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TRANSFORMATION OF 1,2,5,6-TETRA-HYDROPYRIDINES WITH MYCELLAR FUNGI

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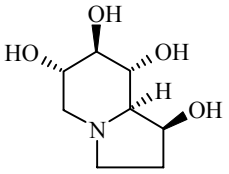
It has been shown that on biotransformation of a series of 1,2,5,6-tetrahydropyridines with strains of the mycellar fungi Cunninghamella verticillata VKPM F-430, Beauveria bassiana ATCC 7159, and Penicillium simplicissimum KM-16, the culture of Cunninghamella verticillata possesses the greatest transforming activity and selectivity. With the aid of the latter practically quantitative oxidation of 1,2,5,6-tetrahydropyridines occurs into the corresponding trans-diol. The structure and spatial disposition of trans-1-benzyl-3,4-dihydroxypiperidine was demonstrated by data of chromato-mass spectrometric analysis and high resolution NMR spectra and was confirmed by comparison with an authentic sample obtained by an alternate synthesis using the oxidation of 1-benzyl-1,2,5,6-tetrahydropyridine with trifluoroperacetic acid.

Keywords: piperidinediol, 1,2,5,6-tetrahydropyridine, mycellar fungi, biotransformation, hydroxylation.

The functional derivatives of piperidine belong to a class of natural and synthetic substances with an extremely wide spectrum of biological activity [1]. Recently polyhydroxylated derivatives of piperidine (azasugars) have attracted particular attention, such as castanospermine **1** and its analogs, in which high anti-HIV activity has been found [2,3].



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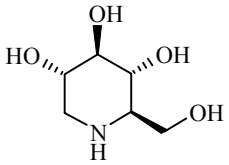

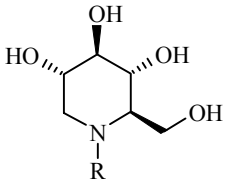

R = Me, Et, Bu

Data are presented in the present work on an investigation of fermentation routes for the synthesis and the stereochemistry of the structurally and stereochemically simpler analogs of castanospermine, the *trans*-3,4-dihydroxypiperidines, by the biotransformation of a series of 1,2,5,6-tetrahydropyridines (THP) with growing cultures of cells of strains of the mycellar fungi *Cunninghamella verticillata* VKPM F-430 (**C**), *Beauveria bassiana* ATCC 7159 (**B**), and *Penicillium simplicissimum* KM-16 (**P**). As substrates 1-benzyl-1,2,5,6- (**2a**), 1-(1-phenylethyl)-1,2,5,6- (**2b**), and 3-methyl-1-propyl-1,2,5,6-THP (**2c**) were selected since fungi of the

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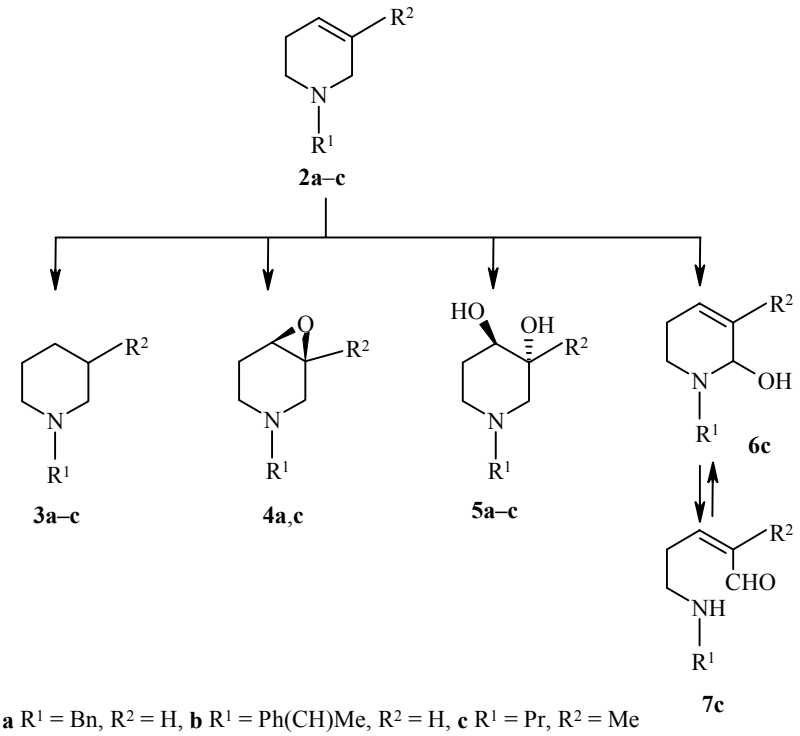
Beauveria, *Cunninghamella*, and *Penicillium*, etc. genus are capable of hydroxylating [4,5] or hydrating [6] the multiple bonds of THP.

Chromato-mass spectral analysis of extracts obtained after incubation with cultures of each of the fungi **C**, **B**, and **P** enabled it to be established that all the N-substituted THP **2a-c** underwent different conversions (Scheme 1).

Scheme 1



a R^1 = Bn, R^2 = H, **b** R^1 = Ph(CH)Me, R^2 = H, **c** R^1 = Pr, R^2 = Me

As is seen from the data given in Table 1, maximum transforming activity and selectivity were observed for strain **C**, which practically completely transformed the three THP **2a-c**. Both compounds **2a** and **2b**, containing a N-aralkyl substituent, were transformed by a culture of fungus **C** practically completely and regioselectively into the *trans*-3,4-dihydroxy derivatives **5a** and **5b**.

TABLE 1. Composition in the Mixture of the Products of Biotransformation of Compounds **2a-c** by Cultures of Fungi **P**, **B**, and **C** (retention time, min)

Fungus	Compound, %								
	3a	**3b**	**3c**	**4a**	**4c**	**5a**	**5b**	**5c**	**6c**
Penicillium (**P**)	—	—	1.0 (3.41)	—	—	—	—	1.0 (7.34)	—
Beauveria (**B**)	2.4 (5.88)	2.0 (5.88)	7.0 (3.41)	0.6 (7.11)	—	—	—	4.0 7.33	—
Cunninghamella (**C**)	—	—	—	—	22.0 (4.50)	97.6 (8.34)	100 (8.46)	19.0 (7.34)	59.0 (4.90)

The selectivity was infringed only for compound **2c** where the formation of three compounds was observed. These were 3,4-epoxy-3-methyl-1-propylpiperidine (**4c**), the product of its hydration (compound **5c**), and 2-methyl-5-propylamino-2-pentenal (**7c**) representing (in the gas phase) the linear tautomeric form of 2-hydroxy-3-methyl-1-propyl-1,2,5,6-THP (**6c**).

The least active proved to be culture **P**, which was completely ineffective on compounds **2a** and **2b**, and compound **2c** was converted to only 1% into *trans*-3,4-dihydroxy-3-methyl-1-propylpiperidine (**5c**) and to 1% into 3-methyl-1-propylpiperidine (**3c**). These same compounds were found among the transformation products of substrate **1c** by a culture of fungus **B**, but in the ratio **2c** : **5c** : **3c** of 89 : 4 : 7. Assumption of the *trans* structure for the dihydroxy derivative **5c** was made in view of the very high stability of its molecular ion [7].

Culture **B** also effected an analogous reduction of the multiple bond to an insignificant extent in the transformation of compounds **2a** and **2b**. The presence of 2% 1-(1-phenylethyl)piperidine (**3b**) was identified among the biotransformation products of **2b** by culture **B**. In the case of compound **2a** 1-benzylpiperidine (**3a**) and also 1-benzyl-3,4-epoxypiperidine (**4a**) were detected in the culture fluid in addition to the initial substrate. The detection of that epoxide enables the assumption that the dihydroxy derivative **5c** was formed by the hydration of the analogous epoxide 1,2,5,6-THP **4c**.

We emphasize that in structure **2c** only aliphatic groups are present and the carbonyl group is absent, which puts in question the hypothesis that for the successful selective transformation by culture **B** of unsaturated heterocycles a carbonyl group and/or an aromatic fragment needs to be present [8-10]. At the same time the complete indifference of culture **B** in attempts to transform 1-benzyloxycarbonyl-1,2,5,6-THP was also established in [6].

Cultures of fungi **P** and **B** are capable of effecting the nonselective transformation of such substrates only to an insignificant extent.

It was found that a culture of *Cunninghamella verticillata* VKPM F-430 (**C**) dihydroxylates the multiple bond in 1,2,5,6-THP **2a-b** regioselectively and in practically quantitative yield. This particular culture of fungus **C** should therefore be used for the preparative biotransformation of THP.

For the strict determination of the spatial structure of diol **5a** we effected an alternate synthesis of *trans*-1-benzyl-3,4-dihydroxypiperidine (**8a**) by the oxidation of compound **2a** fluoroborate with trifluoroperacetic acid. Samples of *trans*-1-benzyl-3,4-dihydroxypiperidine, obtained by biotransformation and synthetically, showed the same chromatographic mobility and practically identical mass and ^{1}H NMR spectra. Comparison of the chemical shifts and their assignment for both samples of the compound are given in Table 2.

The axial orientation of the protons at 3- and 4-H in 3,4-dihydroxypiperidine **5a** is established from the large values of the vicinal coupling constants $^3J_{2a3}$ = 10.3, $^3J_{5a4}$ = 11.1, and $^3J_{34}$ = 8.9 Hz. This determines the diequatorial orientation of the 3,4-dihydroxy groups which indicates the preparation of just the *trans* isomer of synthetic 3,4-dihydroxypiperidine **5a**. An analogous conclusion on the *trans* structure was also made for the biotransformation product **5a**, having practically the same values for the coupling constants $^3J_{2a3}$ = 10.2, $^3J_{5a4}$ = 9.6, $^3J_{34}$ = 9.8 Hz, which demonstrates the identity of both samples.

TABLE 2. ^{1}H NMR Spectra of Samples of Compound **5a**

Compound **5a**	Chemical shifts, δ, ppm							
	2-H	3-H	4-H	5-H	6-H	PhC<u>H</u>	OH	Ar–H
Biotransformation product	*a* 1.92 *e* 2.93	3.52	3.32	*a* 1.55 *e* 1.83	*a* 2.02 *e* 2.73	3.48	4.4	7.3
Synthetic sample	*a* 1.98 *e* 2.94	3.56	3.40	*a* 1.59 *e* 1.90	*a* 2.08 *e* 2.75	*a* 3.51 *b* 3.53	3.2	7.3

It has therefore been shown that N-substituted 1,2,5,6-THP may be converted regioselectively and stereoselectively into *trans*-3,4-diols by biotransformation.

EXPERIMENTAL

The chromato-mass spectra were taken on a HP-5990 instrument with a HP-5972 mass selective detector, a quartz capillary column 30 m x 0.2 mm with stationary phase HP-5MS and temperature programing from 70 to 250°C at a rate of 30°/min.

The ^{1}H NMR spectra were recorded on a Varian VXR-400 (400 MHz) instrument in $CDCl_3$, internal standard was TMS.

1,2,5,6-Tetrahydropyridines 2a,c were synthesized by the sequential quaternization of pyridine and 3-picoline with benzyl chloride and propyl bromide in absolute acetonitrile respectively. The isolated salts, after recrystallization from ethyl acetate–ether were reduced with sodium borohydride in absolute ethanol at 0°C [11,12]. The ethanol was evaporated in vacuum, water was added to the residue, and the mixture twice extracted with ether. The combined extracts were dried with anhydrous $MgSO_4$, filtered, the solvent evaporated, and the residue distilled in vacuum.

Compound 2a. Yield 60%; bp 101-102°C (2 mm Hg), R_f 0.85 (Silufol, benzene–acetone, 2:1). IR spectrum (thin film), ν, cm^{-1}: 1670 (C=C). Mass spectrum, *m/z* (I_{rel}, %) [origin]: 173 (57) $[M]^+$, 96 (60) $[M\text{-}C_6H_5]^+$, 91 (100) $(C_7H_7)^+$, 84 (17), 82 (21) $[M\text{-}C_7H_7]^+$, 70 (16), 65 (14), 58 (14), 44 (28), 42 (23), 41 (19) [13].

Compound 2c. Yield 75%; bp 85-88°C (30 mm Hg), R_f 0.3 (Silufol, benzene–acetone, 2:1). IR spectrum (thin film), ν, cm^{-1}: 1667 (C=C). Mass spectrum, *m/z*: 139 (11) $[M]^+$, 124 (5) $[M\text{-}CH_3]^+$, 110 (100) $[M\text{-}C_2H_5]^+$, 94 (5), 81 (4), 68 (5), 67 (7), 42 (20), 41 (8).

1-(1-Phenylethyl)-1,2,5,6-tetrahydropyridine (2b). Sodium borohydride (0.9 g, 2.4 mmol) was added at 0°C in portions to a solution of 1-(1-phenylethyl)pyridinium chloride (2.6 g, 1,18 mmol) (obtained in 70% yield by boiling in butanol equivalent amounts of 1-(2,4-dinitrophenyl)pyridinium chloride (Zincke salt) with 1-phenylethylamine) in absolute ethanol (30 ml). The mixture was stirred for 30 min, and the ethanol was evaporated in vacuum. Water (20 ml) was added to the residue, the mixture was extracted with methylene chloride (3 × 15 ml), the combined organic extracts were dried with anhydrous $MgSO_4$, the solvent evaporated, and the residue distilled in vacuum. Compound **2b** (1.5 g, 65%) was obtained; bp 95-96°C (2 mm Hg). Hydrochloride has mp 223-224°C. Found, %: C 69.96; H 8.04; N 5.93. $C_{13}H_{18}ClN$. Calculated, %: C 69.79; H 8.10; N 6.26. Mass spectrum, *m/z*: 187 (14) $[M]^+$, 172 (100) $[M\text{-}CH_3]^+$, 118 (16), 110 (24) $[M\text{-}C_6H_5]^+$, 106 (8), 105 (85) $[C_8H_9]^+$, 104 (13), 91 (32), 82 (34) $[M\text{-}C_8H_9]^+$, 79 (26).

1-Benzyl-3,4-dihydroxypiperidine (5a). Boron trifluoride etherate (2.98 ml, 3.28 g, 23.1 mmol) was added to a solution of 1-benzyl-1,2,5,6-THP **2a** (4 g, 23.1 mmol) in absolute CH_2Cl_2 (12 ml) at -50°C. The mixture was stirred for 5 min at –50°C and 20 min at room temperature. Triethylammonium trifluoroacetate (0.9 g, 4.4 mmol) was added, and then a 1.7 M solution of trifluoroperacetic acid in methylene chloride (20 ml). The mixture was boiled for 1 h 30 min. The excess of oxidizing agent was decomposed with dimethyl sulfide (0.86 g, 13.9 mmol), and the solution neutralized at 0°C with 10% NaOH to pH 11. The organic layer was separated, and the aqueous layer was extracted with methylene chloride (5 × 15 ml). The organic extracts were combined, and dried with anhydrous $MgSO_4$. The mixture was filtered, the solvent evaporated, and 1-benzyl-3,4-dihydroxypiperidine (2.38 g, 50%) was obtained as white crystals; mp 96-97°C (ethyl acetate–hexane, 1:1), R_f 0.6 (Silufol, $CHCl_3$–MeOH, 3:1). IR spectrum (CCl_4), ν, cm^{-1}: 3605, 3630 (OH). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.55 (1H, dq, J_{5a6a} = 11.6, J_{5a5e} = 11.9, J_{4a5a} = 11.1, J_{5a6e} = 3.4, 5-H *a*); 1.83

(1H, dq, J_{4a5e} = 3.9, 5-H*e*); 1.92 (1H, t, J_{2a2e} = 10.6, J_{2a3a} = 10.3, 2-H*a*); 2.02 (1H, dt, J_{6a6e} = 11.4, J_{5e6a} = 1.9, 6-H*a*); 2.73 (1H, d, 6-H*e*); 2.93 (1H, dd, J_{2e3a} = 2.9, 2-H*e*); 3.32 (1H, dt, J_{3a4a} = 8.9, 4-H*a*); 3.48 (2H, s, Ph-CH_2); 3.52 (1H, td, 3-H*a*); 4.4 (2H, br. s, OH); 7.3 (5H, m, Ar-H). Found, %: C 69.72; H 8.24; N 6.42. $C_{12}H_{17}NO_2$. Calculated, %: C 69.54; H 8.27; N 6.76.

Biotransformation was effected in a growing cell culture of strains **P**, **B**, and **C** at pH 5.0 by the procedure of [6,8]. Two parallel experiments were carried out for each substrate and culture. The substrate (compounds **2a-c**) for transformation was introduced as the hydrochloride at 100 mg/liter, the volume of culture fluid for one test was 100 ml. After the end of the process the cells were filtered off, the filtrate was acidified to pH 3 with hydrochloric acid, evaporated to a volume of 7-10 ml, and extracted with chloroform (3 × 10 ml) to remove contaminants of a nonbasic nature. The aqueous layer was made alkaline with 10% NaOH solution to pH 11, and extracted with chloroform (6 × 10 ml). The combined chloroform extracts were dried with anhydrous $MgSO_4$, evaporated to 2 ml, and analyzed by chromato-mass spectrometry.

Mass spectra of the compounds identified (Scheme 1):

3a. 175 (26) $[M]^+$, 174 (41) $[M-H]^+$, 146 (5), 132 (4), 98 (27) $[M-C_6H_5]^+$, 91 (100) $[C_7H_7]^+$, 84 (18), 65 (22), 43 (15), 41 (14).

3b. 189 (7) $[M]^+$, 174 (100) $[M-CH_3]^+$, 112 (6), 105 (100) $[C_8H_9]^+$, 91 (4), 84 (3), 77 (3).

3c. 141 (7) $[M]^+$, 140 (4), 112 (100) $[M-C_2H_5]^+$, 84 (3) $[M-C_4H_9]^+$, 70 (3), 43 (4), 42 (5).

4a. 189 (5) $[M]^+$, 146 (3) $[M-C_2H_3O]^+$, 138 (8), 118 (7) $[M-C_4H_7O]^+$, 112 (6) $[M-C_6H_5]^+$, 91 (100) $[C_7H_7]^+$, 89 (10), 77 (12), 65 (27), 63 (15), 55 (26), 51 (24), 41 (32).

4c. 155 (12) $[M]^+$, 126 (95) $[M-C_2H_5]^+$, 116 (34), 115 (39), 98 (100) $[M-C_3H_5O]^+$, 96 (23), 84 (43) $[M-C_4H_7O]^+$, 83 (31), 69 (23), 44 (32), 43 (56), 42 (48), 41 (53).

5a. 207 (22) $[M]^+$, 206 (14), 190 (15) $[M-OH]^+$, 172 (5) $[M-OH-H_2O]^+$, 154 (27), 146 (7) $[M-C_2H_5O_2]^+$, 134 (7), 130 (14) $[M-C_6H_5]^+$, 116 (21) $[M-C_7H_7]^+$, 98 (8), 91 (100) $[C_7H_7]^+$, 70 (27), 65 (10), 43 (7), 41 (10).

5b. 221 (3) $[M]^+$, 206 (100) $[M-CH_3]^+$, 204 (1) $[M-OH]^+$, 144 (20) $[M-C_6H_5]^+$, 116 (5) $[M-C_8H_9]^+$, 106 (6), 105 (51) $[C_8H_9]^+$, 103 (9), 91 (27), 79 (11), 77 (14) $[C_6H_5]^+$.

5c. 173 (95) $[M]^+$, 155 (18) $[M-H_2O]^+$, 143 (8), 142 (11), 138 (51) $[M-H_2O-OH]^+$, 129 (12) $[M-CH_3COH]^+$, 128 (10), 125 (21), 115 (26), 112 (24), 111 (47), 99 (98) $[M-C_3H_6O_2]^+$, 97 (30), 83 (48), 55 (100) $[C_3H_5N]^+$, 43 (58).

6c. 155 (3) $[M]^+$, 126 (100) $[M-C_2H_5]^+$, 116 (4), 115 (6), 112 (3), 98 (2), 84 (11) $[M-C_4H_9N]^+$, 70 (4), 55 (6), 43 (21), 42 (10), 41 (8).

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