The irritating effect was studied according to the method described in [8], the capacity to produce terminal anesthesia was determined with rabbits' eyes by the method of Renier [3], the analgesic activity by using nociceptive irritation with electrical [1], thermal [9] or chemical (3% solution of AcOH, i.p.) [2] modality, and the spasmolytic activity with isolated small intestine of rats according to Magnus [4]. To determine the analgesic activity of the compounds we determined the value of ED_{50} (the dose causing a two-fold increase in the threshold of sensitivity to pain in 50% of the animals) and the therapeutic index of the analgesic activity (TIA). The latter was calculated by dividing LD_{50} by ED_{50}. The anesthetizing activity was compared with that of promedol, and the spasmolytic activity with that of papaverine hydrochloride and of No-Spa. The numerical data of the experiments were processed statistically [6].

LITERATURE CITED

1. N. K. Barkov, Farmakol. Toksikol., No. 1, 36-40 (1961).
2. V. V. Gatsura, Methods for Primary Pharmacological Study of Biologically Active Substances [in Russian], Meditsina, Moscow (1974).
3. V. V. Zakusov, Pharmacology of the Nervous System [in Russian], Leningrad (1953).
4. M. P. Nikolaev, Experimental Bases of Pharmacology and Toxicology (a Practical Guide) [in Russian], Leningrad (1941).
5. K. D. Praltsev, V. K. Yu, R. V. Sharipov et al., Khim.-farm. Zh., **23**, No. 9, 1070-1074 (1989).
6. R. B. Strelkov, Farmakol. Toksikol., No. 4, 100-104 (1986).
7. P. A. J. Janssen, Br. J. Anaesth., **34**, 260-268 (1962).
8. I. Setnikar, Arzneim.-Forsch., **16**, 623-628 (1966).
9. G. Woolfe and A. D. MacDonald, J. Pharmacol. Exp. Ther., **80**, 300-307 (1944).

PYRIDINE DERIVATIVES POSSESSING HYPOGLYCEMIC AND ANALGESIC ACTIVITY

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The need to individualize the treatment of diabetes mellitus with respect to various etiologies, patient age, and the presence of primary and secondary resistance has given rise to the search for antidiabetic agents with differing mechanisms of therapeutic action.

The most efficacious of the existing synthetic antidiabetic preparations are the sulfonylureas [3]. It is well known that nicotinamide is widely used both as a preventative measure and for the treatment of vessel damage due to diabetes mellitus and that it displays weak hypoglycemic activity [4]. This is the basis of our search for substances that lower blood sugar among several of the nicotinamide derivatives and compounds with urea and pyridine fragments within the molecule.

3-Pyridylurea (I) was previously obtained in small yield by heating 3-aminopyridine with urea [1] or by hydrolyzing 1-(N-pyridyl-3)-N'-methoxycarbonylurea [7], and 4-pyridylurea (II) was obtained by heating N-pyridyl-4-ethylurethane with alcoholic solutions of ammonia in an autoclave [8]:

$$RCONHCl \xrightarrow{NH_4OH} \underset{I,\ II}{RNHCONH_2} \xrightarrow{CH_3J} \underset{III,\ IV}{R'NHCONH_2} + J^-$$

$R = 3\text{-}C_5H_4N$ (I), $4 = C_5H_4N$ (II),

$R' = 3\text{-}C_5H_4\overset{+}{N}CH_3$ (III), $4 = C_5H_4\overset{+}{N}CH_3$ (IV)

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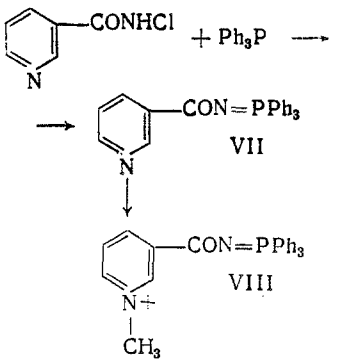
It was found that urea derivatives I and II are easily formed in the reaction of N-monochloramides of nicotinic and isonicotinic acids, respectively, with ammonia (cf. [11]).

The reaction of ureas I and II with methyl iodide produce (1-methyl-pyridinium-3)-urea iodide (III) and (1-methylpyridinium-4)-urea iodide (IV). Analogously, 1-methylpyridium-3-carbamide iodide (VI) is formed from nicotinamide (V) in the presence of methyl iodide.

The N-monochloramide of nicotinamide reacts with triphenylphosphine in the presence of triethylamine to form N-nicotinoyliminotriphenylphosphine (VII), which converts into (1-methylpyridinium-3-carbo)-imino-triphenylphosphine iodide in the presence of methyl iodide (VIII):



N-monochloramides of nicotinic and isonicotinic acid were obtained via the reaction of tert-butyl hypochloride with the amides of the corresponding acids (cf. [10]).

Pharmacological testing of compounds I-VI revealed that several of them possess hypoglycemic and analgesic properties.

EXPERIMENTAL (CHEMICAL)

3-Pyridylurea (I). A suspension of 0.04 mole of N-monochloronicotinamide in 25 ml of an aqueous solution of ammonia was slowly heated at 40-45°C until a homogeneous solution was formed. Compound I crystallized when cooled. Yield 81%. Crystals large, colorless, and prism-shaped (when recrystallized from ethanol). Mp 188-190°C (decomp.); the literature [1] showed 178-179°C. Compound I did not cause melting point depression with samples obtained as in [1]. $C_6H_7N_3O$.

4-Pyridylurea (II) was synthesized in an analogous manner from N-monochloramide of isonicotinic acid. Yield 88%. Prism-shaped crystals (from alcohol). Mp 187°C (decomp.) which corresponds to the data in the literature [8]. $C_6H_7N_3O$.

(1-Methylpyridium-3-)-urea Iodide (III). A 0.01 mole portion of I and 0.03 mole of methyl iodide were boiled in 25 ml of methanol for 6 h. The precipitate that formed after cooling was filtered by suction. Yield 99%. Colorless, prism-shaped crystals (from aqueous ethanol). Mp 204-206°C. $C_7H_{10}IN_2O$.

(1-Methylpyridinium-4)-urea iodide (IV) was synthesized from compound II and methyl iodide as described above. Yield 96%. Prism-shaped crystals (from aqueous ethanol). Mp 208-210°C. $C_7H_{10}IN_3O$.

N-(1-Methylpyridinium-3)-carbamide iodide (IV) was synthesized as described above from nicotinamide (V) and methyl iodide. Yield 93%. Prism-shaped crystals (from aqueous ethanol). Mp 200-202°C. $C_7H_{10}IN_3O$.

N-(Pyridyl-3-carbo)iminotriphenylphsophine (VII). With cooling, 0.01 mole of N-monochloronicotinamide was added to a solution of 0.01 mole of triphenylphosphine and 0.01 mole of triethylamine in 20 ml of benzene. Triethylamine hydrochloride was filtered off and the filtrate was boiled. The residue was treated with ether, and compound VII was filtered off. Yield 76%. Prism-shaped crystals (from ethanol). Mp 198-200°C. $C_{24}H_{19}N_2OP$.

N-(1-Methylpyridinium-3-carbo)iminotriphenylphosphine iodide (VII) was synthesized by boiling 0.01 mole of compound VI with 0.02 mole of CH_3I in 25 ml ethanol for 2 h. Yield 83%. Mp 184-186°C. $C_{25}H_{22}IN_2OP$.

N-Monochloramide of Nicotinic Acid. A mixture of 0.01 mole of nicotinamide and 0.011 mole of tert-butyl hypochlorite in 20 ml of CCl_4 was heated at 60-70°C until a homogeneous solution was formed. The precipitate was

filtered by suction. Fine, colorless, needle-shaped crystals (from dichloroethane) and prism-shaped crystals (from acetonitrile). Mp 110°C (decomp.). $C_8H_5ClN_2O$. (According to [2], N-monochloramide was synthesized with a yield of 35% when chlorine was passed through an alkaline solution of nicotinamide.)

N-Monochloramide of Isonicotinic Acid was formed analogously from isonicotinamide. Yield 82%. Colorless, prism-shaped crystals (from acetonitrile). Mp 163°C (decomp.). $C_5H_5N_2O$.

EXPERIMENTAL (PHARMACOLOGICAL)

The hypoglycemic activity of the test compounds was judged from their effect on the blood glucose level of intact, white, male, nonpedigree mice weighing 170-220 g. The test substances were administered intragastrically via a metal cannula in the form of a 2% aqueous suspension (Tween-80) into rats that had been fasting for 24 h. The dose was 50 mg/kg.

The level of glycemia was determined using the o-toluidine method [9] before administration, as well as over a variety of time intervals after administration of the test substance. It was also used in the control, which consisted of an equivalent quantity of water. Bukarban ("Quinoin," Hungary) was used as the reference drug.

The analgesic activity of the compounds was studied on models of pain reaction in response to thermal (the "hot plate" method) and chemical (the "spasms" method) irritation [5]. In the experiments 18-22 g male and female white, nonpedigree rats were used. The test substances were administered to the test animals of the experimental groups intragastrically at a dose of 20 mg/kg (when the effect of chemical irritation was being measured) and at a dose of 40 mg/kg (when the effect of temperature was being measured) in the form of 0.2% aqueous solutions (water—insoluble substances in the form of aqueous emulsions with Tween-80) 1 h prior to exposure to pain.

The analgesic activity of the compounds was assessed by the reduction in the number of "spasms" of the animals in the experimental groups in comparison to the control and was calculated from the formula

$$A_k = \frac{(K_k - K_0)}{K_k} \cdot 100,$$

where A_k is the activity of the compound and K_k and K_0 are the number of "spasms" in the control and experimental groups, respectively.

In the case of the effect of temperature, the time from the moment the mice were placed on the heated surface until the moment they began licking their appendages or hopping served as the indicator of a pain reaction. The activity of the test compounds was calculated from the formula

$$A_t = \frac{(T_0 - T_k)}{T_k} \cdot 100,$$

where A_t is the activity of the compound and T_0 and T_k are the time (in seconds) from the beginning of a response until the start of a pain reaction in the test and control animals, respectively.

A group of animals injected with an equal volume of solvent was used as a control.

The analgesic activity of the compounds studied was assessed in comparison to one of the most widely used analgesics in medical practice — Analgin.

A study of the toxicity of the compounds in warm-blooded animals was conducted on male and female mice weighing 18-21 g under conditions of intraperitoneal administration at various doses. The test animals were kept under observation continuously for the first day and then once every 24 h for a period of 10 days.

DISCUSSION OF RESULTS

From the data presented (Table 1), it is apparent that the test compounds are not very toxic. Ureas I and II do not possess hypoglycemic activity; however, their derivatives — salts III and IV, which contain a quaternary nitrogen atom in the pyridine ring — show high activity. The hypoglycemic effect of compound III is close to that of the reference compound — Bukarban. There are certain similarities in the dependence of the hypoglycemic properties of the pyridine derivatives on their structure. According to the literature [6], nicotinamide V shows weak hypoglycemic activity. At a dose of 50 mg/kg, which was used to test compounds I-IV, it was inactive, but at the same dose the methiodide of nicotinamide VI also demonstrated noticeable activity. When the hydrogen atoms of the carbamide

TABLE 1. Hypoglycemic Activity and Toxicity of Pyridinecarboxylic Acid Derivatives

Compound	Decrease in blood glucose level, %	LD_{50}, mg/kg
Bukarban	29,3±3,5	2800
I	0	1900 (1600—2300)
II	0	3300 (2900—3700)
III	24,5±2,7	1500 (1300—1800)
IV	12,0±2,9	2100 (1800—2300)
V	0	3100 (2600—3600)
VI	12,7±1,1	2800 (2500—3200)
VIII	0	1500 (1300—1800)

TABLE 2. Analgesic Activity of Pyridinecarboxylic Acid Derivatives under Conditions of Thermally and Chemically Induced Pain

Compound	Thermal effect		Chemical effect	
	reaction time, sec	A_t, %	number of "spasms"	A_K, %
Control	10,5±0.3	—	24,3±1,3	—
Analgin	14,1±1,3*	34,3	16,2±1,9*	33,3
I	10,5±1,1	0,0	23,0±4,7	5,3
II	11,6±1,7	10,5	20,4±3,6	16,0
III	16,4±2,0*	56,2	13,8±1,2*	43,2
IV	14,2±1,4*	35,2	12,4±3,4*	49,0
V	13,1±1,9	24,8	24,9±4,4	—2,4
VI	16,7±2,2*	59,0	19,0±2,9	21,8
VIII	11,4±1,5	8,5	14,1±3,9*	42,0

*The reliability of the difference between the control and the experiment at $p < 0.05$.

group of compound VI are replaced by a triphenylphosphine group (compound VIII), the hypoglycemic activity disappeared.

Several of the test compounds were observed to have analgesic as well as hypoglycemic activity. The data presented in Table 2 show that compound V possesses insignificant analgesic activity in response to temperature and no activity in response to chemical irritation, which, apparently, is an indication of its primary mechanism of action. Replacing the hydrogen atoms in the amino group of nicotinamide by a triphenylphosphoine group (compound VIII) leads to a significant increase in analgesic activity in response to chemical irritation. Yet, in the hot plate experiments, this compound does not differ as much in its analgesic effect.

Replacing the amino group of nicotinamide by a urea fragment (I) leads to a complete loss of analgesic properties in both models. A change in the position of the urea fragment in the pyridine ring (II) had a substantial effect on the change in the activity under investigation.

When compounds V, I, and II were alkylated by methyl iodide, salts III, IV, and VI were formed, which turned out to have the greatest analgesic activity with respect to both models, exceeding the reference drug Analgin in this respect.

As is obvious from the data, a certain degree of parallelism is observed in the dependence of both hypoglycemic and analgesic activity of the test compounds on their structure.

This study has revealed a strong positive correlation (correlation coefficient $r = +0.87$) between the ability of pyridinecarboxylic acid derivatives to reduce the blood glucose level and their ability to produce an analgesic effect in experiments, involving the "hot plate." In the case of the analgesic effect using the "spasms" method, the value of r was +0.66, which is an indication of a weaker correlation between these indicators. This correlation may be responsible for the similarities between the cellular mechanisms that regulate the glucose level and those that elicit the pain reaction [12].

It is well known that in clinical endocrinology hypoglycemics are often used in combination with analgesics in order to suppress pain. This is due to the difficulties involved in achieving an efficient combination of pharmacological agents, particularly in the case of long-term use. In this connection, we believe that the discovery of analgesic properties in hypoglycemic compounds is important and that future research along these lines is merited.

LITERATURE CITED

1. M. P. Gerchuk and S. Z. Taits, Zh. Obshch. Khim., **20**, No. 5, 910-916 (1950).
2. Patent Application No. 53-31666, 1976, Japan; Ref. Zh. Khim., No. 5-0448 (1979).
3. T. A. Maksimov, A. V. Ostashkova, and Z. M. Nakipova, Topical Problems in Endocrinology [in Russian], Frunze, 81-83 (1983).
4. M. D. Mashkovskii, Drugs [in Russian], 11th ed., Vol. 2, Moscow, (1988).
5. Systemic Recommendations Regarding the Experimental (Preclinical) Study of Nonsteroidal Antiinflammatory Pharmacological Substances, Moscow (1983).
6. I. G. Obrosova, N. N. Velikii, and A. S. Efimov, Vopr. Med. Khim., **31**, No. 2, 125-127 (1985).
7. Patent No. 974085, 1951, France; Chem. Abstr., **47**, No. 12421 (1953).
8. Patent No. 324439, 1957, Switzerland; Chem. Abstr. **52**, No. 18475 (1958).
9. Z. I. Tsyukhno, V. N. Slavnova, N. I. Panchenko, et al., Functional Methods of Research in Endocrinology [in Russian], Kiev (1981).
10. B. Altenkirk and S. S. Israelstam, J. Org. Chem., **27**, No. 12, 4532-4534 (1962).
11. G. R. Elliott, J. Chem. Soc., **121**, 202-209 (1922).
12. C. J. Taylor and J. Merritt, Trends Biochem. Sci., **11**, No. 6, 238-242 (1986).