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MICROBIOLOGICAL TRANSFORMATION IN A SERIES OF NITROGEN-CONTAINING HETEROCYCLES (REVIEW)

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We generalize literature data on transformation of aromatic and hydrogenated heterocycles. We show that in some cases, the processes have been accomplished regioselectively and stereoselectively in preparative yields.

By microbiological transformation we mean incomplete conversion of organic compounds by enzymes of microorganisms, accompanied by accumulation of the conversion products in the medium. The reactions are accomplished by one or several enzymes and do not lead to significant changes in the structure of the substrate [1]. The products of partial conversion (transformation) of many organic compounds exhibit valuable practical properties. Furthermore, the chemical industry and biotechnology need to draw on microbiological methods to solve ecology problems on the one hand and on the other hand to search for sufficiently simple methods for synthesis of compounds which are difficult to obtain by chemical means [1, 2].

Biotransformation is less laborious and more economical than chemical reactions, and also ensures high regioselectivity and stereoselectivity of the reactions. Accordingly, capital investment in this area has become more advisable, since the demand is increasing for many compounds obtained by the biotransformation method for organic synthesis, the biotechnology and pharmacology industries (for example, the demand for optically active intermediates) [3-4].

The advantages of the microbiological transformation method over chemical reactions include the following.

1. The specificity of action of enzymes, which makes it possible to accomplish very fine rearrangement of molecules of organic compounds using simple processing schemes, while the analogous chemical conversions usually require laborious multistep syntheses.

2. The "mild" conditions of action of the enzymes, since the latter function usually in aqueous nonaggressive media and at a temperature no higher than 100°C.

3. The moderate amounts of wastes and byproducts which are harmful for the biosphere [5].

Microbiological transformation processes may include oxidation, reduction, decarboxylation, hydrolysis, methylation, condensation, esterification, halogenation, resolution into enantiomers, isomerization, amination, synthesis of nucleotides from precursors [1, 6].

At the present time, transformation of steroids, organic acids, carbocyclic hydrocarbons, terpenes, and aromatic compounds have been best studied [7]. Processes of microbiological transformation of heterocyclic compounds, especially nitrogen-containing heterocycles, have been studied to a much lesser degree. Thus in the book by Fonken and Johnson [8], out of the more than 650 papers mentioned, only about 100 concern transformation of these compounds to any degree. A whole chapter is devoted to this topic in the monograph by Skryabin and Golovleva [1], but even in that chapter the number of cited papers is no more than 150. The last review on this subject [9] was published more than 15 years ago. Moreover, especially in recent years, a number of new investigations have appeared on microbiological transformation, predominantly hydroxylation of azines and some other nitrogen-containing heterocycles.

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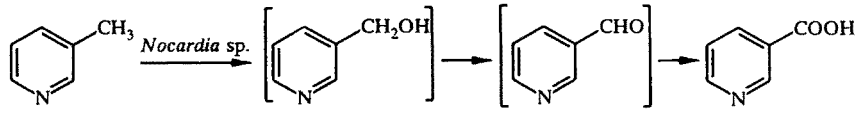
1. HYDROXYLATION OF MONOCYCLIC NITROGEN-CONTAINING HETEROCYCLES

1.1. Hydroxylation of Azines

In 1968, for the first time the possibility was observed for oxidation of the methyl group of 3-methylpyridine to a carboxyl group by cells of *Nocardia*, *Candida*, *Mycobacterium*, *Arthrobacter* from the collection of the Institute of Biochemistry and Physiology of Microorganisms [10].

The microorganisms were cultured on n-paraffins (n-hexadecane and others); the medium for the transformation contained 3-methylpyridine and n-paraffin (cometabolism). In the given case, the n-paraffin was the growth substrate, while 3-methylpyridine (I) was oxidized to nicotinic acid (II) in yield up to 80% [11].

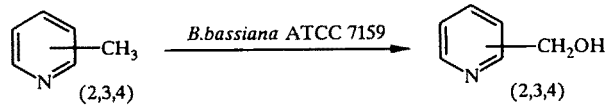


I II

In the absence of n-paraffins, oxidation did not occur.

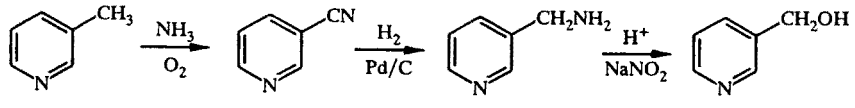
In contrast to the indicated microorganisms, the fungus *Sporotrichum sulfurescens* ATCC 7159 (*Beauveria bassiana* ATCC 7159)* oxidized 3-methylpyridine and its isomers III to the corresponding alcohols IV.

The transformation process is one-step, and the hydroxymethylpyridines obtained were not metabolized further [14-16]:



III IV

Thus, 3-, 2-, and 4-hydroxymethylpyridines are obtained using a washed cell suspension of *B. bassiana* ATCC 7159, cultured on a medium with glucose and corn extract, in yields up to 40% [15]. The chemical synthesis of 3-hydroxymethylpyridine is complicated and is accomplished in three steps [17]:



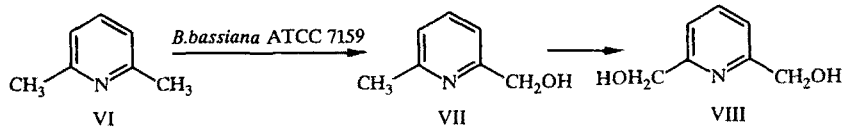
I V

This method requires the use of expensive and aggressive reagents (Pd, H_2O_2), and is unsuitable for industry. Furthermore, the chemical method for obtaining 3-hydroxymethylpyridine is unsuitable for synthesis of analogous compounds containing a second methyl group in other positions of the pyridine ring, since it also can undergo oxidative ammonolysis [15].

We should note that the one-step microbiological synthesis described here is of significant interest because the pyridyl carbinols obtained as a result and their derivatives are physiologically active compounds, for example 3-hydroxymethylpyridine tartrate is the drug Ronicol® (Roche), which regulates lipid metabolism in the human organism [17, 18]. Some derivatives (esters) of 2-hydroxymethylpyridine lower the cholesterol, lipid, and triglyceride level in mice [15].

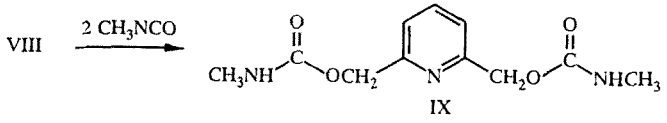
Microorganisms transform not only mono- but also dialkylpyridines. For example, the fungus *B. Bassiana* ATCC 7159, cultured on a medium with glucose and corn extract, hydrolyzes 2,6-dimethylpyridine (VI) with formation of 2-methyl-6-hydroxymethylpyridine (VII) in 88% yield and trace amounts of 2,6-dihydroxymethylpyridine (VIII) [14, 15].

*In 1970, this strain was reclassified and named *Beauveria bassiana* ATCC 7159 or *Beauveria sulfurescens* ATCC 7159 [12, 13]. In the following, we call it *Beauveria bassiana* ATCC 7159.

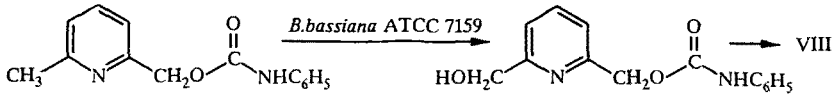


The transformation can be accomplished both by the growing culture and by a suspension of nonreproducing cells in buffer solution.

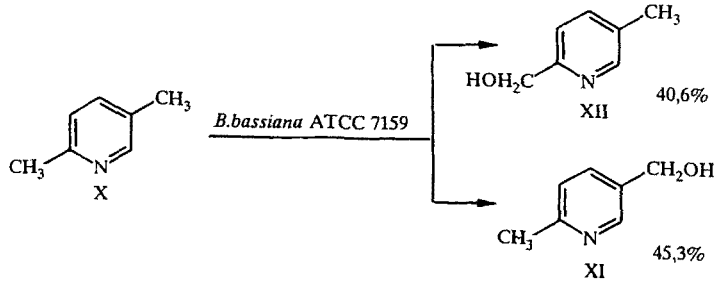
We should note that chemical synthesis of alcohols VII and VIII is a rather complicated multistep process, described in detail in the literature [17]. Both these compounds are of practical interest: the alcohol VII is used in synthesis of crown ethers [19]; the 2,6-dihydroxy derivative of VIII is a key intermediate in synthesis of the antisclerotic drug Parmidine (IX) [20, 21]:



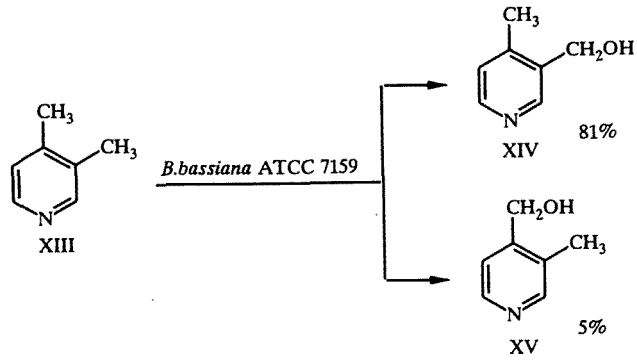
The authors were able to obtain the 2,6-dihydroxymethylpyridine (VIII) needed for synthesis of this drug in 34% yield when using a compound with a modified oxymethyl group as the original substrate: the N-phenylcarbamate of 2-methyl-6-hydroxymethylpyridine [22, 23]. Hydroxylation of the substrate and subsequent hydrolysis is accomplished by cells of *B. bassiana* ATCC 7159 in one step:



Transformation of other isomeric dimethylpyridines by this fungus was studied earlier in a number of papers. In particular, it was established that hydroxylation of 2,5-dimethylpyridine (X) leads to a mixture of 2-methyl-5-hydroxymethylpyridine (XI) and 3-methyl-6-hydroxymethylpyridine (XII) [14, 15]:

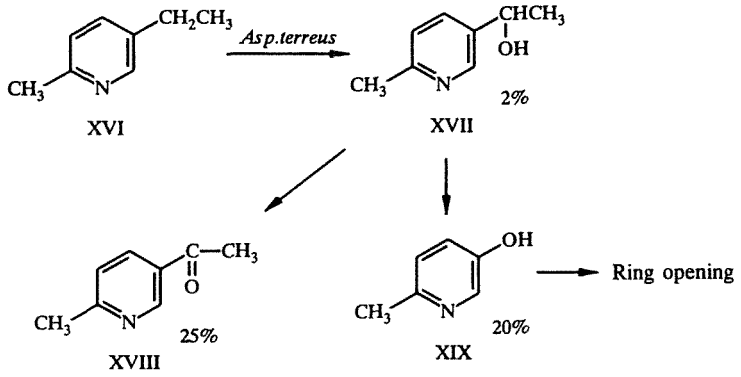


Two isomers are also formed upon oxidation of 3,4-dimethylpyridine (XIII), but the yield of 4-methyl-5-hydroxy methylpyridine (XIV) is significantly higher than the yield of 3-methyl-4-hydroxymethylpyridine (XV):



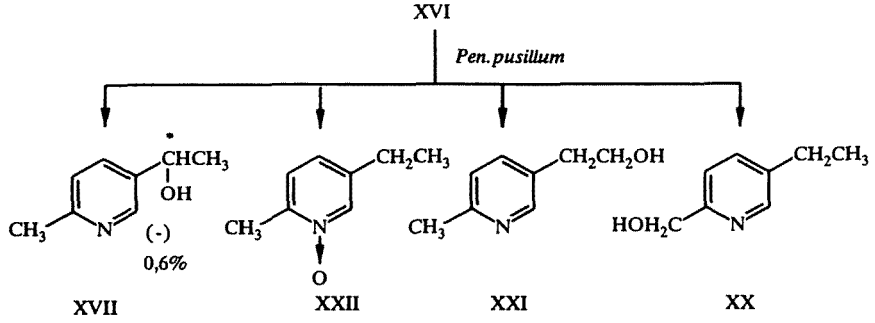
In this case, it has been shown that the products of transformation are not converted further or consumed by the fungus. It is interesting to note that this strain does not hydroxylate the aromatic ring of methylpyridines, but rather oxidizes only the methyl groups; oxidation at the nitrogen atom also has not been noted.

Even earlier it was observed that the fungi *Penicillium pusillum* and *Aspergillus terreus*, isolated by the authors from natural sources and cultured in a medium with glucose, are capable of utilizing 2-methyl-5-ethylpyridine (XVI) as a source of nitrogen [24]. The cultures utilized up to 40% of the substrate. In this case, the *Asp. terreus* culture proved to be more active:



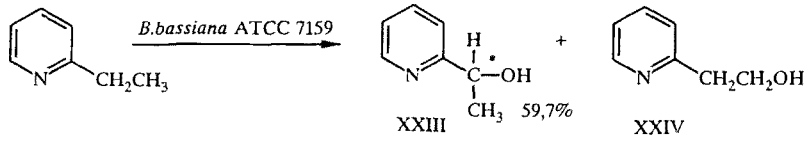
The process occurred with formation of 2-methyl-5-(1-hydroxyethyl)pyridine (XVII) (2% yield), which then was converted to 2-methyl-5-acetylpyridine (XVIII) in 25% yield in the first case, and in the second case 6-methyl-3-hydroxypyridine (XIX) was formed in 20% yield, after which opening of the pyridine ring occurred.

A culture of the fungus *Pen. pusillum* over the course of four days converted 2-methyl-5-ethylpyridine into a mixture of 2-hydroxymethyl-5-ethylpyridine (XX), 2-methyl-5-(2-hydroxyethyl)pyridine (XXI), 2-methyl-5-(1-hydroxyethyl)pyridine (0.6% yield) and the N-oxide of 2-methyl-5-ethylpyridine (XXII):



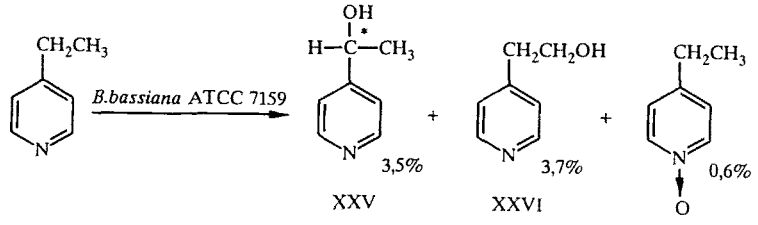
In the case described above (*Asp. terreus*), a secondary alcohol is formed containing an asymmetric carbon atom. However, the presence of optically active compounds was not noted. Moreover, in the literature numerous examples are described of the formation during microbiological transformation of only one or predominantly one enantiomer. In this case, three variants are possible: a) enantioselective hydroxylation of one of the enantiomers of the racemic mixture; b) stereoselective hydroxylation of the prochiral center of the substrate; c) enantioselective conversion of one of the enantiomers of the racemate to an inactive state [25]. Two of these possible conversions were encountered by the authors when investigating transformation of 2-methyl-5-ethylpyridine by the fungus *Pen. pusillum*, preferentially hydroxylating the methylene group with formation of the levorotatory enantiomer [26]. Furthermore, the presence in the culture liquid of the product of further oxidation of the secondary alcohol to 2-methyl-5-acetylpyridine stimulated the authors to investigate the possibility of obtaining the dextrorotatory enantiomer of the same alcohol when culturing the same fungus on racemic 2-methyl-5-(1-hydroxyethyl)pyridine as the substrate. In fact, in this situation the levorotatory isomer was preferentially oxidized to the ketone, and its (+)-stereoisomer accumulated in the mixture [27]. In both cases, the yield of optically active carbinols was 17-25%. We note that the β-dialkylaminoethyl esters of pyridyl carbinols, analogous to 2-methyl-5-(1-hydroxyethyl)pyridine, display antihistaminic activity, while 6-methyl-3-hydroxypyridine is promising as a geroprotector [9].

Later it was shown that the strain *B. bassiana* ATCC 7159 is capable of even more stereoselectively hydroxylating 2-methyl-5-ethylpyridine, and also isomeric 2- and 4-ethylpyridines [28, 29]. When using 2-ethylpyridine, the optically active (−)-2-(1-hydroxyethyl)pyridine (XXIII) was isolated as the major transformation product in 60% yield, and it was 100% optically pure. 2-(2-Hydroxyethyl)pyridine (XXIV) was formed in trace amounts [29]:



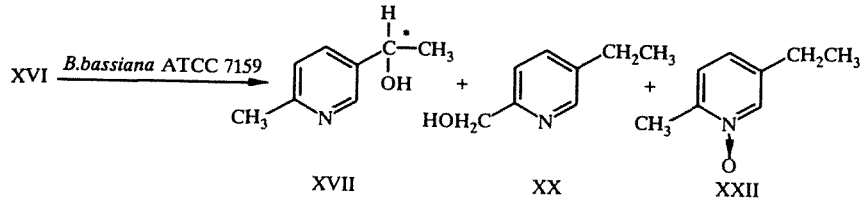
XXIII XXIV

During microbiological transformation of 4-ethylpyridine by the same strain, optically active (−)-4-(1-hydroxyethyl-pyridine (XXV) was also obtained (in no higher than 4% yield), which according to literature data had the S configuration [30]. The second hydroxylation product was isolated in comparable amounts: 4-(2-hydroxyethyl)pyridine (XXVI), also of practical interest [31]. The N-oxide of 4-ethylpyridine was isolated in trace amounts. Thus in the case of 4-ethylpyridine, the process occurred nonregioselectively [29]:



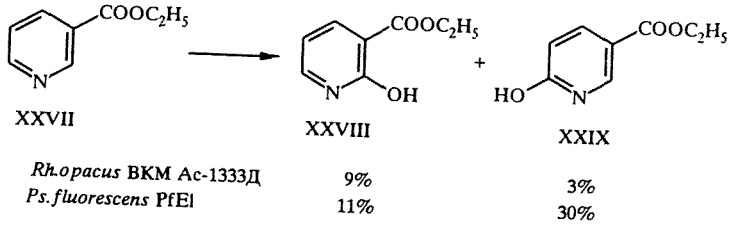
XXV XXVI

Transformation of 2-methyl-5-ethylpyridine by the fungus *B. bassiana* ATCC 7159 led to oxidation of the methylene group of this compound, and the optically active (−)-2-methyl-5-(1-hydroxyethyl)pyrimidine (XVII) was obtained in 10% yield. Its specific rotation was comparable with known data for the structurally similar (−)-3-(1-hydroxyethyl)pyridine [30, 32], which suggests high optical purity of the compound obtained. In contrast to the transformation of 4-ethylpyridine, no products of terminal oxidation of the ethyl group of 2-methyl-5-ethylpyridine were observed. At the same time, the methyl group in the 2 position of the pyridine ring was also hydroxylated, which led to formation of 2-hydroxymethyl-5-ethylpyridine (XX), the yield of which was comparable with the yield of the secondary alcohol XVII. As in the case of 4-ethylpyridine, upon oxidation of 2-methyl-5-ethylpyridine a small amount of the corresponding N-oxide was formed [29]:

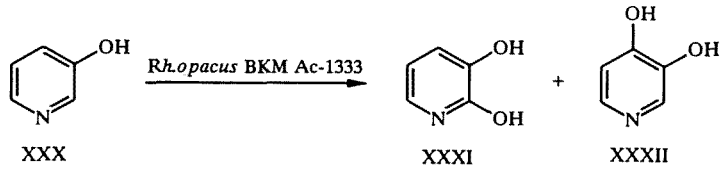


Thus in transformation of 2-methyl-5-ethylpyridine, as in the case of 2,5-dimethylpyridine, both alkyl substituents undergo oxidation.

In later investigations [33], pyridine carboxylic acids were used as the transformation substrates, since hydroxy derivatives of isomeric pyridine carboxylic acids display valuable pharmacological properties [34]. It has been established that dissociated R-variant cells of the strain *Rhodococcus opacus* VKM Ac-1333D and the strain *Pseudomonas fluorescens* PfE1, utilizing pyridine and naphthalene as the growth substrate, are capable of hydroxylating the ethyl ester of nicotinic acid (XXVII) to the isomeric 2- and 6-hydroxy derivatives (XXVIII and XXIX) in a suspension of nonreproducing cells [33]:



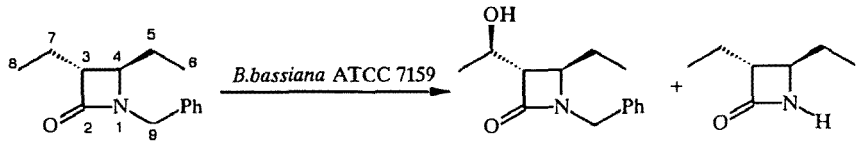
Furthermore, it has been established [33] that dissociated S- and M-variant cells of *Rh. opacus* VKM Ac-1333D carried out hydrolysis of the ester linkage with formation of nicotinic acid, then M-variant cells transformed nicotinic acid to 3-hydroxypyridine (XXX), the subsequent oxidation of which led to formation of the isomeric diols 2,3- and 3,4-dihydroxypyridines (XXXI and XXXII) in 12% and 34% yields respectively [33, 35]:



1.2. Transformation of Saturated Monocyclic Nitrogen-Containing Heterocyclic Compounds

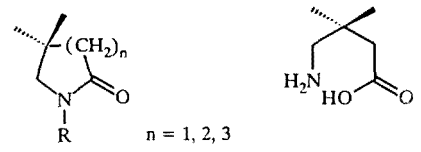
Among reduced nitrogen-containing heterocycles, there is great interest in transformation of monocyclic β-lactams, since they often display interesting pharmacological properties. Furthermore, such rings are contained in the structure of a number of antibiotics.

Upon transformation of the monocyclic β-lactam XXXIII by the fungus *B. bassiana* ATCC 7159, growing on a medium with glucose and corn extract [25], the hydroxyl group was introduced into the 7 position. The yield of the hydroxy derivative XXXIV was 10%; the second transformation product XXXV was formed upon cleavage of the benzyl radical in 20% yield [25]:



XXXIII XXXIV XXXV

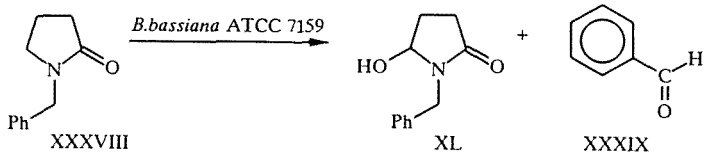
From a pharmacological viewpoint, hydroxy compounds having five or more atoms in the heterocyclic ring with general formula XXXVI are of significant interest:



XXXVI XXXVII

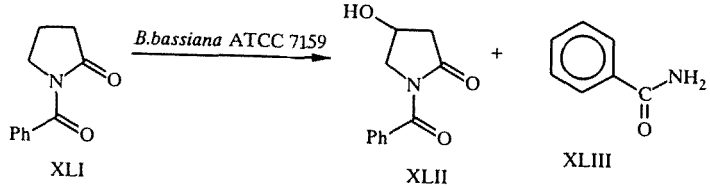
They can be considered as analogs of γ-amino-β-hydroxybutyric acid (XXXVII) [36], having important medicinal value. Furthermore, the pyrrolidine heterocycle is part of the molecule of the alkaloid nicotine, the piperidine ring is part of the molecule of the alkaloid anabasine [37]. These nitrogen-containing heterocycles are parts of the molecules of many other natural alkaloids [38] and toxins, such as lobeline, hyoscyamine, scopolamine [39], batrachotoxin, pumiliotoxins A and C, histriotoxins etc. [40-43]. So microbiological methods for obtaining hydroxylated derivatives of pyrrolidine and piperidine may be of pharmacological importance.

The possibility of hydroxylation of N-substituted pyrrolidines, pyrrolidones, piperidines, piperidones, and their analogs by the fungus *B. bassiana* ATCC 7159 has been studied in [36, 44]. When carrying out the process in a medium with corn extract, hydroxylation of 1-benzyl-2-pyrrolidone (XXXVIII) was not observed, and the transformation product was the benzaldehyde (XXXIX). When accomplishing the transformation in a medium with soy flour and yeast extract, the optically active 1-benzoyl-5-hydroxy-2-pyrrolidone (XL) was formed in 12% yield and benzaldehyde was formed in 2% yield:



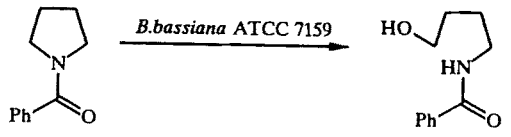
XXXVIII XL XXXIX

In the case of 1-benzoyl-2-pyrrolidone (XLI) in a medium with corn extract and glucose, transformation products were not observed; when carrying out the process in a medium with soy flour and yeast extract, the optically active 1-benzoyl-4-hydroxy-2-pyrrolidone (XLII) was observed in the incubation mixture (21% yield) in a mixture with benzamide (XLIII) [44]:



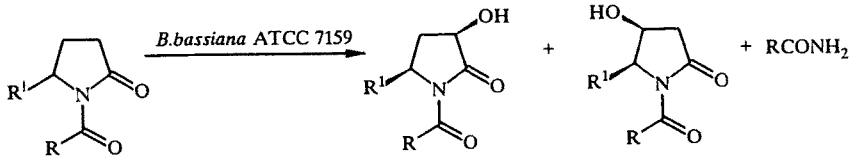
XLI XLII XLIII

When using 1-benzoylpyrrolidine (XLIV) as the substrate in a medium with soy flour and yeast extract, hydroxylation of the carbon atom in the 2 position occurred with opening of the ring and formation of N-(4-hydroxybutyl)benzamide (XLV) in 8% yield [36]:



XLIV XLV

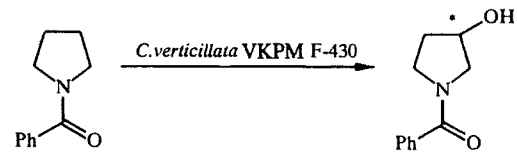
Further study of hydroxylation of a series of substituted pyrrolidones XLVIa-b by a culture of *B. bassiana* ATCC 7159 [44] showed that in a medium with soy and yeast extracts, both the 3 position (XLVIIa-b) and the 4 position (XLVIIIa-b) of the heterocycle undergo stereoselective hydroxylation, but the yields of the corresponding optically active alcohols are no greater than 10-12% [44]:



XLVI a,b XLVII a,b XLVIII a,b

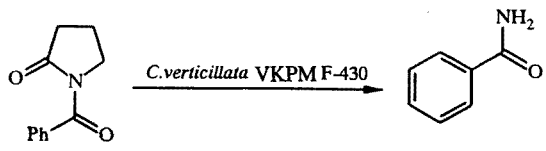
XLVI a R = CH_2Ph, R^1 = H; XLVII a R = CH_2Ph, R^1 = H(10%); b R = Ph, R^1 = CH_3 (5%);
XLVIII a R = CH_2Ph, R^1 = H(11%); b R = Ph, R^1 = CH_3 (12%)

At the same time, using the fungus *Cunninghamella verticillata* VKPM F-430 for transformation of 1-benzoylpyrrolidine (XLIV) and 1-benzoyl-2-pyrrolidone (XLI), isolated from natural sources [45], led to different results. The first of these compounds ensured obtaining optically active (−)-1-benzoyl-3-hydroxypyrrolidine (XLIX) in 38% yield [46]:



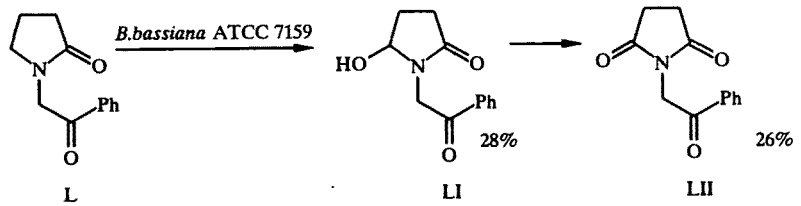
XLIV XLIX

The process occurred in preparative yield only when using a growing culture, since in a suspension of nonreproducing cells the yield of the alcohol XLIX was only 13%. Upon transformation of 1-benzoyl-2-pyrrolidone (XLI) by the same fungus, in the incubation mixture only the benzamide (XLIII) and a wide range of products were identified, which indicates nonspecificity of the process accompanied by degradation of the substrate [46]:



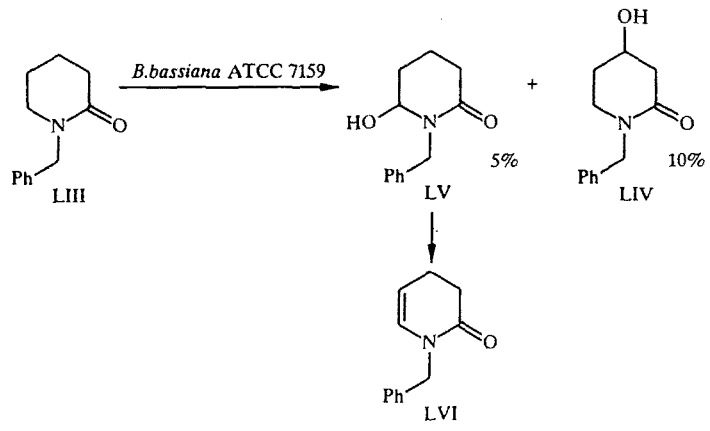
XLI XLIII

Upon hydroxylation of 1-phenacyl-2-pyrrolidone (L) by the fungus *B. bassiana* ATCC 7159, the process occurred through a stage of formation of the intermediate 1-phenacyl-5-hydroxy-2-pyrrolidone (LI), and the end product of the reaction 1-phenacylpyrrolidinedione (LII) (N-phenylsuccinimide) was formed in 23% yield [44]:



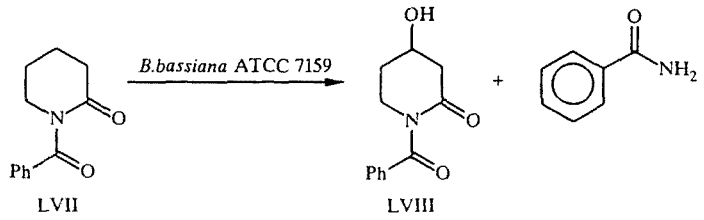
L LI LII

Enlargement of the heterocycle by one CH_2 group leads to a partial change in the orientation of the introduction of the hydroxyl group. Thus when culturing the same fungus in the presence of 1-benzylpiperidone (LIII) (in a medium with corn extract), along with 1-benzyl-4-hydroxy-2-piperidone (LIV) (10% yield), the unstable 1-benzyl-6-hydroxy-2-piperidone was observed in the culture liquid (5% yield), which then was spontaneously converted to its dehydration product (1-benzyl-2-oxo-1,2,3,4-tetrahydropyridine) (LVI) [36]:



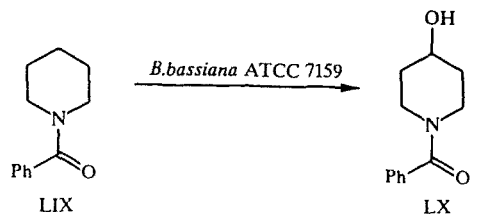
We note that in a medium with soy and yeast extracts, the process proceeds with formation of only a single unstable compound LV.

Upon hydroxylation of 1-benzoyl-2-piperidone (LVII) by the fungus *B. bassiana* ATCC 7159 in a medium with corn extract, along with benzamide the optically active 1-benzoyl-4-hydroxy-2-piperidone (LVIII) was isolated from the reaction mixture in 27% yield [36]:

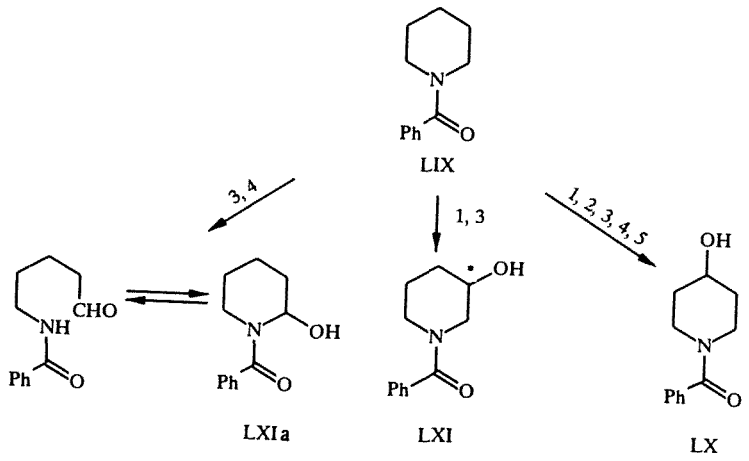


When the process was carried out in a medium with soy and corn extracts, the hydroxylation product was also 4-piperidinol (26% yield), but the benzamide was isolated along with it from the culture liquid in 6% yield.

The use of 1-benzoylpiperidine (LIX) as the substrate did not change the orientation of hydroxylation. In both media mentioned, 1-benzoyl-4-hydroxypiperidine (LX) was isolated in low yield (7%). Similar results were obtained more than 20 years ago in [47], but when carrying out the process in a medium including glucose and corn extract, the yield of alcohol reached 18%:



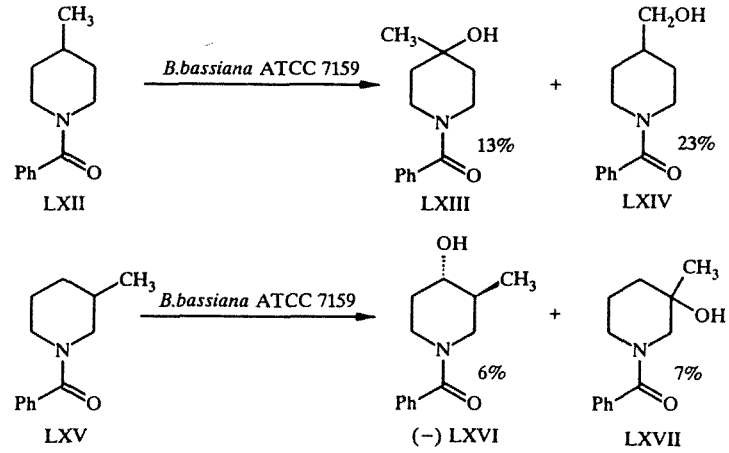
When using other strains of microorganisms for transformation of 1-benzoylpiperidine (LIX), it was established that in addition to the 4-hydroxy isomer LX, the optically active (+)-3-hydroxy-1-benzoylpiperidine (LXI) and the 2-hydroxy-1-benzoylpiperidine (LXIa) are also formed [46, 48]:



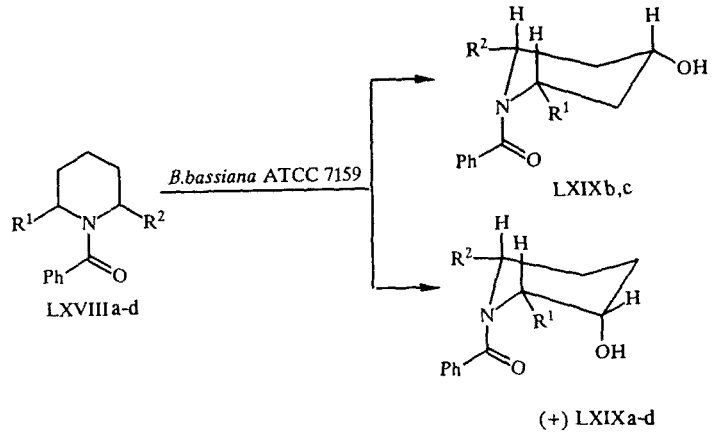
1 — *Beauveria bassiana* VKM F-3111D; 2 — *Aspergillus niger* VKM F-1119;
3 — *Penicillium simplicissimum* KM-16; 4 — *Aspergillus awamori* VKM F-758;
5 — *Cunninghamella verticillata* VKPM F-430

In this case, only two of the investigated strains (*Asp. niger* VKM F-1119 (2) and *C. verticillata* VKPM F-430 (5)), accomplished the process regioselectively with formation of the 4-hydroxy isomer LX in 80% and 20% yields respectively. It is important to note that the strains *B. bassiana* VKM F-3111D (1), *Asp. niger* VKM F-1119 (2), and *Asp. awamori* VKM F-758 (4) are more active in the growing culture than in a suspension of nonreproducing cells, which made it possible to increase the yield of 1-benzoyl-4-hydroxypiperidine to 60%, 80%, and 34% respectively.

When replacing the substrate by 1-benzoylalkylpiperidines, the transformation process proceeds in more different ways. Thus in the case of 1-benzoyl-4-methylpiperidine (LXII), along with the expected 4-hydroxy derivative LXIII we see that 1-benzoyl-4-hydroxymethylpiperidine (LXIV) was obtained, and the isomeric 1-benzoyl-3-methylpiperidine (LV) was hydroxylated both in the 4 position (LXVI) and in the 3 position (LXVII) of the heterocycle, and 1-benzoyl-4-hydroxy-3-methylpiperidine (LXVI) was formed as the optically active levorotatory enantiomer [49]:



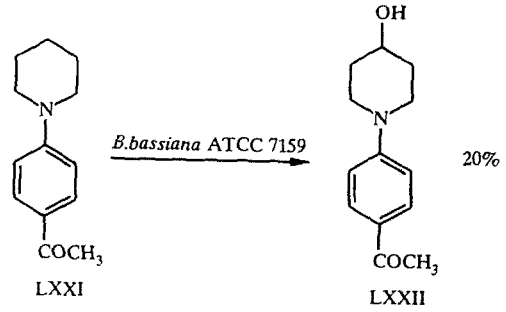
Similar orientations of predominantly 3-hydroxylation by this fungus were retained in principle also in the case of 1-benzoyl-2-alkylpiperidines (LXVIIIa-d), and the yields of the cis-3-hydroxy isomer LXXa-d (always optically active) decrease with an increase in the length of the chain of the substituent R^1, while the yields of the racemic cis-4-hydroxy isomer LXIXb,c do not change. We note that in the case of 1-benzoylconiine (R = C_3H_7), a small amount (2%) of racemic 1-benzoyl-2-(2-hydroxypropyl)piperidine was also observed in the incubation mixture [49]:



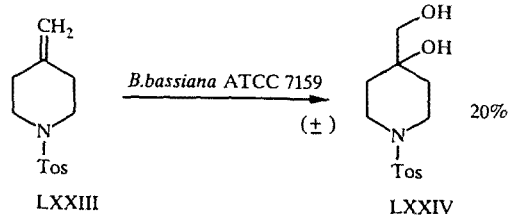
LXIX a $R^1 = CH_3$, $R^2 = H$; b $R^1 = C_2H_5$, $R^2 = H$ (8 %); c $R^1 = C_3H_7$, $R^2 = H$ (8 %); d $R^1 = R^2 = CH_3$; LXX a $R^1 = CH_3$, $R^2 = H$ (36%); b $R^1 = C_2H_5$, $R^2 = H$ (20%); $R^1 = C_3H_7$, $R^2 = H$ (14 %); d $R^1 = R^2 = CH_3$ (49%)

In later investigations [50], the transformation of 1-(4-acetylphenyl)piperidine (LXXI) and 4-methylene-1-tosylpiperidine (LXXIII) by a growing culture of *B. bassiana* ATCC 7159 was studied under standard conditions in [47].

The process of transformation of compound LXXI occurred regioselectively with formation of 4-hydroxy-1-(4-acetylphenyl)piperidine (LXXII) in 20% yield [50]:



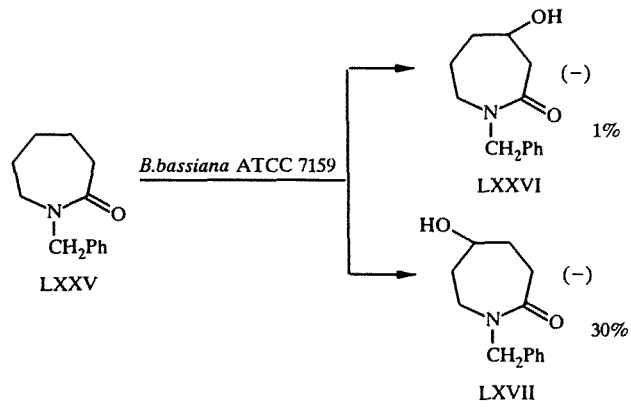
Upon transformation of piperidine LXXIII, the process also occurred regioselectively with formation of racemic 4-hydroxy-4-hydroxymethyl-1-tosylpiperidine (LXXIV) in 20% yield [50]:



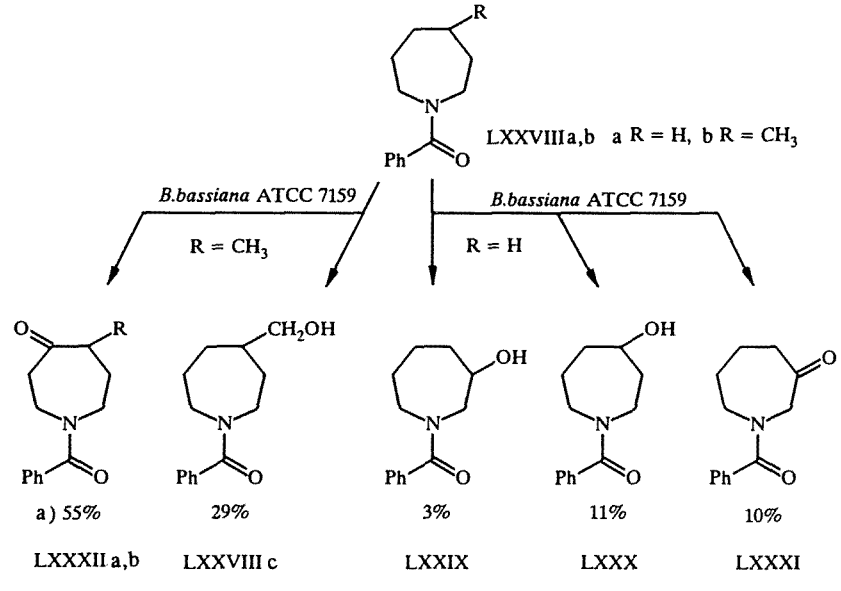
The steady interest in hydroxypiperidines is obviously connected with the fact that a significant number of physiologically active drugs are observed among the piperidinols [20, 51], and recently it has been established that they can be used as components of cosmetics [52].

Thus the fungus *B. bassiana* ATCC 7159 and some other fungi and bacteria accomplish hydroxylation of derivatives of piperidine preferentially at the 3 and 4 positions, and in a number of cases the process occurs stereoselectively.

It has been established that the process of hydroxylation of 1-benzylcaprolactam (LXXV) by the fungus *B. bassiana* ATCC 7159 occurs analogously, as a result of which the optically active isomeric hydroxy derivatives LXXVI and LXXVII are formed, the yield of which is higher when cells of the fungus are cultured in a medium with corn extract than when culturing the same fungus on soy and yeast extracts [36]:



1-benzoylhexamethylenimine (LXXVIII) was oxidized similarly by the fungus *B. bassiana* ATCC 7159 (independently of the culture medium) to the two optically active 3- and 4-hydroxy derivatives LXXIX and LXXX, and in lower yields [36]:



Moreover, according to data obtained 18 years earlier [47], this fungus oxidized the same substrate LXXVIIIa to a mixture of 3- and 4-oxo-1-benzoylhexamethylenimines LXXX and LXXXIa, and the yield of transformation products was significantly higher than for subsequent authors. Possibly such a marked difference in the nature of the processes of transformation of compound LXXVIIIa by the same strain in work by different authors is connected with a decrease in the hydroxylating activity of the enzyme system of the fungus *B. bassiana* ATCC 7159 over time, since in [53] it was established that the given strain loses its hydroxylating activity upon reseeding and depending on the storage conditions.

A similar mixture of 3- and 4-oxo isomers (in 1:3 ratio) is obtained upon microbiological oxidation by the fungus *B. bassiana* ATCC 7159 of compounds with eight-membered and nine-membered rings, 1-benzoylheptamethylenimine and 1-benzoyloctamethylenimine. At the same time, upon introduction of a methyl group at the 4 position of the heterocycle (compound LXXVIIIb), along with the oxo derivative LXXXIIb (11% yield), a second product LXXVIIIc was observed with a hydroxymethyl group (29% yield 11% yield) [47].

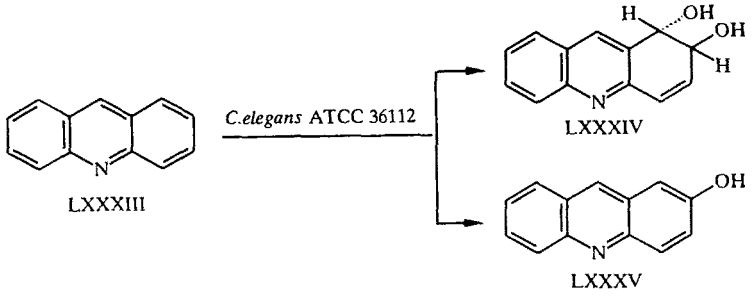
2. HYDROXYLATION OF POLYCYCLIC NITROGEN-CONTAINING HETEROCYCLIC COMPOUNDS

2.1. Condensed Azines

2.1.1. Quinoline, Acridine, and Its Derivatives

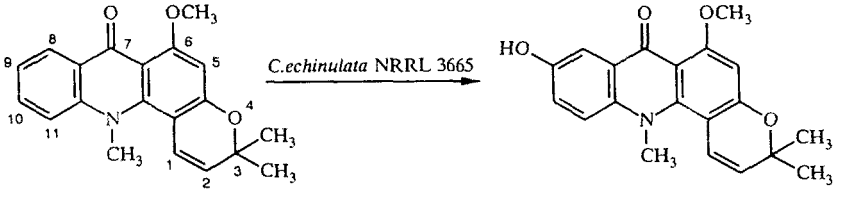
Degradation of the quinoline ring by microorganisms occurs through a stage of hydroxylation of the pyridine ring in the 2 position with subsequent oxidative hydrolytic cleavage of the hetero- and carbocyclic rings [54, 55], although microbiological oxidation of these heterocycles to N-oxides has also been described [56]. However, processes of microbiological transformation of alkyl-substituted quinolines are connected with hydroxylation of both the aromatic ring and the substituents.

Thus, recently American scientists showed that transformation of acridine (LXXXIII) by a growing culture of the fungus *Cunninghamella elegans* ATCC 36112 leads to formation of predominantly acridine-trans-1,2-dihydrodiol (LXXXIV) and small amounts of 2-hydroxyacridine (LXXXV) [57]:



LXXXIII — LXXXIV — LXXXV

Hydroxylation of the benzene ring was also observed upon oxidation of the antitumor drug acronycine LXXXVI by fungi of the *Cunninghamella* genus. In this case, the most active strain *Cunninghamella echinulata* NRRL 3665 converted acronycine (30% yield) to 9-hydroxyacronycine (LXXXVII) [58]:

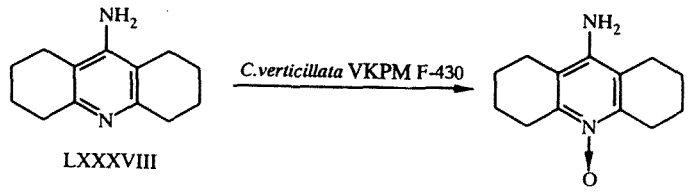


LXXXVI — LXXXVII

The strain *Cunninghamella bainieri* ATCC 9244 transformed compound LXXXVI, forming along with the phenol LXXXVII a whole series of metabolites: 11-hydroxyacronycine, 9,11-dihydroxyacronycine, 3-hydroxymethyl- 11-hydroxy-acronycine. All these compounds are observed in animals as the products of metabolism of compound LXXXVI [59]. This is not surprising, since it has been shown that the strain *C. bainieri* ATCC 9244 exhibits monooxygenase activity, similar to the activity of the microsomal fraction of rat liver in oxidation of different aromatic compounds [60].

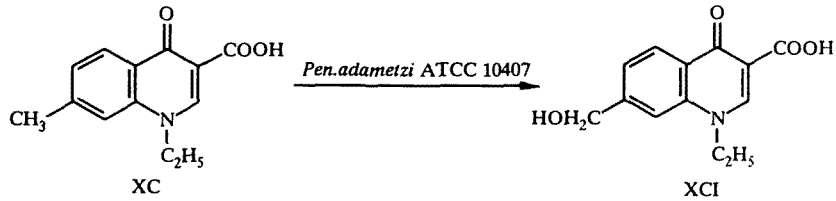
As a result, the reaction of microbiological oxidation of acronycine to compound LXXXVII on the one hand models the process of conversion of the drug in humans and animals and on the other hand is a method for obtaining preparative amounts of this potentially active metabolite [58].

We note that partially hydrogenated 9-amino-1,2,3,4,5,6,7,8-octahydroacridine (LXXXVIII), which is an analog of the drug Tacrine, was converted by a suspension of nonreproducing cells of the fungus *C. verticillata* VKPM F-430 only to its 10-oxide LXXXIX in 90% yield [61, 62]:



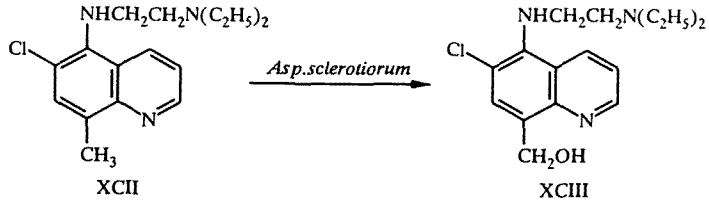
LXXXVIII LXXXIX

On the other hand, in a growing culture of the fungus *Pen. adametzi* ATCC 10407, preferential oxidation of the methyl group of 3-carboxy-7-methyl-1-ethyl-4-quinolone (XC) to the alcohol (compound XCI) is observed [63], while the aromatic carbon atoms are not involved:



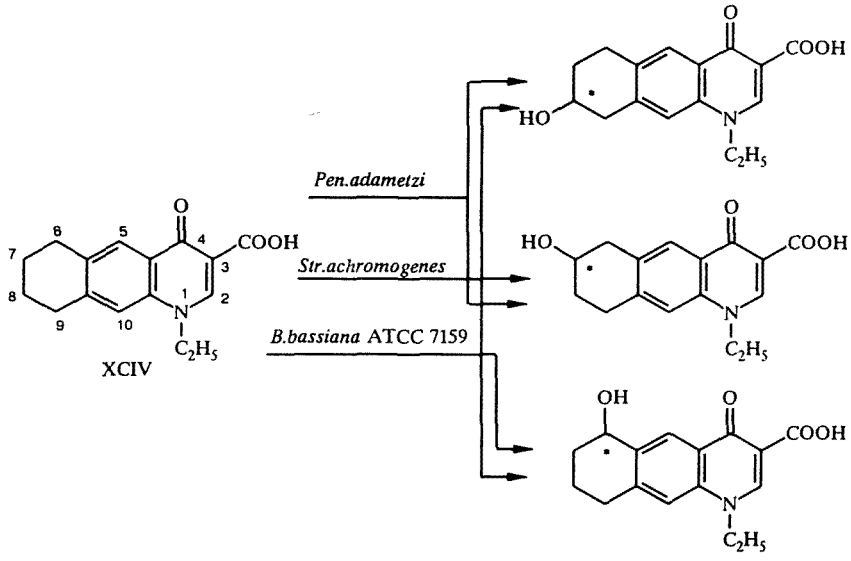
XC XCI

Upon oxidation of compound XCII (an antimalarial drug) by the fungus *Aspergillus sclerotiorum*, also only the methyl group of the benzene part of the molecule is hydroxylated, with formation of the alcohol XCIII [64]:



XCII XCIII

A similar pattern is observed upon oxidation by various microorganisms of a more complex quinoline derivative containing a hydrogenated ring condensed with benzene: 1-ethyl-4-oxo-1,4,6,7,8,9-hexahydrobenzo-[γ]-quinoline-3-carboxylic (XCIV) acid [63]:



XCIV

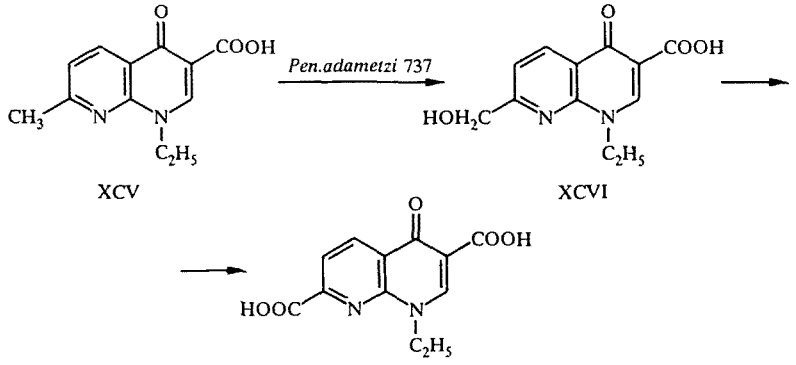
The fungus *Pen. adametzi* introduces a hydroxyl group at the 7 and 8 positions, cells of *Streptomyces achromogenes* introduces it at the 6, 7, and 8 positions, while the fungus *B. bassiana* ATCC 7159 introduces it only at the 6 position. In all cases, the methylene group of the hydrogenated ring are oxidized. The strain *B. bassiana* ATCC 7159 carries out the reaction of hydroxylation regiospecifically, while cells of other microorganisms catalyze formation of two or three isomeric compounds.

Thus upon microbiological oxidation of quinoline, acridine, and their substituted derivatives, we observe both hydroxylation of the pyridine and benzene rings and oxidation of the nitrogen atom of the heterocycle or different alkyl substituents, and in the latter case the regiospecificity of the oxidation depends on the microorganism used for the transformation.

2.1.2. Naphthiridines

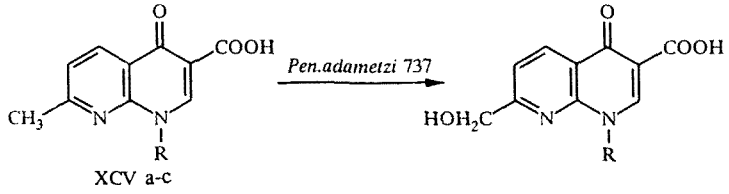
With the goal of studying conversions in the human organism of nalidixic acid (an antibiotic used for treatment of urinary tract infections), its transformation by microorganisms was investigated.

The fungus *Pen. adametzi* 737 converts nalidixic acid (XCV) to the hydroxymethyl derivative XCVI in a yield reaching 60%, but its further oxidation leads to formation of the 3,7-dicarboxylic acid XCVII [65]:



XCV XCVI XCVII

Hydroxylation of nalidixic acid with formation of the carbinol XCVI also occurs in the human organism, and this alcohol displays *in vitro* the same order of activity as the original substrate [66], i.e., this is a long-acting drug. Subsequent oxidation of the acid XCV to the dicarboxylic acid XCVII, which can be accomplished by (in addition to *Pen. adametzi* 737) forty-seven fungi from six orders, models the pathways for its metabolism in the human organism [65]. Hydroxymethyl derivatives are also formed upon oxidation by the fungus *Pen. adametzi* 737 of analogs of nalidixic acid XCVa-c [8]:



XCV a-c

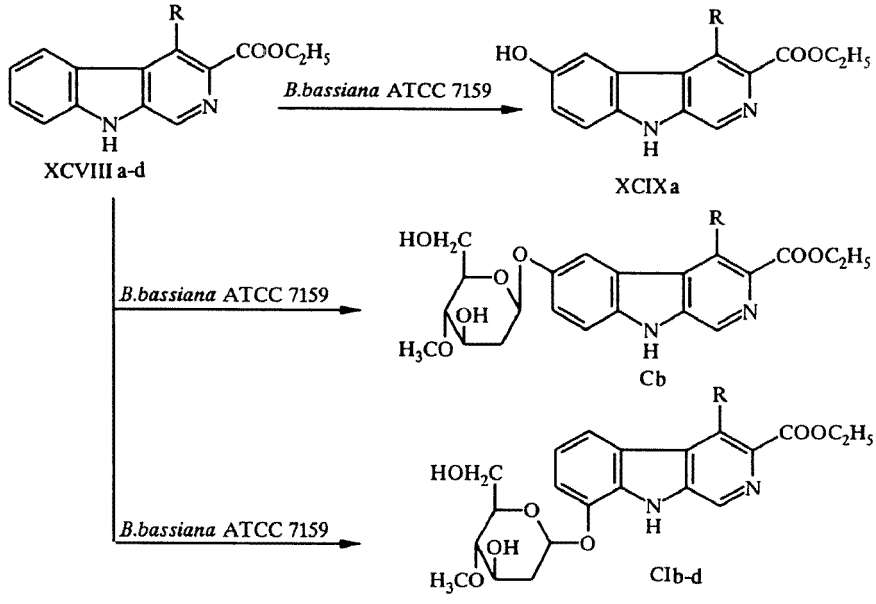
R a $CH_2CH{=}CH_2$ (62%); b $CH_2CH_2CH_3$ (62%); c $(CH_2)_5CH_3$ (17%)

In all cases, the methyl group is oxidized but the yield of hydroxylation product decreases with an increase in the length of the chain of the substituent at the 1 position.

2.1.3. β-Carboline, Its Derivatives, and γ-Carboline

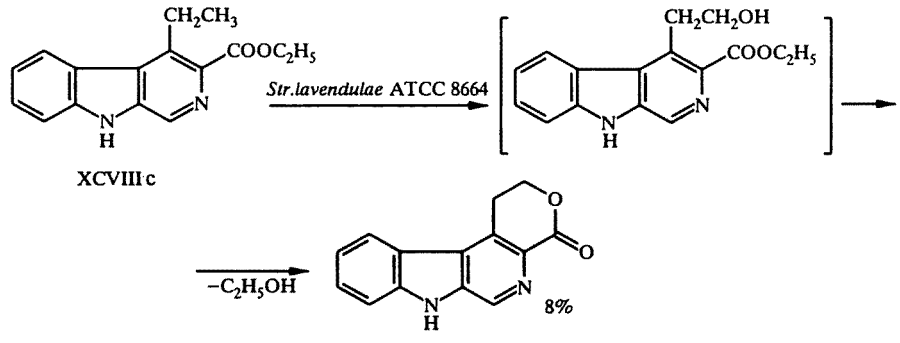
Within a plan for studying the functions and metabolic pathways for β-carboline and its derivatives in the human organism, the conversions of these compounds by fungi and actinomycetes were studied. It has been observed that cells of the microorganism *B. bassiana* ATCC 7159, *Streptomyces lavendulae* ATCC 8664, *Streptomyces griseus* ATCC 10137 display a capability for hydroxylating 3-ethoxy-carbonyl-4-R-β-carbolines XCVIIIa-d in preparative yields [67]. The most active proved to be the fungus *B. bassiana* ATCC 7159. Such a case of hydroxylation of a benzene ring is unusual for this strain.

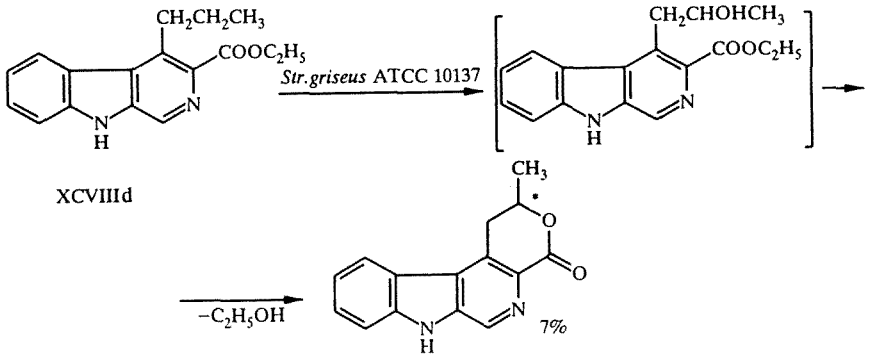
As a rule, *B. bassiana* ATTC 7159 hydroxylates only the alkyl substituents of different substrates, without involving the aromatic ring. Oxidation of β-carboline and other compounds in this series is a unique example of the introduction of a hydroxyl group into an aromatic ring. Some of the hydroxylation products can be isolated from the incubation mixture in the form of glycosides, and the culture uses the components of the growth medium for glycosylation. The unglycosylated 6-hydroxy derivative XCIXa is formed in 62% yield only from the 4-unsubstituted carboline XCVIIIa. Upon introduction of an alkyl substituent into the substrate molecule (compounds XCVIIIb-d), the hydroxylation products are completely glycosylated and are represented by 6- and 8-glycosides (Cb and CIb-d respectively). Furthermore, an increase in the length of the chain of the alkyl substituent in the 4 position leads to preferential formation of 8-glycoside.



XCIX a R = H (62%); C b R = CH_3 (20%); CI b R = CH_3 (18%), c R = C_2H_5 (70%), d R = C_3H_7 (68%)

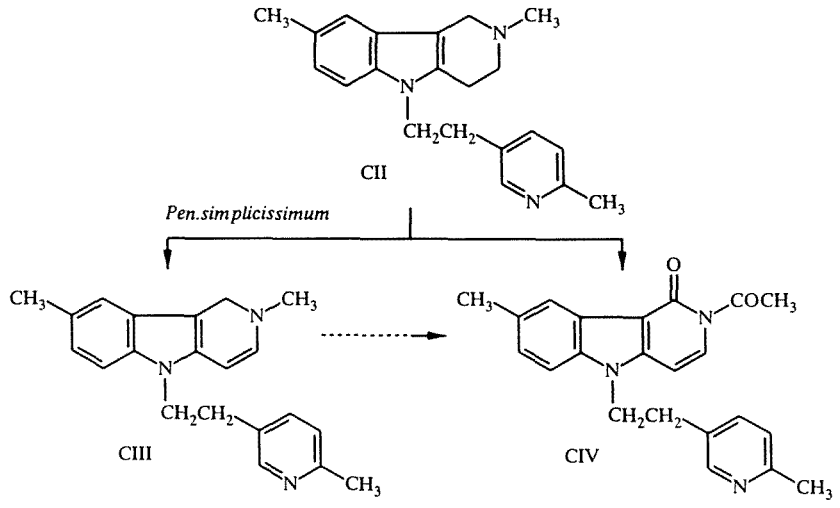
In contrast to the fungus *B. bassiana* ATCC 7159, cells of the actinomycetes *Str. lavendulae* ATCC 8664 and *Str. griseus* ATCC 10137 accomplish hydroxylation of the alkyl substituent of compounds XCVIIIc and d at the β-position of the alkyl group, which is accompanied by formation of the corresponding lactones due to subsequent transesterification. The product yield of such reactions is not high, 7-8% [67]:





Thus the processes of microbiological hydroxylation of condensed heterocycles containing an indole and a pyridine ring occur preferentially at the alkyl groups of the latter (at the α- and β-position relative to the ring), although in a number of cases introduction of an hydroxyl group into the aromatic ring is also possible.

In studying the process of microbiological transformation of 3,6-diethyl-9-[2-(2-methylpyridine-5)-ethyl]-1,2,3,4-tetrahydro-γ-carboline dihydrochloride (CII) (the effective antihistaminic drug Dimebon (USSR)) by different fungi it was established that the strains *Aspergillus awamori* VKM F-758, *Aspergillus niger* VKM F-1119, *B. bassiana* VKM F-3111D, and *Cunninghamella verticillata* VKPM F-430 do not transform this substrate, and only the fungus *Penicillium simplicissimum* (both in a suspension of nonreproducing cells and in a growing culture) accomplishes the process of transformation of Dimebon with formation of compounds CIII and CIV, each in 10% yield [68]:

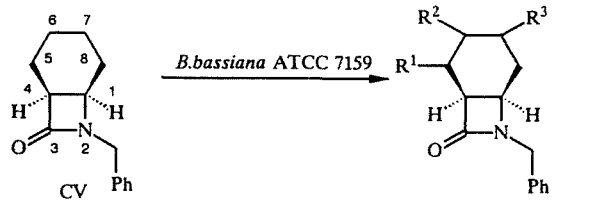


We can say that compound CIV was formed as a result of further demethylation and oxidation of the dehydro derivative CIII with subsequent acetylation of the nitrogen atom of the pyridone ring.

2.2. Saturation of Polycyclic Nitrogen-Containing Heterocycles. Hydroxylation of Bicyclic and Tricyclic Lactams and Amides

As has already been noted, the β-lactam ring is included in the structure of many antibiotics [25]. Accordingly, there is great interest in hydroxylation of bicyclic and tricyclic β-lactams, since introduction of an hydroxyl group into the antibiotic molecule may impart to it new antimicrobial properties. Furthermore, in this case we may obtain a compound having optical activity.

Upon hydroxylation of the bicyclic β-lactam (CV) by the fungus *B. bassiana* ATCC 7159 growing on a medium with glucose and corn extract, three optically active hydroxy derivatives are formed, containing the OH group in the 5 position (CVI) (8% yield), the 6 position (CVII) (23% yield), and the 7 position (CVIII) (15% yield) [25]:

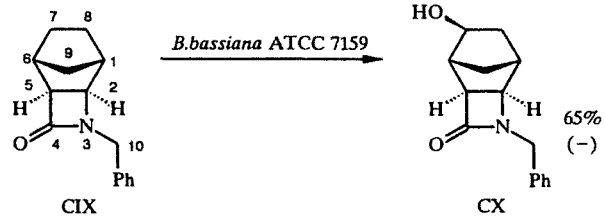


(+)-CVI $R^1 = OH, R^2 = R^3 = H$

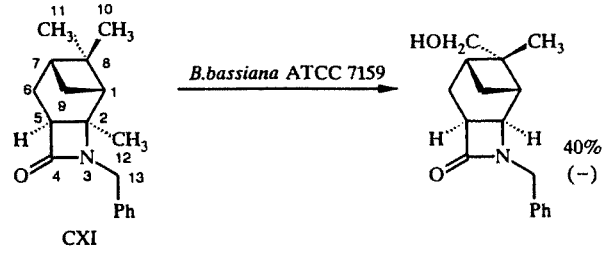
(+)-CVII $R^2 = OH, R^1 = R^3 = H$

(+)-CVIII $R^3 = OH, R^1 = R^2 = H$

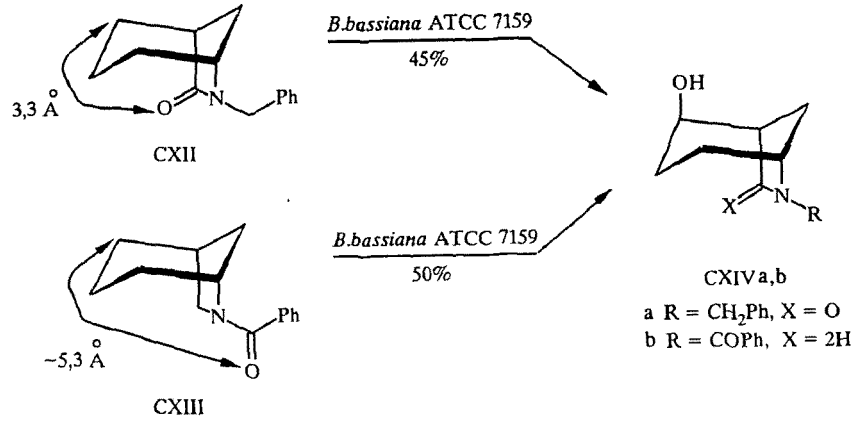
The tricyclic β-lactam CIX of the norbornane type is stereoselectively and regioselectively hydroxylated to an alicyclic ring with formation of a single levorotatory alcohol CX in 65% yield:



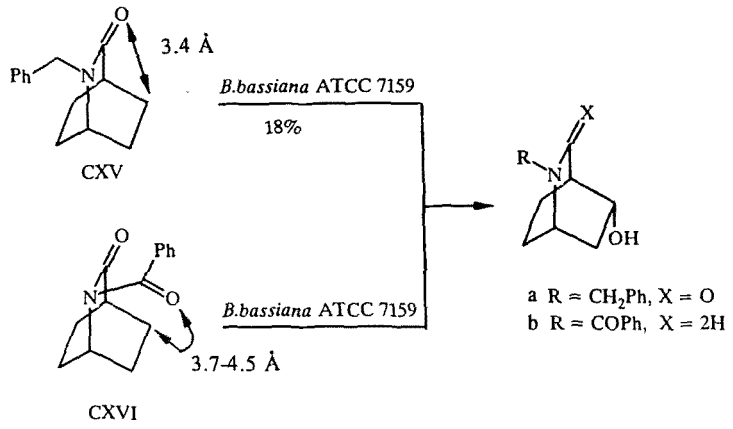
In the case of the trimethyl-substituted tricyclic lactam CXI, including a moiety with the pinane structure, whose molecule in the authors' opinion is oriented at the active center of the enzyme according to the pinane type, only the endo (with respect to the bridge) methyl group is hydroxylated strictly regioselectively and stereoselectively [25]:



French scientists [69], having investigated the hydroxylating activity of the fungus *B. bassiana* ATCC 7159 for the example of amides and lactams of the azabicyclooctane group, established that when using both 2-benzyl-2-aza-3-oxo-bicyclo-1,3,2-octane (lactam) (CXII) and 2-benzoyl-2-azabicyclo-1,3,2-octane (amide) (CXIII), the process occurs strictly regioselectively with formation of the 6-endo-hydroxy derivatives CXIVa-b in preparative yields:

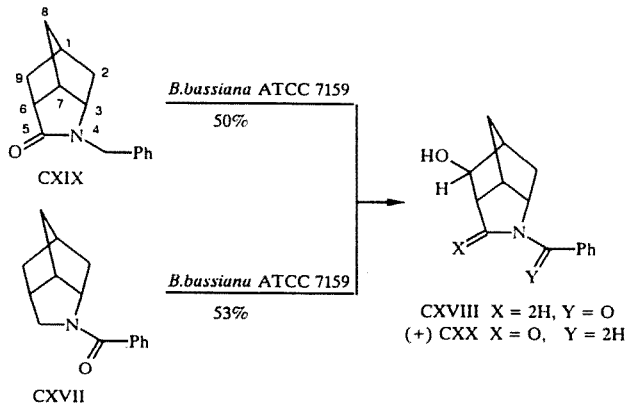


Analogous endo-hydroxy derivatives are formed when using the "lactam" CXV and the "amide" CXVI of the isomeric 2-azabicyclo-2,2,2-octane as the substrate [69]:



Earlier the hypothesis was advanced that the regioselectivity of hydroxylation of monocyclic amides depends on the distance between the carbonyl oxygen and the hydroxylated carbon atom, which is equal to 5.5 Å [70]. The distance between the carbonyl oxygen atom and the hydroxylated carbon atom in the given examples, according to data from the authors of [69], is 3.3-3.4 Å for lactams and 3.7-5.3 Å for amides, which allowed the authors to reject the hypothesis above as ungrounded.

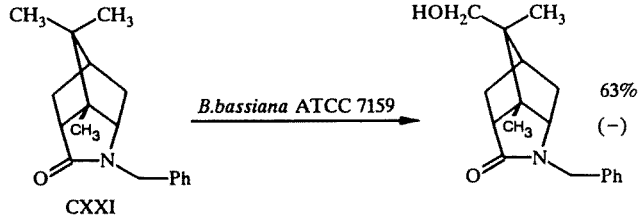
Interesting results were obtained upon hydroxylation of condensed amides and lactams, derivatives of 7-azabrendane and 6-azatwistane, by the fungus *B. bassiana* ATCC 7159, growing in a medium with glucose and corn extract [71]. It was determined that these compounds undergo regioselective endo-hydroxylation at the unactivated carbon atom in the 9 position. For example, an "amide" of the CXVII type is oxidized to the racemic carbinol CXVIII in 53% yield [71], while the "lactam" CXIX is stereoselectively and regioselectively hydroxylated to the dextrorotatory tricyclic alcohol CXX in 50% yield:

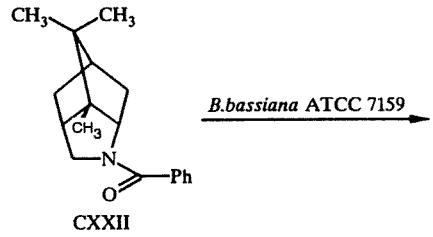


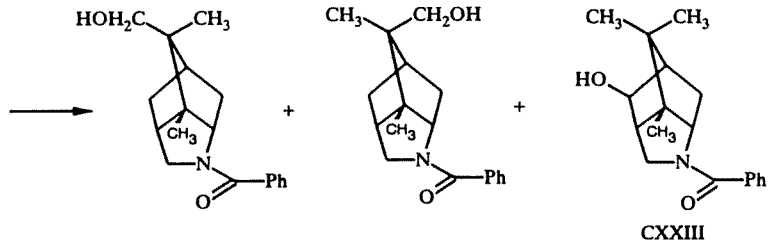
Thus, despite the marked steric hindrance, these structures are good substrates for hydroxylating fungi, and the different position of the carbonyl oxygen for "amides" and "lactams" does not affect the regioselectivity of hydroxylation, but in the case of the "lactam" the process occurs stereoselectively [71, 36].

In later papers, the named authors showed that the regioselectivity of hydroxylation of these substrates increasingly depends on the distance ($\sim 3.4 \pm 0.6$ Å) from the hydroxylated carbon atom to the nitrogen atom [72, 73] and, as follows from the facts presented, the position of the oxygen atom of the carbonyl group in the ring or outside the ring does not affect the regioselectivity of the process, but affects its enantioselectivity.

Differences in the stereoselectivity are connected with the possibility of rotation for the amides about the CO—N bond and the absence of such for the lactams [72]. However, such a viewpoint obviously does not correspond to the true situation, since later the same authors showed that the same strain accomplishes regioselective and stereoselective hydroxylation not only of 3-benzyl-1,8,8-trimethyl-3-aza-4-oxobrendane (CXXI) but also 3-benzoyl-1,8,8-trimethyl-3-azabrendane (CXXII); and the methyl groups in the 8 position preferentially undergo hydroxylation, while the yield of the 6-hydroxy derivative CXXIII is no greater than 5% [25]:

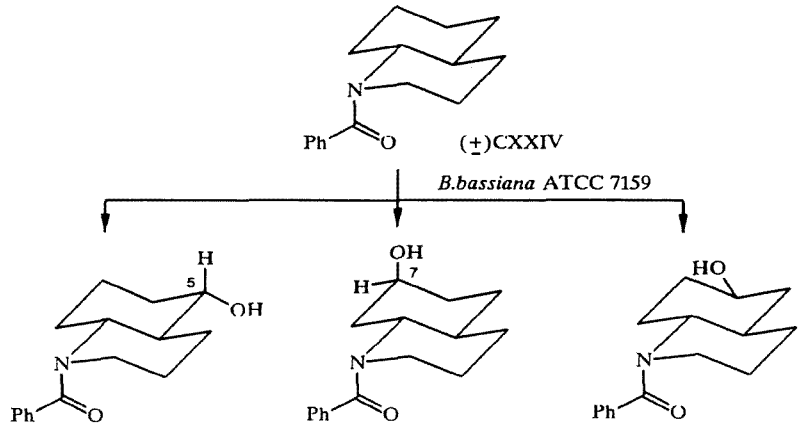






Yield, %	30	30	5
Optical purity, %	85	90	

The less sterically hindered trans-1-benzoylperhydroquinoline (isomeric to azabrendane) (CXXIV) (racemic) is converted to a mixture of two optically active 5- and 7-hydroxy derivatives CXXV and CXXVI with impurity of the racemic 6-hydroxy compound CXXVII with overall yield 80-90% [74]:



From comparison of data on analogous hydroxylation of each of the enantiomers of the substrate, the authors arrived at the conclusion that the levorotatory enantiomer is hydroxylated preferentially at the 5 position and to a lesser degree at the 6 position. At the same time, the (+)-enantiomer forms somewhat preferentially the 6-hydroxy derivative and less probably the 7-enantiomer [74].

Thus, from the material presented it follows that among the diverse microorganisms accomplishing hydroxylation of nitrogen-containing heterocycles, the enzyme system of the fungus *B. bassiana* ATCC 7159, displaying the greatest regiospecificity and stereospecificity, is the most universal and has the most diverse action on organic compounds.

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