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ORGANIC SYNTHESIS AND INDUSTRIAL ORGANIC CHEMISTRY

Synthesis, Isolation, and Purification of Benzylpenicillin β-Diethylaminoethyl Ester Hydroiodide

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Abstract—Processes of synthesis, isolation, and purification of benzylpenicillin β-diethylaminoethyl ester hydroiodide were studied. The optimal conditions of benzylpenicillin esterification with chloroethyldiethylamine in dimetylformamide and acetonitrile were found. Processes for isolation of the target product were developed and its physicochemical properties were studied. Product stabilization conditions providing a stable pH value in solutions of benzylpenicillin β-diethylaminoethyl ester hydroiodide were found.

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Benzylpenicillin β-diethylaminoethyl ester hydroiodide (penetamate hydroiodide) is a semisynthetic antibiotic exhibiting high activity in curing of a number of diseases caused by penicillin-sensitive microorganisms. A specific feature of this preparation, due to the cationic nature of the active component of the molecule, consists in its capacity to concentrate in lactation organs and in lungs. As a result, penetamate hydroiodide has recently found wide use as a means for curing mastites and lung infections, primarily in veterinary medicine.

Benzylpenicillin β-diethylaminoethyl ester hydroiodide is [2s-(2α,5α,6β)]-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabiscyclo-[3,2,0]-heptane-2-carboxylic acid 2-(diethylamino)ethyl ester monohydroiodide (Scheme 1).

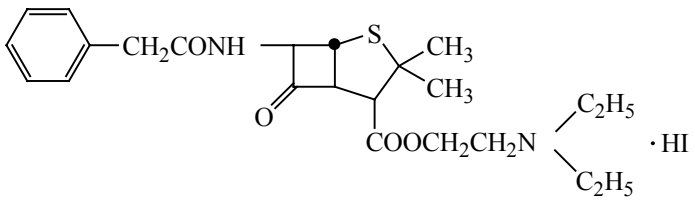
The evidence about methods for synthesis of benzylpenicillin diethylaminoethyl ester salts are limited to several patents [1–3] whose use on the industrial scale fails to provide the required quality of the target product or involves gross technological expenditures because of the low yield and numerous stages of the process.

The goal of our study was to obtain data on technological specific features of synthesis of benzylpenicillin β-diethylaminoethyl ester and on processes of its isolation from the reaction mass and purification.

EXPERIMENTAL

We synthesized penetamate hydroiodide from a sodium salt of benzylpenicillin, which satisfied the requirements of FSP (Pharmacopoeia Standard of Manufacturer) 42-2804–07 and a mass fraction of water not exceeding 0.5%.

Scheme 1.



The esterification was made with *N*-(2-chloroethyl)-diethylamine hydrochloride (diethylaminoethyl chloride) manufactured by Medilux laboratories Pvt. Ltd (India), as the iodine-containing component served sodium iodide dihydrate [GOST (State Standard) 8422–76], with a 99.0% mass fraction of the main substance.

The mass fraction of the main substance in the product was quantitatively determined by iodometric titration and high-performance liquid chromatography (HPLC) on a Waters chromatograph, with a sample from Schweizerhall Pharma GmbH as reference.

Both the methods furnished identical results.

We studied the polarimetric characteristics of the preparation in various solvents: in water, acetonitrile, and dimethylformamide. This was done using an A1-EPO automated photoelectric polarimeter at a working wavelength of 589.3 nm, with an admissible error of ±0.01°. It was found that, because of the poor solubility of penetamate hydroiodide in water, the specific rotation in this solvent can be determined with a gross error and difficulties in preparing a sample of the aqueous solution. The optimal way to determine the specific rotation of the penetamate is to use a 1% solution of the preparation in dimethylformamide. Table 1 lists the results of measurements of the optical rotation of a 1% solution of penetamate hydroiodide samples in dimethylformamide. It follows from the data in Table 1 that the specific rotation of penetamate hydroiodide is linearly related to the activity of the preparation. In accordance with data on the theoretical activity of the preparation (1058 U mg^{-1}) and the experimentally measured values of the specific rotation, the specific rotation was found to be 160°C. The specific rotation of the preparation was determined inconformity with the requirements of GF (State Pharmacopoeia) XI [4].

With the specific rotation of penetamate hydroiodide determined, the concentration of the preparation in technical solutions can be determined by polarimetry with a comparatively small error. We found that, on passing from dimethylformamide solutions to solutions in other polar solvents, the specific rotation of penetamate hydroiodide changes only slightly. Thus, rough technological calculations in synthesis and isolation of the product can be based on the results of a polarimetric determination of the concentration:

$$A = \frac{1058\,aP}{160}$$

where A is the activity of a preparation sample under study (U mg^{-1}); α, optical rotation of a solution of the preparation (deg); 1058, theoretical activity of the penetamate (U mg^{-1}); and 160, specific rotation of the penetamate (deg).

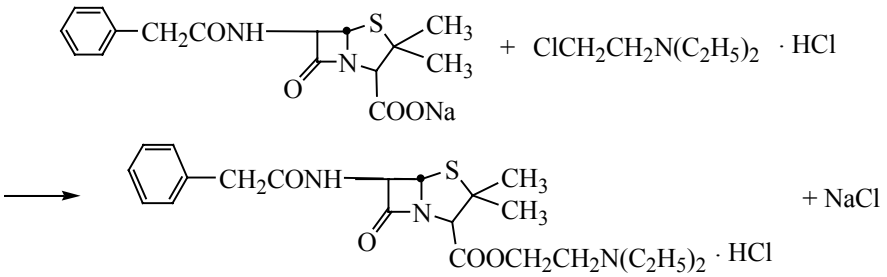
Table 1. Specific rotation of 1% penetamate hydroiodide solutions

Sample no.	α_D^{20}, deg	Activity A, U mg^{-1}
Reference sample	151	1005
1	147	990
2	148	995
3	156	1045
4	152	1030
5	150	1020

When developing a technological process for production of benzylpenicillin diethylaminoethyl ester, we considered as the main variant of synthesis the esterification of *N*-benzylpenicillin with *N*-(2-chloroethyl)-diethylamine hydrochloride in dimethylformamide or acetonitrile (Scheme 2).

Alternative variants, synthesis of diethylaminoethyl ester from benzylpenicillin chloroanhydride [1] or from

Scheme 2.



a mixed anhydride of benzylpenicillin and aliphatic carboxylic acid [2], gave the product in a substantially lower yield, compared with Scheme 2.

Benzylpenicillin diethylaminoethyl ester was synthesized by the following method [5]: a 1000-ml three-necked flask was charged with 200–300 ml of dimethylformamide and 20 g of a sodium salt of benzylpenicillin (56.1 mmol). The suspension was agitated at room temperature for 30 min, and then 7.8–14.5 g of chloroethyldiethylamine hydrochloride (44.8–84.15 mmol) preliminarily dissolved in 200 ml of dimethylformamide at a temperature 45–50°C was added in the course of 3–3.5 h. Upon introduction of chloroethyldiethylamine hydrochloride, the mixture was additionally kept under agitation at 20–80°C for 20–60 min and a sample was taken to determine the degree of conversion. The degree of conversion was found by determining the ratio between the amount of benzylpenicillin diethylaminoethyl ester formed and the corresponding theoretical value. The concentration of the target product was determined by HPLC after a preliminary filtration of an aliquot and its dilution with dimethylformamide and then with water. The results of the synthesis are listed in Table 2.

It follows from the data in Table 2 that the optimal ratio between the components entering into the reaction is the stoichiometric ratio or a small excess of chloroethyldiethylamine hydrochloride, and the optimal reaction temperature is 40–60°C. At higher temperatures, thermal destruction of benzylpenicillin presumably occurs. At the optimal parameters, the esterification reaction is complete in 30–0 min.

Because the subsequent isolation of the target product includes evaporation of the solvent from the reaction mass, we considered the possibility of replacing dimethylformamide with a solvent with a lower boiling point and, specifically, acetonitrile.

The method for esterification in acetonitrile is similar to that described above for dimethylformamide. The relative amounts of the components of the reaction mixture and the temperature-and-time parameters of the process of esterification in acetonitrile correspond to the optimal range found for the esterification in dimethylformamide. The degree of conversion in acetonitrile was 75–80%.

The benzylpenicillin diethylaminoethyl ester hydrochloride formed in the esterification reaction should be converted to a hydroiodide salt. This was done by taking advantage of the different solubilities of chlorides and iodides in organic solvents [6, 7]. It follows from the results of [6, 7] that the solubility of iodides in organic solvents substantially exceeds that of chlorides. This property was used to separate chlorides from the reaction mass obtained.

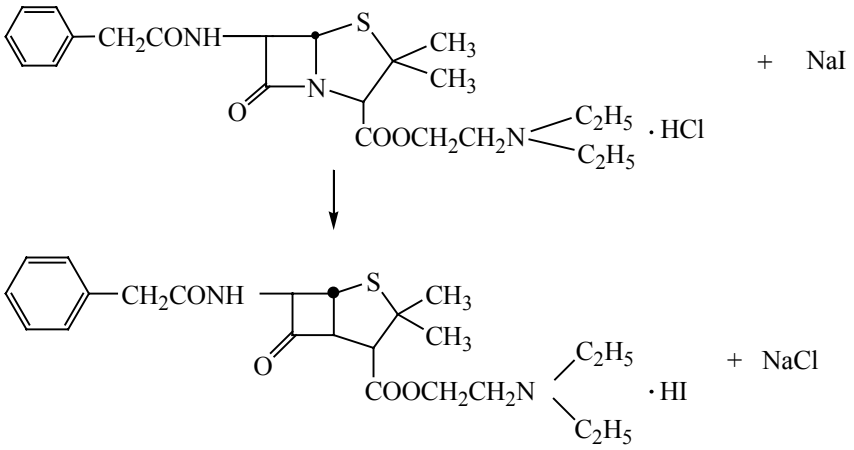
On charging chloroethyldiethylamine hydrochloride and agitating the reaction mass for 30–45 min, we introduced sodium iodide into the solution in a stoichiometric amount with respect to benzylpenicillin. After sodium iodide was dissolved, an exchange reaction occurred by Scheme 3.

Introduction of iodide ions into the reaction mass

Table 2. Esterification of a sodium salt of benzylpenicillin with diethylaminoethyl chloride hydrochloride

Benzylpenicillin : chloroethyldiethylamine molar ratio	Volume ratio of DMFA and charged Na-salt of benzylpenicillin, ml : g	T, °C	τ, min	Conversion, %
1 : 1	200 : 1	20	30	12
	200 : 1	20	60	14
	200 : 1	60	60	75
	300 : 1	50	45	70
1 : 0.8	200 : 1	40	45	60
	250 : 1	50	60	58
1 : 1.5	250 : 1	80	50	70
	250 : 1	50	45	80
	250 : 1	30	60	45

Scheme 3.



simultaneously promotes a more complete esterification because of the catalytic effect of I– anions in substitution reactions [8, 9].

The separation completeness of chlorides was monitored by determining the amount and composition of the precipitate obtained upon filtration of the reaction mass (Table 3).

The target product was isolated from the reaction mass by the method of salting-out crystallization. This method includes crystallization of the target product from solutions by diminishing the solvent activity in solution to a value lower than that in a saturated solution. In this case, the required effect is achieved by introducing into the solution an auxiliary solvent and, in particular, acetone, in which the target product is poorly soluble. This procedure is similar to the "cold" recrystallization technique [10, 11].

On performing the esterification process and separating sodium chloride, we partly evaporated from the resulting solution the solvent (acetonitrile or dimethylformamide), in which benzylpenicillin diethylaminoethyl ester hydroiodide is well soluble, and then acetone was introduced into the distillation residue and the product was crystallized in a mixture of acetone and acetonitrile (or acetone and dimethylformamide).

Processes for isolation of penetamate hydroiodide were for the most part tested for solutions obtained in esterification in acetonitrile. Commonly the evaporation of acetonitrile begins at a vapor temperature of about 20°C. and an insignificant overheating of the reaction mass. As the solvent is evaporated and the concentration of benzylpenicillin diethylaminoethyl ester hydroiodide in the reaction mass increases, the degree of overheating grows. As the volume of the distillation residue decreases to 25–30% relative to the initial value, the reaction mass temperature increases to 35–40°C. Because of this circumstance, the degree of evaporation of the reaction mass is limited by the maximum possible temperature of the distillation residue (40°C), commonly being 65–70%.

When studying the solubility of benzylpenicillin diethylaminoethyl ester hydroiodide in acetonitrile, we found that the high solubility of benzylpenicillin diethylaminoethyl ester in acetonitrile gives no way of

Table 3. Characterization of the sediment formed upon filtration of the reaction mass of benzylpenicillin diethylaminoethyl ester hydroiodide

Charged, mg-mol		Sediment mass, g	Mass fraction of NaCl, %	Molar ratio between NaCl formed and NaI charged
chloroethyldiethylamine hydrochloride	NaI			
56.1	56.8	8.2	81	2 : 1
56.1	55.9	8.29	78	1.99 : 1
56.1	56.0	8.5	74	1.92 : 1

isolating the product from the reaction mass even on evaporating the solvent to 10–15% relative to the initial volume of the reaction mass. Isolation of the product from the reaction mass concentrated by evaporation is hindered not only by the high solubility, but also by the formation of penetamate hydroiodide solvates with the solvent, which results in that solutions of penetamate hydroiodide in acetonitrile are formed as a thick syrupy fluid. In the method we developed, the solvation shell is disintegrated, with the product solubility in the solution simultaneously lowered, by introducing acetone into the concentrated reaction mass.

The kinetics of the crystallization process is shown in Fig. 1, whence follows that evaporation of the acetonitrile solution of the penetamate to 30–35% relative to the initial volume and a 2–2.5-fold dilution of the distillation residue enable isolation of the product in a 50–55% yield. The crystallization of penetamate hydroiodide is complete in 2–2.5 h; to achieve a more complete recovery of the product from solution, the suspension is cooled to 8–10°C.The precipitated benzylpenicillin diethylaminoethyl ester hydroiodide is a crystalline product of white color or white with a slight yellowish tint. The penetamate hydroiodide paste produced on washing with acetone and squeezing to remove the solvent contains no more than 10% volatiles.

The drying mode of benzylpenicillin diethylaminoethyl ester hydroiodide was studied at temperatures of 25 to 110°C. It was found that, at temperatures higher than 40°C, the outward appearance of penetamate hydroiodide changes because of the oxidation of iodide ions: the white or nearly white powder becomes yellow. The optimal mode of drying of the preparation is that in a vacuum at a residual pressure not exceeding 50 mm Hg at temperatures of 25–35°C. It was found that, after 1.5–2 h of drying in this mode, the content of volatiles in the product does not exceed 1.5%.

We studied on model solutions the possibility of sorption recovery of benzylpenicillin diethylaminoethyl ester from acetonitrile–acetone and acetone mother liquors with an KB-4P-2 carboxy cation-exchanger resin. The high sorption selectivity of organic compounds of this kind from aqueous solutions was noted in [12, 13].

Figure 2 shows outlet elution curves for the benzylpenicillin diethylaminoethyl ester cation sorbed on the Na-form of the KB-4P-2 carboxy cation exchanger. It can be seen that capacity of KB-4P-2 to penetamate breakthrough is 150 g l^{-1} in sorption from an acetonitrile–acetone solution and about 300 g l^{-1} in sorption from an acetone solution. These values of the sorption capacity are sufficient for solving the practical problem of the product recovery from mother liquors. Because there are no competing cations in the solution, the value of the selectivity coefficient is unimportant in this case.

The desorption of penetamate from the carboxy cation-exchanger phase was studied using a 0.2 M solution of hydroiodic acid in acetone as eluent. It was shown that using an acetone solution of hydroiodic acid as eluent provides a product yield of about 75–80% in the active fraction of the eluate. We recommend

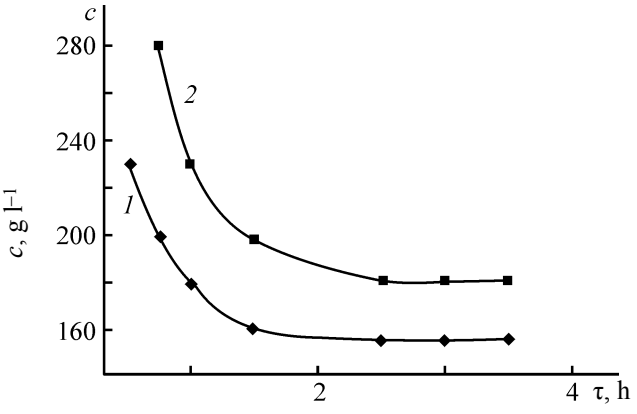


Fig. 1. Kinetics of penetamate hydroiodide crystallization in a 1 : 1 acetonitrile–acetone system. (*c*) Penetamate hydroiodide concentration and (τ) crystallization duration. *T* (°C): (*1*) 8–10 and (*2*) 25–30.

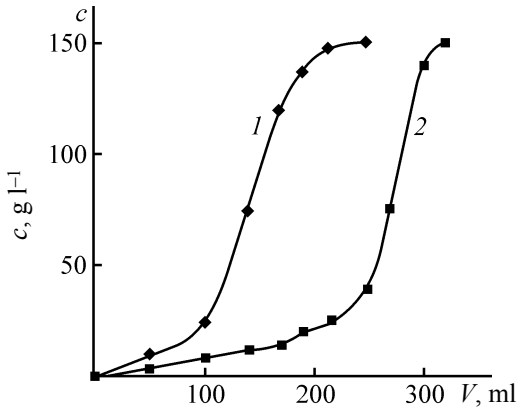


Fig. 2. Elution curves for penetamate hydroiodide sorbed by the sodium form of KB-4P-2. Sorbent volume in the column 10 ml. (*c*) Penetamate hydroiodide concentration at the column outlet and (*V*) filtrate volume. Sorption: (1) from a 1 : 1 acetonitrile–acetone solution and (2) from an acetone solution.

Table 4. Quality characteristics of penetamate hydroiodide samples[a]

Characteristic	Property and standard	Test results for samples				
		no. 1	no. 2	no. 3	no. 4	no. 5
Outward appearance	Crystalline powder of white color or white with a yellowish tint	+	+	+	+	+
Content of the main substance, U mg–1	950–1110	990	1020	1050	990	1010
mp, °C	173–179, within 2°C	174–174.5	174.5–175	175–176	173.5–174	174.5–175
Specific rotation, deg	145–160	149	154	158	150	152
Mass fraction of water, %, not more	1.5	0.40	0.20	0.15	0.35	0.3
pH value of the aqueous suspension, 106 U in 30 ml of water						
at the instant of suspension preparation	5.0–7.0	6.25	6.0	6.5	6.0	5.9
in 72 h	no less than 3.5	3.8	3.6	4.05	3.7	3.7
Identity reaction for iodides	Positive for iodide ions	+	+	+	+	+
Mass fraction of benzylpenicillin, %, not more	0.5	0.15	0.18	0.27	0.31	0.36
Mass fraction of solvents, %, not more,	0.1	0.082	0	0.041	0.052	0
including acetonitrile	0.005	0.002	0	0.001	0.002	0
Solubility	Poorly soluble in water, readily soluble in dimethylformamide	+	+	+	+	+
Permeability of a preparation suspension through a needle	No hindrance	+	+	+	+	+
Identity	Agreement with PMR spectra of a standard sample of the preparation	+	+	+	+	+
Stability of the suspension during 72 h at room temperature	Stable	+	+	+	+	+

[a] "+" corresponds to the standard.

to perform the following procedures for recovery of penetamate hydroiodide from the eluate: (1) neutralization of the excess amount of hydroiodic acid in the eluate with a 20% solution of sodium hydroxide (with the solution cooled to 2–5°C under agitation) until the pH of the medium reaches a values of 6.5–7.0; (2) evaporation of acetone from the neutralized eluate to a distillation residue constituting 1/4–1/5 of the initial volume; (3) crystallization of the product from the resulting distillation residue upon its cooling to 2–5°C and agitation for 2 h; (4) filtration and washing of the resulting precipitate of penetamate hydroiodide; and (5) recrystallization of the product obtained in acetonitrile in case of its insufficient quality.

Table 4 lists the rated quality parameters of benzylpenicillin diethylaminoethyl ester hydroiodide

and results of tests of preparation samples produced by the technique described above.

Benzylpenicillin diethylaminoethyl ester hydroiodide is unstable in aqueous solutions. Hydrolysis of the preparation leads to a decrease in the pH of the medium. The hydrolysis rate strongly depends on specific features of how the product is obtained, especially in final stages. It was found that the purer the product, the higher its stability in aqueous solutions and suspensions. A rated parameter for penetamate hydroiodide is a pH value of no less than 3.5 after 72 h of storage of the suspension. In the course of storage, the suspension is periodically shaken.

Figure 3 shows how the pH value varies in storage of the penetamate suspension in water in the course of 48–96 h. It can be seen that an unstabilized penetamate hydroiodide sample has a lower pH value both at the instant of suspension preparation and in the course of its exposure. After 48 h of storage, the suspension of unstabilized penetamate hydroiodide has a pH value of 2.8, which is lower than the rated value.

To obtain satisfactory results for the "pH of the aqueous suspension, " a procedure for neutralization of the excess amount of the acid with phosphoric acid salts has been introduced [14]. The optimal way is to introduce a mixture of KH_2PO_4 and Na_2HPO_4 at a 15 : 85 mass ratio of the components. The stabilizing additives are introduced into the reaction mass after a vacuum-evaporation of acetonitrile, and its dilution with acetone. The amount of phosphates introduced into the reaction mass is 0.75% relative to the amount of the sodium salt of benzylpenicillin, taken for synthesis of penetamate hydroiodide.

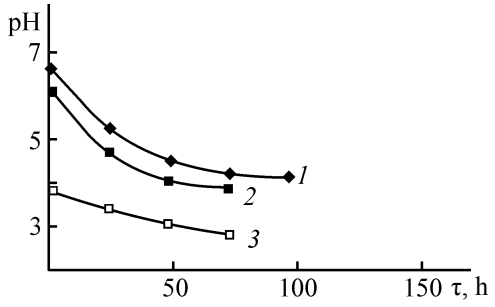


Fig. 3. Variation of the pH value of an aqueous suspension of penetamate hydroiodide. (τ) Exposure duration. Suspension: (*1, 2*) penetamate stabilized in the course of isolation and (*3*) unstabilized penetamate.

The neutralization is necessary because chloroethyldiethylamine hydrochloride used in synthesis contains a certain excess amount of the hydrochloride with respect to the stoichiometry. Introduction of phosphoric acid salts as a neutralizing agent does not contaminate the product with toxic substances and is not prohibited by regulations [15].

Neutralization of the suspension of penetamate hydroiodide in the course of its recovery from the reaction mass provides a higher pH value both at the instant of sample preparation and during the time of observation.

CONCLUSIONS

(1) Benzylpenicillin diethylaminoethyl ester hydroiodide was synthesized with a degree of conversion of up to 80% by esterification of a sodium salt of benzylpenicillin with chloroethyldiethylamine hydrochloride at the stoichiometric ratio between the components.

(2) The conditions of isolation of the target product from the reaction mass by salting-out crystallization from an acetone–acetonitrile solution were determined.

(3) It was found that the optimal variant of product desorption from the KB-4P-2 carboxy cation exchanger is elution with an acetone solution of hydroiodic acid.

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