MICROBIOLOGICAL TRANSFORMATION OF DERIVATIVES OF 4-PHENYL-2-PYRROLIDONE BY MYCELIAL FUNGI

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In the microbiological transformation of 4-phenylpyrrolidine by growing strains of the fungi Cunninghamella, Beauveria, and Penicillium, its 1-ethyl and 1-acetyl derivatives are formed; these are subsequently oxidized at positions 2 and 3. In contrast, 1-benzoyl-4-phenylpyrrolidine is hydroxylated by those same cultures in positions 3 and 5. For the corresponding 2-pyrrolidone derivatives, the 4-phenyl-2-pyrrolidone is not transformed by these fungi, while the 1-benzoyl-2-pyrrolidone is hydrolyzed at the benzamide group under the same conditions. The structures of the products from these transformations were determined by mass spectrometry.

Among the derivatives of pyrrolidine and 2-pyrrolidone (the cyclic form of \(\gamma\)-aminobutyric acid, GABA), there are several preparations with high biological activity, for example, piracetam [1] Fenibut, \(\beta\)-phenyl-\(\gamma\)-aminobutyric acid [2, 3], and others. On the other hand, there is interest in hydroxypyrrolidines [4], which are cyclic analogs of ethanolamine, a compound that plays a multifaceted role in the human organism.

We had previously established [5, 6] that a culture of the fungus Cunninghamella verticillata VKPM F-430 can accomplish the enantioselective hydroxylation of N-benzoylpyrrolidine in position 3.

In the work reported here, we identified all of the nitrogen-containing products formed in the culture fluid in the transformations of 4-phenylpyrrolidine (I), 4-phenyl-2-pyrrolidone (II), 1-benzoyl-4-phenylpyrrolidine (III), and 1-benzoyl-4-phenyl-2-pyrrolidone (IV) by cultures of the fungi Cunninghamella verticillata VKPM F-430, Beauveria bassiana ATSS 7159, and Penicillium simplicissimum KM-16.

The transformation was carried out in a growing culture of cells of these fungus strains at pH 5.0, by means of a procedure that has been described previously [7, 8]. The substrate for the transformation was introduced in amount 100 mg/liter. The transformation products were recovered from the culture fluid at pH 10.0 by three extractions with chloroform. The extracts were evaporated to dryness, and the residue was dissolved in a small quantity of methanol and analyzed in a chromatograph/mass spectrometer HP-5890 Series II/HP-5972, with a quartz capillary column 30 m \(\times\) 0.2 mm with stationary phase HP-5MS, temperature programmed from 70° to 250°C at a rate of 30°C/min. The results from the gas chromatography and mass spectrometric analysis of the culture fluid enabled us to draw the following conclusions.

1. All of the strains that were investigated did transform these particular substrates to one degree or another. However, in none of the experiments did we find any evidence that either of the phenyl rings (that in the benzyl group and that in position 4) was involved in the transformation.

2. In the case of 4-phenylpyrrolidine (I) with an unsubstituted NH group, this group was generally methylated, ethylated, or acetylated. Such processes had been observed previously in examples of other substrates [9-11]. The N-substituted derivatives of pyrrolidine that were formed then underwent partial hydroxylation. Thus, in the extracts of the culture fluid of the fungus Beauveria bassiana ATSS 7159 (substrate I), we identified (Scheme 1) the following products of transformation, along with the respective retention times (min) and mass spectra, as indicated by values of m/z (relative peak intensity, %) and path of formation of the ion: 4-phenyl-1-ethylpyrrolidine (V): 6.52; 175 (26) (M), 174 (15) (M-H), 160 (70) (M-C\(_2\)H\(_5\)), 131 (8) (M-C\(_3\)H\(_7\)), 117 (14) (M-C\(_3\)H\(_7\)), 91 (22) (C\(_7\)H\(_7\)), 71 (100) (M-C\(_6\)H\(_5\)CH==CH\(_2\)); 2-hy-
droxy-4-phenyl-1-ethyl-3-pyrrolidone (VI): 8.11; 205 (22) (M), 190 (29) (M-CH₃), 131 (10) (M-C₆H₂NHCHOH), 130 (18) (M-C₆H₄NHCH₂OH), 118 (25) (C₆H₆CHO), 117 (40) (C₆H₅CO₂H), 101 (100) (M-C₆H₄C₂H₃), 91 (24) (C₆H₇), 77 (10) (C₆H₄); 1-acetyl-2-hydroxy-4-phenyl-3-pyrrolidone (VII): 8.32; 219 (36) (M), 190 (100) (M-C₆H₂), 131 (12) (M-C₃H₇NHCHOH), 118 (22) (C₆H₄CO₂H), 117 (35) (C₆H₅CO₂H), 115 (70) (M-C₆H₄C₂H₃), 104 (17) (C₆H₅C₂H₃), 91 (27) (C₆H₄), 77 (12) (C₆H₂). The ratio of the chromatographic peak areas for compounds V–VI–VII was 1:3:6.

Among the products of transformation of the pyrrolidine (I) by the strain *Penicillium simplicissimum* KM-16, we found only traces of compound VII, a considerable quantity of the pyrrolidine V, and the original substrate. The ratio of chromatographic peak areas for compounds I–V–VII was 15:14:1.

The strain *Cunninghamella verticillata* VKPM F-430 transformed the substrate (added to the medium) by approximately 30-40%; in the reaction mixture, we identified not only compound V, but also its dehydro analog, probably 4-phenyl-1-ethyl-4,5-dihydropyrrole (VIII): 7.32; 173 (52) (M), 172 (15) (M-H), 171 (16) (M-2H), 158 (100) (M-CH₃), 156 (17) (M-2H-CH₃), 144 (11) (M-H-C₆H₄), 143 (17) (M-3H-C₂H₄), 115 (32) (C₆H₅C₃H₂), 91 (22) (C₂H₂). We also found in the reaction mixture compound IX, isobaric to the pyrrolidine V, but having a longer retention time (8-10 min) and differing sharply in character of fragmentation, so that we could tentatively assign the structure of 4-phenylsuccinimide: 175 (100) (M), 174 (8) (M-H), 147 (5) (M-CO), 130 (30) (M-CH₂NO), 118 (28) (C₆H₅CO₂H), 117 (45) (C₆H₅CO₂H), 115 (17) (C₆H₅C₂H₃), 104 (17) (C₆H₅C₂H₃), 71 (50) (C₆H₂NO₂). The ratio of chromatographic peak areas for compounds I–V–VIII–IX was 7:1:1:1. It is interesting to note that the pyrrolidone II proved to be "hard to chew" for all three strains of the fungi; in the culture fluids, we could not detect even traces of any of the products of transformation.

1-Benzoyl-4-phenylpyrrolidine (III), in comparison with the compound that is not substituted in the heterocycle [8], is consumed by these fungi to an appreciably greater degree; as a result, the content of the original substrate in the mixture was 60-70%. Nonetheless, two strains — *Cunninghamella verticillata* VKPM F-430 and *Penicillium simplicissimum* KM-16 — were almost equally effective in accomplishing hydroxylation of the pyrrolidine ring in position 3 with the formation (Scheme 2) of 1-benzoyl-3-hydroxy-4-phenylpyrrolidine (X): 11.55; 267 (17) (M), 266 (30) (M-H), 162 (65) (M-C₆H₄CO), 147 (8) (M-C₆H₅C₂H₂OH), 134 (12) (C₆H₅CONHCH₂), 133 (13) (C₆H₅CONCH₂), 105 (100) (C₆H₅CO), 77 (55) (C₆H₃). For these two fungi, the reaction mixtures also contained two isomeric compounds with molecular mass 249, in approximately equal quantities. In all probability, these substances — 1-benzoyl-4-phenyl-4,5-dihydropyrrole (XI) (t = 11.35 min) and 1-benzoyl-4-phenyl-2,5-dihydropyrrole (XII) (t = 1.20 min) — are products of dehydration of the carbinol X. Fragmentation of their molecular ions was very similar for these two compounds, so that there was no possibility of establishing the position of the multiple bond. The ratio of chromatographic peak areas for the compounds III–X–XI–XII was 8:2:1:1.

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Scheme 1

![Scheme 1](image-url)
The enzyme system of the strain *Beauveria bassiana* ATSS 7159 also accomplished hydroxylation of the heteroring; however, along with the initial substrate III plus the dihydropyrroles XI and XII and the dihydroxy derivative X, we found in the reaction mixture two isomers of the hydroxypyrrolidine X: 1-benzoyl-2-hydroxy-3-phenylpyrrolidine (XIII): 11.77; 267 (24) (M), 249 (5) (M−H₂O), 176 (5) (M−C₇H₅), 134 (4) (C₆H₅CONHCH₂), 117 (15) (C₆H₅C₃H₄), 105 (100) (C₆H₅CO), 104 (38) (C₆H₅C₂H₂), 91 (7) (C₂H₇), 77 (40) (C₆H₅) and its acyclic tautomeric form, 4-benzoylamino-2-phenylbutanal (XIV): 12.91; 267 (87) (M), 162 (7) (M−C₆H₅CO), 147 (8) (M−C₆H₅CO), 134 (25) (C₆H₅CONHCH₂), 120 (10) (C₆H₅C₂H₂OH), 105 (100) (C₆H₅CO), 77 (45) (C₆H₅). The ratio of chromatographic peak areas for the compounds III−(XI, XII)−X−XIII−XIV was 22:1:11:8:3; i.e., the main products of the transformation were still the two hydroxy compounds.

The fourth substrate that we investigated, which contained a carbonyl group on the heteroring and also in the substituent at the nitrogen atom, was less subject to degradation by these particular strains of fungi. The main process of biotransformation involved splitting out the benzoyl group and the formation of the N-unsubstituted pyrrolidone II. Here, the fungus *Beauveria bassiana* ATSS 7159 accomplished hydrogenolysis of the amide bond with reduction of the C=O group, as indicated by the fact that we found benzyl alcohol in the reaction mixture. Also, the strain *Cunninghamella verticillata* VKPM F-430 produced hydrolytic cleavage of the benzoylamide fragment of compound IV, so that benzoic acid accumulated in the reaction mixture. We were unable to evaluate the character of transformation of substrate IV by cells of the fungus *Penicillium simplicissimum* KM-16, since the process was extremely limited, and we found only traces of compound II in the reaction mixture.

In this communication we have intentionally omitted any discussion of the stereochemistry of the compounds that were formed, since a great amount of time and effort would have been required to isolate these compounds in individual form and then to identify the compounds and prove the fine structure. Nonetheless, we should not ignore the possibility that, at least in the case of the last substrate IV, the process of cleaving the amide bond by the enzyme system of the microorganisms may proceed enantioselectively. We hope that further studies will enable us to confirm or refute this hypothesis.

*This work was financed by the Russian Fund of Fundamental Research (Grant 93-03-08887), for which the authors express their profound gratitude.*

REFERENCES


