REACTION PRODUCTS OF 4-(δ-DIETHYLAMINO-α-METHYLBUTYLAMINO)-7-CHLOROQUINALDINE WITH o-CHLOROBENZALDEHYDE

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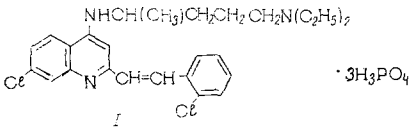
The condensation of 4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinaldine with o-chlorobenzaldehyde is the final stage of the synthesis of the original chemotherapeutic preparation aminoquinol. It is the triphosphate of 2-(2'-chlorostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinoline (I) [1-4] and is used for the treatment of protozoal infections such as cutaneous Leishmaniosis, toxoplasmosis, and also certain collagenoses.



I

The aim of the present investigation was the study of the structure of the by-products formed on condensing chromatographically pure (content of main substance 99%) 4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinaldine (II) with o-chlorobenzaldehyde in the presence of acetic anhydride. In addition the influence of minor quantities of these contaminants on the toxicological characteristics of the preparation and primarily on the most vulnerable organs (target organs) was studied. Doses significantly exceeding therapeutic doses were used in the toxicological investigations.

For the semiquantitative assessment of by-products of the reaction of (II) with o-chlorobenzaldehyde TLC on Silufol UV-254 plates (Czechoslovakia) was used. On chromatography of samples of aminoquinol, synthesized under standard conditions, in the system methanol—aqueous ammonia solution (97:3) and detection by UV light, spots of R_f 0.15 and 0.72 were detected (both colored blue) and of R_f 0.05 (intense yellow coloration) in addition to the spots for the main aminoquinol (R_f 0.30, dark violet coloration). On a two-dimensional chromatogram using ethyl acetate as mobile phase for the second development it was discovered that the spot with R_f 0.72 was not homogeneous and there were at least two substances present in it having R_f 0.52 and 0.67 in ethyl acetate.

The total amount of these products in aminoquinol did not exceed 2% and for their identification a preparative chromatographic separation was carried out on plates of silica gel followed by elution and mass-spectrometric analysis.

The substance with R_f 0.15 was characterized in the mass spectrum by a molecular peak with m/z 314* (containing two atoms of chlorine and two atoms of nitrogen) and by the presence of fragments $(M-H)^+$ and $(M-Cl)^+$ which is in agreement with its structure as 2-(2'-chlorostyryl)-4-amino-7-chloroquinoline (III).

The products having R_f 0.72 in the system methanol—aqueous ammonia where the two compounds were detected by two-dimensional chromatography were substance (IV) and substance (V) with R_f 0.67 and 0.52, respectively, in ethyl acetate. The following mass spectral data were obtained. Substance (IV) had a molecular ion 382 containing two atoms of chlorine and two of nitrogen. Under electron impact its fragmentation was characterized by the stepwise

*Here and subsequently mass numbers are given for ions containing ^{35}Cl.

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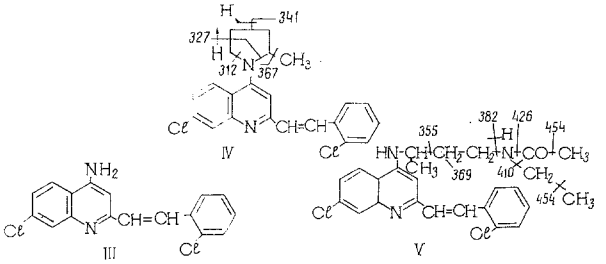
elimination of fragments of α-methylpyrrolidine and also by fission from the molecular ion of a chlorine atom (fragment with m/z 347) and a pentenyl fragment (fragment with m/z 312). The representation on formula (IV) of the outline of the main mass spectral decompositions of this compound is in good agreement with its constitution as 2-(2'-chlorostyryl)-4-(2"-methylpyrrolidyl-1")-7-chloroquinoline.



Substance (V), giving a molecular ion with m/z 469 containing two atoms of chlorine and an odd number of nitrogen atoms, had fragment peaks with m/z 382, 369, 355, and 341 in the mass spectrum characteristic of aminoquinol. In the large mass number region in addition to peaks for ions $(M-CH_3)^+$, $(M-C_2H_5)^+$, and $(M-Cl)^+$ similar to the peaks for aminoquinol, an intense peak was observed for the ion $(M-43)^+$ which was absent from the spectrum of aminoquinol. Analysis of these data showed that substance (V) and aminoquinol (I) differ in the structure of the terminal portion of the side chain. The schematically represented breakdown of substance (V) under electron impact is in good agreement with the structure 2-(2'-chlorostyryl)-4-[δ-N(ethyl-N-acetylamino)-α-methylbutylamino]-7-chloroquinoline.

The structure of the compound having R_f 0.05 and an intense yellow color was not established by mass spectrometry due to its low volatility.

The formation of compounds (III), (IV), and (V) as contaminants in aminoquinol occurred seemingly in the high-temperature (150-155°C, 3 h) condensation of (II) with o-chlorobenzaldehyde in the presence of acetic anhydride as a result of the partial degradation of the labile diethylaminoisopentylamino chain. Variation of the temperature regimen and reaction time with the aim of securing an unambiguous course for the process did not give positive results. Consequently the only means of obtaining aminoquinol with a content of main substance greater than 99% was purification of the final product of synthesis from the accompanying contaminants.

The main difficulty in the resolution of this problem was the need to find a method for the fairly complete separation at one stroke of several substances contained in minor amounts which were similar to aminoquinol.

Study of the solubility and chromatographic mobility of aminoquinol and of the bases isolated from it showed that it was most expedient to use column chromatography on silica gel for this purpose and to apply the system methanol—25% ammonia solution (97:3) as mobile phase. This system gave the best results in the course of analytical and preparative separation of substances by TLC.

A study of the various types of adsorbent made it possible to establish that silica gel of type ASMK manufactured by the Voskresensk Chemical Combine proved to be closest to Silufol in its characteristics in the process under consideration.

A check on the course of the separation of products was carried out using TLC to determine the amounts of aminoquinol base and contaminants in the eluate. Substances (IV) and (V) were contained mainly in the first portions of eluate. Then aminoquinol base began to be eluted together with the indicated substances. Fractions of eluate containing mainly aminoquinol and not more than 1% contaminants were selected and used for obtaining the triphosphate. After washing out the main quantity of the preparation, substance (II) began to be eluted, of which no more than 1% in relation to aminoquinol base was permitted in portions selected for the isolation of triphosphate. The contaminant which was characterized by an intense yellow color was practically insoluble in the mobile phase and remained on the column. The substance obtained by the indicated method had a content of main product of not less than 99% and the overall amount of contaminants was less than 1% (by TLC). Without counting fractions containing more than 1% contaminants in aminoquinol base, yields were 75-77%.

In order to check the process at the stage of isolating the triphosphate a quantitative determination of aminoquinol base in methanol solution was developed using nonaqueous titra-

tion with perchloric acid with addition of a mixture of formic acid and acetic anhydride. This procedure was subsequently used, together with the titration for phosphoric acid described in the Pharmacopoeia, for the quantitative determination of the content of main substance in the aminoquinol preparation.

The obtained samples of aminoquinol, both without purification and with additional chromatographic purification, were no different in acute toxicity in experiments in mice. Study of the subacute toxicity showed that both samples at a dose of 100 mg/kg, which exceeds the daily treatment 20 times, on once daily internal administration for ten days did not cause any deviations from normal.

Study of the chronic toxicity in rats showed that on peroral administration for 1 month at a dose of 100 mg/kg both samples were no different. On administration at a dose of 200 mg/kg for 1-1.5 months to animals receiving aminoquinol not subjected to chromatographic purification, the extent of liver pathology was more marked, body weight was reduced to a greater extent, and the number of animals dying was greater. Pathomorphological investigation of the liver carried out one month after finishing the preparation showed that in the group of rats receiving purified aminoquinol regeneration of injured tissue occurred significantly more rapidly. Thus, the regulatory requirements inserted at the present time into the pharmacopoeia document for aminoquinol for the chromatographic purification of the preparation are very important from the point of view of improving its toxicological characteristics on extended application of this drug.

EXPERIMENTAL TECHNOLOGY

Determination of Minor Contaminants in Aminoquinol. Ethyl alcohol (95%) was passed along a Silufol UV-254 chromatographic plate of size 8 × 15 cm. When the solvent front reached the end of the plate it was removed from the chamber, about 1 cm was cut off both ends across the whole width, the plate was dried for 10 min at 100°C, after which it was used for chromatography.

A previously ground sample (0.1 g) of aminoquinol was dissolved in water (30 ml) in a separatory funnel of capacity 100 ml. After complete solution of the substance a 25% aqueous solution of ammonia (0.1 ml) was poured in, the mixture shaken for 2 min, then chloroform (10 ml) was added, and the mixture shaken for 10 min. After being left to stand for 10 min the lower chloroform layer containing aminoquinol base was separated.

The chloroform solution (0.02 ml: 200 μg calculated as aminoquinol) was applied to the start line of the chromatographic plate. Beside it as reference was applied a 0.02% solution of aminoquinol in chloroform (0.01 ml and 0.02 ml: 1 and 2 μg). Chromatography was by the ascending method in freshly prepared system methanol–25% aqueous ammonia solution (97:3). When the solvent front was at a distance of 1 cm from the edge of the plate the latter was removed from the chamber and scanned in UV light. Spots of contaminants in total must not exceed in size and intensity the coloration of the reference spots (no more than 1%).

On two-dimensional chromatography using the solvent system indicated above as mobile phase new spots did not appear on the chromatogram. This indicated the presence of the contaminants in the sample being analyzed and that they were not formed in the course of the analysis.

On two-dimensional chromatography using ethyl acetate as mobile phase in the second chromatography, the plate was dried for 3 h at 100°C after the first chromatography, turned through 90°, and chromatographed by the ascending method in ethyl acetate. When the solvent front was at a distance of 1 cm from the edge of the plate, the latter was removed from the chamber, dried at room temperature, and scanned in UV light.

Preparative Isolation of Minor Contaminants. This was carried out by the method described above using thirty Silufol UV-254 plates and applying the product to the start line as a band and not as separate spots. After chromatography and detection in UV light the appropriate bands were cut off, the substances eluted with methanol, and the methanolic solutions evaporated. For the separation of substances (IV) and (V) additional chromatography was carried out of the evaporated eluate having R_f 0.72 on Silufol UV-254 plates with ethyl acetate as mobile phase. Elution of substances (IV) and (V) after chromatography and cutting out of the appropriate bands was also carried out with methanol. The substance was not subjected to additional purification for mass spectral analysis. Similar results were then obtained in the preparative isolation of substances (III), (IV), and (V) in the purification

TABLE 1. Dynamics of Weight Gain, Duration of Hexenal Anesthesia, and Amount of Leukocytes in Rats Receiving Aminoquinol for One Month

Aminoquinol sample	Dose mg/kg	Duration of anesthesia, min	Amount of leukocytes, thousands	Time of investigation, weeks			
				1	2	3	4
				dynamics of weight change of rats, % of initial			
A	200	37,92 (16,21—59,72)	36,3 (28,0—44,6)	19,6	39,2	43,1	55.8
B	200	41,16 (26,06—56,26)	35,4 (14,8—55,0)	23,6	30,7	35,0	33,3
A	100	41,9 (15,2—68,6)	28,5 (18,4—38,6)	26,5	43,3	52,2	66,3
B	100	30,79 (10,19—51,39)	28,1 (16,8—39,4)	18,3	37,5	46,6	61,6
Control	—	16,26 (12,76—19,76)	18,2 (15,3—21,1)	26,4	43,8	61,1	73,8

Note. A) Aminoquinol purified additionally by chromatography; B) aminoquinol not subjected to additional purification. Limits of variation are given in parentheses.

of aminoquinol by the procedure described below by chromatography on a column of silica gel of type ASMK. For the preparative isolation of contaminants the appropriate fractions containing a certain amount of aminoquinol base were subjected to a second chromatography on columns of smaller dimensions. Mass spectra of isolated compounds were obtained on an MX-1303 mass spectrometer fitted with a system for direct insertion into the ion source at 30 and 12 eV.

Chromatographic Purification of Aminoquinol on a Silica Gel Column. A chromatographic column of diameter 3.5 cm and height 120 cm was packed with silica gel (400 g) of type ASMK GOST 3956—76 previously suspended in a mixture (500 ml) of methanol—25% aqueous ammonia solution (97:3), the height of the silica gel core was 95 cm. After loading the column the stopcock was closed with a liquid column of height 10 cm above the silica gel. Then 25% aqueous ammonia solution (8 ml) was added, aminoquinol (10 g) was loaded through a wide-necked funnel, more concentrated ammonia solution (3 ml) was poured in, and then further aminoquinol (10 g) was loaded. The stopcock was opened 15 min after applying aminoquinol to the column and chromatography was begun without additional quantities of mobile phase until a layer of liquid of height 10 mm remained above the silica gel. Further chromatography was carried out by adding to the column fresh portions of a mixture of methanol and 25% aqueous ammonia solution (97:3). Selection of fractions was checked by TLC. The flow rate of eluent was 1 drop per sec. The first 550 ml eluate containing substances (IV), (V), and traces of aminoquinol base were separated. Then three fractions of about 20 ml were collected, 1000 ml solution separated, and 3 more fractions of about 20 ml. The ratio of the amount of aminoquinol base and contaminants (substances III, IV, and V) was determined in the obtained fractions. Fractions containing less than 1% contaminants were combined and evaporated on a rotary evaporator at 10-15 mm Hg and a bath temperature no greater than 60°C. The remaining fractions were combined separately and were used in the purification of further portions of aminoquinol. After evaporation of the pure product aminoquinol base (10.6g: 86.6%) was obtained which was dissolved in methanol (50 ml) and converted into the triphosphate. The total yield of aminoquinol with a content of 99.4% of the main substance and less than 1% contaminants was 76.9% (without allowing for the aminoquinol containing a larger amount of contaminants which was used in subsequent stages of purifying the preparation).

EXPERIMENTAL TOXICOLOGY

When studying acute toxicity the preparation was administered to white random bred mice of mass 17-18 g as a single oral dose of from 750 mg/kg. Observation of the condition of animals was carried on for 7 days. The LD_{50} was calculated according to the method of Litchfield and Wilcoxon.

Death of animals occurred in the first four days after administration of the preparation beginning with a dose of 900 mg/kg. At high doses to mice flaccidity and slowing down were

recorded, and tremor was caused in individual animals. On autopsy of dead animals swelling of the intestines was discovered and there was hemorrhage along the whole length of the intestines. The picture of acute toxic effects was the same for both samples. The LD_{50} for a sample purified on a chromatographic column was 1600 (1350-1888) mg/kg and for a sample not subjected to additional purification it was 1550 (1156-2066) mg/kg.

Investigation of subacute toxicity was carried out under conditions of a ten-times-repeated once daily administration of the preparation to mice at doses of 100, 300, and 600 mg/kg. Comparative assessment was carried out on the basis of survival of animals, the dynamics of their weight, and of results of pathomorphological and electron microscopic investigation of internal organs.

In mice receiving the preparation at a dose of 100 mg/kg for ten days no toxic phenomena were detected. A comparative morphological study of internal organs (liver, kidneys, thymus, adrenals) did not reveal any differences from normal. On ultrastructural study of the liver a reduction was observed in the number of ribosomes in hepatocytes and also a weakly expressed edema in the Disse's spaces.

Toxic properties were displayed in mice receiving both samples at doses of 300 and 600 mg/kg. Dry gangrene of the auricle was noticed. At a dose of 600 mg/kg death (about one from each group) and a fall in weight relative to the starting weight was observed.

Comparative pathomorphological study of internal organs showed that the character of the changes after administration to animals of both samples was identical and the extent of their severity correlated with the dose of the preparation. The mean weight of the internal organs of mice was the same in both groups. Both samples at doses of 300 and 600 mg/kg caused involution of the thymus with delymphatization of its tissue. The mean weight of the adrenals after administration of aminoquinol at a dose of 300 mg/kg was somewhat greater in comparison with control as a result of hyperplasia of the cortical layer.

The main pathological changes after tenfold administration of both samples of aminoquinol at doses of 300 and 600 mg/kg were displayed in the liver cf animals. In the overwhelming majority of mice yellow-white foci were distinguished macroscopically in the parenchyma of the organ. Microscopic investigation revealed substantial protein dystrophy of liver parenchyma up to necrobiosis and necrosis of it. With the increase of dose, the frequency and extent of necrosis grew causing total disease of the liver in several cases.

On electron-microscopic study of the liver of animals receiving both samples of aminoquinol an increase was recorded in the content of dense osmiated phagosomes in the cytoplasm of heptatocytes. In animals receiving aminoquinol at a dose of 600 mg/kg marked dystophic changes took place in the tissue of the organ with the development of lipophanerosis and myelinic degeneration of cells with death of the latter.

Chronic toxicity was studied in rats on administering samples of the preparation orally at doses of 100 and 200 mg/kg as a suspension in starch paste for 1 and 1.5 months. In all 80 animals were used.

During the experiment observations were made of the overall condition and behavior of animals, their weight gain, consumption of water and food, and the blood picture (leukocytosis). In view of the marked hepatotoxicity of the preparation a hexenal test was carried out at the end of the experiment (hexenal was administered intravenously at a dose of 50 mg/kg). The obtained results are given in Table 1.

As is evident from Table 1, during the first week experimental animals were practically no different from controls in external appearance and behavior. Weight gain was also approximately the same. After two weeks the difference in weight gain was statistically significant and appeared to be dose dependent. In rats receiving the preparation at a dose of 200 mg/kg the weight gain was less. After three and four weeks this difference had developed distinctly and rats receiving unpurified aminoquinol had clearly fallen behind in weight from the controls and from rats receiving the chromatographically purified preparations at the same dose. Among the rats receiving both samples of aminoquinol at a dose of 100 mg/kg there were practically no differences. There was the same lag in weight from control rats in both groups.

A clearly marked leukocytosis appeared on hematological investigation, no dose dependence was followed.

Intravenous administration of hexenal caused an increase in the duration of anesthesia in animals of all experimental groups independent of the dose or purity of the preparation.

On pathomorphological investigation of animals receiving aminoquinol at a dose of 200 mg/kg for 1 and 1.5 months the presence of certain changes was detected macroscopically in liver having the appearance of yellow-grayish particles of size from 0.5 × 0.5 to 1.0 × 2.0 mm. The extent of liver disease was larger in rats receiving the preparation not subjected to additional purification; in a portion of the animals a suppurative melting of liver tissue was noticed. One month after giving the preparations an appearance of regeneration was observed but on application of the preparation purified additionally by chromatography these processes developed more rapidly.

Microscopic investigations showed that in rats on administration of both purified and unpurified aminoquinol at a dose of 100 mg/kg in individual portions of liver tissue there were disturbances of the "girder" structure and anemia with marked collapse of sinusoids. There were dystrophic processes in hepatocytes, individual cells were found in a state of necrobiosis. In animals receiving the preparation at a dose of 200 mg/kg there was necrotic detritus at the center of tissue zones which were becoming gangrenous, then a demarcation zone containing a large number of round-celled elements. Foci of necrosis surrounded a capsule consisting of filamentous fibrous tissue along the periphery of which multinuclear immature liver cells were found. The "girder" structure of the tissue was lost in places. There was protein dystrophy in cells and there were single foci consisting of dark hepatocytes with hyperchromic nuclei.

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SYNTHESIS OF NITAZOLE AND ITS ANALOGS

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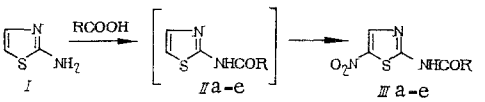
2-Acetylamino-5-nitrothiazole (nitazole; IIIa), used in medicine, is obtained by acetylation of 2-aminothiazole (I) with acetic anhydride, followed by nitration of the 2-acetylaminothiazole (IIa) formed by a mixture of nitric and sulfuric acids [1, 2], or by a rearrangement in concentrated sulfuric acid of 2-aminothiazole nitrate into 2-amino-5-nitrothiazole, followed by its acetylation with acetic anhydride [3]. We have already shown [4] that Ia is readily acetylated by carboxylic acids in oleum to form the corresponding 2-acylthiazoles.



I — IIa-e — IIIa-e

a: R=CH3 b: R=C2H5 c: R=C3H7 d: R=C4H9 e: R=iso-C4H9

The present work presents the results of studies on the synthesis of IIIa and its analogs (IIIb-e) by acylation of I by carboxylic acids in oleum, followed by nitration, without isolation of 2-acylaminothiazoles (IIa-e). We used sodium nitrate, potassium nitrate or ammonium nitrate as nitrating agents. We did not find any appreciable differences in the yields of the end products when the nitrating agent was changed. Since ammonium nitrate is

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