STUDY OF THE CHEMICAL PROPERTIES OF ANALGIN IN 50% AQUEOUS SOLUTION FOR INJECTIONS

R. D. Greshnykh, N. N. Khrakovskaya, and V. E. Chichiro

UDC 615.212.3:615.456.014.45.011.5

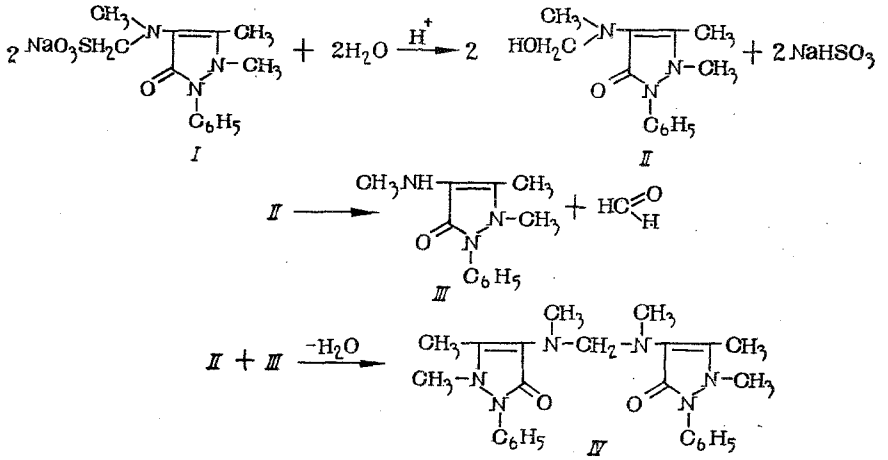
It is known that solutions of analgin for injections tend to change color. The intensity of the color of these solutions changes even during the preparation itself. After sterilization the intensity of color decreases appreciably, and then during a certain time (up to 25 days), it continued to decrease, but after prolonged storage it may increase again. Moreover, we noted that irrespective of the conditions under which the ampules were sealed (in the current of nitrogen, carbon dioxide, or in the absence of inert gases), the color of the analgin solution for injection changes on storage.

There is literature information stating that colored compounds in the injection solutions of amidopyrine have a fairly marked toxicity and influence the hematopoietic system negatively [1].

Analgin is more reactive in an aqueous solution than amidopyrine because of the presence of a polar sulfinate group $-CH_2SO_3Na$ in the 4-position of the molecule. It was found that derivatives of 5-pyrazolone with such a substituent in the 4-position are hydrolyzed in aqueous solution [2-4].

It was interesting to clarify whether analgin hydrolyzes in a 50% aqueous solution, how completely the hydrolysis proceeds, and whether the hydrolysis products may be one of the reasons causing a change in color of the analgin solutions on storage.

During the investigation of the hydrolysis of analgin in an aqueous solution in the pH range from 2.0 to 12.0, the hydrolysis products of analgin were detected in the whole pH range [3]. From the data on electrophoresis and paper chromatography, as well as from data of elementary analysis of the products of hydrolysis isolated, we formulated the mechanism of the acid catalyzed hydrolysis of analgin.



The above scheme shows that one of the hydrolysis products of analgin (I) is monomethylaminoantipyrine (III), which in aqueous solutions readily condensed with N-hydroxymethyl-N-methylaminoantipyrine (II) to form N,N'-methylene-bismethylaminoantipyrine (IV).

By using thin layer chromatography (TLC), liquid extraction, IR and PMR spectroscopy, in 50% solutions of I we detected hydrolysis products corresponding to the above scheme.

Scientific-Research Institute of Standardization and Control of Drugs, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 13, No. 3, pp. 68-73, March, 1979. Original article submitted August 2, 1977.

0091-150X/79/1303-0289$07.50

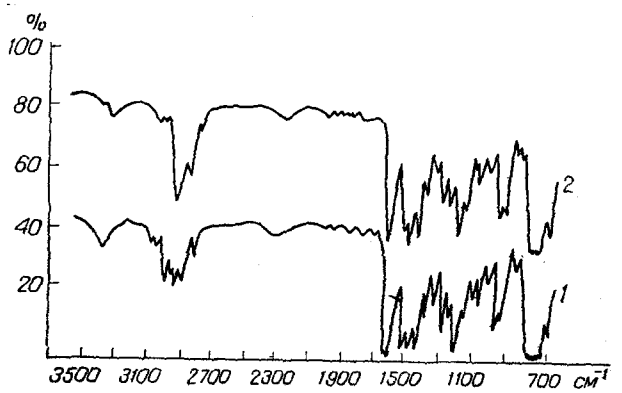


Fig. 1. IR spectra of an impurity extracted by chloroform from an aqueous solution of analgin (1) and monomethylaminoantipyrine (2).

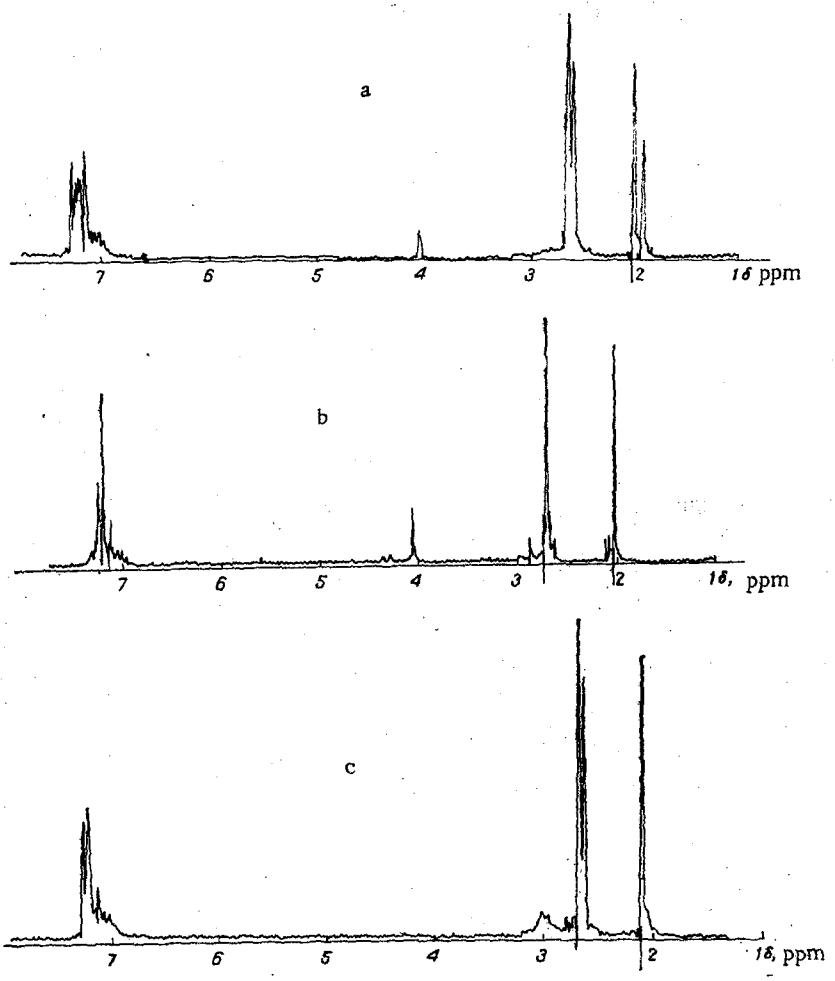


Fig. 2. PMR spectra of an impurity extracted by chloroform from an aqueous solution of analgin (a), monomethylaminoantipyrine (b) and N,N'-methylene-bismethylaminoantipyrine (c).

Thus, in the IR spectrum of the impurity extracted by chloroform from an aqueous solution of I, we observed the following characteristic bands: 3370 cm^{-1} (stretching vibrations of the secondary amine NH group), 3100-3000, 1600, 1500 cm^{-1} (aromatic ring vibrations), 1685 cm^{-1} (cyclic amides), 1360-1310 cm^{-1} (aromatic tertiary amines) [5, 6]. Therefore, this impurity apparently contains compound III. A comparison of the spectrum of the isolated impurity with that of compounds which could be formed during hydrolysis according to the above

scheme showed that the IR spectrum of the impurity includes all the absorption bands characteristic of the IR spectrum of compound III run under the same conditions (Fig. 1).

We considered that the chloroform extract of a 50% aqueous solution of I may contain not only III but also compound IV, the identification of which is difficult from the IR spectra because of the presence of compound III, and so we studied the PMR spectra of the isolated impurity also.

From the chemical shifts observed in the PMR spectra [δ, ppm, 1.95-2.03 (CH_3—CH=C—), 2.60-2.65 (>N—CH_2—), 4.05 (the —CH_2— group), 6.8-7.3 (benzene and pyrazole rings)], it can be assumed that the chloroform extract from the 50% solution of I contains also IV. Comparison of the PMR spectrum of the isolated impurity with the PMR spectra of compounds III and IV showed identity of the chemical shifts in the spectra of the impurity and of compound IV (Fig. 2).

Thus, the data of IR and PMR spectroscopy and also TLC made it possible to establish the presence of the hydrolysis products of I, not only in a 50% solution after prolonged storage, but also in a freshly prepared solution. It was found that during multiple chloroform extractions from a 50% solution of I, no decrease in the intensity of color or decrease in the concentration of the hydrolysis products occurred with passage of time (3 h). The reason is that the hydrolysis of I takes place partially immediately after dissolution, and later, as follows from the literature [7], a dynamic equilibrium is established between the reaction products.

The products of the hydrolysis of I were isolated and separated by the method of two-dimensional chromatography in a thin layer of sorbent (Fig. 3) (in the literature, the R_f values of these compounds in the above systems are fairly close [8]). Compound III is an oily bright-yellow substance, which is rather unstable, and has not yet been isolated in crystal form, with a mp about 70°C. Compound IV forms almost colorless crystals, crystallizes readily from water and benzene, and melts at 171-173°C (this agrees with the literature data [3]).

Since one of the products of the hydrolysis is a bright-yellow substance, we can conclude that the color intensity of the analgin solution depends directly on the direction in which the equilibrium between the hydrolysis products, compounds III and IV, is shifted under the action of external factors, according to the following scheme:

$$IV \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} 2III + CH_2O.$$

It was interesting to determine the degree of decomposition of a 50% solution of I, and to find whether the decomposition products accumulate with time, i.e., on storage taking into account the effect of external factors (temperature, absorption of light, etc.).

For the investigation, we used samples of a freshly prepared and yellow-colored 50% solution of I after different storage periods. All the solutions of I were stored in ampules of colorless glass and were protected from light.

The study of the hydrolysis in aqueous medium was carried out by the method of liquid extraction in combination with UV spectrophotometry. The products of the hydrolysis of I were extracted by chloroform. The presence of an absorption maximum of the hydrolysis products of I at 257-259 nm (in 0.01 N hydrochloric acid), in contrast to the absorption maximum of I (without decomposition products) at 265 nm (in methanol), made it possible to calculate the percent of the hydrolytic cleavage of I.

It was found that in a 50% solution for injections, there are 4% of decomposition products one month after preparation, 5.4% after four to five months, and 6.9% after five years.

There is information in the literature stating that the most effective stabilizer for a 50% solution of I for injections is rongalite at a concentration of 0.5% [9]. Experimental verification showed that during chromatography of solutions of I stabilized by rongalite in the thin layer of the sorbents on the chromatograms additional compounds were observed together with the products of hydrolysis. Hence, although rongalite decolorizes the solution of the preparation, it does not prevent hydrolysis of I in an aqueous medium. Moreover, the possibility of a reaction between rongalite, a strong reducing agent, and I or its hydrolysis products cannot be excluded.

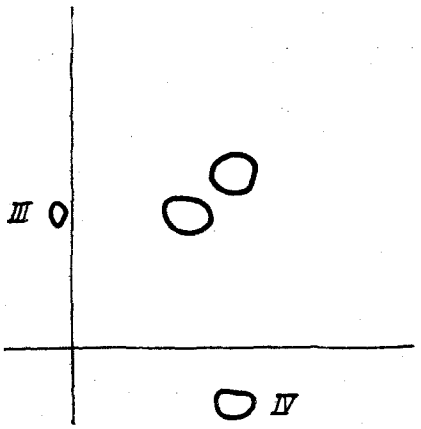


Fig. 3

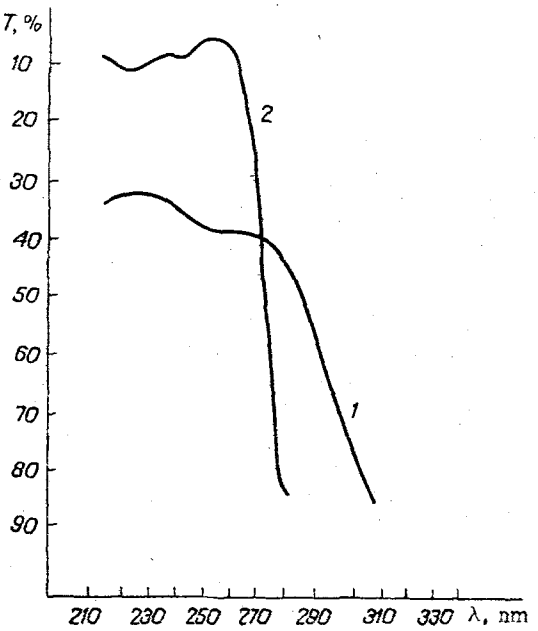


Fig. 4

Fig. 3. Two-dimensional chromatogram in a thin layer of sorbent of impurities extracted by chloroform from a 50% solution of analgin for injections in the presence of reference spots.

Fig. 4. UV spectra of hydrolysis products of analgin in 0.01 N hydrochloric acid (1) and in methanol (2).

EXPERIMENTAL

The products of the hydrolysis of I were detected by the following procedure.

A 5-ml portion of a 50% solution of I was placed in a separatory funnel, 10 ml of chloroform were added, and the mixture was shaken for 10 min. The mixture was left to settle and the chloroform extract was filtered through a paper filter with anhydrous sodium sulfate. The extract was evaporated on a water bath to dryness. To the residue 5 ml of chloroform were added. A 5-μl portion of the solution was deposited on Silufol plates. The chromatography was carried out by the ascending method in a chloroform–acetone–methanol 25% ammonia (40:20:10:3) system of solvents. As reference spots we deposited 10-μl portions of 0.1% solutions of compounds III and IV. The compounds were detected on the chromatogram by means of a freshly prepared 10% solution of phosphomolybdic acid in 95% alcohol, which formed blue-violet zones, darkening with time. The sensitivity of the detection of the hydrolysis products on the chromatograms was 0.05 μg.

After the removal of chloroform on a water bath, the IR spectra of the compounds extracted by chloroform from the aqueous solution were run in a carbon tetrachloride solution on a UR-20 spectrophotometer with a diffraction grating in the 3800-700 cm^{-1} region. The PMR spectra of the compounds were run in a solution of deuterated dimethyl sulfoxide with the addition of carbon tetrachloride on the "Geol" (Japan) 4H-100 apparatus, with tetramethylsilane as the internal standard.

The hydrolysis of I was followed on a SF-16 spectrometer at wavelength of 257-259 nm in a cuvette with optical path of 1 cm by the following method.

A 1-ml portion of a 50% solution of I for injections was placed in a 100-ml volumetric flask, and the solution was brought up to the mark with 0.01 N hydrochloric acid. A 0.5-ml-portion of the solution was placed in 100-ml volumetric flask, and the solution was brought up to the mark with 0.01 N hydrochloric acid (solution A).

A 1-ml portion of a 50% solution of I for injections was placed in a separatory funnel, 10 ml of chloroform were added, and the mixture was shaken for 5 min. After settling, the chloroform extract was filtered through a paper filter with anhydrous sodium sulfate into a flask, and then 10 ml of 0.01 N hydrochloric acid were added. The chloroform was evapo-

rated on a water bath and the remaining hydrochloric acid solution was quantitatively transferred to a 100-ml volumetric flask, and the solution in the flask was brought up to the mark with 0.01 N hydrochloric acid. A 5-ml portion of this solution was transferred to a 100-ml volumetric flask, and the solution in the flask was brought up to the mark with 0.01 N hydrochloric acid (solution B). The optical density of solutions A and B was measured.

First, a calibrated graph was plotted for the dependence of the optical density of the given solution on the concentration at 257-259 nm in 0.01 N hydrochloric acid. The total concentration of I with the hydrolysis products and the concentration of the hydrolysis products were determined from the graph. The degree of hydrolytic decomposition of I, X (in %) was determined from the following equation

$$\frac{C_B \cdot 100}{C_A \cdot 10},$$

where C_A is the total concentration of I with the decomposition products (mg/liter); C_B is the concentration of the products of hydrolysis of I (mg/liter).

Thus, from our studies, we found that in a 50% aqueous solution compound I hydrolyzes; in a freshly prepared solution of I the hydrolysis products amounted to 4%; after four to five months of storage, 5.4%; and after five years, 6.9%.

The change in the color of solution I is mainly due to hydrolysis products of the preparation.

LITERATURE CITED

1. L. Donok, E. Szaboles, and G. Vastagh, Acta Pharm. Hung., 29, 249 (1959).
2. M. Tiffenau, Bull. Sci. Pharmacol., 21, 71 (1914).
3. G. Wagner, Acta Pharmacol. (Berl.), 289, 121 (1965).
4. T. Takahashi and K. Kanematsu, Chem. Pharm. Bull., 6, 98 (1958).
5. K. Nakanishi, Infrared Spectra and Structure of Organic Compounds [Russian translation], Moscow (1965).
6. L. Bellamy, Infrared Spectra of Complex Molecules, Halsted Press, New York (1957).
7. F. Pechthold, Arzneim. Forsch., 14, 1056 (1964).
8. W. Awe and H. G. Tracht, Pharm. Ztg., 108, 1365-1370 (1963).
9. S. A. Minina and R. P. Lukashevichene, Khim. Farm. Zh., No. 3, 106-110 (1977).

INFLUENCE OF VARIOUS CONCENTRATIONS OF Ca^{2+} AND Mg^{2+} IONS ON THE VITAL ACTIVITY OF *Actinomyces nodosus*, A PRODUCER OF AMPHOTERICIN B

V. V. Borzov and E. N. Bol'shakova

UDC 576.852.18.095.18:[546.41]+546.46

To solve one of the main problems of antibiotic production — increasing the output of the preparations — in addition to new selectively bred highly active strains, the achievements in the field of biosynthesis should be used more widely, in connection with which it is necessary to know the concrete influence of one factor or another on the biosynthetic activity of the producer. This pertains primarily to factors of the nutrient medium: composition of the medium, concentration of each component, pH, etc. There are data on the influence of the quantitative ratio of the components of the nutrient medium used to store producers of biologically active substances on the subsequent biosynthetic activity of the culture [1].

Many elements of mineral nutrition (although in very small quantities) are necessary for the growth of all microorganisms. It has been established, for example, that the addition of Mg to minimal synthetic medium has a stimulating effect on the growth of a culture of *Thermoactinomyces vulgaris* and lowers the depth of dormancy of spores formed on a syn-

All-Union Scientific-Research and Technological Institute of Antibiotics and Enzymes for Medical Purposes, Leningrad.

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 13, No. 3, pp. 73-76, March, 1979. Original article submitted September 14, 1978.

0091-150X/79/1303-0293$07.50