tained by maintaining the compounds on a cold stage at −70°.

Polarographic Procedure.—A Sargent Model XXI polarograph was used to obtain the current–voltage curves. The polarographic H-cell used has been previously described.[15] The capillary had m and t values of 3.09 mg. per second and 1.54 seconds, respectively, yielding a capillary constant of 2.28 mg.$^{2/3}$ sec.$^{-1/2}$. These values were obtained using an open circuit with the capillary immersed in the non-aqueous electrolyte solution maintained at 25 ± 0.1°. The electrolytic solution was 0.3 M lithium chloride in equal volumes of absolute methanol and benzene. Solutions of known concentrations (0.6 to 3.0 × 10^{-3} M) of the peresters and peroxides were prepared, aliquots transferred to the H-cell and polarograms obtained. Three to six solutions of different concentrations were prepared for each compound and the average values for the half-wave potentials and diffusion current constants were then obtained. To minimize the interference of extremely high maxima exhibited by concentrated solutions of peroxides, dilute solutions were used. At these low concentrations the maxima were sufficiently suppressed so that reliable wave height measurements could be obtained provided the wave heights were measured in the flat portion of the curves.

Acknowledgments.—The authors thank D. A. Lutz for determining the X-ray diffraction patterns, Drs. Clyde L. Ogg and John G. Miller for helpful discussions and T. S. Michener, Jr., and A. J. Menna for the schematic representations.

PHILADELPHIA 18, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA]

Physicochemical Study of Metrazole and of Metrazole Derivatives

BY ALEXANDER I. POPOV AND ROGER D. HOLM

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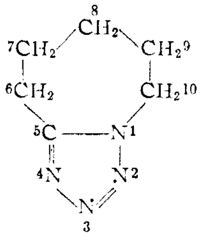
Stability of silver complexes formed by metrazole, 8-*t*-butylmetrazole, 8-*sec*-butylmetrazole, 7-methyl-9-isopropylmetrazole, 7-methyl-10-isopropylmetrazole and 1-cyclohexyl-5-methyltetrazole were determined potentiometrically in acetonitrile solutions. These complexes, of the general formula $(Met)_2Ag^+$, were all more or less equally unstable with the pK_d values being 2.14, 2.14, 2.18, 2.22, 2.19 and 2.26, respectively. No correlation was observed between the convulsive properties of the metrazoles and their complexing ability with silver ion. Solid metrazole–silver nitrate complex was prepared and its infrared spectrum was determined in the 5000–450 cm.$^{-1}$ region and was compared with the spectrum of metrazole. Polarographic study on cadmium-, thallium- and cobalt–metrazole complexes in aqueous solutions showed that complexes were essentially completely dissociated. Metrazole was found to have a weak proton affinity and can be titrated potentiometrically in glacial acetic acid with perchloric acid solutions.

Introduction

It was pointed out in the previous communication,[1] that although metrazole is known to be a strong convulsive agent and has had a long clinical history, its chemical and especially its physicochemical properties have not been investigated to any significant extent. While substituted metrazole derivatives are less well known, Gross and Featherstone[2] reported in a number of papers their studies on the pharmacological properties of a number of such compounds. These authors have found that an addition of a methyl group to the pentamethylene chain of metrazole increases the stimulating action of the parent compound. As the methyl group is moved progressively toward the symmetrical 8-position, the stimulation becomes more pronounced. An increase in the size of the substituent group in the 8-position shows a variable effect; thus while 8-isopropylmetrazole has a minimum convulsant dose of 3 mg./kg. (as compared with 25 mg./kg. for metrazole), 8-*sec*-butylmetrazole and 8-*t*-amylmetrazole show a depressant rather than a stimulating activity. Substitution of larger groups in other positions of the pentamethylene chain likewise decreases the convulsant activity of metrazole; for example, 7-methyl-10-isopropylmetrazole has a minimum convulsant dose of 750 mg./kg. Obviously, the physico-chemical properties of substituted metrazoles are not known any better than those of the parent compound.



It was of interest to continue the study of nucleophilic properties of metrazole and of its derivatives, especially with compounds of varying convulsant activity, in order to see whether there is any correlation between these properties and pharmacological action. Silver ion usually forms quite stable complexes with heterocyclic amines, but while some solid silver–metrazole complexes have been reported[3] in the literature, formation of such complexes in solution as well as their dissociation constants have not been determined. This was the main object in the present study.

Experimental Part

Reagents.—Metrazole (Knoll's "Metrazol") was obtained from the Knoll Pharmaceutical Company, Orange, N. J., and was purified as described in the previous publication.[1] Metrazole derivatives were obtained from the same source and were as follows: 8-*sec*-butylmetrazole, m.p. 68–69°, lit. val.[4] 70°; 8-*t*-butylmetrazole, m.p. 130–132°,

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(1) A. I. Popov, C. Castellani-Bisi and M. Craft, THIS JOURNAL, **80**, 6513 (1958).

(2) E. G. Gross and R. M. Featherstone, *J. Pharmacol. Expt. Therap.*, **87**, 291, 299 (1946); **88**, 353 (1946); **92**, 323, 330 (1948).

(3) (a) A. Dister, *J. pharm. Belg.*, **3**, 190 (1948); (b) J. J. L. Zwikker, *Pharm. Weekblad.*, **71**, 1170 (1934); (c) H. Rheinboldt and H. Stettiner, *Bol. fac. filosof. cien., Univ. Sao Paulo, Quimica*, **1**, 27 (1942); *C. A.*, **40**, 1502 (1946).

(4) E. K. Harvill, C. W. Roberts and R. M. Herbst, *J. Org. Chem.*, **15**, 58 (1950).

lit. val.[4] 132.5–133°; 7-methyl-9-isopropylmetrazole, m.p. 132–133°, lit. val.[4] 135°; 7-methyl-10-isopropylmetrazole, m.p. 46.5–47.5°, lit. val.[4] 49–49.5°. Also a convulsant tetrazole derivative, 1-cyclohexyl-5-methyltetrazole, with m.p. 124.5–125.5°, lit. val.[5] 129–129.5°, was investigated. All of these compounds were obtained in small quantities and had to be used without further purification.

Reagent grade silver nitrate was purified according to the directions of Rosa and Vinal.[6] Anhydrous sodium and silver perchlorates (G. Frederick Smith Chemical Co.) were dried *in vacuo* at 25°. Cadmium nitrate, cobaltous nitrate, thallous nitrate, glacial acetic acid and perchloric acid were all of reagent grade quality. Perchloric acid solutions in glacial acetic acid were prepared according to the directions given by Fritz.[7] The acid solution was standardized with NBS potassium hydrogen phthalate.

Acetonitrile was purified as described earlier,[8] and the upper limit of specific conductance for purified solvent was 1×10^{-7} ohm^{-1} cm.$^{-1}$. Ordinary distilled water was further purified by passing it through a mixed bed ion-exchange column containing IR-120 and IRA-400 resins (Rohm and Haas Co.). The specific conductance of deionized water was approximately 5×10^{-7} ohm^{-1} cm.$^{-1}$. Potassium argentocyanide was prepared according to directions given by Brown.[9]

Preparation of Metrazole–Silver Nitrate Complex.—The complex was obtained in the form of a crystalline precipitate from mixtures of concentrated aqueous solutions of the two components. It was found to be quite difficult to obtain the crystals as the solution showed a marked tendency to supersaturate, and it is important to concentrate the solution by evaporation at a very slow rate. Once the first crop of crystals is obtained, subsequent crystallizations are facilitated by seeding.

Elemental analysis gave the following results: 31.7, 31.9 and 31.9% C; 4.65, 4.78 and 4.75% H; 24.41 and 24.16% Ag. These results were surprising since they did not correspond to 1:1 complex which Rheinboldt and Stettiner[3a] claim to have obtained by the same technique. Recalculations for $(\mathrm{Met})_2\mathrm{AgNO}_3$ complex gave 32.30% C, 4.48% H and 24.17% Ag, in good agreement with results obtained in this investigation and it can be safely concluded that in the solid state we have a complex formed by two molecules of metrazole with one silver ion. It is interesting to note that the previous investigation[3a] reported only the nitrogen analysis for this compound.

Potentiometric Studies.—A Leeds and Northrup type K2 potentiometer and a Rubicon galvanometer with a sensitivity of 0.0004 microampere per mm. of scale division were employed. Two concentration H-type cells were employed with a fine sintered glass disc separating the two compartments. They were of such dimensions that one or two ml. of solution per compartment could be used, respectively.

The silver electrodes were made as follows. Two platinum wires, fused into glass tubes, were cleaned in nitric and hydrochloric acids. The wire tips were fused to provide a smooth end. A potassium argentocyanide solution containing 10 g. of salt per liter was used as platinizing bath. An electrolysis cell was used which had the cathode and the anode compartments separated by a medium porosity sintered glass disc. A 0.2 milliampere current was run for 14$^1/_2$ hr.; the current density was 0.9 milliampere per square cm. The electrodes were washed and placed for 1.5 hr. in ammonium hydroxide solution then washed again for 4 hr. in deionized water after which they were immersed in 0.2 *M* aqueous silver nitrate solution and short circuited.

When the electrodes were immersed in the concentration cell a reasonably steady potential was obtained after 30 minutes. The fluctuations did not exceed 0.2 millivolt. Calibration curves were obtained by plotting the e.m.f. *vs.* silver ion concentration in one-half of the cell, the reference solution in the other arm being 1×10^{-4} *M* in silver ions. Calibration curves were obtained for aqueous as well as for acetonitrile solutions.

Because of the very limited solubility of metrazole derivatives in water, most of the measurements were carried out in acetonitrile solutions. However, it was possible to determine the formation constant of metrazole–silver complex in aqueous solution.

Determination of Formation Constants.—A solution of known total concentration in heterocyclic amine and in silver was placed in one arm of the cell, the other arm containing reference silver solution; the e.m.f. of the cell was measured and the amount of uncomplexed silver ion was established from the calibration curve. It had to be assumed that the liquid junction potential remained constant during a series of measurements.

The instability constant of the complex is given by the equation

$$K = \frac{(\mathrm{Ag}^+)(\mathrm{Met})^2}{(\mathrm{Met}_2 \cdot \mathrm{Ag}^+)} \times \frac{f_{\mathrm{Ag}^+} f^2_{\mathrm{Met}}}{f_{\mathrm{Met}_2 \cdot \mathrm{Ag}^+}}$$

Since the concentrations of Ag^+ and of $(\mathrm{Met})_2\mathrm{Ag}^+$ ions were low, it could be assumed that $f_{\mathrm{Ag}^+} = f_{(\mathrm{Met})_2\mathrm{Ag}^+}$ and that $f_{\mathrm{Met}} = 1$. In order to check the validity of this assumption, measurements were carried out at a constant ionic strength provided by 0.1 *M* NaClO_4, but the results were identical within experimental error to those obtained with solutions void of extra salt.

Polarographic Study.—Polarographic measurements were made with a Sargent Model XXI Recording Polarograph. Potentials were checked with a Leeds and Northrup type K potentiometer. The cell was of conventional type. Beckman saturated calomel electrode was used as reference. A 0.005% gelatin solution was used for maxima suppression, and the solutions were degassed with purified nitrogen before each measurement.

Acid–Base Titration.—Potentiometric titration in glacial acetic acid was made on a Beckman Model G *p*H meter equipped with an external glass–calomel electrode pair. Connection between the acetic acid solution and aqueous calomel cell was made by means of a fine-fritted tube containing 0.2 *M* sodium perchlorate in glacial acetic acid.

Spectroscopic Studies.—Infrared spectra of metrazole and of metrazole–silver complex were determined in the cesium bromide region on a Perkin–Elmer Model 12C spectrometer modified for single beam-double pass measurements. The compounds were dispersed in a Nujol mull and run between silver chloride windows. In the sodium chloride region the metrazole spectrum was determined with a Perkin–Elmer Model 21 spectrometer in potassium bromide pellets, containing 2 mg. of compound per 400 mg. of KBr. The spectrum of the silver complex had to be determined in a Nujol mull since the complex seemed to react with KBr.

Measurements in the ultraviolet region were made with a Cary recording spectrometer, Model 11, in quartz cells of 1.00 ± 0.01 cm. path length.

Results and Discussion

Silver Complexes.—When metrazole or one of the substituted metrazoles was added to one-half of the silver concentration cell, a change in potential was observed, from which the concentration of free silver ion present in solution could be calculated. In all cases the change in potential was quite small and approximately 0.1 *M* metrazole solution had to be employed in order to produce an e.m.f. of about 50 mv. The silver concentration was varied between 1×10^{-4} and 1×10^{-2} *M*; the results are given in Table I. The complexes were found to be quite unstable, and a very large excess of the heterocyclic amine was needed to bind even a portion of the silver ion present. As was mentioned above, the solid silver–metrazole complex contained two molecules of metrazole per silver ion. It was assumed that the complex was formed in solution in the same proportions, and the dissociation constants reported in Table I were calculated on this basis. It is, of course, possible that the actual complex is

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(5) E. K. Harvill, R. M. Herbst, E. C. Schreiner and C. W. Roberts, *J. Org. Chem.*, **15**, 662 (1950).

(6) E. B. Rosa and G. W. Vinal, *Bull. Bur. Stand.*, **13**, 479 (1916–1917).

(7) J. S. Fritz, "Acid Base Titrations in Nonaqueous Solvents," The G. Frederick Smith Chemical Co. Publication, Columbus, Ohio, 1952, p. 13.

(8) A. I. Popov and W. A. Deskin, THIS JOURNAL, **80**, 2976 (1958).

(9) A. S. Brown, *ibid.*, **56**, 646 (1934).

TABLE I

DISSOCIATION CONSTANTS OF SILVER–METRAZOLE COMPLEXES IN ACETONITRILE SOLUTIONS AT 25°

Total silver concn. × 10⁴	Total ligand concn. × 10²	E.m.f. (mv.)	Free silver × 10⁴	pK
Metrazole (min. conv. dose 25 mg./kg.)[a]				
55.6	9.98	76.0	28.0	2.20
22.2	7.79	57.4	12.7	2.14
111.2	10.05	92.0	56.0	2.09
4.45	9.208	17.0	2.3	2.14
11.1	9.90	36.3	5.2	2.12
			$pK_{\text{Av.}}$	2.14
8-*t*-Butylmetrazole (min. conv. dose 3 mg./kg.)				
5.56	8.00	20.5	2.65	2.24
44.5	8.046	73.0	24.9	2.13
66.7	8.569	83.4	39.0	2.04
			$pK_{\text{Av.}}$	2.14
8-*sec*-Butylmetrazole (min. conv. dose 750 mg./kg.)				
5.56	10.944	67.0	19.2	2.18
11.1	11.994	34.6	4.8	2.18
5.56	10.763	17.6	2.31	2.19
			$pK_{\text{Av.}}$	2.18
7-Methyl-10-isopropylmetrazole (min. conv. dose 500 mg./kg.)				
55.6	11.58	68.0	20.0	2.28
11.1	9.024	36.7	5.3	2.31
5.56	10.30	18.3	2.4	2.08
5.56	10.30	17.0	2.29	2.19
			$pK_{\text{Av.}}$	2.22
7-Methyl-9-isopropylmetrazole (min. conv. dose 300 mg./kg.)				
5.56	10.17	20.4	2.64	2.20
11.1	10.43	35.7	5.00	2.16
55.6	11.79	74.0	26.00	2.20
			$pK_{\text{Av.}}$	2.19
1-Cyclohexyl-5-methyltetrazole (min. conv. dose 6 mg./kg.)				
5.56	8.765	17.7	2.33	2.29
11.10	11.81	37.6	5.46	2.22
			$pK_{\text{Av.}}$	2.26

[a] Minimum convulsant dose is quoted from ref. 2.

formed in 1:1 proportions, but because of the instability of the complex, it is impossible to vary the concentration of metrazole so as to produce meaningful changes in the e.m.f. In order to get the concentration of bound metrazole, one is forced to observe small differences between large numbers and, therefore, sacrifice the accuracy of determination. Actually, assumption of a 1:1 complex leads to consistent series of pK values, which are largely independent of variation in the silver ion concentration, but because of the composition of the solid complex and also since silver has a coördination number of two, it seems more probable that the complex is $(\text{Met})_2\text{Ag}^+$. There does not seem to be any easy way to differentiate between the two possibilities.

The infrared absorption spectra of solid metrazole and of the complex did show a considerable difference in structure, both in the sodium chloride and in the cesium bromide regions, as shown in Figs. 1 and 2. The spectra are too complex for any definite assignments of the vibrational frequencies, but at least they indicate quite unambiguously the existence of a definite chemical interaction between silver ion and metrazole.

It is obvious from the above results that the donor properties of the ligands investigated, at least with respect to the silver ion, are largely dependent on the tetrazole ring and are not influenced to any significant extent by either the substituents on the pentamethylene ring or even by the absence of the ring as evidenced by the pK of the 1-cyclohexyl-5-methyltetrazole. As shown in Table I, there is no correlation between the convulsant activity and the silver ion affinity of these substances. Since metrazole is soluble in water, the stability of its silver complex was determined in aqueous solutions by the same technique. The pK_A values obtained were 2.71, 2.89, 2.81, 2.52 and 2.69, with an average value of 2.72, indicating that the complex is more stable in aqueous solutions than in acetonitrile. This is not unexpected, since it has been shown by Pleskov[10] that acetonitrile forms stronger solvates with silver ion than water.

Polarographic Study of Cd, Tl and Co Complexes.—While the reduction potential of the silver ion is too positive to be used for polarographic determination of silver complex formation, with a dropping mercury electrode, the half-wave potentials of Cd^{++}, Tl^+ and Co^{++} ions are readily accessible. Solid compounds of metrazole with cadmium salts have been reported in the literature,[3a,b] and an attempt to establish their stability in aqueous solution seemed justified.

The half-wave potentials of cadmium nitrate solutions (with 0.5 M potassium nitrate as supporting electrolyte) were -0.583 v. *vs.* S.C.E., (lit.[11] -0.58 v.). A fifty-fold excess of metrazole was added, but the potential was -0.584 v. Evidently, if a cadmium–metrazole complex is formed in solution, it is too weak to be determined polarographically.

A 3.83×10^{-4} M thallium nitrate solution had a half-wave potential of -0.462 v. (lit.[11] -0.460 ± 0.005). A solution of the same thallium concentration and 2.31×10^{-2} M in metrazole (a 58-fold excess) had a half-wave potential of -0.467 v. In the case of cobaltous nitrate the observed half-wave potential of -1.130 v. (lit.[11] $-$ 1.20 v.) remained constant when a 97-fold excess of metrazole was added (4×10^{-4} M Co^{++}, 3.87×10^{-2} M metrazole). On the basis of the above results, it is seen that if complexes are formed, they are too unstable to be determined polarographically.

Titrimetric Study.—While neither Dister nor Zwikker[3a,b] have observed any basic character in metrazole solution, the existence of a solid complex with sulfuric acid[3a] indicates at least a possibility of such interaction. An acid–base titration on metrazole solution in glacial acetic acid was done potentiometrically, and the titration curve is given in Fig. 3. It is seen that there is some interaction between metrazole and the proton but that the basic strength of metrazole is rather low. It is impossible to obtain K_b of metrazole in glacial acetic acid from the above measurements because of the

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(10) V. A. Pleskov, *Zhur. Fiz. Khim.*, **22**, 351 (1948).

(11) L. Meites, "Polarographic Techniques," Interscience Publishers, Inc., New York, N. Y., 1955.

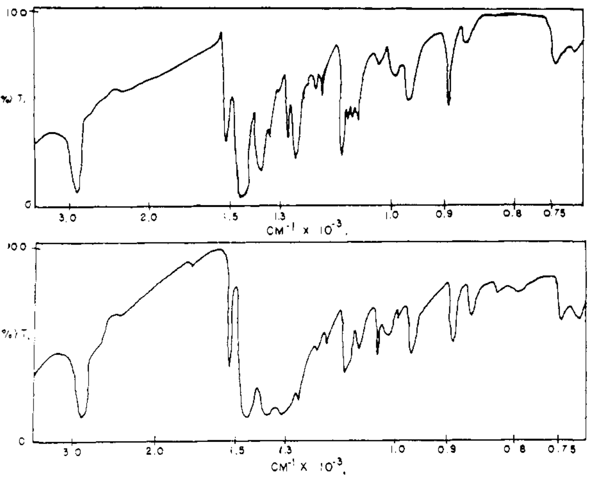

Fig. 1.—Infrared spectra of metrazole (top) and of metrazole–silver nitrate complex (bottom) in the NaCl region.

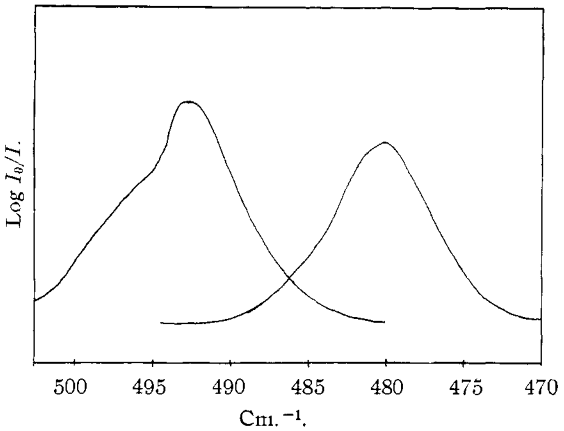

Fig. 2.—Hypsochromic shift of the infrared band of metrazole upon complexation with silver ion.

ion-pair formation in this solvent. Ultraviolet absorption spectrum of metrazole shows a "toe" starting at approximately 270 mμ (for a 10^{-2} M solution) and gradually increasing to the limit of instrument which, in this case, was 220 mμ. However, even at this limit the absorption was very weak and the value of the molar absorptivity was $\sim$1.0. Metrazole spectra were also run in dilute and in concentrated sulfuric acid, but no appreciable change in them was observed.

The above results do not indicate that metrazole has any strong nucleophilic properties either toward the proton or toward metal ions (at least in the case of Ag^+, Tl^+, Cd^{++} and Co^{++}). This is somewhat contrasted by the fairly appreciable tendency of metrazole to form complexes with the halogens.[1] There is no correlation between the convulsant ability of metrazole and of metrazole derivatives and the formation constants of the silver complexes.

It should be noted, however, that these results do not indicate that all of the compounds investigated necessarily have the same nucleophilic properties. In fact, such a generalization on the basis of the strength of silver complexes alone would be quite unwarranted. For example, it has been pointed out by Bruehlman and Verhoek[12] that a change in the configuration of an amine which greatly affects its coördination tendency with a proton has very little influence on its ability to coordinate with a silver ion. Similarly, it was pointed out in the previous communication[1] that the coordinating tendencies of metrazole and of 2,2′-bipyridine are approximately equivalent toward iodine monochloride, while the proton affinity of the bipyridine is much higher than that of metrazole. Steric effects may likewise be important as was shown by the comparison of the coördinating tendencies of pyridine, 2-picoline and 2,6-lutidine with respect to halogens and halogen halides.[13] Of course, in the present case, the structure of the silver complexes with metrazoles is unknown, but it is being presently investigated in this Laboratory.

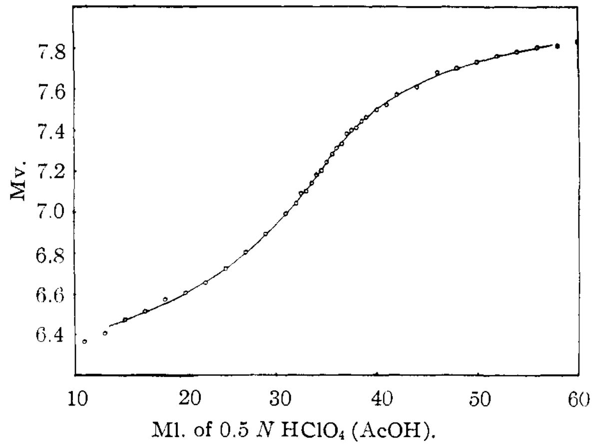

Fig. 3.—Titration curve of metrazole with perchloric acid in glacial acetic acid.

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[12] R. J. Bruehlman and F. H. Verhoek, THIS JOURNAL, **70**, 1401 (1948).

[13] A. I. Popov and R. H. Rygg, *ibid.*, **79**, 4622 (1957).