practically up to 300°C. The inappreciable amount of water determined in the product is possibly due to the presence of free CD in this sample.

It can thus be concluded that the product obtained is an inclusion compound of CoQ_9 with CD. Certain properties (stability, composition, solubility) of the compound obtained and the CoQ_{10}—CD inclusion compound are similar. It should be noted that in experiments on animals, it was shown that absorption of CoQ_{10} introduced in the form of a complex with CD, substantially increases in comparison with the case of free CoQ_{10} [5]. It is possible that the use of CoQ_9 in the form of an inclusion compound will enable production of a new ready-for-use medicinal form of the CoQ_9 preparation, characterized by a high bioaccessibility.

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SYNTHESIS OF AMINAZINE

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The final stage in the synthesis of aminazine (I·HCl) is alkylation of 2-chlorophenothiazine (II) with dimethylaminochloropropane (III). The optimum process conditions have been selected; reagents II and III are boiled in a mixture of toluene and chlorobenzene in the presence of powdered caustic soda and a 90% yield of I is attained [1]. Conducting the reaction in a similar manner but in the presence of a phase transfer catalyst (PTC) does not offer any obvious advantages — the yield of I amounts to 78% [5]. Under milder conditions typical for a phase transfer reaction (80°C, 25 mole % PTC, benzene—water solution of NaOH) it was not possible to obtain compound I [4],* although under the same conditions

*It was mistakenly claimed in [2] that aminazine I base was obtained with a yield of 20% in [4]. In actual fact, by using a benzyltriethylammonium salt as phase transfer catalyst, Masse [4] obtained 10-benzyl-2-chlorophenothiazine (IV) with a yield of 20% instead of the intended product I.

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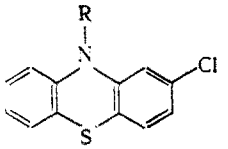
phenothiazine II is alkylated by benzyl chloride and ethyl bromide. The inferior ability of III as an alkylating agent has been noted [4, 5], but no explanation has been given.

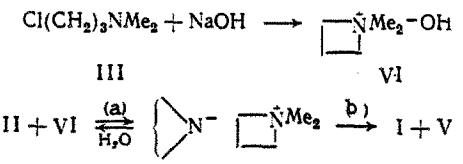


I:R = $(CH_2)_3NMe_2$; II:R = H; IV:R = CH_2Ph; V:R = Me; VII:R = $CH_2CH=CH_2$

I, II, IV, V, VII

To study the dependence of the quality of aminazine on the quality of the starting material used at the alkylation stage, we carried out 15 experiments according to the standard method in [1] and in each experiment detected a small amount of 10-methyl-2-chlorophenothiazine (V) in the crude product by means of TLC. We were able to isolate and identify V from its melting point [3] and mass spectrum.

The formation of the methylated compound V suggests that during reaction the alkylating agent is not III but dimethylazetidinium hydroxide (VI) formed from it.* The 2-chlorophenothiazine anion attacks the azetidinium ion on the carbon atoms of the ring and to a lesser degree on the methyl group carbons, which leads to the formation of a mixture of phenothiazines I and V:



It is logical to assume that alkylation with hydroxide VI proceeds in two stages (a, b). In stage (a) hydroxide VI acts as a PTC, assisting the ionization of the N–H bond in molecule II. In stage (b) N-alkylation of 2-chlorophenothiazine occurs and it is this stage that dictates the required temperature and duration of the process. Introduction of an extraneous PTC [4, 5] assists the ionization of N–H bonds but cannot have any significant effect on the rate-limiting stage (b).

When the reaction mixture was analyzed by means of GLC it was possible to detect another product, 10-allyl-2-chlorophenothiazine (VII), which was identified by chromatography–mass spectrometry. We established that the conversion I → VII does not occur during analysis; hence compound VII is formed during reaction. According to the GLC data, the ratio of I:V:VII in the reaction product is 30:1:2.

EXPERIMENTAL

GLC analyses were carried out on Varian-3700 (USA) and Chrom-5 (Czechoslovakia) chromatographs with flame ionization detector. The columns were of glass or steel (100 × 0.3 cm) filled with sorbent (5% OV-17) on 'N-super' Chromatone. Replacement of the glass columns by metal ones and also changing the temperature of the vaporizer (210-350°C) did not lead to thermocatalytic decomposition of aminazine I base; the ratio of the peaks on the chromatograms did not change and there were no additional peaks. Conditions for analysis were: column temperature 210°C, vaporizer temperature 250°C, detector temperature 250°C, carrier gas (nitrogen) rate 30 ml/min; retention time of products V, VII, and I were 3, 3.8, and 7 min, respectively.

Electron impact mass spectra were obtained on a MAT_112 (Varian, FRG) chromatograph-mass spectrometer with electron ionization energy of 70 eV. Samples from the reaction mixture were introduced via a Varian Aerograph 1440 chromatograph. The columns and their operating conditions were the same as for the GLC analysis.†

2-Chloro-10-(γ-dimethylaminopropyl)phenothiazine Hydrochloride (I·HCl) and 2-Chloro-10-methylphenothiazine (V). Reaction of II (0.05 mole) with III was carried out according to [1], with evaporation of water lasting for 4.5 h. The reaction mixture was then washed

*The possible participation of azetidinium ions in aminopropylation reactions has been discussed in [6].

†Mass spectra were obtained by V. V. Chistyakov, to whom the authors express their thanks.

with water, the mixture of solvents was distilled off under vacuum, the residue was dried to a constant weight (16.13 g) and analyzed by GLC.

The mixture obtained was dissolved in 70 ml of toluene, a solution of HCl in ethanol was added, the solution was evaporated under vacuum to a volume of 30 ml, and the precipitated crystals were filtered off; after recrystallization from a toluene–isopropanol (4:1) mixture 10.92 g of I·HCl (61.4%) was obtained.

After separation of the crude I·HCl the mother liquor was washed with water, the toluene was distilled off, and the residue thoroughly dried under vacuum and analyzed on a chromatographic-mass spectrometer. Mass spectrum m/z (I_{rel}): V – 247 (100), 232 (65), 215 (12), 212 (19); VII – 273 (11), 247 (8), 233 (95), 232 (100), 198 (39). After recrystallization twice from hexane 0.1 g of V was obtained, mp 81-83°C.

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INFLUENCE OF THERMAL STERILIZATION OF CULTURE MEDIA USED IN THE BIOSYNTHESIS OF PENICILLIN ON THE CONTENT OF AMINO ACIDS IN THEM.

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The most widely used technological method for the sterilization of culture media is heat treatment by indirect steam heating and direct (live) steam heating.

In many processes of microbiological synthesis, natural media are conventionally used, including components such as soybean flour and corn extract. Despite the long time since these media have been employed, the dynamics of their composition and properties, depending on thermal sterilization conditions have not been sufficiently well investigated. It is known that thermal stailization of culture media appreciably influences the composition, form and color of the medium, as well as the level of antibiotic formation [4].

The main components of the culture media used for the biosynthesis of biologically active compounds, including antibiotics, are carbohydrates, mineral salts, organic protein-type biopolymers, and amino acids in both the free and bonded states, which play the role not only of nitrogen sources, but also of growth stimulators in the biosynthesis.

Since the preservation of the initial properties of the fermentation media during the thermal sterilization is one of the most important conditions for constant reproduction of the results in the biosynthesis, the aim of the present work was to evaluate the influence of the thermal sterilization parameters on the content of amino acids in corn extract and in the culture medium containing it.

In the investigation, one of the variants of the culture medium for the synthesis of penicillin was used [5], which included lactose (4%), glucose (4%), corn extract (3.2%),

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