AZACYCLOALKANES.

XXIV. CHEMICAL AND PHYSICAL PROPERTIES OF NONACHLAZINE

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Nonachlazine — 10-{β-N-(1,4-diazabicyclo[4.3.0]nonyl)propionyl}-2-chlorophenothiazine dihydrochloride (I) — is a new Soviet preparation used in the treatment of ischemic heart disease [1]. In connection with the introduction of I into therapy we have examined its physicochemical properties and stability under various conditions (solvent, pH, temperature).

Nonachlazine is a yellowish crystalline substance with molecular weight 486.88 and decomposition temperature 219-228°C (in the range 2°C), soluble in water and aqueous alcohol; the 5% aqueous solution has pH 2.0-2.1 (potentiometric measurement).

Its UV spectrum has two maxima at λ 226 nm (ε 24000) and 263 nm (ε 12000), which are also present in the spectrum of the base and are typical of 2-chlorophenothiazines (Fig. 1).

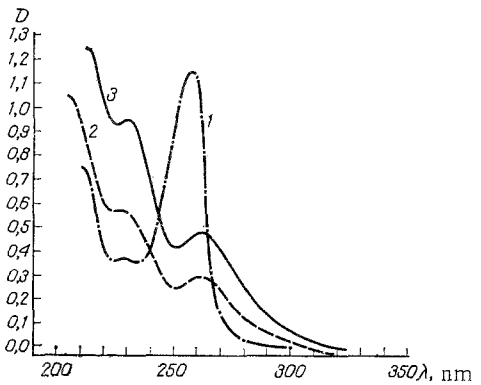

Fig. 1. UV spectra: 1) 2-chlorophenothiazine, c $2.5 \cdot 10^{-5}$ M (alcohol); 2) I·2HCl, c $2.5 \cdot 10^{-5}$ M (70° alcohol); 3) base I, c $3.0 \cdot 10^{-5}$ M (alcohol).

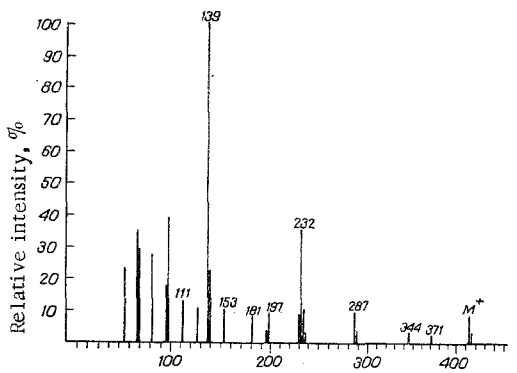

Fig. 2. Mass spectrum of I.

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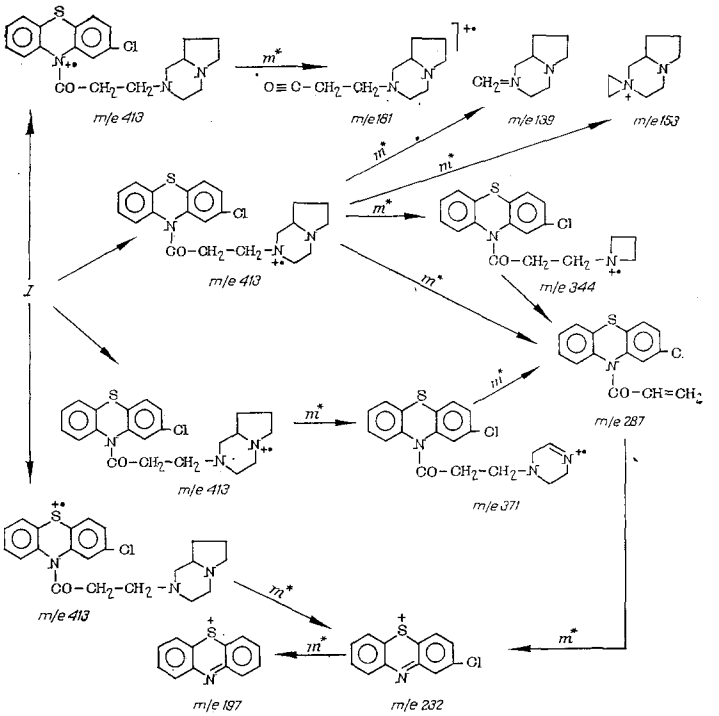
The IR spectrum of I contains bands at 1673 cm^{-1} due to the amide carbonyl, and 2910 cm^{-1} due to stretching of the methylene hydrogens in the chain.

The mass spectrum of nonachlazine (Fig. 2) has the molecular ion M^+ 413 and the corresponding doubly charged ion at m/2e 206.5. The observed metastable ions correspond to the following fragmentation scheme. The elimination of 42 and 69 mass units from the molecular ion is typical of the fragmentation of the diazabicyclononane unit. The mass spectrum also has a peak at m/e 287, formed by loss of the ion of 1,4-diazabicyclo[4.3.0]nonane (II) from the molecular ion. The peak at m/e 232 belongs to the 2-chlorophenothiazine fragment (III), which is formed by cleavage of the amide bond. The mass spectrum also contains peaks at m/e 181, 153, and 139, representing fragments containing the diazabicyclic system. The base peak at m/e 139 is due to the fragment of the N-methyl derivative of II. The fragmentation scheme of nonachlazine can be used in work on the possible pathways of its metabolic transformations [2].



We tried thin-layer chromatography (TLC), gas–liquid chromatography (GLC), and high-pressure liquid chromatography (HLPC) for examination of the degradation of nonachlazine under various conditions (the analytical conditions are described in the experimental section; HPLC determinations were carried out quantitatively). Thin-layer chromatography of nonachlazine on Silufol and Kieselgel gives a single spot with R_f 0.21 (Silufol) and 0.76 (Kieselgel). Gas–liquid chromatography of nonachlazine under the experimental conditions produces peaks with retention times 4 min 48 sec and 18 min 18 sec. Chromatographic–mass spectrometric analysis at various injector temperatures revealed that the eluates are diazabicyclononane (II) and 10-acryloyl-2-chlorophenothiazine (IV) respectively (comparison with authentic samples). This implies that under the chromatographic conditions thermal β-elimination of the diazabicycle II from the nonachlazine molecule takes place. The sole peak eluted after liquid chromatography of nonachlazine with retention time 9 min 20 sec was identified as nonachlazine base from mass spectrometric data.

Examination of the stability and decomposition pathways of nonachlazine under various conditions is relevant to development of its dosage forms and determination of it and its possible metabolites in biological media.

We established by TLC and HPLC that a 5% aqueous solution of I·2HCl is stable at pH 2.1 when stored for 2 months at room temperature. After acidification of a 5% aqueous solution to pH 1.13 HPLC reveals a second peak with retention time 2 min 42 sec, identified as III by mass spectrometric analysis. About 1% of I·2HCl has been hydrolyzed after 48 h and 6-7% after 20 days. When a 5% aqueous solution of I·2HCl is heated at pH 1.13 at 100°C for 3 h the content of III increases to 15-17%.

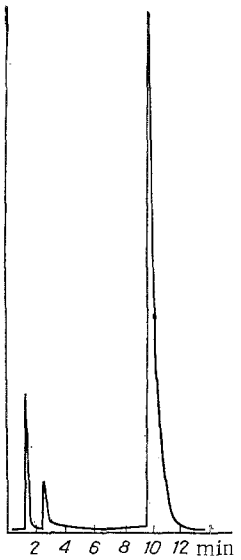


Fig. 3

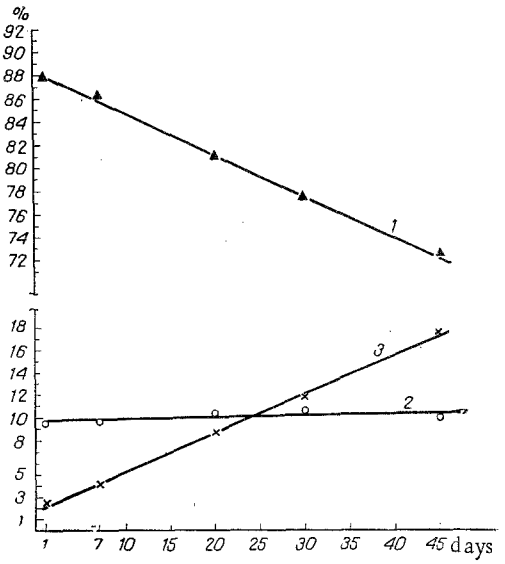


Fig. 4

Fig. 3. Liquid chromatography of a 5% alcoholic solution of nonachlazine base (after 24 h standing at 20°C).

Fig. 4. Conversion of I to III and IV (5% alcoholic solution of base I at 20°C); 1) content of I; 2) content of III; 3) content of II.

We also examined the stability of I as the base in various solvents, since it is isolated from biological materials at pH > 7.0 in pharmacokinetic work. In aprotic solvents such as ether, tetrahydrofuran, benzene, or toluene, nonachlazine base is unchanged after 1 month. The base is less stable in protic solvents; after 3 h reflux of an emulsion of nonachlazine base in water, gas chromatographic–mass spectrometric analysis of the silylated sample revealed the presence of a peak with retention time 10 min 59 sec (M^+ 270), representing trimethylsilyl β-N-(1,4-diazabicyclo[4.3.0]nonyl)propionate (Vc), and a peak with retention time 15 min 30 sec (M^+ 305), due to the trimethylsilyl derivative of III.

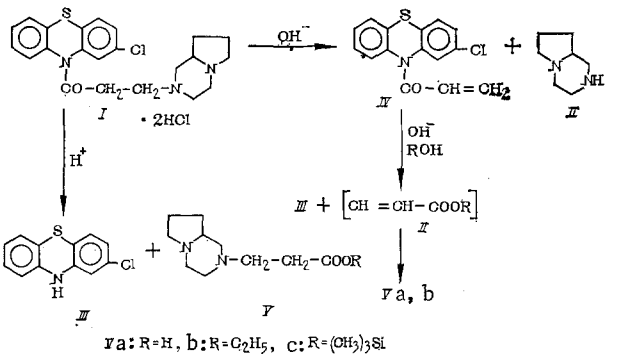
We examined the stability of alcoholic solutions of the base in more detail. Chromatography of a 5% alcoholic solution of the base of I (pH 7.45) after standing for 24 h on Silufol revealed a spot with R_f 0.21 (I) and a green spot with R_f 0.91 due to III. Liquid chromatography of this solution eluted three peaks (Fig. 3) with retention times 1 min 25 sec (IV), 2 min 42 sec (III), and 9 min 20 sec (I). Chromatography of this solution at intervals over a period of 1.5 months revealed that the absolute content of IV in the solution remains almost constant (9-10%), whereas that of III rises from 2.7 to 17.5% (the absolute quantities of I, III, and IV were determined from the calibration curves). This suggests that in an alcoholic solution of the base of I (pH 7.45) the diazabicycle II is eliminated first, forming the acryloyl derivative IV, which in turn in alkaline medium undergoes alcoholysis of the amide bond to form compound III. Since the content of the acryloyl derivative IV remains almost constant, its formation and alcoholysis may well proceed at roughly the same rate (Fig. 4). Alcoholysis of the acryloyl derivative takes place only at alkaline pH; we found that an alcoholic solution of the acryloyl derivative of IV can be stored without change for 1 month, whereas, after addition of the diazabicycle II (1.1 moles of II per mole IV) to this solution and the concomitant increase in pH to 9.75, after 48 h the acryloyl derivative IV has almost completely decomposed to form III.

The use of HPLC with a UV detector did not make it possible to detect the other degradation products of I (diazabicyclononane II and its derivatives) and we used TLC and GLC for their determination. Thin-layer chromatography on Kieselgel of an alcoholic solution of the base of I after 6 days standing gave several spots. To identify them we synthesized compound IV and β-N-(1,4-diazabicyclo[4.3.0]nonyl)propionic acid (Va) as possible standards. The spot with R_f 0.86 corresponded to IV; that with R_f 0.79 to base I, that with R_f 0.72 to III, and that with R_f 0.35 to the diazabicycle II. The GLC analysis of this alcoholic solution revealed the presence of another peak with retention time 10 min 50 sec (M^+ 226), whose mass spectrum corresponded to the ethyl ester of acid Vb. Ester Vb is probably formed

by the reaction of diazabicycle II with the ethyl acrylate formed by alcoholysis of the acryloyl derivative IV; as a result of the equivalent reactions the hydrolysis of base I forms acid Va, which we detected in the form of the trimethylsilyl ester (Vc) by mass spectrometric analysis.

We also established that when base I is heated at 150°C the C–N alkyl bond and the amide bond are cleaved. Analysis by HPLC revealed that after 2 h heating about 15% of base I has decomposed (the mixture contained 8% of the acryloyl derivative IV and 7% III).

Thus our results reveal that the dihydrochloride of I is reasonably stable in aqueous solutions, whereas solutions of base I in protic solvents undergo quite rapid change. The major pathways of the transformation of I 2HCl are hydrolysis of the amide bond in acidic medium and in alkaline medium β-elimination of diazabicycle II, forming the acryloyl derivative IV, and solvolysis of this to form III and acid Va or its ester Vb.



EXPERIMENTAL

The UV spectra were recorded on a Perkin-Elmer-402 spectrophotometer in solutions in absolute and 70% alcohol in a 10 mm cell. The IR spectrum was recorded with a Perkin-Elmer 457 spectrometer in a potassium bromide tablet with 1.5 mg of the compound in 300 mg potassium bromide.

Chromatography–mass spectrometry was carried out on a Varian MAT-112 instrument with a glass column (1.5 m) with 3% OV-1 on support W (100-120 mesh), carrier gas (helium) flow rate 20 ml/min, oven temperature 100-250°C (10°C per min), injector temperature 250°C, and ionization energy 80 eV. The metastable transitions were determined by the DAD method [3]. Silylation was carried out with N,O-bis(trimethylsilyl)trifluoroacetamide in heptane at 100°C under nitrogen for 15 min.

Thin-layer chromatography was carried out on Silufol UV-254 (Czechoslovakia) in benzene–absolute alcohol–25% ammonia (95:15:1) and on Merck 60 F-254 Kieselgel in chloroform saturated with a mixture of ammonia–heptane (17:3); visualization utilized iodine vapor.

Liquid chromatography was carried out on a Varian 8500 chromatograph with a UV detector at 260 nm using gradient elution with two systems — heptane–5% absolute alcohol (A) and chloroform–15% absolute alcohol–0.5% acetic acid (B); elution proceeded in system A for 4 min and then for the next 10 min in system A with the addition of system B at 6% per minute. The mobile phase flow rate was 40 ml/h. The Micro Pak-NH_2-10 column length was 25 cm, the inlet pressure 3.5 atm.

The calibration graphs for the quantitative determination of I, II, and IV were derived with instrument sensitivity 500 mV using alcoholic solutions of the standards in the range 1.5-30 μg. In this concentration range the peak area is linearly dependent on the amount of substance injected.

10-Acryloyl-2-chlorophenothiazine (IV). A toluene solution of β-chloropropionyl-2-chlorophenothiazine (0.02 mole) and triethylamine (0.05 mole) was refluxed for 1 h and filtered after cooling. The filtrate was evaporated to dryness and the residue (a yellow oil) was crystallized from alcohol (30 ml) to give IV, mp 121.5-123°C, yield 83.8%. Found, %: Cl 11.97; N 4.92. $C_{15}H_{10}ClNOS$. Calculated, %: Cl 12.32; N 4.87.

β-N-(1,4-Diazabicyclo[4.3.0]nonyl)propionic Acid (Va). A mixture of the methyl ester of acid Va (0.05 mole) [4] and concentrated hydrochloric acid (0.2 mole) was refluxed for 1 h and then repeatedly evaporated (five times) with distilled water (20 ml) and twice with

benzene. The residue was crystallized from alcohol to give the dihydrochloride of acid Va, mp 167-169°C, yield 81%. Found, %: C 41.19; H 7.67; N 9.69; Cl 24.55; H_2O 6.47. $C_{10}H_{20}Cl_2O \cdot H_2O$. Calculated, %: C 41.53; H 7.67; N 9.69; Cl 24.52; H_2O 6.23.

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MASS SPECTRA AND TAUTOMERIC EQUILIBRIUM OF HETEROCYCLIC SULFANILAMIDES

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In recent years studies have appeared in which the method of mass spectrometry has been used to investigate tautomeric equilibria of various classes of compounds [1-4]. The mass spectral data expand our information on the equilibrium systems obtained in an investigation by traditional spectral methods. The use of mass spectrometry also seems promising in that the substances are analyzed in the gas phase in this case, which permits exclusion of solvation and association effects.

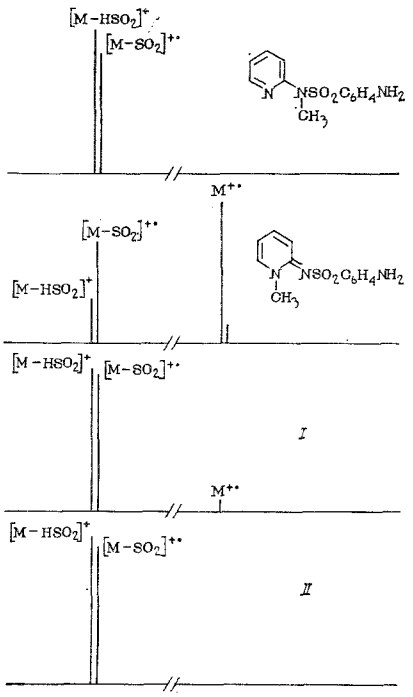

Fig. 1. Mass spectra of sulfanilamide of pyridine and their N- and N'-methyl derivatives.

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