NONACHLAZINE: A NEW PREPARATION FOR TREATING ISCHEMIC HEART DISEASE

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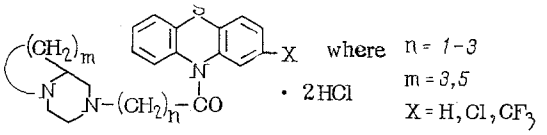
The search for new preparations for treating ischemic heart disease is one of the most important problems of modern medicine.

In the search for new antianginal drugs,* several 1,4-diazabycyclo[4,m,0]alkanyl derivatives of 10-acylphenothiazines have been synthesized at the Institute of Pharmacology of the Academy of Medical Sciences of the USSR [1]:



During the pharmacological study of these compounds, two active compounds were discovered which were called nonachlazine and nonaftazine [2]. Pharmacological studies, followed by clinical investigation, showed that nonachlazine (I; n = 2, m = 3, X = Cl) is characterized by a more marked antianginal effect than nonaftazine (X = CF_3). At present, nonachlazine is recommended for use in the medicinal practice.

In pharmacological experimentation, it was found that nonachlazine appreciably increases the blood supply to the heart [3, 4]. In experiments on cats (5 mg/kg intravenously), it increases the coronary blood flow by 65%, on an average. The absorption of oxygen by the heart does not increase much under the influence of nonachlazine. As a result, the oxygen reserve of the heart increases [3]. Particularly, nonachlazine increases the blood supply to ischemized sections of the myocardium [5]. This is indicated by the data of epicardial electrograms and the results of the determination of the lactate content in the blood flowing from the ischemic center. In its ability to redistribute the blood flow by improving the blood supply to the ischemized sections, nonachlazine is similar to β-adreno-blocking agents and nitroglycerin, and differs favorably from dipyridomol and oxyfedrine (the latter increase the blood supply mainly to the healty sections of the myocardium). Since in the pathogenesis of the attack of stenocardia, an acutely developing cardial insufficiency is important, the ability of nonachlazine to increase the contractility of the myocardium [4] should be especially noted. The mechanism of the influence of nonachlazine on the tonus of the coronary vessels and contractility and metabolism of the myocardium is highly related to the ability of the preparation to affect the adrenergic processes: Nonachlazine increases the content of catecholamines in the myocardium [6] and blocks the backward transport of noradrenaline into the sympathetic nervous extremities [7]; the β-blocking agent propranolol eliminates the cardiotonic effect of nonachlazine and the ability of the latter to increase the coronary blood flow and to activate phosphorylase in the myocardium [6]. At the same time, in contrast to many β-stimulants, nonachlazine does not cause tachycardia and arrhythmia (their development is probably prevented by the negative chronotropic action and the antiarrhythmic properties of the preparation). Nonachlazine has no pronounced influence on the arterial pressure. The preparation has weak sedative, tranquilizing, and analgesic properties [8]. It has a fair therapeutic scope. The LD_{50} for mice with intravenous administration of nonachlazine is 55 mg/kg. The preparation does not cause side effects [9] on pro-

*Antianginal drugs are preparations for treating Angina pectoris.

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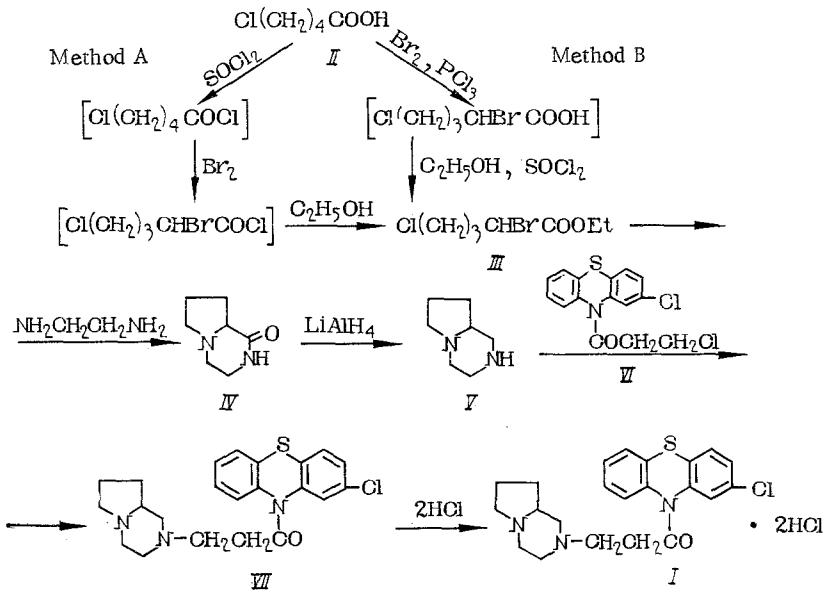
longed application (the chronic toxicity was studied on three animals for 6 months). During the study of the dynamics of the distribution of nonachlazine [10], it was shown that during oral administration to rats in a dose of 20 mg/kg in a conventional aqueous solution, the preparation is rapidly absorbed (during the first 15 min) in the blood. The maximum nonachlazine content in the blood and in the heart was noted after 1 h, and then the content of the preparation in the heart gradually decreased.

In clinics, nonachlazine is used in two medicinal forms: in tablets in doses of 30 mg each, and as a 1.5% solution. The clinical tests were carried out at the following: A. L. Myasnikov Cardiology Institute of the All-Union Cardiological Scientific Center of the Academy of Medical Sciences of the USSR [11]; All-Union Scientific-Research Institute of Clinical and Experimental Surgery of Ministry of Public Health of the USSR [12]; the hospital therapy department of the N. I. Pigorov II Moscow Medicinal Institute [13]; the clinical sector of the Scientific-Research Institute of Biological Testing of Chemical Compounds [14]; the clinical pharmacology department of the Central Scientific-Research Laboratory; internal diseases department of the I. M. Sechenov I Moscow Medical Institute [15]; the cardiology therapy department of the Tbilisi Institute of Advanced Training for Physicians [16]; the hospital therapy department of the Tbilisi Medical Institute [17]; the 1st and 2nd therapy departments of the Central Institute for Advanced Training for Physicians; the hospital therapy department of the Aktyub Medical Institute; the Central Polyclinic and Central Hospital of the Ministry of Health of the USSR; Hospital No. 1 of the 4th Central Board of the Ministry of Public Health of the USSR; the Hospital No. 52 in Moscow; and several other medical institutions [18]. Up to the present, nonachlazine has already been given to more than 1500 patients. In clinical tests it has been found that nonachlazine is a highly effective drug for treating various forms of ischemic heart disease (stenocardia in the state of tension as well as in the state of rest, and also in stenocardia with a background of previous or current infarct of the myocardium). After nonachlazine tablets have been taken (in doses of 30 mg, 3-6 times per day), the preparation decreases or completely eliminates attacks of stenocardia 2 to 5 days after beginning of the treatment. The number of nitroglycerin doses sharply decreases, and in many cases the patients no longer need this. The resistance to physical stress considerably increases [12, 19]. The best results were noted in patients with the hypokinetic form of chronic ischemic disease of the heart. The effectiveness of nonachlazine in patients with stenocardia in the state of tension is higher than that in stenocardia in the state of rest. Depending on the severity of the patient's condition, a positive effect is observed in 60-95% of cases [13]. In patients receiving nonachlazine, it is possible to note an improvement in the general and local contractility of the myocardium [19] and the rheological properties of the blood [13]. Disturbances in the heart's rhythm, cardial insufficiency, arterial hypertonia and hypotonia are not counterindications for the application of the preparation. If we take into account the action mechanism of nonachlazine, the combination of this drug with β-adreno-blocking agents should be considered as counterindicative. It is recommended that nonachlazine in tablet form be used for 1-2 months, and the course of treatment be gradually stopped by decreasing the daily dose for 3-4 days. After a break of 1 month, the course of treatment with nonachlazine in tablet form can be renewed.

Since the effect of nonachlazine during application in tablet form is noted only 2-5 days after the beginning of the treatment, the preparation cannot be used in this form for curing a developing attack of stenocardia. To use nonachlazine as a drug for curing an attack of angina, a 1.5% solution has been suggested. Besides nonachlazine, this solution contains ingredients which ensure its rapid absorption during oral administration, and also stability of the solution on storage: ethyl alcohol, ascorbic acid, sodium metabisulfite, sodium chloride, and distilled water [20]. It was shown in clinical tests [11-19] that in most of the patients, the nonachlazine solution stops the attack of stenocardia 3-5 min after oral administration in a dose of 5-10 ml. When there are no changes in the coronary arteries (according to the data of coronarography), and also if only one of the main arteries has been affected, the solution of nonachlazine has been found effective in 90-94% of cases; when two arteries have been affected, in 67% of cases, and when three arteries have been affected, in only 33% of cases [12]. In very severely ill patients with a very acute atherosclerosis of all the principal trunks of the coronary arteries, with an extremely low resistance to physical stress, the solution of nonachlazine sometimes in less effective than nitroglycerin. At the same time, nonachlazine solution has certain advantages over nitroglycerin (and other preparations in the group of nitrates and nitrites): It does not cause

headaches, dizziness, decrease in the arterial pressure, changes in the intraocular pressure (and therefore it can be applied in patients suffering from a combination of stenocardia and glaucoma), and also has a much more prolonged action than nitroglycerin. Thus, at the Institute of Clinical and Experimental Surgery of the Ministry of Public Health of the USSR, very severely ill patients who received up to 60 and more tablets of nitroglycerin per day, in several cases passed over to 3-4 doses of the nonachlazine solution. Apart from its use in curing the attacks of stenocardia, it is desirable to use the nonachlazine solution (in doses of 5 ml, 3-4 times per day) during the first three days of a systematic nonachlazine treatment to achieve a more rapid therapeutic effect. When a positive effect is reached, from the fourth day it is possible to treat with nonachlazine tablets.

For the preparation of nonachlazine, the scheme already proposed previously in [21-23] has been mainly used. When the process was carried out under industrial conditions, several changes were introduced into the separate stages.



δ-Chlorovaleric acid (II) was brominated with bromine in the α-position in the presence of catalytic amounts of phosphorus trichloride, and the α-bromo-δ-chlorovaleric acid thus obtained was esterified, without isolation, by ethanol in the presence of thionyl chloride to the corresponding ethyl ester of α-bromo-δ-chlorovaleric acid (III) in a yield of 83% (method A). For the industrial preparation, it is more convenient to use the following variant, which makes it possible to carry out all the stages of the transition from acid II into the ester III in one single apparatus (method B). By this method, the action of thionyl chloride on acid I leads to the corresponding acid chloride, which is then brominated, and the acid chloride of α-bromo-δ-chlorovaleric acid is treated with ethanol to give ester III; this method also gives a yield of 80%. Condensation of ester III with ethylenediamine in the presence of catalytic amounts of potassium iodide leads to the formation of 5-keto-1,4-diazabicyclo[4,3,0]nonane (IV). It was shown that a commercial 70% aqueous solution of ethylenediamine can be used in this reaction (comp. [21]). The reaction product is purified by filtering its chloroform solution through a layer of activated carbon. Lactam IV, obtained in a yield of 60%, is then reduced by lithium aluminum hydride in absolute ether to 1,4-diazabicyclo[4,3,0]nonane (V) [22]. The yield of amine V was 75-80%.

The nonachlazine base (VII) is prepared by heating a toluene solution of the bicyclic amine V and β-chloropropionyl-2-chlorophenothiazine (VI) at 80°C in the presence of triethylamine as an acceptor of hydrogen chloride, which separates out [2]. Base VII, without isolation, is converted into the dihydrochloride I by the action of an alcoholic solution of hydrogen chloride. The crude product I is obtained in a yield of 90-95%, calculated on the starting compound VI. The crude product is purified either by boiling in absolute ethanol, or by reprecipitation with acetone from an aqueous solution of I.

The medicinal preparation of nonachlazine and the methods of its analysis were developed in the laboratory of finished medicinal forms of the Experimental-Technological Depart-

ment of the Institute of Pharmacology of the Academy of Medical Sciences of the USSR. Nonachlazine (Temporary Pharmacopoeia Article [TPA] 42 No. 374-74) is sold in the form of tablets, in doses of 0.03 g each (TPA 42 No. 404-75). The preparation should be stored with care (list B) in a dry place, at low temperatures, and protected from light.

EXPERIMENTAL

Identification Reactions. A. A 1-ml portion of bromine water is added to 5 ml of 1% aqueous solution of the preparation, and the mixture is heated to boiling. A transparent lilac-colored solution is obtained (derivatives of phenothiazine).

B. A 10-ml portion of water is added to 1 ml of a 1% aqueous solution of the preparation. The mixture is cooled to 10°C, and then 1 ml of concentrated nitric acid is added. No color should appear (in contrast to the alkyl derivatives of phenothiazine).

C. To 1 ml of a 1% aqueous solution of the preparation are added 5-6 drops of a Bromphenol Blue solution. After stirring, 2 ml of benzene are added, and the mixture is shaken; the benzene layer acquires a yellow color (in contrast to other preparations in the phenothiazine series, except for etaperazine and meterazine).

D. After the nonachlazine base is separated by adding a solution of sodium hydroxide, the aqueous layer gives the characteristic reaction for chlorides. The decomposition temperature of nonachlazine is 219-228°C (in a range of 2°).

Purity tests. A solution of 0.2 g of the preparation in 10 ml of a mixture of 95% alcohol and water (1:1) should not exceed the turbidity standard No. 3; the color of the solution should not be more intense than that of standard No. 3b; the pH of this solution should be 2.0-2.5 (potentiometrically); the loss in weight on drying at 105°C should not exceed 1%; content of sulfate ash should be not more than 0.1%, and of heavy metals, not more than 0.001%.

The content of impurities consisting of 2-chlorophenothiazine and VI is determined by GLC on Silufol plates. To dissolve nonachlazine, a mixture of 95% alcohol and chloroform (1:1) is used. As the mobile phase the chloroform–benzene system of solvents (5:1) is used. The preparation and the impurities are identified by spraying the plate with an aqueous-alcoholic solution of sulfuric acid (10 ml of 50% sulfuric acid and 10 ml of 95% alcohol), followed by holding the plates for 5 min in a desiccator at 80-90°C. Thus, nonachlazine and the impurities appear in the form of red spots with the following R_f values: 2-chlorophenothiazine, 0.65; VI, 0.45, with the nonachlazine spot situated on the starting line. Temporary Pharmacopoeia article (TPA) permits the presence of 2-chlorophenothiazine impurity in an amount not exceeding 0.4%, and VI impurity, not more than 0.8%; i.e., during chromatography of 25 mg of nonachlazine, no spots of these impurities should appear.

The content of the impurity consisting of the hydrochloride of V is also determined chromatographically on Silufol plates. Nonachlazine and the impurity are deposited on the plates as bases, obtained by the action of a concentrated solution of ammonia on nonachlazine, and are extracted by ether.

The chloroform–ether–95% alcohol (10:2:1) system is used as the mobile phase. The compounds are identified by bromine vapors, followed by the action of iodine vapors. Nonachlazine and V form yellow spots with R_f 0.5 and 0.0 (on the starting line), respectively.

The Temporary Pharmacopoeia article permits the presence of not more than 0.4% of the V impurity; i.e., during chromatography of 50 mg of nonachlazine, no spot should appear on the starting line. The quantitative determination of nonachlazine is carried out by the method of anhydrous titration with 0.1 N perchloric acid in glacial acetic acid in the presence of mercuric acetate and Crystal Violet as indicator. A 1-ml portion of 0.1 N perchloric acid corresponds to 0.02434 g of nonachlazine, which, recalculated as dry substance, should be not less than 98.5%.

Nonachlazine in tablet form is analyzed in the same way as the compound itself. The quantitative content of nonachlazine in tablets is determined spectrophotometrically by measuring the optical density of the sample and standard solutions in cuvettes with an optical path of 1 cm at a wavelength of 262 nm. The content of nonachlazine in one tablet varies from 0.027 to 0.033 g.

Ethyl Ester of α-Bromo-δ-chlorovaleric Acid III. Method A. A mixture of 136.5 g of acid II, 207.8 g of dry bromine, and 4 ml of phosphorus trichloride is heated at 80°C for 16 h, and then for 2 h at 100°C. The reaction mixture is cooled and washed several times with water. The lower organic layer is separated, and the aqueous layer extracted with benzene (3 × 80 ml). The benzene extracts are combined with the organic layer, and dried over calcium chloride. Benzene is distilled, and 208 g of crude II are obtained. The material is dissolved in 580 ml of absolute ethanol, and 130.9 g of thionyl chloride are added dropwise to this solution at a temperature not higher than 0°C. After 48 h, the alcohol and excess of thionyl chloride are distilled off, the residue is distilled *in vacuo*, and the fraction boiling at 88-90°C (1 mm) is collected; n_D^{20} 1.4738. Yield, 202 g (82.9%). Found, %: (Cl + Br) 47.64. $C_7H_{12}BrClO_2$. Calculated, %: (Cl + Br) 47.38.

Method B. A 158-g portion of thionyl chloride is added dropwise at 25°C to a solution of 146 g of acid II in 100 ml of chloroform. The temperature is raised to 85-90°C, the mixture is held at this temperature for 4 h, and excess of thionyl chloride is distilled *in vacuo* at 45°C. To the residue, 214 g of bromine are added dropwise at 25°C, and the reaction mixture is held for 1 h at 45°C, and then for 4 h at 85°C. Then, 210 ml of absolute alcohol are gradually added to the reaction mixture cooled to 25°C, so that the temperature of the mixture does not rise above 60-65°C. The solution is heated to boiling for 2 h, and then cooled, 260 ml of distilled water are added, and the mixture is extracted with chloroform (3 × 80 ml). The combined chloroform extracts are washed with a 5% aqueous solution of sodium bicarbonate and water, and dried over magnesium sulfate. The solvent is distilled, the residue distilled *in vacuo*, and the fraction boiling at 112-118°C (10 mm) is collected; n_D^{20} 1.4738. Yield 208 g (80%).

5-Keto-1,4-Diazabicyclo[4,3,0]nonane (IV). A 120-ml portion of a 70% aqueous solution of ethylenediamine and 0.5 g of potassium iodide are added, with stirring, to a solution of 151 g of III in 500 ml of absolute ethanol, and the mixture is boiled, with stirring, for 15 h. When cool, the precipitate is filtered, the alcohol is distilled, and 50 ml of water are added to the residue. Compound IV is extracted with chloroform. The chloroform solution of IV is filtered through a layer of activated carbon (40 g), and evaporated. Crude lactam IV is obtained, which melts at 66-73°C. Literature data [22]: mp 80-81°C (from anhydrous acetone).

1,4-Diazabicyclo[4,3,0]nonane (V). A 340-ml portion of a 15% solution of IV in absolute benzene (the solution contains 46 g of IV) is added dropwise, with stirring, to a suspension of 16.2 g of lithium aluminum hydride in 520 ml of absolute ether, and the mixture is boiled for 15 h. Then, the reaction mixture is cooled and, with stirring, 26 ml of water, 24 ml of a 20% solution of sodium hydroxide, 70 ml of water, and 90 ml of 40% sodium hydroxide are successively added. The reduction product V is extracted with ether, the ether–benzene solution is dried over sodium hydroxide, evaporated, and distilled at 62-66°C (10-12 mm). Yield, 31 g (75%).

10-[β-N-(1,4-Diazabicyclo[4,3,0]nonanyl-4)propionyl-2-chlorophenothiazine Dihydrochloride (I). A 21.7-g portion of triethylamine is gradually added to a solution of 70 g of VI and 28.3 g of amine V in 450 ml of dry toluene. The mixture is heated with stirring for 25 h at 80°C, cooled, and filtered. To the filtrate consisting of a toluene solution of VII, a 20% solution of hydrogen chloride in absolute alcohol is added to pH 1.0. The mixture is left to stand at 10-15°C for 12 h, and then compound I is filtered. Yield, 100 g (94%). A 100-g portion of the crude product is dissolved in a mixture of 400 ml of acetone and 117 ml of water, the solution is stirred for 30 min, boiled for 15 min with 6.5 g of activated carbon, and filtered. To the filtrate, 735 ml of acetone are added with stirring, and the mixture is stirred for 6 h at 5-10°C. Compound I is filtered, washed with ether, and dried at 50-60°C to yield 80 g (80%) of purified nonachlazine, corresponding to the Temporary Pharmacopoeia article specifications.

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