SYNTHESIS AND PROPERTIES OF QUATERNARY SALTS OF DERIVATIVES OF 1-PHENAZINECARBOXYLIC ACID

V. S. Mokrushin, Z. V. Pushkareva,
T. K. Pashkevich, and E. N. Rysakova

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1-Phenazinecarboxylic acid (I), its amide (II), the amide free radical (chlororafin), 2-hydroxy-1-phenazinecarboxylic acid, and also 1-carboxy-6-amino-10-methylphenazine and 1-carboxy-3-sulfo-6-amino-10-methylphenazine (eroginosins A_2-III and B), isolated from the culture Pseudomonas aeruginosa and Pseudomonas aureofaciens [1-4], posses bacteriostatic activity [3, 6, 7] and (II) is able to inhibit the growth of certain experimental tumors [8].

We have prepared a series of quaternary salts of 1-phenazine-carboxylic acid derivatives for biological investigation. Alkylation of the methyl ester of 1-phenazinecarboxylic acid (IV) with dimethyl sulfate or diethyl sulfate and subsequent conversion of the methylmethosulfate and ethylethosulfate into the iodides gives the 10-iodomethylate (V) and 10-iodoethylate of the methyl ester of 1-phenazinecarboxylic acid (VI). The 10-iodomethylates of amide (VII), diethylamide (VIII), and phenylamide of 1-phenazinecarboxylic acid [14] were synthesized analagously from the corresponding amides prepared according to Rozum [9]. Since, according to Vol' [1, 10], 1-phenazinecarboxylic acid is obtained in a yield of not more than 7%, we developed a method which gives a yield of 18%. Treatment of a sodium carbonate solution of (I) with dimethyl sulfate gives (IV) in almost quantitative yield. The oxidative amination reaction [11-13] of (Va), the methylmethosulfate of (IV), was used to prepared analogs of (III). The aminating agents used were ammonia, methylamine, ethylamine, dimethylamine, diethylamine, aniline, p-anisidine, and morpholine. The obtained methylmethosulfates were converted into the iodomethylates [compounds (X-XVII), correspondingly]. As in the amination of phenazine 1-methoxy-10-ethylmethosulfate, formation of two isomers is possible here: the 10-methylmethosulfates of the 6-amino and the 3-amino derivatives:

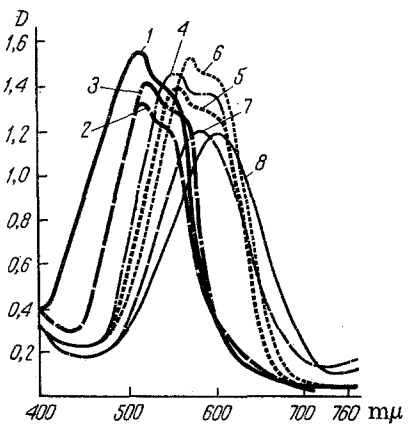
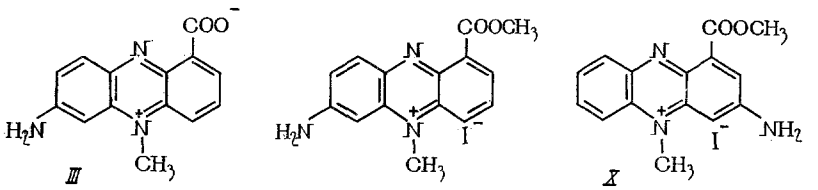


Fig. 1. Absorption spectra of the 10-iodomethylates of 3-amino, alkylamino, and arylamino derivatives of the methyl ester of 1-phenazinecarboxylic acid: 1) (X); 2) (XI); 3) (XII); 4) (XIII); 5) (XIV); 6) (XVII); 7) (XV); 8) (XVI).



The demethylation reaction [4, 12, 14] known for quaternary salts of N-methylphenazine was used to demonstrate the structure of the obtained products. Treatment of the primary amine (X) with dilute base yielded a compound identical in melting point, λ_{max} (in alcohol and base), and R_f with 1-carboxy-3-aminophenazine synthesized by the method of Holliman [15]. On the basis of these data and also by comparison of visible absorption spectra (see Fig. 1) to compounds (X-XVII) were assigned the structure of the 10-iodomethylates of 3-amino, alkylamino, and arylamino derivatives of the methyl ester of 1-phenazinecarboxylic acid.

Upon examination of the bacteriostatic and bactericidal activity of compounds (V), (VII), (XI), (XV), it was found that (V), (VII), and (XI) show a weak activity. Compound (XV) has a bacteriostatic effect on staphylococcus at a dilution of 1:16,000, on streptococcus at a

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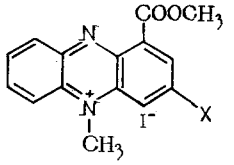
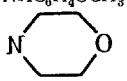
TABLE 1. Properties of Compounds of the General Formula



Compound	X	Mp (deg)	Yield (%)	Found, %			Empirical formula	Calculated, %		
				C	H	N		C	H	N
X	NH_2	208—9	42	45,82	3,69	10,80	$C_{15}H_{14}IN_3O_2$	45,56	3,58	10,63
Xa*	NH_2	218—9	73	49,24	3,83	11,10	$C_{15}H_{14}ClN_3O_6$	48,99	3,84	11,42
XI	CH_3NH	198	49	47,18	4,11	10,04	$C_{16}H_{16}IN_3O_2$	46,96	3,96	10,27
XII	NHC_2H_5	195	38	48,29	4,30	10,41	$C_{17}H_{18}IN_3O_2$	48,24	4,29	9,94
XIII	$N(CH_3)_2$	182	27	47,96	4,14	—	$C_{17}H_{18}IN_3O_2$	48,24	4,29	9,94
XIV	$N(C_2H_5)_2$	178—9	49	50,22	5,11	9,67	$C_{19}H_{22}IN_3O_2$	50,56	4,92	9,31
XV	NHC_6H_5	168	50	53,76	3,91	9,12	$C_{21}H_{18}IN_3O_2$	53,51	3,84	8,91
XVI	$NHC_6H_4OCH_3$	166	11	52,60	4,14	—	$C_{22}H_{20}IN_3O_3$	52,69	3,99	8,37
XVII		195—7	19	48,60	4,41	9,34	$C_{19}H_{20}IN_3O_3$	49,04	4,33	9,03

* Perchlorate.

dilution of 1 : 138,000, on Esherichia coli at a dilution of 1 : 16,000, and on hay bacillus at a dilution of 1 : 64,000. The bacteriocidal activity on the same microorganisms was correspondingly equal to 1 : 16,000, 1 : 32,000, 1 : 16,000, and 1 : 1000. The 10-iodomethylate of 1-phenazinecarboxylic acid amine inhibited the growth of Mycobacterium tuberculosis without serum at a dilution of 1 : 512,000 and at a dilution of 1 : 128,000 with serum.

EXPERIMENTAL

Spectral characteristics were obtained on a SF-10 instrument with ethanol as solvent; concn. $5 \cdot 10^{-4}$ / mole/liter.

1-Phenazinecarboxylic Acid (I). We heated 35 g of anthranilic acid and 70 ml of nitrobenzene to 140°C. With intense stirring in small portions was added 50 g of powdered KOH, not letting the mixture warm up above 150°. After addition of the total amount of KOH the reaction mass was heated at 150° for 30 min. The nitrobenzene and azobenzene were steam-distilled. The aqueous solution was evaporated to 150 ml, cooled, the precipitated K salt was separated, washed with 30% KOH and acetone, dissolved in a small amount of water, acidified with HCl, and precipitated (I) was washed with acetone. The material was precipitated from base with acid. Yield 10.5 g (18%). Mp 237-239°. Literature data [1], mp 239°.

Methyl Ester of 1-Phenazinecarboxylic Acid (IV). To 5 g of (I) in 40 ml of a saturated solution of sodium carbonate at 60° was added dropwise with intense stirring dimethyl sulfate to pH 7.0. The mixture was maintained for 0.5 h and filtered. Yield 4.7 g (87%). Mp 121-123°. Literature data [16], mp 123°.

10-Iodomethylate of the Methyl Ester of 1-Phenazinecarboxylic Acid. To 2.5 g of (IV) in 3.5 ml of nitrobenzene at 120° was added 3.33 g of dimethyl sulfate; after 10 min the mixture was cooled and the methylmethosulfate precipitate was washed with absolute ether, dissolved in a minimum amount of water and a saturated solution of KI was added. Yield 1.85 g. Mp 154-155° (from alcohol). Found, %: C 47.45; H 3.54; N 6.96. $C_{15}H_{13}IN_2O$. Calculated, %: C 47.40; H 3.62; N 7.35.

10-Methylperchlorate of the Methyl Ester of 1-Phenazinecarboxylic Acid. The material was prepared analagously to (V) and the methylmethosulfate was transformed into the prechlorate using $NaClO_4$. Yield 2.01 g. Mp 194° (from alcohol). Found, %: C 51.21; H 3.83; N 7.70. $C_{15}H_{13}Cl\ N_2O_6$. Calculated, %: 51.01; H 3.71; N 7.94.

10-Iodoethylate of the Methyl Ester of 1-Phenazinecarboxylic Acid (VI). To 2.5 g of (IV) in 3.5 ml of nitrobenzene at 120° was added 4.5 g of diethyl sulfate. The mixture was kept at the same temperature for 5 h, cooled, and the quaternary salt was precipitated with absolute ether, dissolved in a minimum amount of water and a saturated solution of KI was added. Yield 1.82 g. Mp 168-170° (from alcohol). Found, %: C 48.70; H 3.92; N 6.83. $C_{16}H_{15}IN_2O_2$. Calculated, %: C 48.73; H 3.81; N 7.10.

10-Iodomethylate of the Amide of 1-Phenazinecarboxylic Acid (VII). To 1 g of (II) in 15 ml of nitrobenzene at 140° was added 2.66 g of dimethyl sulfate, the mixture was maintained for 10 min, cooled, and the quaternary salt was precipitated with absolute ether and transformed into the iodide. Yield 0.95 g. Mp 185-188° (from alcohol). Found, %: C 46.40; H 3.51; N 11.48. $C_{14}H_{12}IN_3O$. Calculated, %: C 46.02; H 3.29; N 11.50.

10-Iodomethylate of the Diethylamide of 1-Phenazinecarboxylic Acid (VIII). The compound was prepared analagously to (VII). Yield 0.2 g. Mp 167-168° (from abs. alcohol). Found, %: C 51.27; H 4.83; N 9.68. $C_{18}H_{20}IN_3O$. Calculated, %: C 51.50; H 4.71; N 9.99.

10-Iodomethylate of the Phenylamide of 1-Phenazinecarboxylic Acid was prepared analagously to (VII). Yield 0.48 g. Mp 180-181° (from alcohol). Found, %: C 54.19; H 3.75; N 9.50. $C_{20}H_{16}IN_3O$. Calculated, %: C 54.42; H 3.64; N 9.53.

Amination of the Methylmethosulfate of (IV) (Va). Through a solution of 1 g of (Va) (0.0027 mole) in 50 ml of alcohol was bubbled over 20 h a mixture of air and 0.0135 mole of the amine (nonvolatile amines were added directly to the reaction flask). The alcohol was distilled, the residue was dissolved in water, and a saturated solution of KI was added (in the preparation of the perchlorate $NaClO_4$ was added). The precipitate was washed on the filter with a small amount of water, dried, and crystallized from alcohol (see Table 1).

Demethylation of (X). To 0.67 g of (X) in 5 ml of water was added 3 ml of 10% HCl and the mixture was boiled for 30 min. The mixture was cooled, 10 ml of 15% KOH was added, and the mixture was boiled for 1.5 h and acidified to pH 6.0 with HCl. Yield 0.4 g. Mp 302-304° (dec., from alcohol). λ_{max} 500 nm (in alcohol), 473 nm (in 2 N KOH solution). Using thin-layer chromatography on Al_2O_3 (Grade II) and the following systems: a) butanol–acetic acid–water (4 : 1 : 1); b) water–alcohol (1 : 2); c) butanol–water (20 : 1), for the demethylation product of (X) and 1-carboxy-3-aminophenazine were found R_{fa} 0.74, R_{fb} 0.1, and R_{fc} 0.12. The obtained compound did not give a melting point depression with 1-carboxy-3-aminophenazine.

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