesterone receptor (progesterone = 100)	Estrogen receptor (estradiol = 100)
J 540	**IIa**	3.4 ± 0.2 (4)	30 ± 3 (5)
J 1201	**IIb**	1.0 ± 0.1 (4)	28 ± 4 (5)
IIIa, in mixture with **IIIb** (c. 1:1)		<0.1 (3)	0.28 ± 0.04 (5)
J 1358	**IIIb**	<0.1 (4)	0.12 ± 0.01 (4)
J 1113 (not found as metabolite)	**I**	<0.2 (5)	<0.01 (3)
For comparison: Dienogest	DNG	10.5 ± 2.5 (10)	<0.1 (5)
STS 528 (17α-hydroxymethyl-17β-hydroxyestr-4-en-3-one)		0.65 ± 0.25 (4)	<0.01 (3)

the side chain. It was tentatively identified as a lactone resulting from complete hydrolysis of the 17α-cyanomethyl group to the carboxyl acid and from 15- (or 16-) hydroxylation [15]. This compound is analogous to a lactone metabolite of 17α-cyanomethylestradiol 3-methyl ether produced in the rat [17].

The metabolization of the side chain of DNG to 17α-acetamido compounds, as found here, is a possible, but probably not important pathway. It does not result in hormonally active metabolites which would exhibit high receptor binding affinities. On the contrary, the 17α-acetamido compounds exhibit negligible receptor binding, and even the synthetically prepared 17α-acetamido analog of dienogest J 1113 (compound **I)**, is inactive at the hormone receptor level and in mice. This finding is not surprising. The mammalian progesterone receptor preferentially binds nortestosterone derivatives bearing hydrophobic 17α-substituents. Thus, 17α-methyl and ethinyl [18], even 17α-chloromethyl [19], or 17α-iodovinyl substituents [20] were tolerated. More polar 17α-substituents were barely tolerated. Correspondingly, the 17α-hydroxymethyl compound STS 528, another potential DNG metabolite tentatively characterized in rabbit urine [14], exhibited only marginal receptor affinity.

Thus, the high efficacy of dienogest as a progestin in vivo cannot be explained by hormonal activity of metabolites with a hydrolyzed 17α-cyanomethyl side chain. Rather, it may be explained by the high levels of free, meaning active, DNG in plasma [16], by the influence of the conjugated 9(10) double bond inhibiting some metabolization pathways [21], and by the pharmacokinetic parameters of this progestin.

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References

[1] Hübner M, Ponsold K, Oettel M, Freund R. Eine neue Klasse hochwirksamer Gestagene: 17α-CH_2X-substituierte Gona-4,9(10)-diene. Arzneim-Forsch-Drug Res 1980;20:401–4.
[2] Oettel M, Carol W, Kaufmann G, Moore C, Römer W, Klinger B, Schneider B, Schröder J, Sobek L, Walter F, Zimmermann H. A 19-norprogestin without a 17α-ethinyl group. II: Dienogest from a pharmacodynamic point of view. Drugs of Today 1995;31:517–36.
[3] Juchem M, Schaffrath M, Pollow K, Hoffmann G, Kaufmann G. Dienogest: Bindungsstudien an verschiedenen Rezeptor- und Serumproteinen. In: Teichmann AT, editor. Dienogest. Präklinik und Klinik eines neuen Gestagens, 2th Ed. Berlin: De Gruyter, 1995. pp. 119–33.
[4] Oettel M, Kaufmann G, Kurischko A. Das endokrinologische Profil von Metaboliten des Gestagens Dienogest. Pharmazie 1993;48:541–5.
[5] Hübner B, Böhme M, Lebentrau B, Müller M, Häfner B, Juhr E, FZ Biotechnik Berlin GmbH. Mikroorganismus und Verfahren zur mikrobiellen Herstellung eines Nitrilhydratase-/Amidase-Enzymkomplexes. Germ Patent DE 43 13 649 C1. 1993 April 21.
[6] Nawaz MS, Franklin W, Campbell WL, Heinze TM, Cerniglia CE. Metabolism of acrylonitrile by *Klebsiella pneumoniae*. Arch Microbiol 1991;156:231–8.
[7] Kobayashi M, Yanaka N, Nagasawa T, Yamada H. Nitrilase-catalyzed production of pyrazinoic acid, an antimycobacterial agent, from cyanopyrazine by resting cells of *Rhodococcus rhodochrous* J1. J Antibiot Tokyo 1990;43:1316–20.
[8] Effenberger F, Böhme J. Enzyme-catalysed enantioselective hydrolysis of racemic naproxen nitrile. Bioorg Med Chem 1994;2:715–21.
[9] Menzenbach B, Hübner M, Ponsold K. Untersuchungen zur Bromierung/Dehydrobromierung von 17α-Cyanomethyl-17β-hydroxyöstr-5(10)-en-3-on. J Prakt Chemie 1984;326:893–8.
[10] Menzenbach B, Hübner M, Sahm R, Ponsold K. Synthese potentieller Metaboliten der STS 557 (Dienogest). Teil 3: 17α-Hydroxymethyl-17β-hydroxyestra-4,9-dien-3-on. Pharmazie 1984;39:496–7.
[11] Hübner M, Menzenbach B, Röhrig H, Ponsold K. Synthese potentieller Metaboliten der STS 557 (Dienogest). Teil 2: 17α-Cyanomethylestra-1,3,5(10),9(11)-tetraen-3,17β-diol. Pharmazie 1984;39:496.
[12] Groh H, Schön R, Ritzau M, Kasch H, Undisz K, Hobe G. Preparation of 3-ketodesogestrel metabolites by microbial transformation and chemical synthesis. Steroids 1997;62:437–43.
[13] Hobe G, Schön R, Hörhold C, Hübner M, Schade W, Schubert K. Microbial transformation of 17α-cyanomethyl-17-hydroxy-4,9-estradien-3-one (STS 557) and 17α-cyanomethyl-19-nortestosterone by Mycobacterium smegmatis. Steroids 1982;39:399–409.
[14] Hobe G, Schön R, Wehrberger K, Schade W, Ritter P, Hillesheim HG. Studies on the biotransformation of the progestagen dienogest in the rabbit. Exp Clin Endocrinol 1989;94:203–10.

[15] Hobe G, Schön R, Schade W, Wehrberger K, Knappe R, Claussen C, Carol W, Klinger G. Zur Biotransformation von Dienogest beim Menschen. In: Teichmann AT, editor. Dienogest. Präklinik und Klinik eines neuen Gestagens, 2th Ed. Berlin: De Gruyter, 1995. pp. 105–17.

[16] Oettel M, Bervoas-Martin B, Elger W, Golbs S, Hobe G, Kaufmann G, Mathieu M, Moore C, Puri C, Ritter P, Reddersen G, Schön R, Strauch G, Zimmermann H. A 19-norprogestin without a 17α-ethinyl group. I: Dienogest from a pharmacokinetic point of view. Drugs Today 1995;31:499–516.

[17] Hobe G, Schön R, Wehrberger K. Species differences in biotransformation of 17α-cyanomethylestradiol 3-methyl ether. Steroids 1980; 36:131–47.

[18] Delettré J, Mornon JP, Lepicard G, Ojasoo T, Raynaud JP. Steroid flexibility and receptor specificity. J Steroid Biochem 1980;13:45–59.

[19] Bohl M, Simon Z, Vlad A, Kaufmann G, Ponsold K. MTD calculations on quantitative structure-activity relationships of steroids binding to the progesterone receptor. Z. Naturforsch 1987;42c:935–40.

[20] Ali H, Rousseau J, van Lier JE. Synthesis of (17α,20E/Z)iodovinyl testosterone and 19-nortestosterone derivatives as potential radioligands for androgen and progesterone receptors. J Steroid Biochem Mol Biol 1994;49:15–29.

[21] Schubert K, Schumann G, Kaufmann G. Influence of a 9-double bond on stereospecific microbial 4,5-reductions. J Steroid Biochem 1983; 18:75–80.