MICROBIOLOGICAL TRANSFORMATION OF NITROGEN-CONTAINING HETEROCYCLIC COMPOUNDS. 3.* MICROBIOLOGICAL SYNTHESIS OF HYDROXY DERIVATIVES OF 1-BENZOYLPIPERIDINE AND 1-BENZOYLPYRROLIDINE

I. A. Parshikov, L. V. Modyanova, E. V. Dovgilivich, P. B. Terent'ev, L. I. Vorob'eva, and G. V. Grishina

The microbiological transformation of 1-benzoylpiperidine and 1-benzoylpyrrolidine by cultures of the fungi Aspergillus niger *VKM F-1119 and* Cunninghamella verticillata *VKPM F-430 proceeds regio- and stereospecifically and leads respectively to the 4- and 3-hydroxy derivatives in 40-80% yield. The spectrometric properties of these compounds were studied.*

As is known, the hydroxy derivatives of 1-substituted piperidines and pyrrolidines are synthons for the synthesis of many physiologically active substances [1-4]. Recently polyhydroxypiperidines and pyrrolidines were shown to have antivirus, and in particular anti-HIV, activity [5, 6]. The chemical synthesis of such compounds is complex and, depending on the target structure and the synthetic strategy, requires from 3 to 15 steps [3, 7].

The microbiological transformation of a series of saturated nitrogen-containing heterocycles has been investigated previously and the possibility of introducing a hydroxyl group onto the heterocyclic ring was shown [8, 9]. Transformation of 1-benzoylpiperidine by a culture of *Beauveria bassiana* ATCC 7159 gave as sole product 1-benzoyl-4-hydroxypiperidine in 18% yield [8]. Transformation of 1-benzylpyrrolidine by the same culture led to opening of the heterocyclic ring [10]. These studies were not carried further.

The purpose of this study was to investigate the hydroxylation of 1-benzoylpiperidine and 1-benzoylpyrrolidine by several cultures of microscopic fungi to obtain preparative amounts of the hydroxy derivatives.

For the transformations, museum cultures of *Aspergillus niger* VKM F-1119 and *Aspergillus awamori* VKM F-758 were used as well as cultures, isolated from natural sources, of *Beauveria bassiana* VKM F-3111D, *Penicillium simplicissimum,* and *Cunninghamella verticillata* VKPM F-430.

Transformations were carried out in growing cultures as well as in suspensions of stationary-phase cells in pH 6.0 buffer by the method described earlier [11]. The separation of the individual transformation products has been described earlier [12]. The reaction products were identified by analysis of their aggregate physicochemical and spectral characteristics and by countersynthesis. 1-Benzoyl-4-hydroxypiperidine was obtained by reduction of 1-benzoylpiperidone-4 [13] with lithium aluminum hydride, and 1-benzoyl-3-hydroxypiperidine by benzoylation of commercially available 3-hydroxypiperidine by a known method [14]; 1-benzoyl-3-hydroxypyrrolidine was synthesized analogously from 3-hydroxypyrrolidine.†

It was found that the transformation of 1-benzoylpiperidine gave achiral 1-benzoyl-4-hydroxypiperidine (I), optically active (+)-1-benzoyl-3-hydroxypiperidine $[\alpha]_D^{20}$ +124° (II), and 1-benzoyl-2-hydroxypiperidine (III), which due to ring-chain tautomerism was separated as a racemate. The site of hydroxylation and the yields of products depended on the microbial strains used and the reaction conditions (Table 1). The results indicate that only two cultures carried out the reaction regioselectively to give the 4-hydroxy isomer, formed in 80% yield in the case of *Aspergillus niger* VKM F-1119. The use of cultures of *Beauveria bassiana* VKM F-3111D and *Penicillium simplicissimum* gave the optically active 3-hydroxy isomer as well, though in minor yield. The presence of 1-benzoyl-2-hydroxypiperidine was noted in the case of *Penicillium simplicissimum* and *Aspergillus awamori* VKM F-758, also in small yield. It should be noted that this compound is quite chemically unstable.

The structures of the separated hydroxylation products were assigned on the basis of their mass spectral fragmentation (Schemes 1-3).

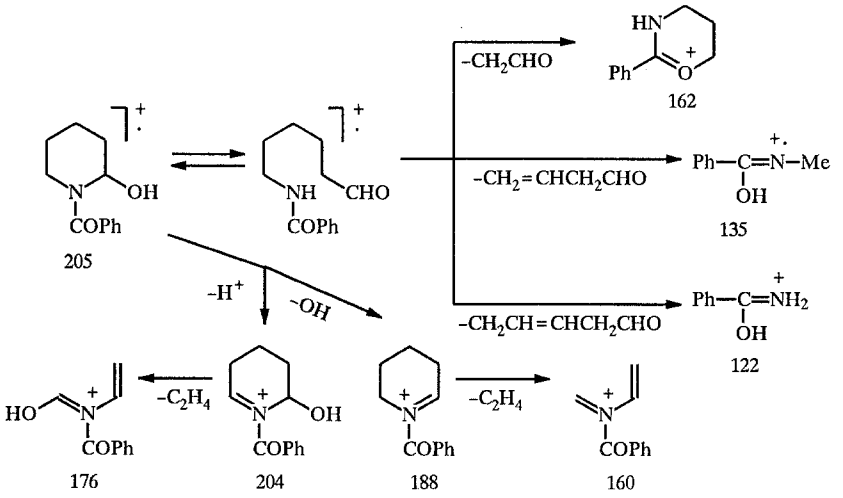
*For Communication 2, see *Biotekhnologiya,* No. 4, 24, 1990.

†We thank A. V. Dovgilevich for the kind provision of chemically pure 3-hydroxypyrrolidine.

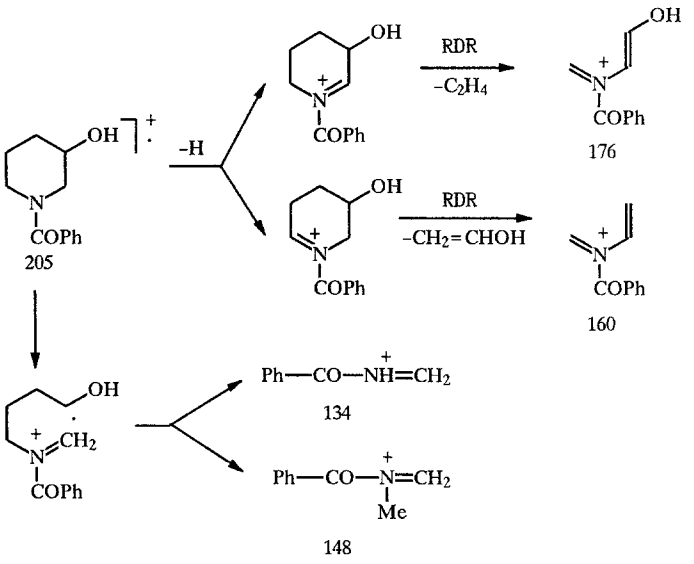
M. V. Lomonosov Moscow State University, Chemical and Biological Faculties. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 2, pp. 195-199, February, 1992. Original article submitted December 12, 1990.

0009-3122/92/2802-0159$12.50

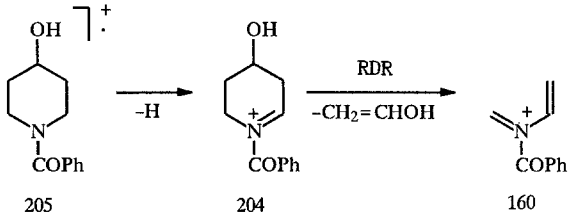
Scheme 1



Scheme 2



Scheme 3



One of the criteria of structure assignment for the three regioisomers separated was the differences in likelihood of hydroxyl group cleavage from the molecular ion. In the mass spectra of all three compounds were observed, besides the molecular ion, ion peaks for $(M-H)^+$, $(M-OH)^+$, and $(M-H_2O)^+$. However, while the loss of the OH group from the molecular ions of I and II was a minor reaction, in the mass spectrum of the 2-hydroxy derivative this peak was second in intensity after the m/z 105 peak of the benzoyl cation,* as is characteristic of 2-substituted piperidines [15]. This compound, as to be expected, exists partly in the gas phase in linear form as ω-benzoylaminovaleric aldehyde, since only its mass spectrum shows ion peaks at m/z 162 $(m-CH_2CHO)^+$, 122, and 135, characteristic of 1-alkylbenzamides [15] (see Scheme 1).

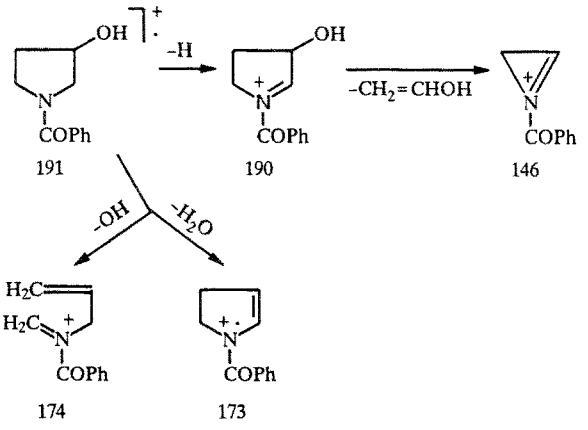
*This abundance of the benzoyl cation confirmed the site of hydroxylation on the heterocycle and not the benzyl fragment.

The mass spectral fragmentation of all three compounds is characterized by retrodiene decomposition of the tetrahydropyridinium ions formed by primary loss from the M^+ ions of a hydrogen atom (or a hydroxyl) from the position α to the nitrogen. Depending on the position of the hydroxyl group on the ring, hydroxyl-containing fragments (m/z 176) or ions lacking the hydroxyl (m/z 160) are formed (see Schemes 1-3). The first of these is absent from the mass spectrum of I, while both are present in the mass spectra of II and III. The structures of the isomeric hydroxy derivatives separated are thus unambiguous. Finally, the retention times on HPLC of 3- and 4-hydroxy-1-benzoylpiperidines coincided for the transformation products and the synthetic checks.

Only the culture of *Cunninghamella verticillata* VKPM F-430 was able to perform the transformation of 1-benzoylpyrrolidine. *Beauveria bassiana* VKM F-3111D, *Aspergillus awamori* VKM F-758, and *Aspergillus niger* VKM F-1119 did not transform the substrate at all, while *penicillium simplicissimum* gave trace quantities of products. We were able to isolate in 40% yield optically active (—)-1-benzoyl-3-hydroxypyrrolidine (IV). As with the transformation of 1-benzoylpiperidine, the process furnished preparative yields only with a growing culture. In a stationary-phase cell suspension the transformation proceeded analogously but with only a 13% yield of the 3-hydroxy derivative.

The structure of the separated compound follows from its PMR spectrum as well as the nature of its molecular-ion decomposition on electron impact. Thus the mass spectrum of 1-benzoyl-3-hydroxypyrrolidine displayed ions at m/z 174 and 173 (M—OH, M—H_2O) as well as 190 (M—H) and 146 (M—H—CH_2=CH—OH), confirming the presence of a hydroxyl group in the structure as well as its β-position on the heterocycle (Scheme 4). The PMR spectrum of this compound in the high-field region shows two groups of methylene-proton multiplets at 1.76-2.16 ppm (H-3 and H-4) and 3.40-3.83 (H-2 and H-5), as well as the hydroxyl proton singlet at 4.45 ppm and an aromatic proton singlet near 7.50 ppm.

Scheme 4



The HPLC retention times of the transformation product and the synthetic 1-benzoyl-3-hydroxypyrrolidine were identical.

In neither of the examples was the formation of polyhydroxy derivatives of 1-benzoylpiperidine or pyrrolidine observed. The one-step microbiological method of hydroxylation of 1-benzoylpiperidine and benzoylpyrrolidine is thus competitive with the multistep chemical synthesis of the corresponding hydroxy derivatives.

EXPERIMENTAL

IR spectra were recorded on a UR-20 spectrometer in vaseline. PMR spectra were taken on a Tesla BS-467 (60 MHz) instrument with TMS as internal standard, in $CDCl_3$. Mass spectra were taken on a Varian MAT III at ionization energy 80 eV with direct introduction of the sample into the ion source. The presence of transformation products was determined by TLC on Kieselgel 60 F 254 Merck 5583 plates in a hexane—ethyl acetate—ethanol, 10:10:2 system. The individual compounds were separated by flash chromatography on Merck 60.0.013 mM silica gel with elution by he same system. Purity and quantity of transformation products were determined by high-performance liquid chromatography on a Milikhrom instrument with UV detector (λ = 220 nm); eluent flow 100 μliter/min, sample size 2 μliter, sensitivity 0.4, time of measurement 0.6 s. After separation on the column, the proportions of regioisomers were determined by their mass. Specific rotation was measured on EPO-1 VNII Prodmash and Perkin—Elmer 141 spectropolarimeters.

1-Benzoyl-4-hydroxypiperidine (I). Mp 82-84°C. R_f 0.24. Retention time (R_t) 225 s. IR (cm^{-1}, film): 1680 (C=O), 3400 (OH). PMR: 1.36-2.18 (4H, m, 3-H and 5-H), 2.86-4.36 (6H, m, 2-H, 6-H, 4-H, and O—H), 7.40 ppm (5H, s, C_6H_5). Mass spectrum, m/z (I_{rel}, %): 205 (30) M^+, 204 (50), 188 (3), 187 (5), 186 (8), 160 (4), 149 (4), 105 (100), 100 (8), 78 (4), 77 (55).

TABLE 1. Transformation of 1-Benzoylpiperidine by Microscopic Fungi

Culture	Yield of transformation products, %					
	I*	I**	II*	II**	III*	III**
Beauveria bassiana VKM F-3111D	21	60	3	5	—	—
Aspergillus niger VKM F-1119	13	80	—	—	—	—
Penicillium simplicissimum	3	3	1	—	12	—
Aspergillus awamori VKM F-758	7	34	—	—	4	—
Cunninghamella verticillata VKPM F-430	22	19	—	—	—	—

*With cell suspension in buffer.

**With growing culture.

1-Benzoyl-3-hydroxypiperidine (II). Mp 80-81°C. R_f 0.30. R_t 185 s. Mass spectrum, m/z (I_{rel}, %): 205 (16) M^+, 204 (28), 188 (6), 187 (9), 186 (5), 177 (3), 176 (6), 160 (2), 149 (4), 148 (8), 134 (6), 105 (100), 100 (20), 78 (4), 77 (64). $[\alpha]_{600}^{20}$ +124.48° (C 0.45, methanol).

1-Benzoyl-2-hydroxypiperidine (III). R_f 0.35. Mass spectrum, m/z (I_{rel}, %): 205 (9) M^+, 204 (16), 188 (42), 187 (6), 186 (3), 177 (4), 176 (6), 162 (11), 149 (18), 135 (6), 134 (8), 122 (6), 105 (100), 100 (2), 83 (9), 77 (37).

1-Benzoyl-3-hydroxypyrrolidine (IV). Mp 115-116°C. R_f 0.25. R_t 235 s. IR spectrum (vaseline): 1680 (C=O), 3370 cm^{-1} (OH). PMR: 1.76-2.16 (3H, m, 3-H and 4-H), 3.40-3.83 (4H, m, 2-H and 5-H), 4.45 (1H, s, OH), 7.45 ppm (5H, s, C_6H_5). Mass spectrum, m/z (I_{rel}, %): 1.91 (30) M^+, 190 (8), 174 (3), 173 (2.5), 162 (2.5), 146 (9), 114 (5), 105 (100), 99 (20), 86 (7.5), 85 (10), 77 (50), 70 (70), 57 (15), 56 (25), 55 (15). $[\alpha]_D^{20}$ —46.56° (C 1.89, ethanol).

LITERATURE CITED

1. Yu. A. Ovchinnikov, *Bioorganic Chemistry* [in Russian], Prosveshchenie, Moscow (1987), pp. 644, 662.
2. M. S. Malik, N. K. Sangwan, and S. N. Rastogi, *Chim. Acta Turc.*, **14**, No. 3, 307 (1986).
3. D. M. Flanagan and M. M. Joullie, *Heterocycles*, **26**, No. 8, 2247 (1987).
4. A. D. Cale, US Patent 4,705,853 (1987); *Chem. Abstr.*, **109**, 231,085 (1988).
5. G. W. J. Fleet, A. Karpas, R. A. Dwek, L. E. Fellows, A. S. Tyms, S. Petursson, S. K. Namgoong, N. G. Ramsden and P. W. Smith, *FEBS Lett.*, **237**, No. 1/2, 128 (1988).
6. B. D. Walker, M. Kowalski, W. C. Goh, K. Kozarsky, M. Krieger, C. Rosen, L. Rohrschneider, W. A. Haseltine, and J. Sodroski, *Proc. Natl. Acad. Sci. USA*, **84**, No. 22, 8120 (1987).
7. N. Hamana, N. Ikota, and N. Ganem, *J. Org. Chem.*, **52**, 5492 (1987).
8. R. A. Johnson, M. E. Herr, H. C. Murray, and G. S. Fonken, *J. Org. Chem.*, **34**, No. 8, 3198 (1968).
9. A. Archelas, R. Furstoss, D. Srairi, and G. Maury, *Bull. Soc. Chim. Fr.*, No. 2, 234 (1986).
10. D. Srairi and G. Maury, *Bull. Soc. Chim. Fr.*, No. 2, 297 (1987).
11. L. V. Modyanova, L. I. Vorob'eva, O. I. Shibilkina, E. V. D ovgilevich, P. B. Terent'ev, and A. N. Kost, *Biotechnologiya*, No. 3, 24 (1990).
12. A. N. Kost, L. I. Vorob'eva, P. B. Terent'ev, L. V. Modyanova, O. I. Shibilkina, and L. A. Korosteleva, *Prikladn. Biokhim. Mikrobiol.*, **13**, No. 5, 696 (1977).
13. S. M. McElvain and R. E. McMahon, *J. Am. Chem. Soc.*, **71**, 901 (1949).
14. B. P. Mundi, B. R. Larsen, L. F. McKenzie, and G. Braden, *J. Org. Chem.*, **37**, No. 10, 1635 (1972).
15. P. B. Terent'ev and A. P. Stankyavinus, *Mass Spectrometry of Biologically Active Nitrogenous Bases* [in Russian], Mokslas, Vilnius (1987), p. 76.