Reviews

Microorganisms as reagents for transformations of 5α-steroids

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Microbial activation of rings A, B, and C of 5α-steroids is reviewed in connection with the prospects for the use of 5α-steroids as a new starting material for the manufacturing of steroidal medicinal preparations. The combination of chemical synthesis and microbial methods of steroid transformation allows the successful solution of the problem of selective functionalization of steroid molecules, provided the suitable microorganisms are chosen and the conditions of their cultivation are optimized.

Key words: 5α-pregnanes, 9α-hydroxysteroids, dehydrogenation, *Arthrobacter*, *Nocardia*, *Rhodococcus*.

1. Introduction

In our previous review[1] we demonstrated how widely and efficiently modern chemistry applies living biocatalysts, *e.g.*, fungi, to the hydroxylation of 5α-steroids, thus opening the way to manufacturing new precursors required in the synthesis of corticoid hormones. The possibilty of the use of 5α-steroids in steroidal drug production has also been considered in this review. However, in the majority of cases fungi do not perform the dehydrogenation of ring A to form Δ^4- or $\Delta^{1,4}$-3-oxo derivatives, which is necessary to make the steroids biologically active. The ability to conduct such a transformation is characteristic of bacteria and is discussed in the present review, which is the logical continuation of our previous paper.[1] The literature data on the dehydrogenation of 5α-steroids published up to 1994, which have not yet been summarized, except in two reviews[2,3] by Chinese authors dealing with their own works published in national journals, virtually beyond the reach of the great majority of investigators, are discussed. Reactions that accompany dehydrogenation or proceed independently under the specific conditions are also reviewed in the present paper.

One of these reactions is 9α-hydroxylation, which follows the formation of Δ^4-3-oxo derivatives from 5α-steroids. It is catalyzed by a culture of a new *Rhodococcus sp. IOKh-77* strain[4] isolated by the authors. The transformations carried out using this strain, which make it possible to simultaneously activate rings A and C of saturated 5α-pregnanes, have no parallels in the literature and are discussed, as far as possible, in relation to the transformations of the better studied Δ^5-3β-hydroxysteroids.[5–12]

2. Directed oxidation of 5α-steroids depending on the transforming bacterial species and the structure of the substrate

Unlike the hydroxylation of 5α-steroids under the action of fungi,[1,13–20] the dehydrogenation of ring A is performed by microorganisms belonging mainly to the class *Schizomycetes* (Table 1). The table comprises all the kno wn species of 5α-steroid-transforming microorganisms,[21–95] excluding the fungi species which conduct hydroxylation alone and which have been

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discussed in the previous review.[1] One can see that some representatives of *Fungi Imperfecti*, in particular, of the *Septomyxa* genus, unlike the majority of hydroxylating cultures,[1] also transform 5α-steroids in a fashion similar to that for Δ^4-3-oxosteroids,[13–20] *i.e.*, by the introduction of a double bond into ring A. *Septomyxa* was found to exhibit the unique ability to introduce the double bond into ring A of A/B-*trans* as well as of A/B-*cis*-fused pregnanes as far back as 1958.[93] These fungi differ from the bacteria by the absence of Δ^4-5β-dehydrogenase.[96] As a rule, they manifest 1,2-dehydrogenase activity and, more rarely, Δ^4-5α-dehydrogenase activity.[96] As is seen from the table, some other species of fungi belonging to different classes (*Ascomycetes, Deuteromycetes,* and *Phycomycetes*) also catalyze the dehydrogenation of 5α-steroids but they do not perform hydroxylation.[42,44–46,48–55,90,91]

The data listed in Table 1 show that only two bacterial species (*Corynebacterium simplex*[44] and *Rhodococcus sp. IOKh-77* (Refs. 5—12)) carry out the dehydrogenation and hydroxylation of 5α-steroids simultaneously. But *Corynebacterium simplex*, contrary to *Rhodococcus sp.*

Table 1. Microorganisms with an established ability to oxidize, hydrolyze, and reduce 5α-steroids as well as oxidize them with fission of the C—C bond

Microorganism	The reaction catalyzed*									References
	(1)			(2)	(3)	(4)	(5)	(6)	(7)	(8)	
	1,2	4,5	1,2; 4,5								
Aphanocladium album							+				21
Arthrobacter simplex			+	+		+	+	+	+		22—30
Arthrobacter sp.	+		+								31, 32
Arthrobacter sp. 9-2			+	+			+	+	+		33—38
Bacillus sphaericus	+										23, 39—41
Calonectria decora			+		+						42, 43
Corynebacterium simplex	+				+						44
Didymella lycopersici			+								42, 45, 46
Flavobacterium dehydrogenans							+				47
Fusarium caucasicum				+				+			48, 49
Fusarium solani				+				+			49—51
Gymnoascus reesii						+		+			52
Gymnoascus umbrinus		+						+			53, 54
Hypomyces haematococcus			+					+			55
Hypomyces rosellus		+	+					+			54, 55
Hypomyces solani			+					+			54, 55
Micromonospora chalcea			+								56
Mycobacterium flavum			+								23
Mycobacterium fortuitum								+			57
Mycobacterium phlei			+	+				+			25, 58—62
Mycobacterium smegmatis			+	+							29, 30, 63
Mycobacterium sp.								+			64
Mycobacterium vaccae		+						+			57, 65
Nocardia blackwelli			+								66
Nocardia globerula			+								67
Nocardia restrictus			+	+				+		+	25, 49, 60, 68—75
Nocardia sp.		+	+	+				+			26—28, 31, 32, 76—81
Penicillium lilacinum								+			82
Protaminobacter alboflavum			+								83
Protaminobacter rubrum			+								83
Proactinomyces globerulus			+								23
Pseudomonas testosteroni	+		+			+					84—86
Rhodococcus sp. IOKh-77		+		+	+	+		+			5—12
Rhodotorula mucilagenosa							+				87
Saccharomyces cerevisiae							+				88, 89
Scopulariopsis capsici		+						+			90
Septomyxa affinis	+		+								23, 73, 91—93
Septomyxa corni	+										91
Septomyxa salicina	+										91
Septomyxa tulasnei	+										91
Stichococcus bacillaris		+			+						94
Syncephalastrum racemosum			+								95

*Dehydrogenation (1), hydrolysis (2), hydroxylation (3), 3-OH → 3-CO (4), CO → C—OH (5), side chain splitting (6), epoxidation (7), epimerization (8).

IOKh-77, affords a mixture of 9α-hydroxy-5α-androstane derivatives with a predominance of the Δ^1-product.[44] The *Rhodococcus sp. IOKh-77* culture transforms 5α-pregnanes only into practically useful Δ^4-3-oxo or 9α-hydroxy-Δ^4-3-oxo derivatives.[5–12]

2.1. Stereochemistry and mechanism of dehydrogenation of the saturated ring A

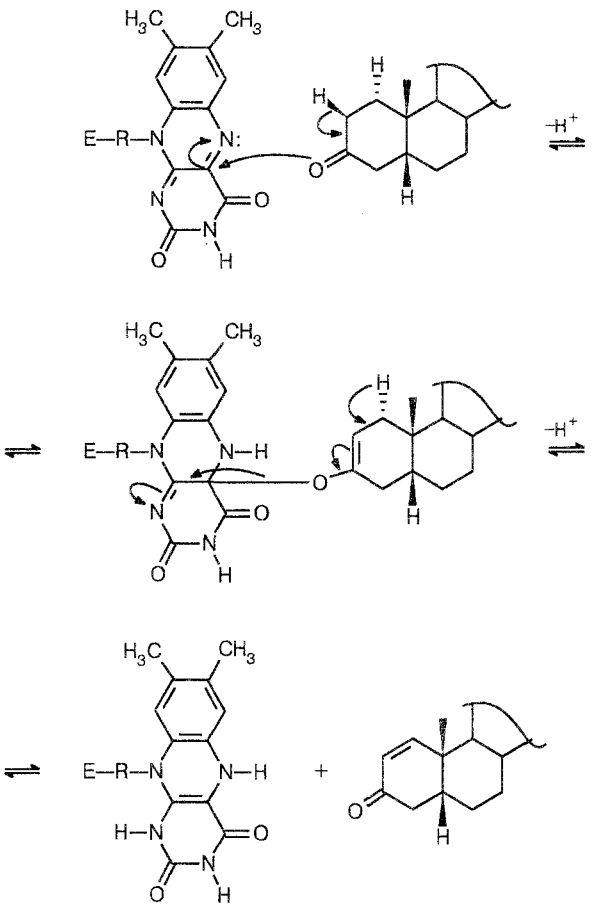
Microbial dehydrogenation of ring A occurs intracellularly under the action of induced 1,2-, 4,5α-, and 4,5β-dehydrogenases. These enzymes catalyze real dehydrogenation but not hydroxylation followed by dehydration.[14] The mechanism and the stereochemistry of enzymatic 1,2-dehydrogenation have been investigated using labelled5α- and5β-androstane-3,17-diones[41,74,96–98] and 5β-pregnane-3,11,20-trione[99], which were transformed by *Bacillus sphaericus, Nocardia restrictus,* and *Septomyxa affinis*. It was established that 1,2-dehydrogenation proceeds via *trans*-diaxial elimination of the 1α- and 2β-hydrogen atoms. The resemblance of the enzymatic 1,2-dehydrogenation of 5β-steroids to that of their 5α- and Δ^4-3-oxo analogs[74,96,100] is confined to the stereospecific elimination of 1α-hydrogen atoms and the preferential elimination of 2β-hydrogen atoms.[74] The dehydrogenation of Δ^4-3-oxosteroids occurs according to Ringold's[96,101] mechanism, *i.e.*, through the *trans*-diaxial elimination of the 1α- and 2β-hydrogen atoms proceeding via the stage of $\Delta^{2,3}$-enolization. In the 5β-pregnane series these atoms are *trans*-diequatorially oriented and their elimination can proceed according to a mechanism[99] that involves *a*) the formation of a 3-ketone—enzyme covalent bond, which favors enolization and elimination of the 2β-proton; *b*) 1α-H deprotonation due to the 1,2-shift; *c*) cleavage of the covalent bond with the enzyme (Scheme 1).

The stereochemistry of enzymatic 4,5-dehydrogenation of 5α- and 5β-steroids has also been investigated using the transformations of steroids of the androstane and pregnane series with a cell-free extract of *Nocardia restrictus* bacteria capable of the simultaneous introduction of double bonds into positions 1,2 and 4,5.[76] The enzymatic 4,5-dehydrogenation of 5α-steroids is considered to be *cis*-elimination of the 4α- and 5α-hydrogen atoms; with 5β-steroids, the process proceeds through the stereospecific elimination of the 4α- and 5β-hydrogen atoms.[73–75,98] Dehydrogenation with the introduction of a 4,5-double bond most likely proceeds similarly to the formation of the 1,2-double bond .

2.2. Substrate specificity of dehydrogenation

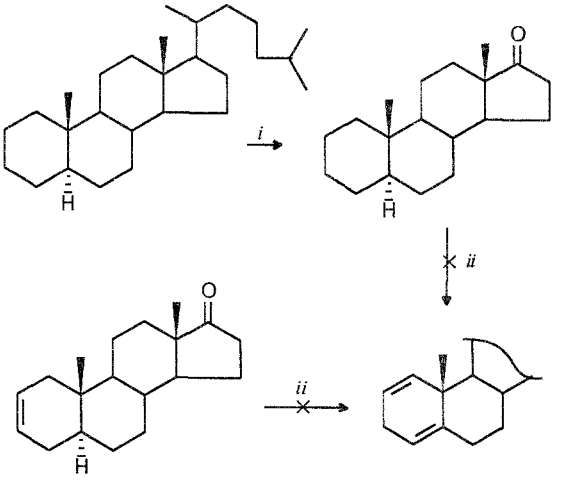
The transformation of C(4)-substituted 5α-steroids by a *Nocardia restrictus* preparation affords solely Δ^4-ketones, whose yields depend on the stereochemistry of the substituent (72 % from 4β-substituted and 10—15 % from 4α-substituted androstanes, respectively).[75] This implies that 4-substituted androstanes cannot serve as the substrates for 1,2-dehydrogenase of *Nocardia*

Scheme 1



Note. A probable mechanism of enzymatic 1,2-dehydrogenation of 5β-steroids.
E — is an enzyme.

Scheme 2



i — Nocardia sp., ii — Syncephalastrum racemosum

restrictus. The 1α-methyl group prevents the introduction of the 1,2-double bond by the bacterium,[64] but the 1β-methyl group does not.[39] The elimination of the 4α- and 5α-hydrogen atoms seems to be impeded by the substituent at C(6). In any case, *Mycobacterium vaccae* forms Δ^4-3-oxosteroid from 5α-cholestane-2α,3α-diol[65] but not from its 6-oxo analog.[57]

For steroid-dehydrogenases to act, the substrate must possess an oxygen function at C(3), since no dehydrogenation takes place when it is absent (Scheme 2). This requirement is equally necessary for fungal (*Syncephalastrum racemosum*) and for bacterial (*Nocardia sp.*) enzymes.[80,95] The inability to dehydrogenate the steroid ring A may have no connection with the molecule structure, as has been noted for marine microorganisms, which only shorten the cholestanol and campestanol side chains to afford the corresponding C(26)- or C(25)-steroids,[103] whereas the soil bacteria transform Δ^5- and 5α-sterols into Δ^4-3,17-diketones.[13–20,102]

The dehydrogenation of ring A of 5α-steroids proceeds more smoothly than that of 5β-compounds, irrespective of the microorganism species used. This rule holds for the introduction of both the 4,5-[48] and the 1,2-double bond. For example, *Septomyxa affinis* forms the Δ^1-derivative of 5α-pregnane-3,11,20-trione in 40—45 % yield, while in the case of the 5β-epimer the yield is only 5—10 %.[93]

2.3. The sequence of microbial modification of the saturated ring A

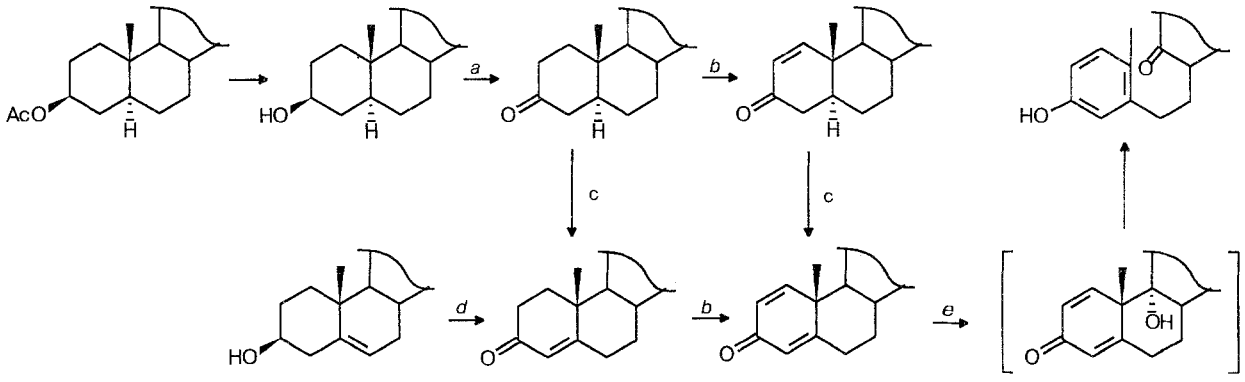
It has been demonstrated for different 3β-hydroxy(acetoxy)-5α-steroids, which were transformed by *Arthrobacter, Nocardia, Mycobacterium,* and *Rhodococcus* cultures,[7,25,28,33,63,68] that the modification of the saturated ring A proceeds via 5α-3-oxo-, Δ^4-3-oxo-, and, finally, $\Delta^{1,4}$-3-oxo-compounds sequentially under the action of a series of enzymes, among which are 3β-hydroxysteroid-dehydrogenase (HSD) [EC 1.1.1.145], 3-oxosteroid-dehydrogenase (4,5-OSD) [EC 1.3.99.5], and 3-oxosteroid-1,2-dehydrogenase (1,2-OSD) [EC 1.3.99.4]. When a 3-acetoxy group is present in the substrate, the first stage of the transformation is catalyzed by an esterase.

The transformation may be stopped at the stages of Δ^1-, Δ^4-, or $\Delta^{1,4}$-3-ketone formation, depending on the capability of the cells to synthesize 1,2-OSD or 4,5-OSD. However, the transformation leading solely to Δ^4-3-ketones with no further Δ^1-dehydrogenation is in fact rather rare. This statement is based on the analysis of literature data on microbial transformations of 5α-steroids. Only the *Rhodococcus sp. IOKh-77* culture is capable of accumulation of Δ^4-3-oxo derivatives from 5α-steroids[5–10,12] and from Δ^5-3β-hydroxysteroids.[104,105] Further transformations of Δ^4-3-ketones proceed in the vast majority of cases according to the scheme proposed for Δ^5-3β-hydroxysteroids that are assimilated by bacteria as a carbon source[63] (Scheme 3). The transformation of Δ^5-3β-hydroxysteroids into Δ^4-3-oxo derivatives occurs with the participation of HSD and 3-oxosteroid-Δ^5→Δ^4-isomerase (OSI) [EC 5.3.3.1].[14] Futhermore, the result of the simultaneous attack on Δ^4-3-oxosteroids by 1,2-OSD and 9α-hydroxylase (steroid-9α-monooxygenase) [EC 1.14.99.24] is the undesirable fission of ring B (Scheme 3).

It is noteworthy, however, that fission of ring B by *Mycobacterium, Nocardia,* and *Rhodococcus* mutants is used to transform sterols into compounds with rings C and D, which are of interest as precursors in the chemical synthesis of retro-steroids, which do not occur in nature.[106–108]

In connection with sterols, we can say that we have deliberately omitted the discussion of transformations of 5α-sterols, since we considered unnecessary the detailed analysis of microbial transformations of 5α-sterols with the main task of the splitting of the side chain, since this aspect has been discussed sufficiently fully in a number

Scheme 3



a — HSD, *b* — 1,2-OSD, *c* — 4,5-OSD, *d* — HSD, OSI, *e* — 9α-hydroxylase.

of monographs and reviews.[14–20,102] Examined below is only the cleavage of the 5α-sapogenin side chain, for it is connected with the possibility of the use of 5α-sapogenins as steroid raw materials in the synthesis of corticoids.*

3. Transformations of compounds of the spirostane, androstane, and pregnane series.

3.1. Transformations of 5α-sapogenins

Modifications of 5α-sapogenins have been carried out using fungi and bacterial cultures. As was stressed above, some fungi catalyze the dehydrogenation of the saturated ring A, instead of its hydroxylation, and simultaneously cleave the side chain of a number of sapogenins. However, unlike bacteria, which cleave 5α-sapogenins like sterols, *i.e.*, to affording 3,17-diketones,[24–26,62] fungi form 3,16-diketones, whose yields depend on the structure of the substrate and the microbial species used and may amount to 60 %, *e.g.*, in the transformation of hecogenine by *Hypomyces solani*.[54] Fungi mostly oxydize ring A of 5α- as well as of Δ^5-steroids to give $\Delta^{1,4}$-diene-3-one or Δ^4-en-3-one. The transformation of 5α-sapogenins and their Δ^5-analogs into androst-4-ene-3,16-dione by different species of *Scopulariopsis, Gymnoascus,* and *Hypomyces* (Table 1) proceeds in 10—50 % yields.[53–55]

Ascomycetes *Gymnoascus reesi* can cleave ring A (as was demonstrated with tomatidine **1**)[52] according to a pathway that is sometimes encountered in the bacterial degradation of cholesterol[14] (Scheme 4).

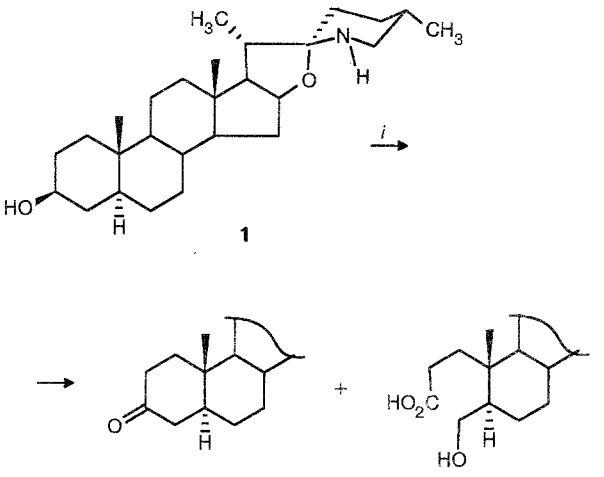
A deeper degradation of the steroidal molecule occurs under the action of unidentified soil bacteria isolated on a medium with diosgenin (**2**). When incubated with hecogenin (**3**), they first afford hecogenon (**4**), which further undergoes cleavage with the removal of the 12-keto group to give the lactone and the ketoacid depicted in Scheme 5. The ketoacid mentioned is also formed in 55 % yield from diosgenin (**2**), *i.e.*, from Δ^5-3β-hydroxysteroid, with a transformation period 2 days shorter than that of 5α-3β-hydroxysteroid.[67]

Fission of rings A and B is inhibited by α,α-dipyridyl or 8-hydroxyquinoline. In the presence of these inhibitors, *Arthrobacter simplex* and *Mycobacterium phlei* transform tomatidin **1**, tygogenin (**5**), and neotygogenin into androsta-1,4-diene-3,17-dione (**6**), though in low yieds (2.5—4 %) (Scheme 6).[24,25]

The report about the transformation of tygogenin **5** into androstenedione (**7**) by the *Hypomyces rosellus* fungus in 30 % yield[54] is so far unique. The *Rhodococcus sp. IOKh-77* bacterium distinguished for its high dehydrogenating activity towards 5α-steroids does not transform tygogenin even in 4 days.[110]

*The structure and the methods of transformation of spirostanols, the main industrial source for manufacturing steroid drugs, are covered comprehensively in Ref. 109.

Scheme 4



i — *Gymnoascus reesii*

The experiments with *Nocardia restrictus* have shown that the presence of a nitrogen atom in a side chain prevents the induction of side chain cleavage-catalyzing enzymes.[68,69] In particular, tomatidin **1** can be degraded into dienedione **6** only using *Arthrobacter simplex* cells grown on a cholesterol-containing medium.[25] Uninduced *Nocardia restrictus* cells transform toatidine **1** and 5α-solasodanol (**8**), which differs from **1** in the stereochemistry of its ring F, by modifying only ring A and affording the corresponding $\Delta^{1,4}$-3-oxo derivatives[25] (Scheme 7).

Seco-ring E compounds are transformed by the *Nocardia restrictus* culture analogously[25] (Scheme 8).

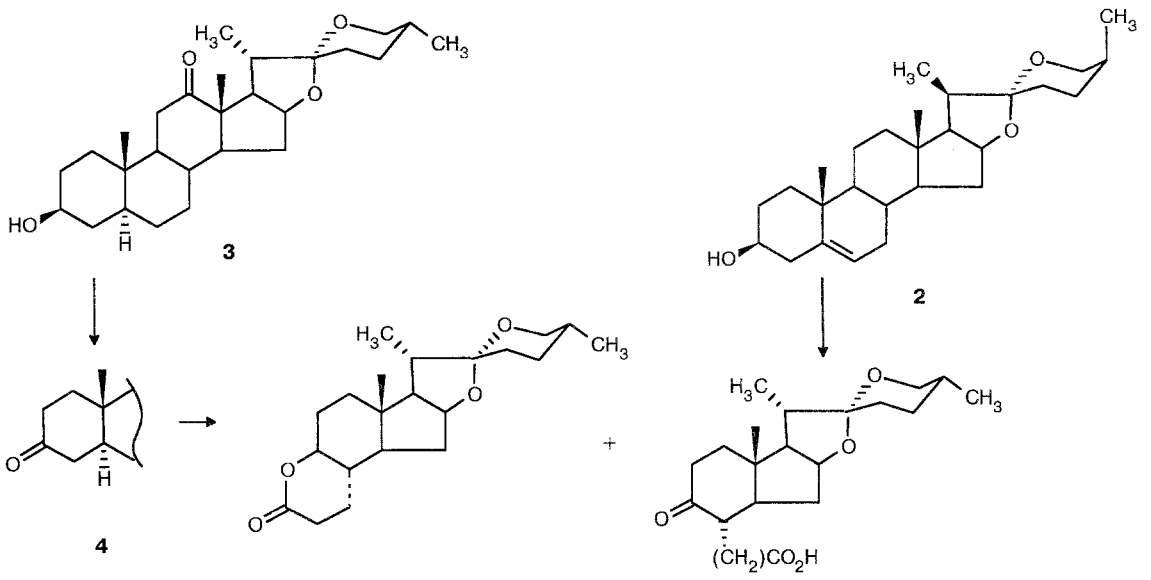
When steroid alkaloids with a tertiary amino group, *i.e.*, *N*-methyl- and *N*-acetyl-5α-tomatanin-3β-ol, demissidin (**9**), and 5α-conanin-3β-ol (**10**), were subjected to the action of *Nocardia restrictus*, they behaved like compounds with a secondary amino group: the side chain was not split off, dehydrogenation of ring A proceeded with difficulty (except for demissidin **9**) and *N*-acetyltomatidin was transformed only into the 3-oxo derivative[71] (Scheme 9).

When the incubation period of 3β-hydroxysteroid alkaloids with *Nocardia restrictus* was reduced to 4 h instead of 3—7 days, 3α-hydroxyalkaloids were obtained in 5—70 % yields (from tomatidin **1**, *N*-acetyltomatidin, dihydrotomatidin, and 5α-solasodanol, but not from demissidin).[70] Microbial epimerization, unlike chemical epimerization, does not require preliminary preparation of 3-oxo compounds and may become a useful tool in the directed synthesis of 3α-hydroxysteroids used for their subsequent chemical 9α-hydroxylation.

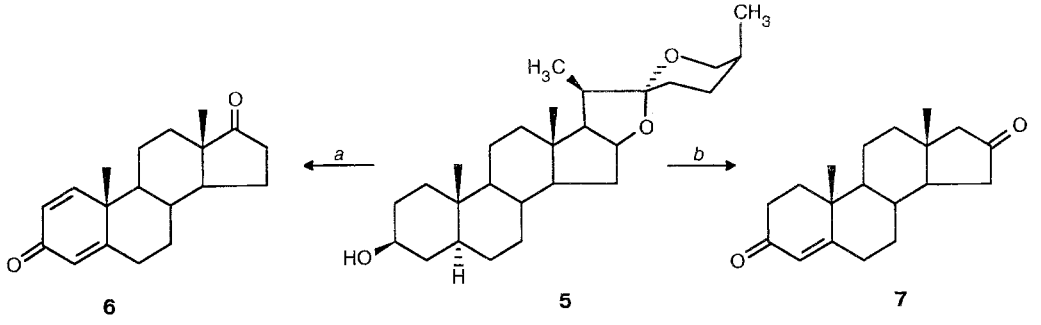
3.2. Dehydrogenation of 17-oxo-5α-steroids

As follows from the review by K. Schubert[63] on dehydrogenation of 17-oxosteroids by mycobacteria, the

Scheme 5

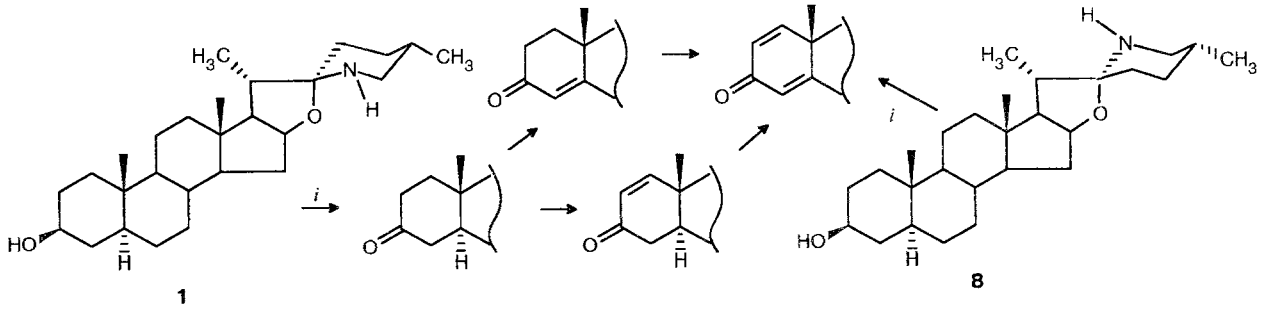


Scheme 6



a — *Mycobacterium phlei* (4%), *b* — *Hypomyces rosellus* (30%)

Scheme 7

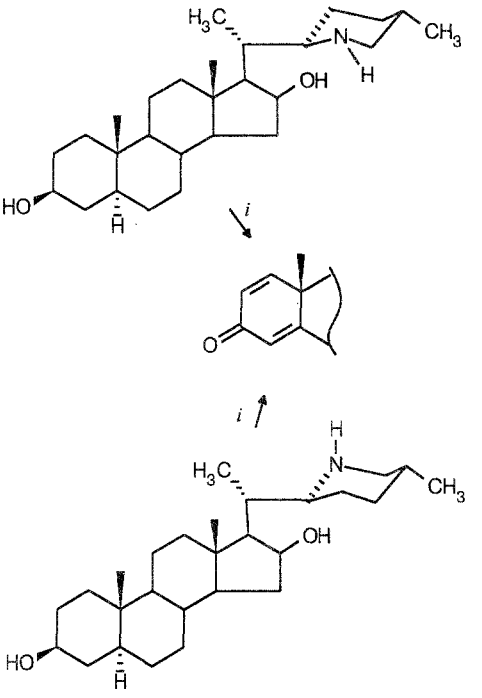


i — *Nocardia sp.*

saturated ring A is not an obstacle to enzyme—substrate interaction. Under the same incubation conditions, *Syncephalastrum racemosum* forms dienedione **6** from androst-4-ene-3,17-dione (**11**) in even smaller yield than from 5α-androstane-3,17-dione[95] (**12**) (Scheme 10).

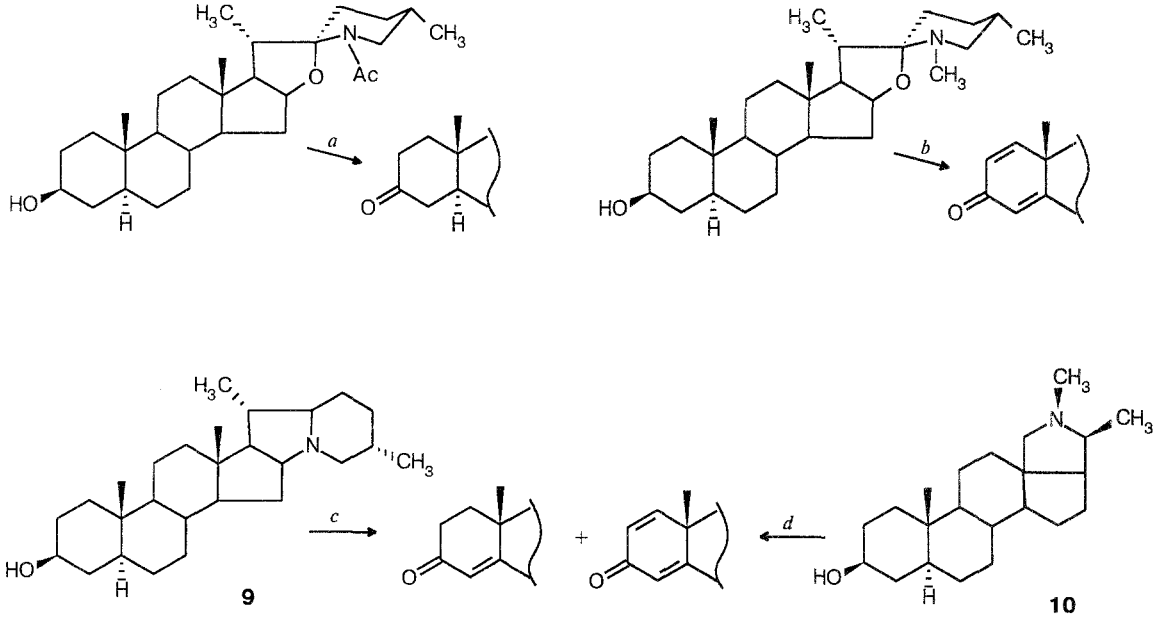
The mode of further modification of Δ^4-3-ketones is determined by the ability of bacteria to synthesize 9α-hydroxylase. As was noted above (Section 2.3), the availability of the latter results in cleavage of the C(9)—C(10) bond in ring B due to simultaneous 1,2- and 4,5-dehydrogenation and 9α-hydroxylation. In the absence of 9α-hydroxylase inhibitors, the complete oxidation of the steroid molecule into CO_2 and H_2O may take place. This is the case when 17-methyl-androstanediol (**13**) is incubated with the *Arthrobacter simplex* bacterium, which was chosen from 62 strains of microorganisms of the *Arthrobacter* and *Nocardia* genera.[33] However, in the presence of 9α-hydroxylase inhibitors (heavy metal salts, α,α-dipyridyl, 8-hydroxyquinoline) dianabol (**14**), a highly effective anabolic, is formed in a high yield[27,33] (Scheme 11). Dianabol **14** has been obtained using both the individual cultures of *Mycobacterium phlei,* and *Nocardia restrictus* bacteria and mixed cultures of *Nocardia sp.* and *Arthrobacter simplex.*[27] *Mycobacterium phlei* perform 1,2-dehydrogenation first, while the sequence of 1,2-double bond formation and 9α-hydroxylation by *Nocardia restrictus* depends on the reaction conditions.[60] The structure of

Scheme 8



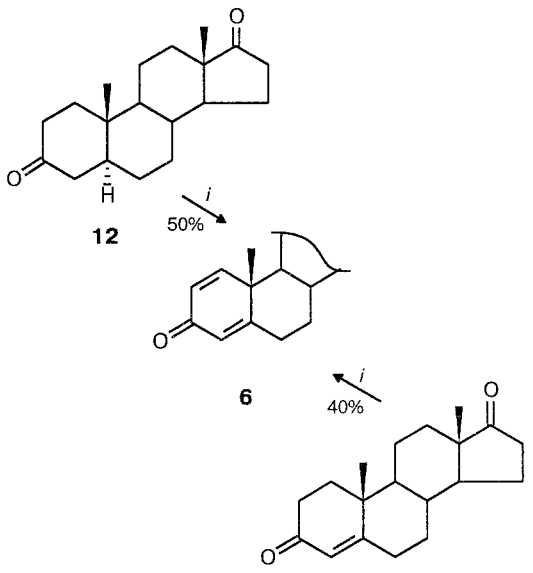
i — *Nocardia restrictus*

Scheme 9



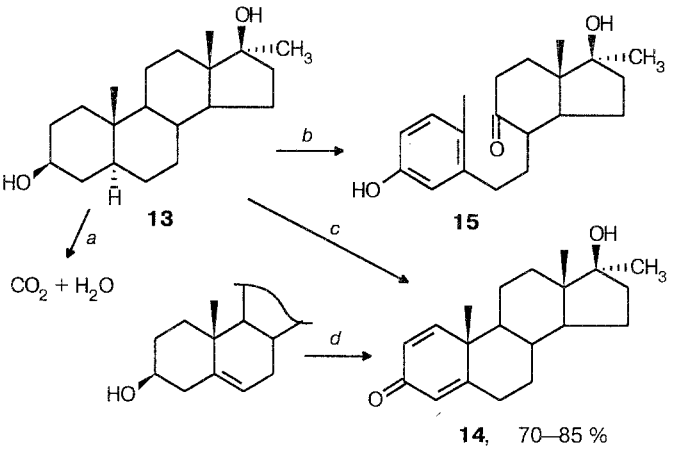
a — *Nocardia restrictus* (7 days), *b* — *Nocardia restrictus* (3 days), *c* — *Nocardia restrictus* (4 h), *d* — *Nocardia restrictus* (7 days).

Scheme 10



i — *Syncephalastrum racemosum.*

Scheme 11



a — *Arthrobacter sp.*, *b* — *Nocardia sp.* +*Arthrobacter simplex*, *c* — *Arthrobacter sp.*, $CoSO_4$, *d* — *Mycobacterium sp.*, $CoCl_2$.

the final product of diol **13** transformation is determined by the composition of the nutrient medium: sometimes the fission of ring B takes place leading to the formation of 9-secodiol[26] (**15**) instead of dianabol **14** (Scheme 11).

We have obtained dianabol **14** from the corresponding Δ^5-3β-hydroxy substrate in a high yield, too, using a mycobacterium culture,[111] which was later identified as a microorganism of the *Rhodococcus* genus according to the Goodfellow[112] classification. In this case, it was also found that the intermediate and the final products of the transformation are more toxic towards the bacteria under investigation than the starting steroid substrate.[113] This fact contradicts one of the hypotheses, which states that the transformation of steroids by microbes is caused by the necessity to detoxify them.[14]

The *Rhodococcus sp. IOKh-77* strain isolated by researchers from the N. D. Zelinsky Institute of Organic Chemistry is of great practical interest. It differs from the above-mentioned *Rhodococcus* species, as well as from all the other microorganisms listed in Table 1 in producing solely Δ^4-, but not Δ^1- or $\Delta^{1,4}$-3-oxo derivatives. Along with the transformation of 5α-steroids into Δ^4-3-oxo compounds this culture also carries out their further 9α-hydroxylaton. Because of the absence of 1,2-OSD in *Rhodococcus sp. IOKh-77* cells, they are the perfect biocatalyst for the preparation of 9α-hydroxy-Δ^4-3-oxosteroids both from 5α-compounds[6—12] and from their Δ^5-3β-hydroxy analogs.[104] *Rhodococcus sp. IOKh-77* is characterized by the high activity of 4,5-OSD and HSD, as well as of OSI, which are necessary for the transformations of 5α- and Δ^5-3β-hydroxysteroids, correspondingly (see Section 2.2). Unlike *Circinella sp.* fungi, which introduce a 9α-hydroxy group into 5α-steroids without modifying ring A,[114] the *Rhodococcus sp. IOKh-77* culture introduces a 9α-hydroxy group only into Δ^4-3-oxo substrates; 9α-hydroxy derivatives are formed from Δ^4-3-oxo- or Δ^5-3β-acetoxy substrates in 70 % yield at a loading of up to 3 g/L.[104]

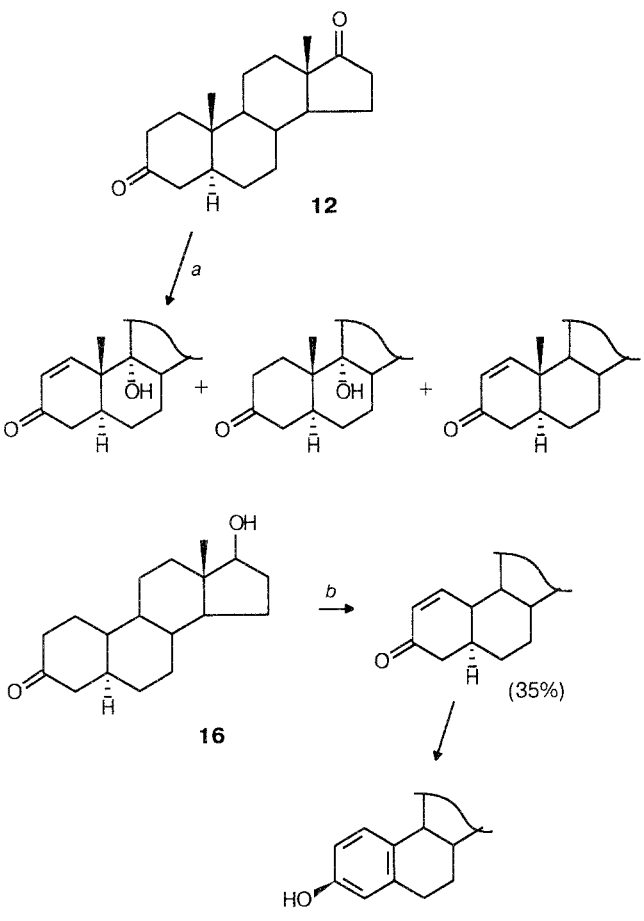
Corynebacterium simplex bacteria do not cleave 5α-androstane-3,17-dione for a different reason. *Rhodococcus sp. IOKh-77* does not synthesize 1,2-OSD, whereas *Corynebacterium simplex* cells do not synthesize 4,5α-OSD and therefore transform the above-named substrate only by the introduction of a 1,2-double bond and by 9α-hydroxylation[44] (Scheme 12). The initial introduction of the 1,2-double bond was observed in the case of the *Septomyxa affinis* culture.[39—41,92,93] In the process of dehydrogenation of 5α-estranol (**16**) by *Pseudomonas testosteroni*, double bonds are also introduced first into the 1,2- and then into the 4,5-positions[84—86] (Scheme 12).

The possibility of the transformation of 3-hydroxy-5α-androstanes into $\Delta^{1,4}$-3-oxo derivatives by a *Nocardia globerula* culture depends on the stereochemistry of the 3-hydroxy group. 3β-Acetoxy-5α-androstanes are converted into their $\Delta^{1,4}$-3-oxo derivatives, whereas 3α-acetoxy-5α-androstanes are only saponified into 3α-alcohols.[72] The introduction of 1,2- and 4,5-double bonds into androstane-3,17-dione by a *Calonectria decora* culture was patented in 1957[42] but in the papers published in 1958 and 1972 this culture was stated to have only hydroxylating ability.[43,115]

3.3. Dehydrogenation of ring A of 5α-pregnanes and concomitant reactions

In the course of the transformations of androstanes discussed in the previous section, the oxidative fission of ring D may occur along with the fission of ring B.[23]

Scheme 12



a — *Corynebacterium simplex*, *b* — *Pseudomonas testosteroni*

It has been found that when soil bacteria transform a steroid molecule to use it as a source of carbon and energy, they "attack" it from two directions.[14] Therefore, the introduction of 1,2- and 4,5-double bonds into ring A during transformations of pregnanes is often accompanied by side chain cleavage similar to that occuring during the fermentation of sterols.[116] Transformations of pregnanes often proceed with the undesirable reduction of the 20-keto group, leading at best to the accumulation of 20-hydroxy derivatives[7,28,38] and at worst to the appearance of the products of the side chain cleavage, *i.e.*, 17-oxosteroids[34,48,51,82] (Scheme 13), to the degradation of the molecule, and to a dramatic decrease in the yield of the target compounds. Cleavage of the side chain can proceed even faster than ring A dehydrogenation.[49] The degradation of the side chain of 5α-pregnanes to give 17-oxosteroids is observed not only in bacteria, but also in some fungi (Table 1).[48,49,51,82,93]

It was noted that protection of the C(20)-keto group as the ethylene acetal efficiently prevents cleavage of the side chain by *Septomyxa affinis* fungi.[92,117] The reduction of the 20-keto group also depends rather substantially on the nature of the substituents in ring D. The comparison of the results of the transformations of 17-hydroxy- and 16,17-disubstituted 21-hydroxypregnanes with an α- or β-CH_3 group at C(16) shows that there is a basis for asserting that the 16-methyl group hinders the reduction of the 20-keto group and the splitting of the side chain. This is indicated by the transformation of 16α-methylpregnane-17α,21-diol by an *Arthrobacter sp.* culture with a 100 % overall yield of 20-oxo products,[37] and of 16β-methylpregnane-17α,21-diol into the target $\Delta^{1,4}$-3,20-diketone in more than 60 % yield.[36]

The comparison of the data on the transformation of different 5α-steroids by the *Rhodococcus sp. IOKh-77* culture enables one to arrange these substrates in the order represented in Scheme 14, which shows a decrease in the efficiency of the 20-keto group reduction as a function of ring D functionalization.[6–10] The destruction of a steroid molecule connected with 20-keto group reduction is determined not only by the structure of the starting substrate, but also by the physiological status of the culture, as well as by the mode of introduction of the steroid into the cultural liquid, and by the aeration conditions.[7]

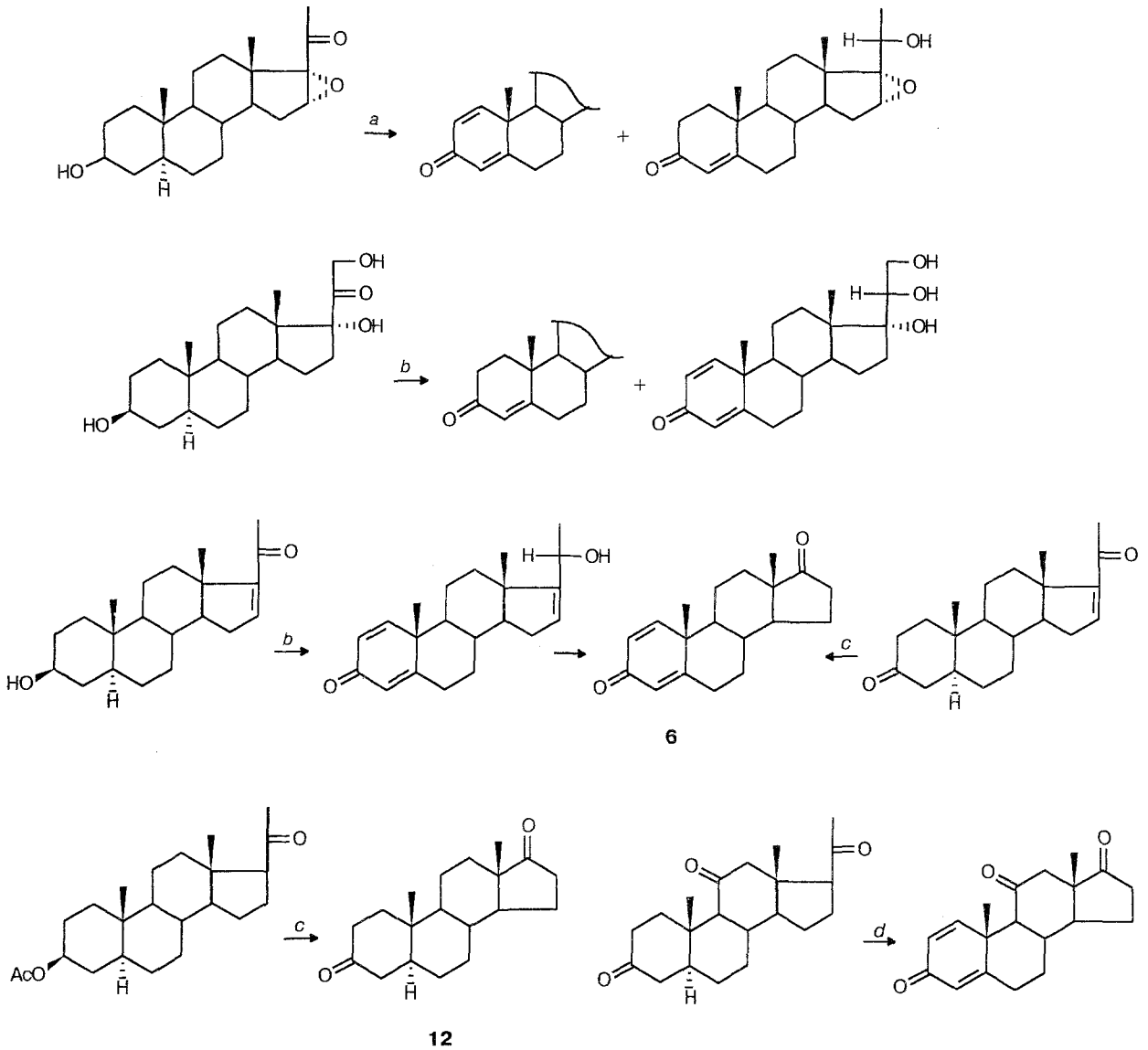
The presence of a nitrogen atom in a substrate molecule is probably an obstacle to its destruction. Thus, 21-azido- and 12a-azapregnanes are transformed into the target dehydro compounds by *Arhtrobacter, Nocardia,* and *Mycobacterium* cultures;[58] *Nocardia* transforms 5α-12a-aza-C-homopregnane (**17**) mainly into $\Delta^{1,4}$-3-ketone, while *Arthrobacter* transforms it into Δ^{1}-3-ketone[31,32] (Scheme 15).

Dehydrogenation of 16α,17α-epoxypregnanes results in a number of products with the predominance of Δ^{4}-3-ketone, if oxydized by *Nocardia*, or of $\Delta^{1,4}$-3-ketone, if oxydized by *Arthrobacter*[28] (Scheme 16). Nevertheless, the ability to initially perform 1,2- or 4,5-dehydrogenation revealed in some bacteria is not a taxonomic feature.

The comparison of the results of the fermentation of 5α- and 5β-pregnane 16α,17α-epoxides by *Nocardia* and *Arthrobacter* shown in Scheme 16 demonstrates that bacteria of both genera readily catalyze the first stages of 5α-steroid ring A oxidation.[28] The same feature is observed in *Septomyxa affinis* fungi, which transform 5α- and 5β-pregnane-3,11,20-triones into $\Delta^{1,4}$-androstan-3-ones in 40—45 % and 5—10 % yields, correspondingly.[93] In addition to Scheme 16, let us point to the fact that the formation of 3-oxo-5α-steroids at the first stage of the oxidation of the 3β-hydroxy derivatives is an argument in favor of the sequence of microbial oxidation of 3β-hydroxy-5α-steroids given in Scheme 3.

From the data mentioned above it can be seen that dehydrogenation of 5α-steroids of the pregnane series definitely proceeds with the preferential formation of $\Delta^{1,4}$-3-ketones, as does dehydrogenation of androstanes. Attempts have been undertaken to transform the 5α-steroids, for example, 3β-hydroxy-17-spirolactone

Scheme 13



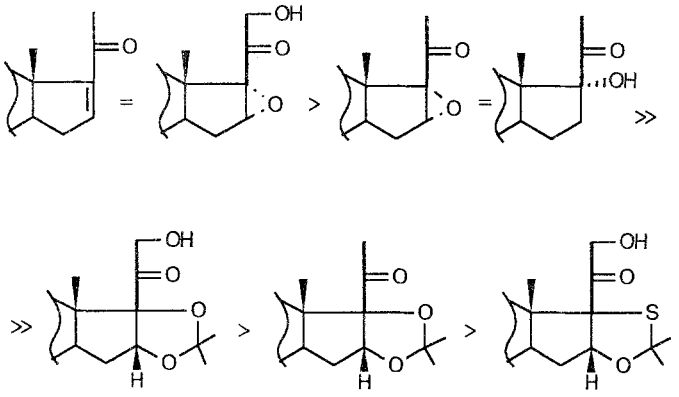
a — Arthrobacter simplex, b — Arthrobacter sp. 9-2, c — Fusarium caucasicum, d — Fusarium solani

(**18**)[79] and the 5,6-dihydro derivative of Reichstein's Substance R (**19**),[77,78] into Δ^4-3-ketones using *Nocardia* (Scheme 17). In the first case, fermentation in a nitrogen-deficient medium affords mainly Δ^4-3-ketone, whereas in a medium enriched in nitrogen the $\Delta^{1,4}$-3-ketone is mainly formed; the yield of Δ^4-3-ketone (Reichstein's Substance S, **20**) in the second case has not been reported.

The authors of the present review have demonstrated that the oxidation of ring A of a number of 5α-pregnanes may result in either Δ^4-3-oxosteroids or their 9α-hydroxy-Δ^4-3-oxo derivatives (see below), if it is conducted using the already mentioned *Rhodococcus sp. IOKh-77* strain isolated from soil contaminated by petrochemicals.[4–12] In particular, incubation of *Rhodococcus sp. IOKh-77* with 3β,17α,21-trihydroxy-5α-pregnan-20-one (**21**) diacetate on a deficient medium affords up to 55 % of cortexolone (Scheme 17).

This activity of *Rhodococcus sp. IOKh-77* bacteria was first revealed in studies of transformations of Δ^5-3β-hydroxysteroids of the pregnane series and of 24-nor- and 21,24-dinorcholanes (with an additional oxygen-containing ring E).[104,105] Their conversion into Δ^4-3-ketones or into the final 9α-hydroxy-Δ^4-3-ketones depended substantially on the functional substituents in ring D and in the side chain remote from ring A. In

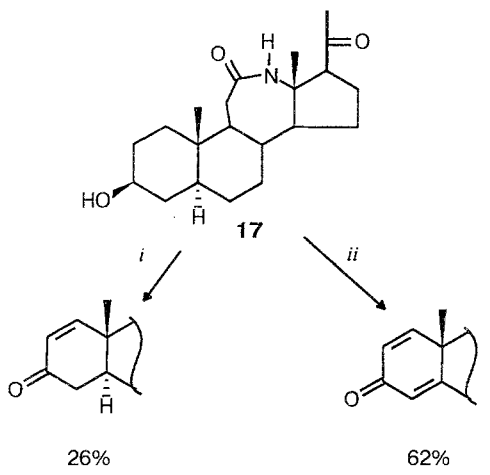
Scheme 14



much the same way, the transformation either stopped at the stage of intermediate Δ^4-3-ketone formation, or proceeded to afford 9α-hydroxy-Δ^4-3-ketone as the final product, depending on the ring E structure. In addition, some Δ^5-3β-acetoxy steroids were only saponified into the corresponding alcohols, though they could be transformed into the 9α-hydroxy derivatives, provided the corresponding Δ^4-3-ketone (obtained using *Corynebacterium mediolanum*) was used as the starting substrate for the transformation by *Rhodococcus sp. IOKh-77*.[118,119]

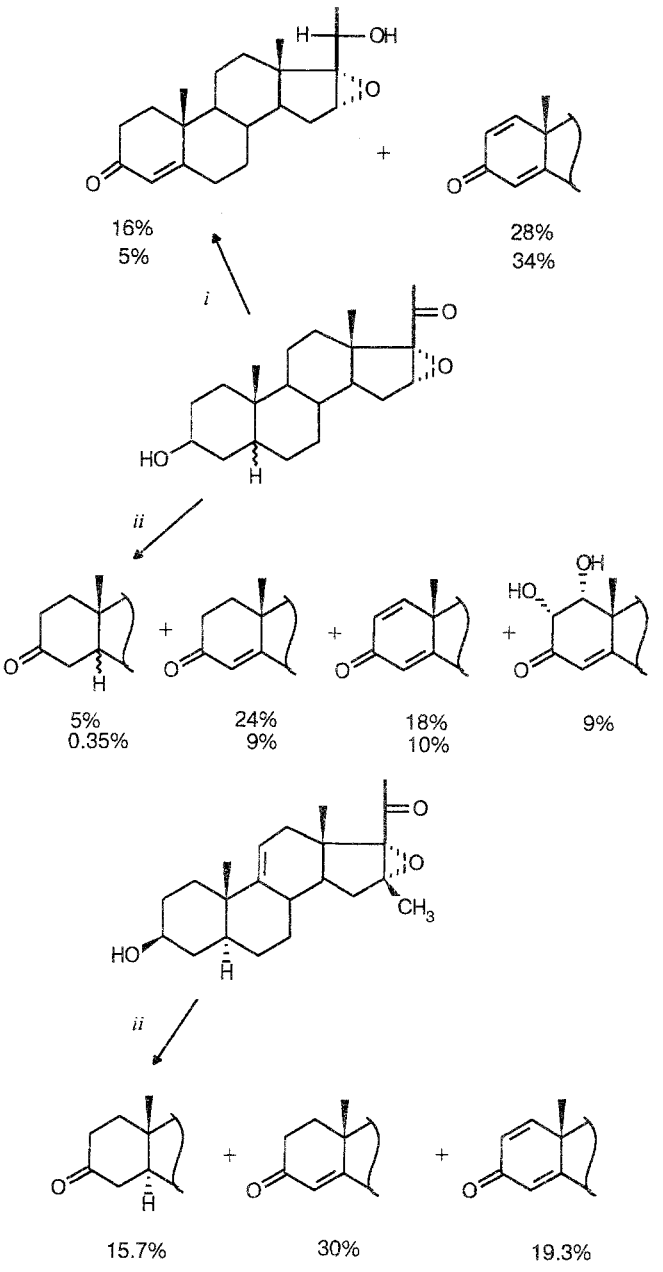
Because *Rhodococcus sp. IOKh-77* performs Δ^4-dehydrogenation and 9α-hydroxylation simultaneously in most cases, it became necessary to separate these processes. This separation was of great preparative interest, since it allowed the preparation of a valuable and hitherto practically unavailable intermediate in

Scheme 15



i — Arthrobacter sp., ii — Nocardia sp.

Scheme 16



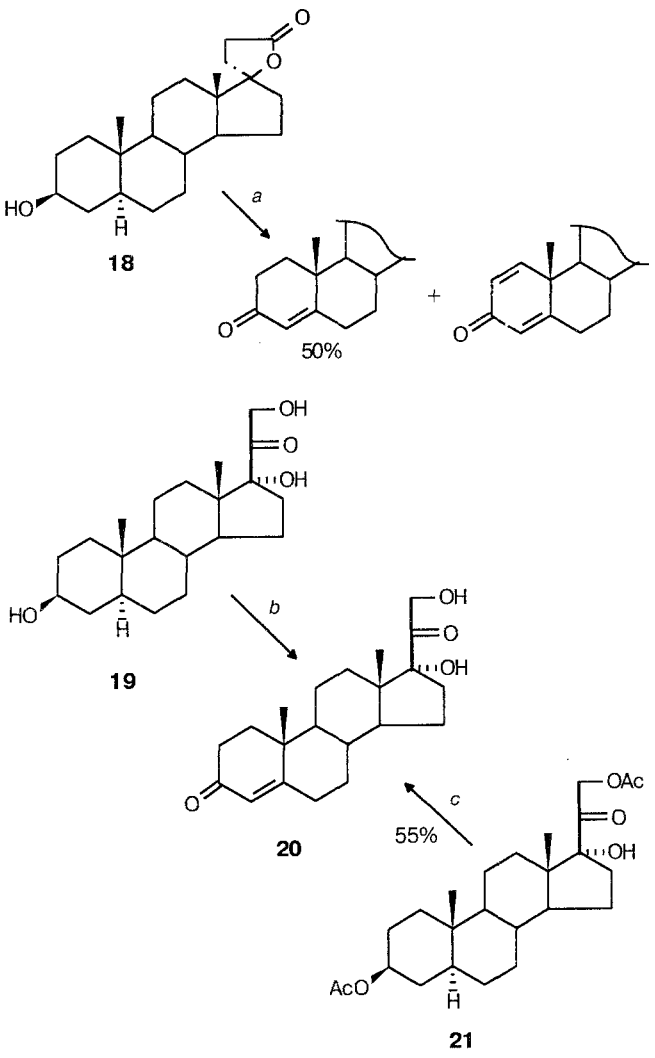
i — Arthrobacter simplex, ii — Nocardia sp.

Note. The upper row of figures refers to the transformations of 5α-steroids, the lower row, to those of 5β-steroids.

corticoid synthesis, viz, 16α,17α-epoxycorticosterone (**22**), from both Δ^5- and 5α-pregnanes (**23**). These could be Δ^4-dehydrogenated to Δ^4-3-ketone (**24**) using *Rhodococcus sp. IOKh-77* (Refs. 6,110) (Scheme 18).

The directed synthesis of compound **25**, the analog of thioxolane **26**, was also of interest, since it could be effectively transformed into the biologically active

Scheme 17



a — Nocardia restrictus, b — Nocardia sp., c — Rhodococcus sp. IOKh-77

11β-hydroxy derivative (**27**) by a *Curvularia lunata* culture[120] (Scheme 19).

The catalytic activity of *Rhodococcus sp. IOKh-77* (illustrated in Scheme 19 for thioxolane **26**) was controlled in different ways depending on the structure of the steroid to be transformed. In the particular example of thioxolane **26**, the direction of transformation, which resulted in only Δ^4-3-ketone **25** or 9α-hydroxy derivative **28**, was controlled by making substantial changes in the reaction conditions, namely, by using bacteria of different ages as the inoculate and by stirring the cultural liquid with different intensities.[8] The second technique consists of conducting the transformation in the presence of an inhibitor, *e.g.*, α,α-dipyridyl. In the same way, 9α-hydroxylation of 16-dehydropregnanolone (**29**) and 16α,17α-epoxypregnenolone (**30**) was entirely blocked, whereas for the analog of the latter, hydroxyacete **23**, this method appeared to be inadequate[7] (Scheme 20). At the concentration necessary for 9α-hydroxylase inhibition the transformation of **23** was hindered at the first stage of the oxidation (Scheme 3), and in the presence of $CoCl_2$ the target Δ^4-3-ketone **24** was formed in a low yield together with the 9α-hydroxy derivative (**31**) (Scheme 20). The directed conversion of substrate **23** into Δ^4-3-ketone **24** needed an essentially different approach to the transformation by *Rhodococcus sp. IOKh-77*, *i.e.*, the use of the resting cells. When isolated from the nutrient medium in the beginning of the stationary growth phase and resuspended in a buffer, the *Rhodococcus sp. IOKh-77* cells afforded the Δ^4-3-ketone in 66 % yield[110] (Scheme 18).

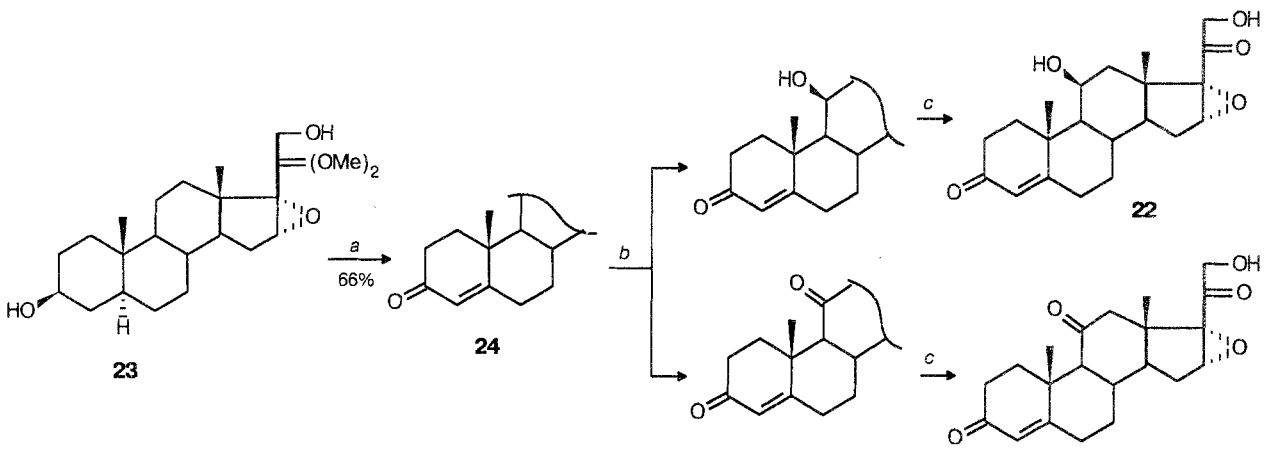
3.4. Bacterial modification of 5α-pregnanes into 9α-hydroxy-Δ^4-3-oxo derivatives

The synthesis of corticoids from 5α-pregnanes suggests the availability of effective methods for the functionalization of the inactivated rings A, B, and C. So far, this could be achieved by combining multi-stage chemical and microbial methods. A dramatic reduction in the number of stages is attained by the application of a novel bacterial strain of *Rhodococcus sp. IOKh-77*, which is characterized by its unique ability to conduct both Δ^4-dehydrogenation and 9α-hydroxylation during the same fermentation process.[5–12] Therefore, the *Rhodococcus sp. IOKh-77* bacterium can be regarded as more promissing than fungi, that manifest 9α-hydroxylating activity, but do not modify ring A of 5α-steroids,[1] and fungi in which 9α-hydroxylase induction occurs only in the presence of a preexisting Δ^4-3-oxo fragment. For example, the fungus *Cunninghamella blakesleeana* BKM 984 introduces a 9α-hydroxy group into 21-hydroxy-16α,17α-epoxypregn-4-ene-3,20-dione (**32**), but 9α-hydroxy derivative (**33**) is produced in no more than 16 % yield (Scheme 21).[5]

The application of *Rhodococcus sp. IOKh-77* bacteria enabled the one-step production of 9α-hydroxy-Δ^4-3-ketones from 5α-steroids. Thus, the transformation of 20,20-dimethoxy-16α,17α-epoxypregnane-3β,21-diol **23** using *Rhodococcus sp. IOKh-77* followed by the removal of the dimethyl acetal protective group from the product **31** resulted in the already mentioned 9α,21-dihydroxy-16α,17α-epoxypregn-4-ene-3,20-dione (**33**),[121] while an attempt at the chemical production of the latter from the corresponding 21-deoxyepoxide (**34**) failed[120] (Scheme 21). Using this example we have first established the important role of the 20,20-dimethyl acetal protective group for the preparative production of a number of 9α-hydroxy-16α,17α-epoxypregnanes.

The experimental data on *Rhodococcus sp. IOKh-77* activity towards 16α,17α-epoxy-5α-pregnanes in comparison with its activity towards their Δ^4- and Δ^5-analogs and with the activity of the other microorganisms (both bacteria and fungi) are summarized

Scheme 18

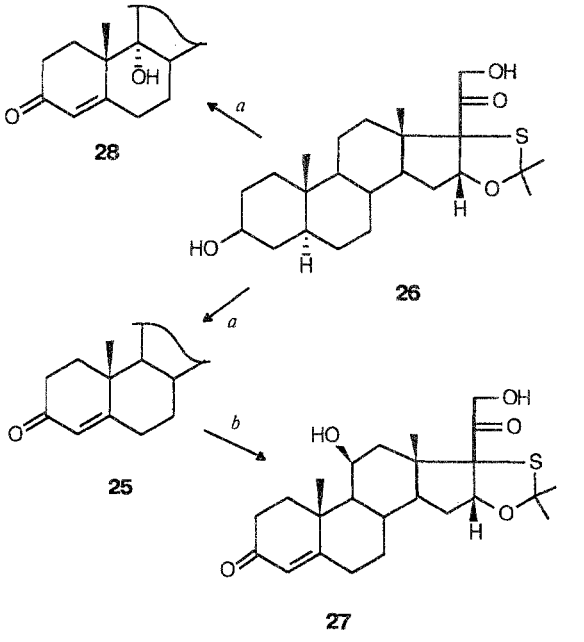


a — *Rhodococcus sp. IOKh-77*, *b* — *Curvularia lunata*, *c* — TsOH, Me_2CO

in Table 2. From this table it follows, first, that the bacterium *Rhodococcus sp. IOKh-77* transforms 5α(H)-16α,17α-epoxides into the 9α-hydroxy-Δ^4-3-oxo derivatives at lesser substrate loadings and for a longer period of time than necessary for their Δ^5-analogs. This phenomenon, observed also in the transformation of 3β-hydroxy- and 3β-acetoxy-16-dehydropregnanes of Δ^5- and 5α-series,[12] is probably connected with the higher hydrophobicity of 5α-steroids and their hindered penetration into a cell. Second, despite the fact that the transformation of 5α- and Δ^5-3β-hydroxysteroids by *Rhodococcus sp. IOKh-77* culture proceeds through the stage of Δ^4-3-ketone formation, the latter are less appropriate substrates for 9α-hydroxylation by *Rhodococcus sp. IOKh-77* than are 3β-hydroxysteroids, except for those Δ^5-3β-acetoxysteroids with an additional ring E.[104]

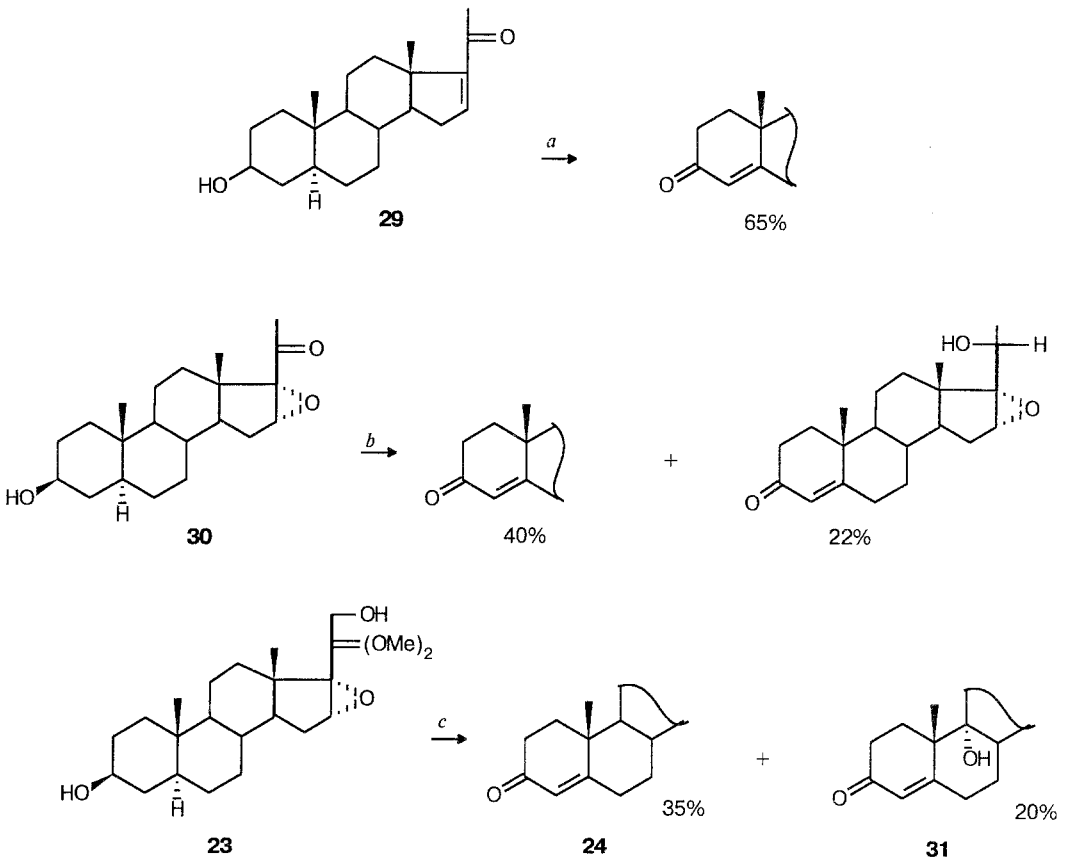
Further, Table 2 makes it possible to correlate the data on the microbial transformation of 20-oxoepoxides of the Δ^5- and 5α-series with those of their 20,20-dimethoxy analogs and to reveal essential differences. Δ^4-Dehydrogenation and 9α-hydroxylation of 20-oxoepoxides are accompanied by reduction of the 20-keto group, which cannot be completely prevented by varying the cultivation conditions of *Rhodococcus sp. IOKh-77* or the mode of introduction of the substrate. Moreover, on passing from 21-unsubstituted substrates to 21-hydroxyepoxides the degradation of the steroid molecule increases. On the other hand, the incubation of *Rhodococcus sp. IOKh-77* with 20,20-dimethoxyepoxides affords the target 9α-hydroxy-Δ^4-3-oxosteroids in 75 and 90 % yields (from 5α- and Δ^5-3β-hydroxyepoxides **23** and **35**, correspondingly) (Scheme 21) owing to the protection of the 20-keto group as dimethyl acetal. The value of the dimethyl acetal group as an effective means for conducting the microbial transformations has also been demonstrated with other cultures. From Table 2 it can be seen that the transformation of 20,20-dimethoxyepoxide **35** of the Δ^5-series using *Corynebacterium mediolanum* results in the formation of the Δ^4-3-ketone in a high yield.[6] In the 11β-hydroxylation by the fungus *Curvularia lunata*, the dimethyl acetal group prevents the formation of a side 14α-hydroxy derivative, so that the target 11β-hydroxy product can be isolated in 75 % yield.[6] According to patent data, a similar transformation of 20-oxoepoxide (**36**) results in a mixture of three products[122] (Scheme 22).

Scheme 19



a — *Rhodococcus sp. IOKh-77*, *b* — *Curvularia lunata*

Scheme 20



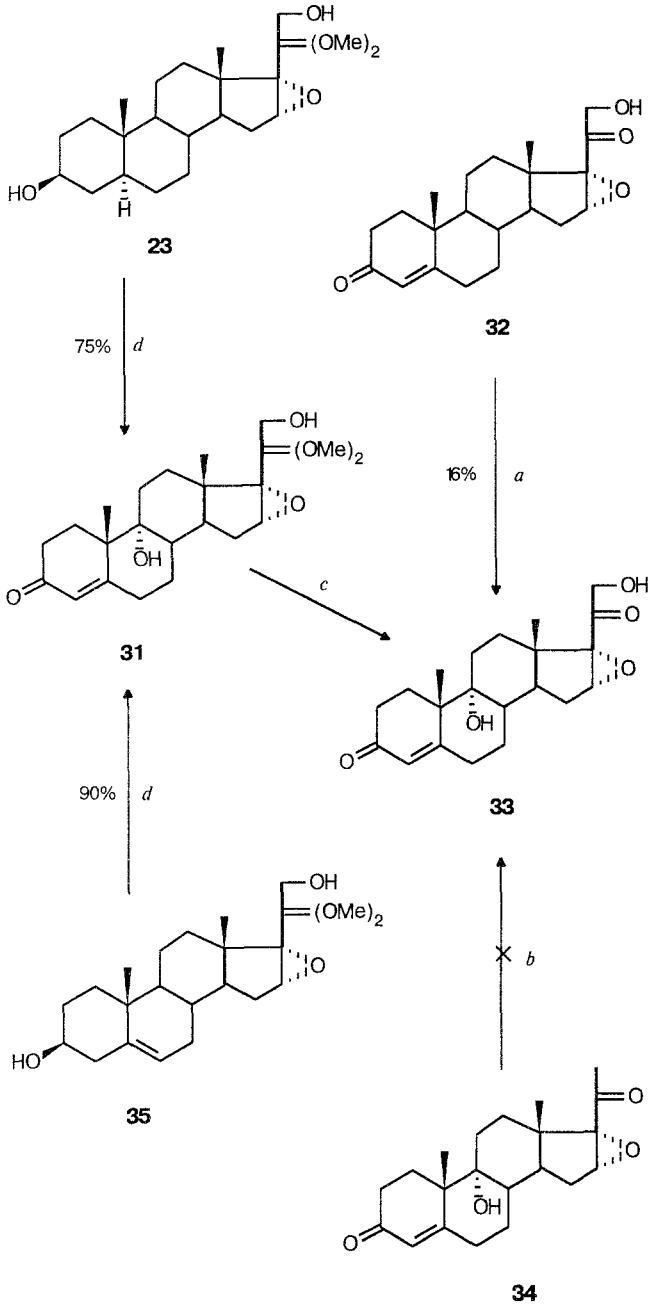
a — 3 g/L **29** *Rhodococcus sp. IOKh-77*, α,α-dipyridyl, 42 h; *b* — 1 g/L **30** *Rhodococcus sp. IOKh-77*, α,α-dipiridil, 40 h; *c* — 0.5 g/L **23** *Rhodococcus sp. IOKh-77*, $CoCl_2$, 12 h;

9α,21-Dihydroxyepoxide **33** isolated in a high yield, owing to the 20,20-dimethyl acetal protection block, profitably serves as a key compound in the chemical synthesis of such valuable precursors of biologically active substances as 9α,21-dihydroxy-16α,17α-thioxolane **26** and 9α,21-dihydroxy-16α,17α-dioxolane (**37**) (Scheme 23). The basic step of the synthesis illustrated in the scheme is the stereospecific *cis*-opening of the epoxide cycle by acetic or thioacetic acid in the presence of ethoxycarbonylhydrazine.[10] As can be seen from Scheme 22, the 16,17-acetonide of 9α,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione (**38**) cannot be obtained directly by microbial oxidation of the corresponding 5α-substrate **37**, for the reaction stops at the stage of formation of the 3-oxo-5α-derivative.[7]

The existence of a direct correlation between the steroid-transforming ability of *Rhodococcus sp. IOKh-77* and the character of the D ring and side chain substitution follows from the microbial transformation of 5α-pregnanes with 16α,17α-dioxolane and 16α,17α-thioxolane fragments. The nature of the heteroatom at C(17) has a great impact on the course of transformation.[7,8] As is shown in Scheme 23, 9α,21-dihydroxy-16α,17α-isopropylidenedioxypregnenedione **38** can be prepared from 9α,21-dihydroxyepoxide **33** only by chemical synthesis, whereas 16α,17α-thioxolane **28** can be obtained by two ways, viz, by chemical (from compound **33**) and microbial synthesis (directly from 16α,17α-thioxolane **26**), the yields in these two ways being equal. The prerequisite for the microbial transformation of 5α-substrate **26** by the *Rhodococcus sp. IOKh-77* culture to be directed towards 9α-hydroxy-Δ^4-3-ketone **28** is the use of an inoculate prepared during the logarithmic phase of growth and intensification of aeration of the medium.[8] The 21-deoxy analog (**40**) of 16α,17α-dioxolane can also be obtained directly from the corresponding 5α-substrate (**39**) using *Rhodococcus sp. IOKh-77*, but the yield in this case is insufficient and therefore its chemical synthesis from 9α-hydroxypregna-4,16-diene-3,20-dione (**41**) seems to us to be more rational.[10]

The problem of the effective conversion of 5α-pregn-16-enolone **29** into 9α-hydroxy-Δ^4-3-ketone **41** using *Rhodococcus sp. IOKh-77* at first seemed insoluble. The

Scheme 21



a — *Cunninghamella blakesleeana*, *b* — $PhI(OAc)_2$, KOH, MeOH, *c* — TsOH, Me_2CO, *d* — *Rhodococcus sp. IOKh-77*

20-keto group was not protected against reduction and bulky substituents in ring D were absent, which made the side chain of 5α-pregnenolone **29** easily degradable during the activation of rings A and B by *Arthrobacter sp.* and *Rhodococcus sp. IOKh-77* bacteria.[7,34] If menadione, which inhibits carbonyl group reduction and degradation of steroid substrates during dehydrogenation of Δ^5-3β-hydroxysteroids by *Arthrobacter globiformis*,[124] is introduced into the cultural liquid, it stops the transformation of 5α-pregnanes by *Rhodococcus sp. IOKh-77* at the stage which has been achieved by that moment.[12]

Frequently the destruction of a steroid molecule is connected with the assimilation of steroids by bacteria as a source of carbon and energy. Microbial transformations, such as dehydrogenation of ring A and 9α-hydroxylation, which are necessary for the synthesis of biologically active compounds, are stages preceding the complete degradation of a steroid skeleton. Therefore, to perform a certain transformation, one selects or obtains microorganisms whose enzymic systems responsible for partial or complete degradation of a steroid substrate are blocked. For example, to accumulate the desired products of the transformation of β-sitosterol by the bacterium *Arthrobacter oxydans*, a strain was designed lacking the plasmide carrying the 1,2-OSD gene. This strain (No. 317) was isolated after treating the parent strain with dodecyl sulfate.[125] The *Rhodococcus sp. IOKh-77* strain has probably lost the plasmide responsible for the ability to form 1,2-dehydrosteroids under unfavorable environmental conditions.

The ability of bacteria of the *Rhodococcus sp.* genus to assimilate steroids is one of the features that is used for their classification.[112] However, the ability to partially or completely degrade the steroids depends not only on the *Rhodococcus* species, but also on the nature of dissociative variants, which appear due to the natural variability of these and the related microorganisms[126] (*Arthrobacter, Corynebacterium,* and *Nocardia*). The cells of S-, R-, and M-variants of *Rhodococcus sp. IOKh-77*, named according to the type of colonies formed by each of them, *i.e.*, smooth, rough, or mucoid,[126] differ in their specific transforming activity towards 3β-acetoxy(hydroxy)-5α-pregnenolone and the corresponding Δ^5-3β-hydroxy analogs and in their ability to degrade the steroid metabolites formed from the above-mentioned substrates.[12] The S- and R-bacteria transform 3β-hydroxypregnenolone **29** to give 80 % and 60 % overall yields of metabolites, the contribution of 9α-hydroxy-Δ^{16}-progesterone **41** being 60 % and 50 %, respectively. 9α-Hydroxy-Δ^{16}-progesterone **41** is used in the chemical synthesis of the 9α-hydroxy analog of 16α,17α-dioxolane **40** according to Scheme 22.[9]

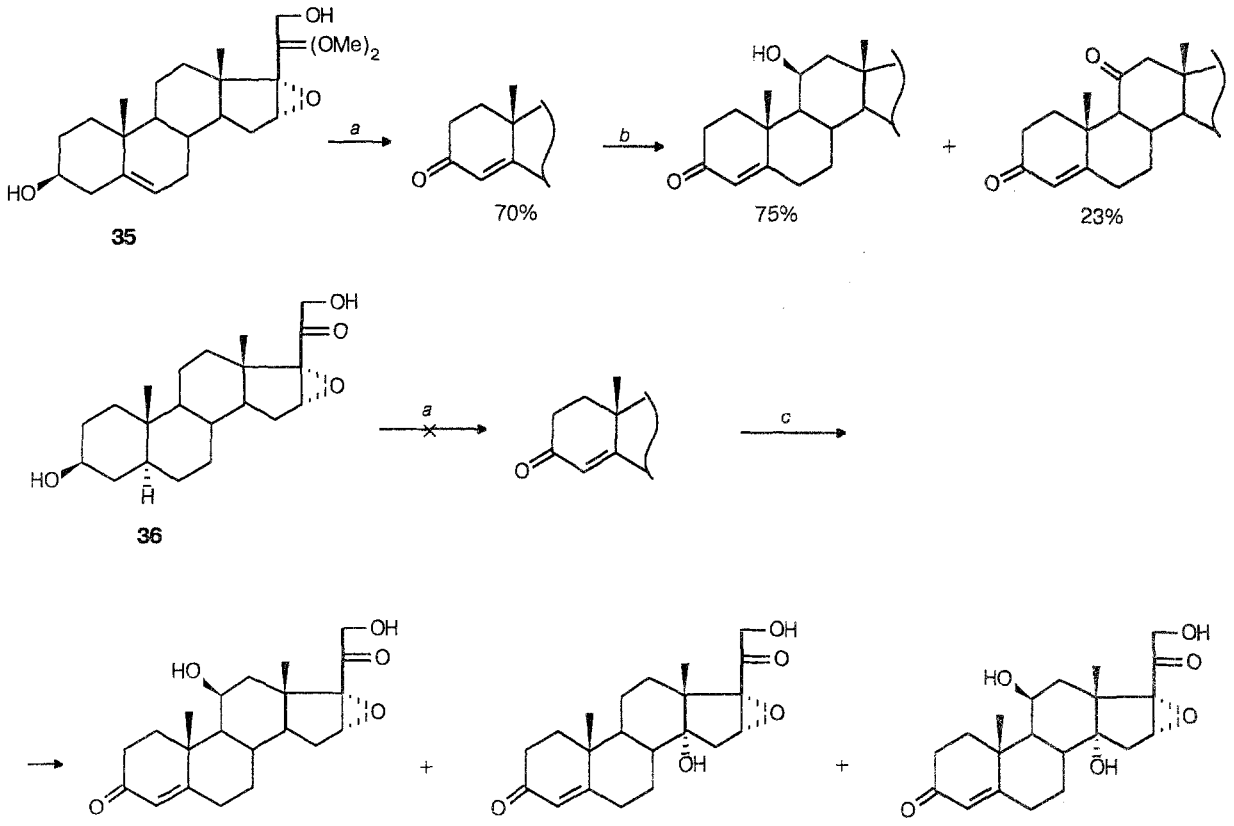
The foregoing data on transformations of different 5α-steroids and Δ^5-acetoxy(hydroxy)steroids by the *Rhodococcus sp. IOKh-77* culture show that 9α-hydroxylation depends mainly on the character of the D ring functionalization and manifests itself after the formation the Δ^4-3-oxo fragment.[7–12,105] It should be taken into account that the ability of *Rhodococcus sp. IOKh-77* to transform Δ^5-3β-hydroxysteroids into the Δ^4-3-oxo derivatives, frequently encountered in bacteria,[13–20] seems not to correlate with its ability to dehydrogenate ring A of 5α-steroids. For instance, neither *Corynebacterium*

Table 2. Microbial modification of 16a,17a-epoxy-20-pregnanes and the 20,20-dimethyl acetals thereof

Ring A; side chain	Microorganism (inhibitor)	Substrate contents / g L^{-1}	Transformation time/h	Target product: Fragments inserted	Target product: Yield (%)	Side product: Fragments inserted	Side product: Yield (%)	References
5α-H,3β-OH; 20-CO,21-CH_3	*Rhodococcus sp. IOKh-77*	1.0	26	9α-OH; Δ^4-3-CO-	28	9α,20β-$(OH)_2$-	28	7
5α-H,3β-OH; 20-CO,21-CH_3	*Rhodococcus sp. IOKh-77* (α,α-dipyridyl)	1.0	40	Δ^4-3-CO-	40	20β-OH-	22	7
Δ^5-,3β-OH; 20-CO,21-CH_3	*Circinella sp.*	0.5	26	9α-OH	55	7α,9α-$(OH)_2$-	5	123
Δ^5-,3β-OH; 20-CO,21-CH_3	*Rhodococcus sp. IOKh-77*	3.0*	40	9α-OH; Δ^4-3-CO-	70	9α,20β-$(OH)_2$-	15	104, 105
Δ^5-,3β-OH; 20-CO,21-CH_3	*Rhodococcus sp. IOKh-77*	1.0	17	9α-OH; Δ^4-3-CO-	16	9α,20β-$(OH)_2$-	18	6
5α-H,3β-OH; 20-CO,21-OH	*Rhodococcus sp. IOKh-77*	1.0	20	9α-OH; Δ^4-3-CO-	6	9α,20β-$(OH)_2$-	50	6
5α-H,3β-OH; 20-CO,21-OH	*Rhodococcus sp. IOKh-77*	3.0*	12	9α-OH; Δ^4-3-CO-	27	9α,20β-$(OH)_2$-	5	6
Δ^4-, 3-CO; 20-CO,21-OH	*Cunninghamella blakesleeana*	0.5	48	9α-OH	20	9α,6β-$(OH)_2$-	15	121
Δ^4-, 3-CO; 20-CO,21-OH	*Curvularia lunata*	0.5	168	11β-OH	–	7α,14α-$(OH)_2$-	10	122
Δ^4-, 3-CO; 20-$(OCH_3)_2$,21-OH	*Curvularia lunata* F-70	0.4	28	11β-OH	75	11-CO	23	6
Δ^4-, 3-CO; 20-$(OCH_3)_2$,21-OH	*Rhodococcus sp. IOKh-77*	1.0	48	9α-OH	62	–	–	7
Δ^4-, 3-CO; 20-$(OCH_3)_2$,21-OH	*Rhodococcus sp. IOKh-77*	2.0	48	9α-OH	19	–	–	7
Δ^5-, 3β-OH; 20-$(OCH_3)_2$,21-OH	*Rhodococcus sp. IOKh-77*	1.2—2.0	12—16	9α-OH; Δ^4-3-CO-	90	–	–	6
Δ^5-, 3β-OH; 20-$(OCH_3)_2$,21-OH	*Corynebacterium mediolanum*	2.0	22	Δ^4-3-CO-	70	–	–	5, 6
5α-H,3β-OH; 20-$(OCH_3)_2$,21-OH	*Corynebacterium mediolanum*	0.5		No transformation	–	–	110	
5α-H,3β-OH; 20-$(OCH_3)_2$,21-OH	*Rhodococcus sp. IOKh-77*	0.5	24	9α-OH; Δ^4-3-CO-	50	Δ^4-3-CO-	8	7
5α-H,3β-OH; 20-$(OCH_3)_2$,21-OH	*Rhodococcus sp. IOKh-77* ($CoCl_2$)	0.5	12	9α-OH; Δ^4-3-CO-	20	Δ^4-3-CO-	35	7
5α-H,3β-OH; 20-$(OCH_3)_2$,21-OH	*Rhodococcus sp. IOKh-77*	1.0*	26	9α-OH; Δ^4-3-CO-	70	–	–	6

*The steroid was added without any solvent, in the form of microcrystals obtained by sonication; in the other examples steroids were added as solutions in DMF.

Scheme 22



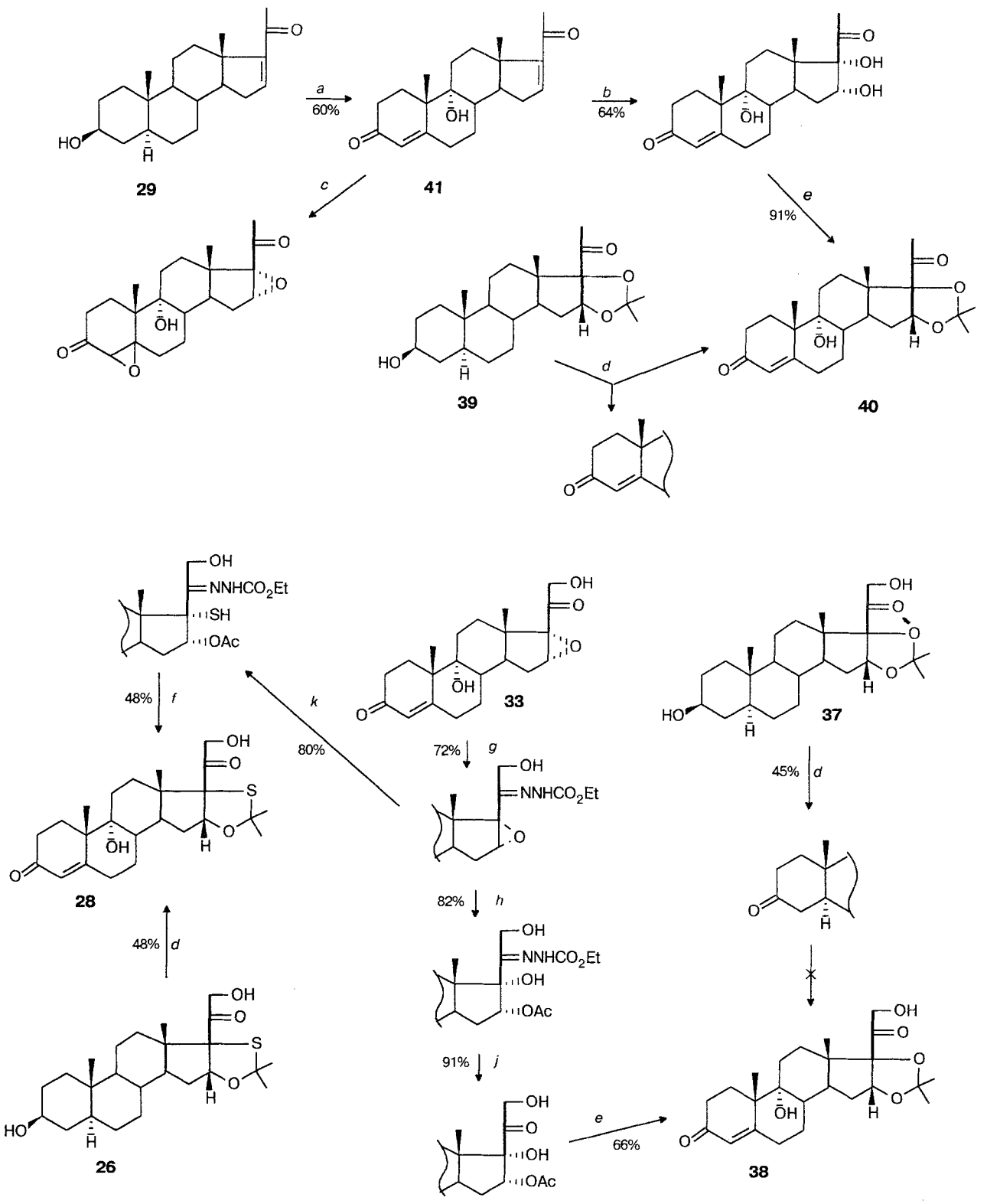
a — *Corynebacterium mediolanum*, *b* — *Curvularia lunata* F-70 (from the All-Union Collection of the Industrial Microorganisms), *c* — *Curvularia lunata*

mediolanum, which is used in the laboratory practice to modify different Δ^5-3β-hydroxysteroids into the corresponding Δ^4-3-oxo analogs[119] (Scheme 22), nor *Flavobacterium dehydrogenans*, which is used in industrial steroid production for the same purpose, introduce the double bond into ring A of 5α-steroids.[6,47] With *Flavobacterium dehydrogenans*, though, the presence of the 6β-methyl group should certainly be taken into consideration, since it may probably hamper the action of 4,5-OSD like the 6-keto group prevents the introduction of a 4,5-double bond by *Mycobacterium vaccae* and *Mycobacterium fortuitum*[57] (provided, of course, 4,5-OSD is synthesized at all in *Flavobacterium dehydrogenans* cells) (Scheme 26).

3.5. 9α-Hydroxylation of steroids by fungi and bacteria

The correlation between the 9α-hydroxylating activities of fungal and bacterial cultures towards the same steroid substrates merits consideration, especially as this has not been done yet. First, the reasons for which steroids are hydroxylated by fungi have not been conclusively established,[13–17,127] as distinct from bacteria, in which 9α-hydroxylation is an intermediate step in the process of assimilation of steroids as a carbon source. Second, hydroxylation of steroids by fungi is only in exceptional cases accompanied by dehydrogenation of ring A, which may be quite possibly explained by bacterial contamination. Third, bacteria introduce the 9α-hydroxy group only when the 3-oxo fragment is formed,[7,44] whereas fungi, *e.g.*, *Circinella*, hydroxylate both Δ^4-3-oxo and 5α-, as well as Δ^5-3β-hydroxysteroids (Scheme 24).[114,123,128,129] And finally, the most crucial distinction in our opinion lies in the modes of action of fungal and bacterial hydroxylases. In fungi, the interaction between the enzyme and the substrate and the subsequent hydroxylation proceed, according to the model proposed by Jones *et al.*[115,130] (see Ref. 1 for a detailed discussion), when the steroid molecule contains two oxygen-containing functions at a certain distance from each other, both of them accessible to the binding sites of the enzyme. Actually, the results of 9α- and 7α-hydroxylation of 20- and 17-oxo-3β-hydroxysteroids by the fungi *Circinella sp.* are in complete agreement with the Jones' model (Scheme 24). When androstenolone ethylene

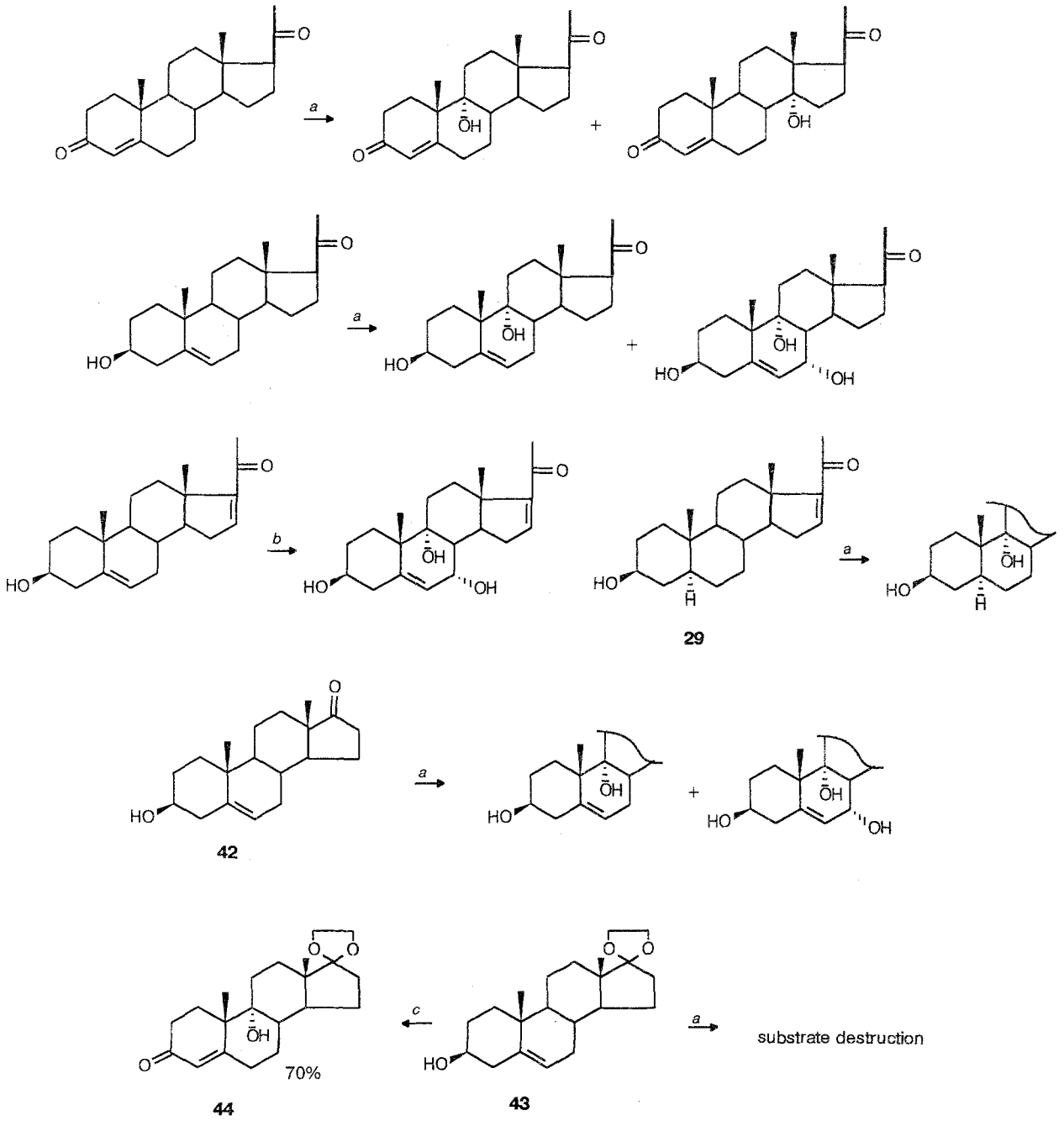
Scheme 23



a — *Rhodococcus sp. IOKh-77*, S-variant, *b* — $KMnO_4$, *c* — H_2O_2, OH^-, *d* — *Rhodococcus sp. IOKh-77*, *e* — Me_2CO, H^+, *f* — Me_2CO, MeOH, $HClO_4$, *g* — H_2NNHCO_2Et, *h* — HOAc, *j* — $(COMe)_2CH_2$, *k* — HSAc

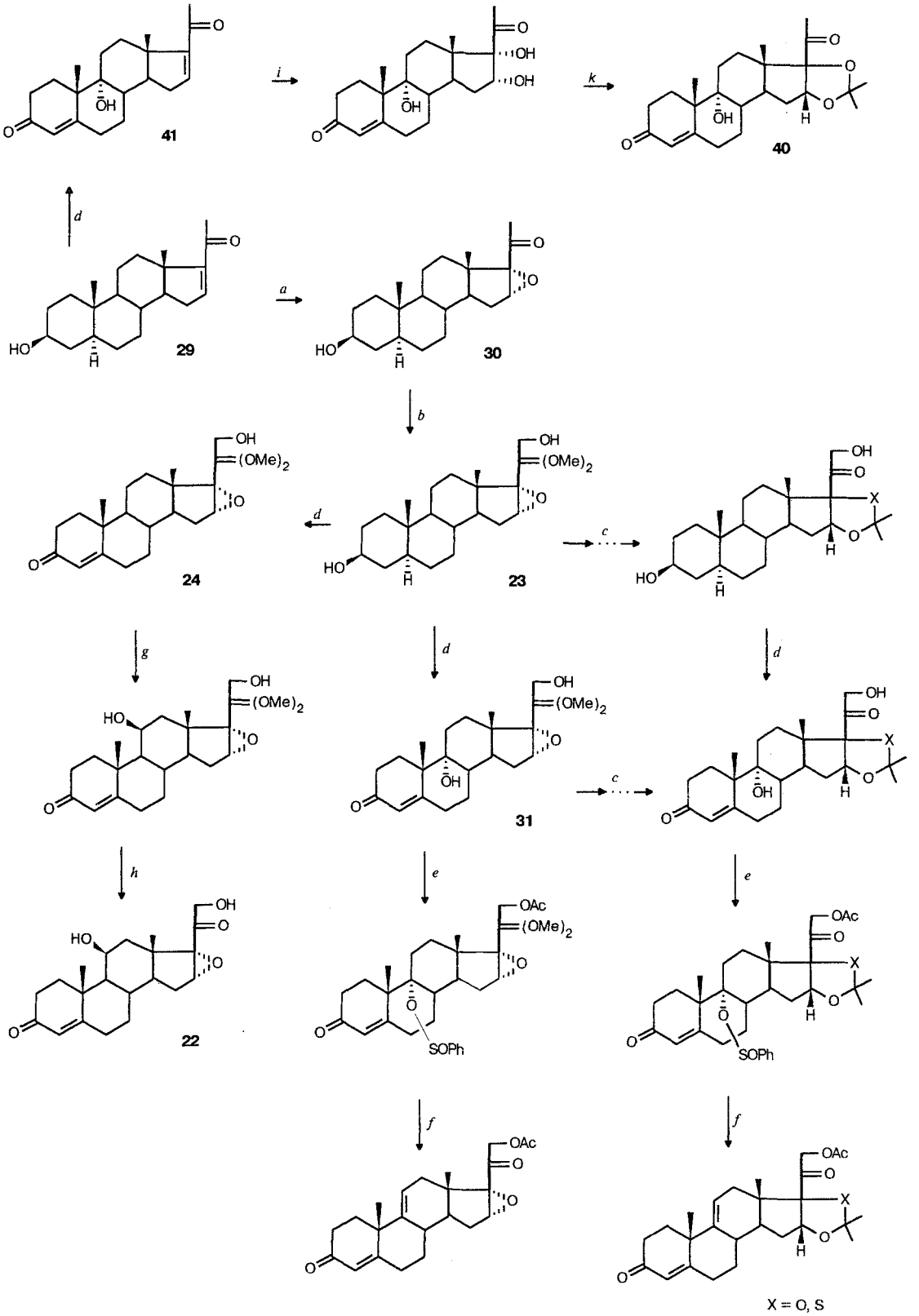
acetal (**43**), that is, a compound with only one oxygen-containing function accessible, is used instead of androstenolone **42** itself, the *Circinella sp.* culture affords a set of unidentified products. Unlike fungi, the bacterium *Rhodococcus sp. IOKh-77* transforms androstenolone ethylene acetal **43** into 9α-hydroxy derivative (**44**) in a high yield.[123] Cobalt salts inhibit bacterial 9α-hydroxylation, as has been already mentioned above, but increase the yield of 9α-hydroxy derivatives from 5α-steroids from 19 up to 49 % in the transformation by the fungus *Absidia regnieri*.[130]

Scheme 24



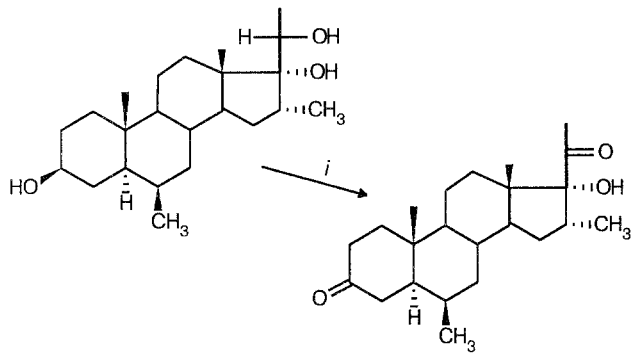
a — *Circinella sp.*, *b* — *Circinella muscae*, *c* — *Rhodococcus sp. IOKh-77*

Scheme 25



a — H_2O_2/OH^-, *b* — $PhI(OAc)_2/KOH/MeOH$, *c* — Scheme 23, *d* — *Rhodococcus sp. IIOX-77*, *e* — PhSOCl, Py, *f* — $TsOH/SiO_2$, *g* — *Curvularia lunata*, *h* — TsOH, Me_2CO, *j* — $KMnO_4$, *k* — Me_2CO, H^+

Scheme 26



i — *Flavobacterium dehydrogenans*

4. Conclusions

The data presented demonstrate that it is possible to selectively produce Δ^4-3-oxo, $\Delta^{1,4}$-3-oxo, and 9α-hydroxy-Δ^4-3-oxo derivatives from 5α-steroids. With this in mind, bacteria may be considered to be the most preferable group of microorganisms for the modification of steroids not only of the Δ^5-3β-hydroxy series, but also of the 5α-series. The undoubted advantages of bacterial species for 5α-steroid transformation into biologically active preparations become obvious on examination of Table 1. Only some fungal species perform dehydrogenation of 5α-steroids, which is additionally accompanied by degradation of a side chain. These fungi genera do not hydroxylate 5α-steroids, nor the metabolites formed from them. On the other hand, the fungi capable of hydroxylation, as well as some streptomycetes, *e.g.*, *Streptomyces sp.*, and *Streptomyces roseochromogenes*, which introduce a 16α-hydroxy group into 5α-pregnanes,[131,133] do not activate ring A. Unicellular algae[94] (24 strains examined) conduct mainly reduction of carbonyl groups, as do yeasts[87–89] (some of algae can hydroxylate steroids into 6α-, 7α-, 11α-, and 9α-positions[94,134]).

The use of the bacterium *Rodococcus sp. IOKh-77*, which differs from the above-mentioned microorganisms by its ability to simultaneously activate rings A and C, allowed us to advance a novel variant of the synthesis of the precursors of corticoid compounds[5–12,135] summarized in Scheme 25. This makes it possible now to consider 5α-steroids as a new source of steroid raw material.

References

1. A. M. Turuta, N. E. Voishvillo, A. V. Kamernitsky, *Usp. Khim.*, 1992, **61**, 1883 [*Russ. Chem. Rev.*, 1992, **61**, 1033 (Engl. Transl.)].
2. L. -G. Zhang, Z. H. Wu, Z. H. Jin, and W. S. Zhou, *Yu Chi Hua Hsuech*, 1981, **3**, 171; *Chem. Abstr.*, 1981, **95**, 93439.
3. L. Zhang, *Jiyao Gongye*, 1985, **16**, 37; *Chem. Abstr.*, 1985, **102**, 168159.
4. N. E. Voishvillo, A. A. Akhrem, *X Int. Congr. for Microbiology*, Abstr., Mexico, D. F. VIII, 1970, 46.
5. N. E. Voishvillo, A. M. Turuta, A. V. Kamernitsky, N.V. Jlantiashvili, A. A. Korobov, *Izv. Akad. Nauk, Ser. Khim.*, 1990, 690 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1990, **39**, 610 (Engl. Transl.)].
6. A. M. Turuta, A. V. Kamernitsky, N. E. Voishvillo, N. V. Jlantiashvili, A. P. Krymov, and N. V. Domrachev, *Mendeleev Commun.*, 1991, 113.
7. N. E. Voishvillo, A. M. Turuta, A. V. Kamernitsky, N. V. Jlantiashvili, V. K.Datcheva-Spasova, *Khim.-Farm. Zhurn.*, 1991, **26**, 64 (in Russian).
8. N. V. Jlantiashvili, A. M. Turuta, A. V. Kamernitsky, N. E. Voishvillo, A. A. Korobov, *Izv. Akad. Nauk, Ser. Khim.*, 1992, 1182 [*Bull. Russ. Acad. Sci., Div. Chem. Sci.*, 1992, **41**, 931 (Engl. Transl.)].
9. A. M. Turuta, N. E. Voishvillo, A. V. Kamernitsky, N. V. Jlantiashvili, A. A. Korobov, *Izv. Akad. Nauk, Ser. Khim.*, 1992, 1898 [*Bull. Russ. Acad. Sci., Div. Chem. Sci.*, 1992, **41**, 1482 (Engl. Transl.)].
10. A. M. Turuta, A. V. Kamernitsky, N. V. Jlantiashvili, A. A. Korobov, N. E. Voishvillo, *Izv. Akad. Nauk, Ser. Khim.*, 1992, 2436 [*Bull. Russ. Acad. Sci., Div. Chem. Sci.*, 1992, **41**, 1916 (Engl. Transl.)].
11. A. M. Turuta, N. V. Jlantiashvili, N. E. Voishvillo, *Izv. Akad. Nauk, Ser. Khim.*, 1993, 983 [*Russ. Chem. Bull.*, 1993, **42**, 946 (Engl. Transl.)].
12. N. E. Voishvillo, A. M. Turuta, A. V. Kamernitsky, E. S. Mil'ko, N. V. Jlantiashvili, O. G. Strelkova, *Prikl. Biokhim. Microbiol.*, 1993, **29**, 424 [*Appl. Biochem. Microbiol.*, 1993, **29**, 321 (Engl. Transl.)].
13. W. Charney and H. L. Herzog, *Microbial Transformation of Steroids*, Acad. Press, New York, 1967, 728 pp.
14. A. A. Akhrem, Yu. A. Titov, *Steroidy i Mikroorganizmy* [Steroids and Microorganisms], Nauka, Moskva, 1970, p. 526 (in Russian).
15. L. L. Smith, *Terpenoids and Steroids*, Ed. K. H. Overton, The Chem. Soc., London, 1974, **4**, 394.
16. C. Vezina and S. Rakhit, *Handbook of Microbiology*, Eds. A. T. Laskin, H. Lephevalier, CRC Press, Ohio, 1974, **4**, 117.
17. K. Kieslich, *Economic Microbiology*, Ed., A. H. Rose, Acad. Press, New York, 1980, **55**, 369.
18. S. B. Mahato and A. Mukherjee, *Phytochemistry*, 1984, **23**, 2131.
19. S. B. Mahato and S. Banerjee, *Phytochemistry*, 1985, **24**, 1403.
20. S. B. Mahato, S. Banerjee, and S. Podder, *Phytochemistry*, 1989, **28**, 7.
21. J. Dmochovska-Gladysz, *J. Basic Microbiol.*, 1991, **31**, 357.
22. D. Kluepfel and C. Coronelly, *Experientia*, 1962, **18**, 441.
23. J. Protiva and V. Schwarz, *Folia Microbiol.*, 1970, **15**, 318.
24. I. Belic, V. Hirsl-Pintaric, H. Socic, and B. Vranjek, *J. Steroid Biochem.*, 1975, **6**, 1211.
25. I. Belic, H. Socic, *Acta Microbiol.*, Acad. Sci, Hung., 1975, **22**, 389.
26. L. Zhang, E. Zhang, Z. Wu, *Acta Pharm. Sin.*, 1981, **16**, 356; *Chem. Abstr.*, 1982, **97**, 70723.
27. M. Chen, Y. Zhou, L. Zhang, *Pharm. Ind.*, 1983, **2**, 7; *Chem. Abstr.*, 1983, **99**, 156756.
28. S.-H. Wu, W.-S. Zhou, *Acta Chim. Sin.*, 1982, **40**, 629.
29. L. Zhang, J. Wang, *Acta Pharm. Sin.*, 1986, **21**, 674.
30. J.-Y. Wang, L.-Q. Zhang, R.-H. Ma, D.-Q. Fei, X.-Y. You, *Acta Pharm. Sin.*, 1987, **22**, 141.

31. US Pat. 3143543 *Chem. Abstrs.*, 1964, **61**, 11298q.
32. R. H. Mazur and R. D. Muir, *J. Org. Chem.*, 1963, **28**, 2442.
33. Y. Fa, S. Xu, *Acta Microbiol. Sin.*, 1980, **20**, 185.
34. S. Xu, Y. Fa, *Acta Microbiol. Sin.*, 1982, **22**, 361.
35. S. Xu, Y. Fa, *Acta Microbiol. Sin.*, 1984, **24**, 46.
36. Y. Fa, S. Xu, *Acta Microbiol. Sin.*, 1984, **24**, 382.
37. S. Xu, Y. Fa, *Acta Microbiol. Sin.*, 1985, **25**, 181.
38. S. Ma, Y. Fa, *Acta Microbiol. Sin.*, 1987, **27**, 73.
39. M. Hyano, H. J. Ringold, and V. Stefanovic, *Biochem. Biophys. Res. Commun.*, 1961, **4**, 454.
40. V. Stefanovic, M. Hyano, and R. G. Dorfman, *Biochim. Biophys. Acta*, 1963, **71**, 429.
41. H. J. Brodie, M. Hyano, and M. Gut, *J. Am. Chem. Soc.*, 1962, **84**, 3766.
42. Pat. 2949405, USA.
43. A. Schubert, R. Siebert, *Chem. Ber.*, 1958, **91**, 1856.
44. W.-S. Zhou, G.-M. Shen, Hua Hsuech Pao, 1979, **38**, 251; *Chem. Abstrs.*, 1980, **93**, 2193021.
45. Brit. Pat. 825210, *Chem. Abstrs.*, 1960, **54**, 14309.
46. Ger. Offen 1020329, *Chem. Abstrs.*, 1960, **54**, 25562.
47. Belg. Pat. 619759, *Chem. Abstrs.*, 1963, **58**, 14071.
48. G. Wix, K. Albrecht, *Acta Microbiol.* Acad. Sci. Hung., 1961, **8**, 339.
49. E. Visher, A. Wettstein, *Experientia*, 1953, **9**, 371.
50. E. Kondo and I. Mitsugi, *J. Am. Chem. Soc.*, 1966, **88**, 4737.
51. US Pat. 2952693, *Chem. Abstrs.*, 1961, **55**, 4880.
52. V. Gaberc-Porekar, H. E. Gottlieb, and M. Mervic, *J. Steroid Biochem.*, 1983, **19**, 1509.
53. Pat. 71.40754 Japan, *Chem. Abstrs.*, 1972, **76**, 71013.
54. Pat. 3575810, USA.
55. Pat. 71.39067, Japan.
56. US Pat. 2890153, *Chem. Abstrs.*, 1959, **53**, 20143.
57. Ger(East) DD 277697, *Chem. Abstrs.*, 1991, **114**, 80070h.
58. US Pat. 2854486, *Chem. Abstrs.*, 1959, **53**, 8209.
59. Pat. 1158866, Great Brit.
60. K. G. Büki, G. Ambrus, A. Szabo, *Acta Microbiol.* Acad. Sci. Hung., 1969, **16**, 253.
61. G. Ambrus, E. Tömorkeny, K. G. Büki, *Experientia*, 1968, **24**, 432.
62. G. Ambrus and K. G. Büki, *Steroids*, 1969, **13**, 623.
63. K. Schubert, K. H. Boehme, Ch. Kuehn, *Monatsber.*, Deut. Acad. Wiss., 1959, **1**, 57.
64. Ger. Offen 2558089, *Chem. Abstrs.*, 1977, **87**, 1659955.
65. Ger(East) DD 279901, *Chem. Abstrs.*, 1991, **114**, 120296.
66. T.-H. Stoudt, W. J. McAleer, M. A. Kozlowski, and V. Marlatt, *Arch. Biochem. Biophys.*, 1958, **74**, 280.
67. R. Howe, R. H. Moore, B. S. Rao, and D. I. Gibson, *J. Chem. Soc. Perkin Trans.*, 1973, **18**, 1940.
68. I. Belic, H. Socic, *Experientia*, 1971, **27**, 626.
69. I. Belic and H. Socic, *J. Steroid Biochem.*, 1972, **3**, 843.
70. I. Belic, R. Komel, and H. Socic, *Steroids*, 1977, **29**, 271.
71. I. Belic, M. Mervic, T. Kastelic-Suhadolc, and V. Kramer, *J. Steroid Biochem.*, 1977, **8**, 311.
72. C. J. Sih, J. Laval, and M. A. Rahim, *J. Biol. Chem.*, 1963, **238**, 566.
73. S. Ikegawa and T. Nambara, *Chem. Ind.*, 1973, **5**, 230.
74. T. Nambara, S. Ikegawa, and C. Takahashi, *Chem. Pharm. Bull.*, 1975, **23**, 2358.
75. J. Abul-Hajj, *Biochem. Biophys. Res. Communs.*, 1971, **4**, 766.
76. C. J. Sih, R. E. Bennett, *Biochim. Biophys. Acta,* 1960, **38**, 378.
77. E. Bean, L. Shang, S. Lu, *Jouji Huaxue*, 1982, **2**, 97; *Chem. Abstrs.*, 1982, **97**, 90332.
78. Y. Fa, S. Ma, Q. Su, S. Huong, *Acta Microbiol. Sin.*, 1981, **21**, 489.
79. Z.-H. Jin, L. G. Zhang, W.-S. Zhoa, *Yao Hsueh Hsueh Pao*, 1980, **15**, 730; *Chem. Abstrs.*, 1981, **95**, 113302.
80. L. J. Mulheirn, J. Van Eyk, *J. Gen. Microbiol.*, 1981, **126**, 267.
81. R. H. Mazur and R. D. Muir, *J. Org. Chem.*, 1963, **28**, 2442, Ref. 32.
82. Pat. 2762747, USA, *Chem. Abstrs.*, 1957, **51**, 5131.
83. Pat. 2876171, USA, *Chem. Abstrs.*, 1959, **53**, 16323.
84. H. R. Levy and P. Talalay, *J. Am. Chem. Soc.*, 1957, **79**, 2658.
85. H. R. Levy and P. Talalay, *J. Biol. Chem.*, 1959, **234**, 2009.
86. H. R. Levy and P. Talalay, *J. Biol. Chem.*, 1959, **234**, 2014.
87. B. Drachynska, E. Tlomak, J. Dmochovska-Gladysz, A. Siewinski, *Bull. Acad. Pol. Sci., Ser. Chem.*, 1982, **30**, 13.
88. B. Camerino, C. G. Alberti, H. Vercellone, *Helv. Chim. Acta,* 1953, **36**, 1945.
89. A. Butenandt, H. Dannenberg, J. Suranyi, *Chem. Ber.*, 1940, **73**, 818
90. Pat. 72.13716, Japan.
91. Pat. 2902410, USA, *Chem. Abstrs.*, 1960, **54**, 1660.
92. Pat. 2981659, USA, *Chem. Abstrs.*, 1961, **55**, 18007.
93. R. C. Meeks, P. D. Meister, S. H. Eppstein, J. P. Rosselet, A. Weintraub, H. C. Murray, O. K. Sebek, L. M. Reineke, and D. H. Peterson, *Chem. Ind.*, 1958, 391.
94. A. Fiorentino, G. Pinto, A. Pollio, and L. Previtera, *Bioorg. Med. Chem. Lett.*, 1991, **1**, 673.
95. R. M. Jankov, G. S. Uroševic, M. Stefanovic, *Bull. Soc. chim. Beograd*, 1977, **42**, 327.
96. H. J. Ringold, M. Hyano, V. Stefanovic, *J. Biol. Chem.*, 1963, **238**, 1960.
97. H. J. Ringold, M. Gut, M. Hyano, and A. Turner, *Tetrahedron Lett.*, 1962, 835.
98. T. Nambara, S. Ikegava, and H. Hosoda, *Chem. Pharm. Bull.*, 1973, **21**, 2794.
99. J. Abul-Hajj, *J. Biol. Chem.*, 1972, **247**, 686.
100. T. Nambara, T. Anjyo, M. Ito, and H. Hosoda, *Chem. Pharm. Bull.*, 1973, **21**, 1938.
101. R. Jerussi and H. J. Ringold, *Biochemistry*, 1965, **4**, 2113.
102. S. Ahmad, S. K. Garg, B. N. Johri, *Biotech. Adv.*, 1992, **10**, 1.
103. J.-L. Boutry and M. Barbier, *Biochem. Syst. Ecol.*, 1981, **9**, 215.
104. V. K. Datcheva, N. E. Voishvillo, A. V. Kamernitzky, R. J. Vlachov, and I. G. Reshetova, *Steroids*, 1989, **54**, 271.
105. V. K.Datcheva-Spasova, N. E. Voishvillo, A. V. Kamernitzky, R. Vlakhov, I. G. Reshetova, avail. *VINITI*, 1988, No 5552-B88 (in Russian).
106. U. Schömer and F. Wagner, *Eur. J. Appl. Microbiol.*, 1980, **10**, 99.
107. Pat. 4176123, USA, *Chem. Abstrs.*, 1980, **92**, 144974.
108. Pat. 80.162738, Japan, *Chem. Abstrs.*, 1981, **95**, 22958.
109. A. V. Kamernitzky, M. B. Gorovitz, Yu. E. Vollerner, N. E. Voishvillo, I. G. Reshetova, V. A. Paseshnichenko, *Khimiya spirostanolov* [Chemistry of Spirostanols], Nauka, Moskva, 1986, 176 pp. (in Russian).
110. N. V. Jlantiashvili. Ph. D. Thesis (chemical sciences), N. D. Zelinsky Institute of Organic Chemistry, Russian

Academy of Sciences, Moscow, 1993.
111. A. A. Akhrem, N. E. Voishvillo, *Prikl. Biokhim. Mikrobiol.*, 1970, **6**, 654 [Appl. Biochem. Microbiol., 1970, **6**].
112. M. Goodfellow, E. G. Thomas, A. S. Ward, and A. L. James, *Zbl. Bacteriol.*, 1990, **274**, 299.
113. A. A. Akhrem, N. E. Voishvillo, *Izv. Akad. Nauk, Ser. Biol.*, 1971, 302 [Bull. Acad. Sci. USSR, Div. Biol. Sci., 1971].
114. N. E. Voishvillo, Z. I. Istomina, A. V. Kamernitzky, I. V. Vesela, I. G. Reshetova, O. G. Strelkova, *Prikl. Biokhim. Mikrobiol.*, 1994, **30**, 617 [*Appl. Biochem. Microbiol.*, 1994, **30**, (Engl. Transl.)].
115. A. M. Bell, W. A. Denny, E. R. H. Jones, G. B. Meakins, and W. E. Müller, *J. Chem. Soc., Perkin Trans, I*, 1972, 2759.
116. C. K. A. Martin, *Advances in Applied Microbiology*, 1977, **22**, 28.
117. G. S. Fonken and H. C. Murray, *J. Org. Chem.*, 1969, **27**, 1102.
118. V. K. Datcheva, A. V. Kamernitzky, R. Vlakhov, N. E. Voishvillo, I. G. Reshetova, V. G. Levy, *Izv. Akad. Nauk, Ser. Khim.*, 1986, 446 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1986, **35**, 413 (Engl. Transl.)].
119. V. Datcheva, A. Kamernitzky, R. Vlakhov, N. Voishvillo, V. Levy, I. Reshetova, I. Chernoburova, *Appl. Microbiol. Biotechnol.*, 1986, **25**, 14.
120. A. P. Krymov, A. V. Kamernitzky, A. I. Terekhina, B. I. Demchenko, I. V. Vesela, A. V. Skorova, G. I. Gritzina, L. I. Ionesyan, V. I. Tropina, N. I. Kislenko, *Khim.-Farm. Zhurn. [Pharm Chem. J.]*, 1988, **22**, 82 (in Russian).
121. A. M. Turuta, A. V. Kamernitzky, A. A. Korobov, V. S. Bogdanov, T. G. Baklashova, G. V. Komarova, K. A. Koscheenko, *Khim.-Farm. Zhurn.*, 1990, **24**, 682 [*Pharm. Chem. J.*,1990, **24**, 440 (Engl. Transl.)].
122. Pat. 2976283, USA.
123. N. E. Voishvillo, Z. I. Istomina, A. V. Kamernitzky, *Izv. Akad. Nauk, Ser. Khim.*, 1994, 737 [*Russ. Chem. Bull.*, 1993, **42**, No. 4 (Engl. Transl.)]
124. V. K. Datcheva, N. E. Voishvillo, A. V. Kamernitzky, R. Vlakhov, I. G. Reshetova, E. I. Chernoburova, *Prikl. Biokhim. Mikrobiol.*, 1987, **23**, 617 (in Russian).
125. R. K. Dutta, M. K. Roy, and H. D. Singh, *J. Basic. Microbiol.*, 1992, **32**, 167.
126. E. S. Mil'ko, *Prikl. Biokhim. Mikrobiol.*, 1990, **26**, 732 (in Russian).
127. H. L. Holland, *Chem. Soc. Rev.*, 1982, **11**, 371.
128. A. Schubert, D. Onken, R. Siebert, R. Heller, *Chem. Ber.*, 1958, **91**, 2549.
129. M. B. Gorovitz, F. S. Khristulas, N. K. Abubakirov, *Khim. Prirodn. Soedin.*, 1970, **6**, 273 [*Chem. Nat. Compd.*, 1970, No. 6 (Engl. Transl.)].
130. A. M. Bell, E. R. H. Jones, G. D. Meakins, *J. O. Miners, and A. L. Wilkins., J. Chem. Soc. Perkin Trans. I*, 1975, 2040.
131. R. Neher, P. Desaulles, E. Vischer, P. Wieland, and A. Wettstein, *Helv. Chim. Acta*, 1958, **41**, 1667.
132. Pat. 3033749, USA.
133. M. Iida, H. Iizuka, *J. Pharmacol. Dyn.*, 1981, **4**, s. 49.
134. Pat. 5215894, USA, *Chem. Abstrs.*, 1993, **119**, 115490.
135. A. M. Turuta, A. V. Kamernitzky, N. V. Jlantiashvili, L. K. Kavtaradze, A. A. Korobov, *Izv. Akad. Nauk, Ser. Khim.*, 1991, 1185 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1991, **40**, 1063 (Engl. Transl.)].

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