The presence of the alcohols (VIII) and ketones (VII) in the catalysates was shown by t the method of adding authentic samples of these compounds, obtained by the hydrogenation of ionone, to the reaction mixture.

SUMMARY

The catalytic hydroamination of a mixture of α- and β-ionones by aliphatic nitriles and amines has been studied. The optimum parameters for the occurrence of the process have been determined. It has been established that the reaction forms a mixture of stereoisomeric N-substituted 1-methyl-3-(2,6,6-trimethylcyclohexyl)propylamines. A scheme of the course of the reaction is proposed.

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SYNTHESIS OF DERIVATIVES OF PENTOPYRANIC ACID

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UDC 547.854'455

N^4-Benzoylcytosine 1-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate) has been obtained with a yield of 70% by the glycosylation of the trimethylsilyl derivative of N^4-benzoylcytosine with methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate in the presence of three equivalents of $SnCl_4$ as condensing agent. Cytosine 1-(β-D-glucopyranosiduronamide) (IV) — the amide of pentopyranic acid — has been synthesized in practically quantitative yield by the ammonolysis of the nucleoside (I).

In a study of the biosynthesis of the antibiotic blasticidin S Seto et al. [1] isolated from the culture liquid of a strain of *Streptomyces griseochromogenes* a whole series of intermediate products, among which pentopyranic acid (I), which is a cytosine nucleoside of D-glucopyranuronic acid, was detected.

In 1976, Fox et al. [2] synthesized this compound by the glycosylation of N^4-acetylcytosine with methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate (II), but the yield of the protected nucleoside was only 20%.

We have found that the glycosylation of the silyl derivative of N^4-benzoylcytosine with compound (II) in the presence of three equivalents of $SnCl_4$ as condensing agent leads to the formation of N^4-benzoylcytosine 1-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate) (III) with a yield of 70%.

The PMR spectrum ($J_{1',2'}$ = 8.5 Hz) and the positive Cotton effect of the B_{2U} band in the CD spectrum of compound (III) show its β-anomeric configuration.

The ammonolysis of compounds (III) gave cytosine 1-(β-D-glucopyranosiduronamide) (IV) — the amide of pentopyranic acid (I) — in practically quantitative yield. (See scheme on page 581).

The spectral characteristics (UV, CD, PMR) of the compounds obtained are in complete correspondence to the structures ascribed to them.

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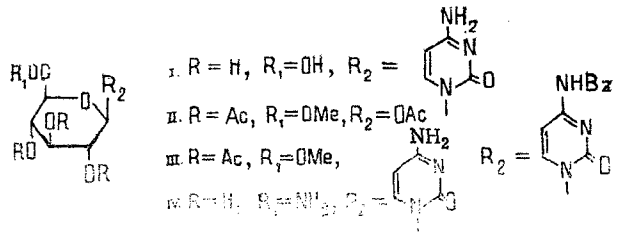

EXPERIMENTAL

The peracetate (II) was obtained from D-glucuronolactone as described by Wyss et al. [3]. PMR spectra were recorded on a JNM PS-100 instrument (the chemical shifts are given in the δ scale relative to the signal of TMS), and UV spectra on a Specord UV-VIS spectrophotometer. Angles of rotation and CD spectra were measured on a JASCO-20 spectropolarimeter. The course of the reactions and the individualities of the compounds obtained were monitored on Silufol UV-254 plates in the chloroform—methanol (8:1) and (4:1) systems. The results of elementary analysis corresponded to the calculated figures.

N⁴-Benzoylcytosine 1-(Methyl 2,3,4-Tri-O-acetyl-β-D-glucopyranosiduronate (III). To a solution of 2.5 g of carefully dried cytosine in 25 ml of anhydrous pyridine was gradually added (the reaction is exothermic) 34 ml of benzoyl chloride. The solution was stirred for another 3 h and then, with cooling, 200 ml of 10% hydrochloric acid was added. The crystalline product was filtered off washed with hot ethanol, and dried, to give 4 g of N⁴-benzoylcytosine. With stirring and without the access of atmospheric moisture, 4.8 ml of chlorotrimethylsilane and 5.2 ml of triethylamine in 30 ml of benzene were gradually added to a suspension of the N⁴-benzoylcytosine. After 7 h, the solution was filtered, and the filtrate was evaporated in vacuum to 6.43 g of an oily product. A solution of 1.80 g (0.005 mole) of this product in anhydrous dichloroethane (30 ml) was added to a solution of 1.3 g (0.004 mole) of the peracetate (II) in dichloroethane (30 ml), and then, with stirring, 1.4 ml (0.012 mole) of $SnCl_4$ was added to the solution. After 2 days, the contents of the flask were poured into a saturated solution of $NaHCO_3$ and were extracted with chloroform, and the extract was washed with water, dried over Na_2SO_4, and evaporated, and the residue was crystallized from chloroform—ethanol. This gave 1.25 g (70%) of the nucleoside (III) with mp 274-276°C, $[\alpha]_D^{20}$ +24.5° (c 1.0; $CHCl_3$).

PMR ($CDCl_3$), ppm: 8.46 (d, 1 H, $J_{5,6}$ = 8.0 Hz, H-6); 8.04 (d, 1 H, H-5); 7.70-7.30 (5 H, Bz); 6.38 (d, 1 H, $J_{1',2'}$ = 8.5 Hz, H-1'); 5.66 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, H-3'); 5.58 (dd, 1 H, H-2'); 5.16 (dd, 1 H, $J_{3',4'}$ = 9.0 Hz, $J_{4',5'}$ = 10.0 Hz, H-4'); 4.84 (d, 1 H, H-5'); 2.10 (s, 6 H, 2 OAc); 1.96 (s, 3 H, OAc).

UV λ_{max}^{MeOH}, nm ($\varepsilon \cdot 10^{-3}$): 230(19.5), 272(9.1).

CD (MeOH) λ, nm ($[\theta] \cdot 10^{-3}$): 220(—7.5), 270(15.0).

Cytosine 1-(β-D-Glucopyranosiduronamide) (IV). A solution of 1.0 g of the nucleoside (III) in 25 ml of methanol saturated with ammonia in the cold was left at room temperature for a day. Then the solution was evaporated in vacuum, the residue was dissolved in the minimum volume of methanol, and ether was added until the product obtained had been completely precipitated. This gave 0.55 g (100%) of the nucleoside (IV) with mp 227-230°C (decomp.).

PMR (DMSO-d_6): 7.62 (d, 1 H, $J_{5,6}$ = 8.0 Hz, H-6); 7.48 (s, 1 H, CONH); 7.24 (s, 3 H, CONH, NH_2); 5.78 (d, 1 H, H-5); 5.50 (d, 1 H, $J_{1',2'}$ = 9.0 Hz, H-1').

UV $\lambda_{max}^{H_2O}$, nm ($\varepsilon \cdot 10^{-3}$): 270(7.0).

CD (MeOH) λ, nm ($[\theta] \cdot 10^{-3}$):215(—2.3), 250, 260(2.0), 308(—1.4).

SUMMARY

The glycosylation of the trimethylsilyl derivative of N⁴-benzoylcytosine with methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate in the presence of three equivalents of $SnCl_4$ is an effective method of synthesizing N⁴-benzoylcytosine 1-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate) the ammonolysis of which leads to the amide of pentopyranic acid.

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ISOLATION AND PURIFICATION OF AN AMINOACYLASE FROM *Aspergillus oryzae*

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An aminoacylase (EC 3.5.1.14) has been isolated from a surface culture of the fungus *Aspergillus oryzae* (amilorizin P10X) with a 764-fold degree of purification, an activity yield of 32.7%, and a specific activity in relation to the hydrolysis of N-acetyl-D,L-methionine of 99.3 a.u./o.u. The scheme of the purification of the aminoacylase from *Aspergillus oryzae* includes: extraction at pH 6.7, precipitation with ammonium sulfate (30 and 80% saturation), gel filtration on Acrilex P-150 (pH 7.5), ion-exchange chromatography on amino-Silochrom Cx-1,5 (mean pore radius 790 Å); the sorption of the enzyme takes place at pH 6.2 and elution with 0.05 M borate buffer, pH 8.0; ion-exchange chromatography on AH-Sepharose 4B at pH 8.0, with elution by a stepwise increase in the concentration of sodium chloride to 0.25 M; and, finally, gel filtration on Sephadex G-200 (pH 8.0). According to the results of disk electrophoresis in 7.5% polyacrylamide gel in a Tris-glycine systems of buffers with a separation pH of 8.9 in the presence of Co^{2+} ions (10^{-5} M) of the *Aspergillus oryzae* aminoacylase, two components possessing enzymatic activity were detected, with R_f 0.53 (major component) and R_f 0.63 (minor component).

The use of an enzyme hydrolyzing acyl-L-(amino acids) — aminoacylase (EC 3.5.1.14) — for the separation of amino acid racemates obtained synthetically into their optical antipodes is well known. Until recently, the asymmetric hydrolysis of various N-acetyl derivatives of amino acids was carried out with the aid of aminoacylase of animal origin [1].

A partially purified aminoacylase of microbial origin was first obtained from the mycelium from a mold fungus, strain No. 9 of *Aspergillus oryzae* [2]. The possibility was shown of using the *Aspergillus oryzae* aminoacylase for the continuous separation of N-acetyl-D,L-(amino acid)s [3, 4]. Recently, it has been possible to obtain an aminoacylase from a commercial preparation of *Aspergillus oryzae* which is homogeneous according to electrophoresis in polyacrylamide gel [5]. The purification of the enzyme included the thermal treatment of the intiial preparation, subsequent precipitation with 5% polyethyleneimine and ammonium sulfate, gel filtration on Sephadex G-150, and preparative disk electrophoresis. A 201-fold purification was achieved with a yield of 14%.

We have previously obtained an aminoacylase from a surface culture of *Aspergillus oryzae* (amilorizin P10X) by the extraction of the initial preparation at pH 8.0, precipitation with ammonium sulfate (80% saturation), ion-exchange chromatography on DEAE- and ECTEOLA-celluloses, gel filtration on Sephadex G-200, and affinity chromatography. The specific activity of the preparation rose by a factor of 800, amounting to 36.5 μmole of L-methionine/mg/min (N-acetyl-D,L-methionine was used as the substrate. We have studied some physicochemical properties of the enzyme preparation obtained: action pH optimum, dependence of the stability on the pH, dependence of the activity of the enzyme on the concentration of Co^{2+} ions, substrate specificity [6].

In the production of a highly purified preparation of the aminoacylase, we came up against a number of difficulties connected with the fact that a surface culture of *Aspergillus oryzae* contains, in addition to a multiplicity of pigments and a large number of products of the

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