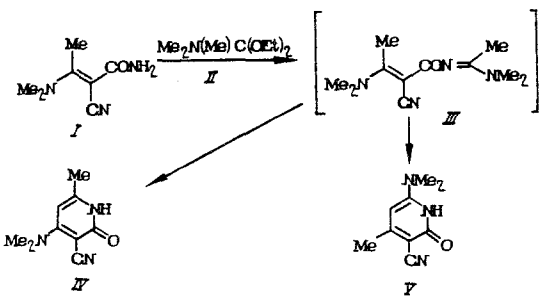
SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF DERIVATIVES OF 3-CYANO-4(6)-METHYL-6(4)-DIMETHYLAMINOPYRIDINE

M. V. Mezentseva, I. F. Faermark,
E. F. Kuleshova, O. S. Anisimova,
G. Ya. Shvarts, V. G. Granik,
and M. D. Mashkovskii

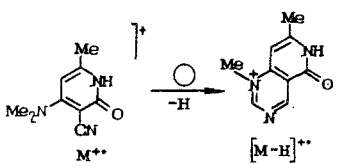
UDC 615.015.8:547.821].012.1.07

It is known that amino derivatives of pyridine have a broad spectrum of biological activities [7, 11]. Among derivatives of 2,4-diaminopyridine we find compounds with antihypertensive and analgetic activities [6]. With this in mind and with the purpose of carrying out pharmacological investigations we have carried out the synthesis of novel derivatives of 2,4-diaminopyridine and 4(6)-aminopyridone-2.

As starting compounds we have selected α-cyano-β-dimethylaminocrotonamide (I) and dimethylacetamide diethyl acetal (II). When a solution of these compounds in DMF is heated, three compounds are isolated, two of which are isomers according to elemental analyses and mass spectra. It is known [2] that cyclization of enamidines of type (III),* which probably are intermediates in the pyridine synthesis under investigation, may proceed by two routes: with participation of the meso 6-carbon atom of the amide moiety and the methyl group at the 2-position with formation of 3-cyano-4-dimethylamino-6-methylpyridone-2 (IV), and by attack of enamino C(2) atom on the 6-methyl substituent, which in our case leads to isomeric 3-cyano-4-methyl-6-dimethylaminopyridone-2 (V).



The mass spectra of compounds IV and V contain peaks of the molecular ions with the same value m/z = 177, but with different decomposition patterns, which points to their isomeric structures [1]. Thus, in the spectrum of compound V we find an intensive ion peak of m/z = 148 (95%), corresponding with elimination of CH_3N from $M^{+\cdot}$, which is evidence of the α-position of the dimethylamino group relative to the nitrogen atom of the pyridine ring [5]. The spectrum of compound V contains an intensive ion peak $[M-H]^+$ of m/z = 176 (52%), the formation of which is caused by the ortho-position of the dimethylamino and cyano groups at the ring, which contributes to the closure of the pyridine ring.



*Initial attack of amide acetals on the NH_2 amide group of enaminoamides has been demonstrated, for example in [5].

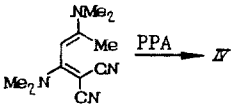
Central Chemical Laboratory Buildings, S. Ordzhonikidze All-Union Scientific-Research Institute of the Chemical and Pharmaceutical Industry, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 25, No. 9, pp. 29-33, September, 1991. Original article submitted September 25, 1990.

TABLE 1. Effect of the Excess of Acetal II on the Yields of Compounds IV, V, and VI

Ratio of the reagents, mole		Yield, %		
enamine I	acetal II	IV	V	VI
1	1	39.5	31.4	5.8
1	1.5	33.9	13.4	12.6
1	2	19.1	—	18.7

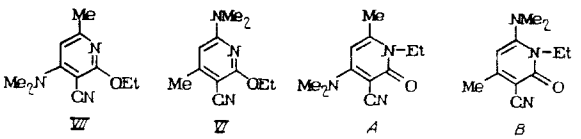
The UV spectrum of isomer IV contains two absorption maxima and that of isomer V three, the long-wave absorption maximum being more intensive and shifted bathochromically by 50.8 nm.

The given data are evidence in favor of the fact that compound IV is a 4-dimethylamino and compound V a 6-dimethylamino derivative. The unambiguous evidence of such a conclusion follows from comparison of the physical and spectral characteristics of compound IV with an authentic sample that we have prepared by retrosynthesis according to [3], which provides a single cyclization path:



It is significant that under the conditions that we have studied compound IV is produced in much higher yield than compound V (see Experimental section and Table 1). This confirms an earlier conclusion [2] that cyclizations that proceed with participation of amide meso carbon atoms are energetically more favorable than in the case of electrophilic attack on the α-C atom of enamines.

According to elemental analyses and mass spectral data, the third of the isolated compounds has the empirical formula $C_{11}H_{15}N_3O$, which may correspond with four structural formulae:



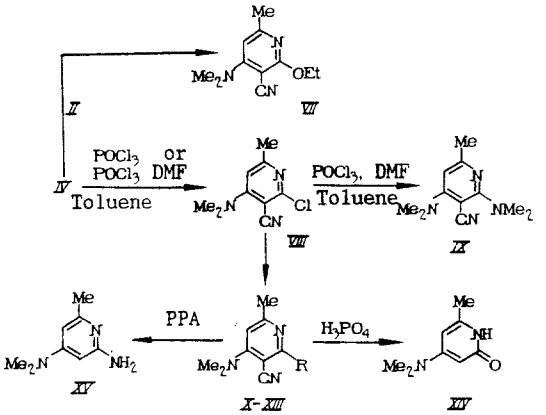
The absence of carbonyl valence vibrations in the IR spectrum and the presence of proton signals of a C_2H_5O group in the PMR spectrum (Table 2) made it possible to exclude structures A and B. The general resemblance of the decomposition of the molecular ion of VI with the decomposition of $M^{+\cdot}$ of compound V [elimination of a CH_3N group from $M^{+\cdot}$ m/z = 176 (37%) and the absence of an $[M-H]^+$ peak] made it possible to assign it the structure of 2-ethoxy-3-cyano-4-methyl-6-dimethylaminopyridine (VI).

We have also found that the yields of compounds IV, V, and VI considerably depend on the amount of acetal II used in the reaction (see Table 1).

It is clear from Table 1 that increasing the amount of acetal II lowers the yields of compounds IV and V and increases the yield of compound VI. Obviously, this results from alkylation of oxo compounds IV and V by the excess of acetal II. However, we have isolated only compound VI from the reaction mixture. Its isomer VII was prepared by heating compound IV with acetal II in DMF. The structure of compound VII was confirmed by the presence of an ion peak $[M-H]^+$ (77%) in the mass spectrum. Reaction of pyridone IV with Vilsmeier reagent leads to formation of chloro derivative VIII, which was also obtained on heating pyridone IV with $POCl_3$.

Attempts to formylate VIII with Vilsmeier reagent in toluene were unsuccessful: the only product isolated in that case was 2,4-bis(dimethylamino)-3-cyano-6-methylpyridine (IX). The mass spectrum of compound IX contains an intensive molecular ion peak 204 (90), and peaks $[M-H]^+$ 203 (73) and $[M-CH_3]^+$ 189 (100).

Treatment of chloro derivative VIII with primary and secondary amines, and also with an alcoholic solution of NH_3, yielded corresponding amides X-XIII.



R=NHCH$_2$Ph(X), piperidino (XI) morpholino (XII), NH$_2$(XIII)

Heating amine XIII with 100% phosphoric acid and with polyphosphoric acid (PPA) leads to elimination of the cyano group and formation of pyridone XIV and amine XV, respectively. In the IR spectra of these compounds the C≡N absorption band has disappeared and there appear a band at 1620 cm^{-1} (C=O) (XIV) and bands at 3460, 3365, 3280, and 3100 cm^{-1} (NH$_2$) (XV).

EXPERIMENTAL (CHEMICAL)

IR spectra were taken on a Perkin–Elmer 457 spectrometer in paraffin oil. UV spectra were recorded on a Specord M-40 spectrometer (Karl Zeiss, Jena) in alcohol. PMR spectra were recorded on a Varian XL-200 spectrometer in DMSO-d_6, standard tetramethylsilane. Mass spectra were taken on a Varian MAT-112 mass spectrometer at an ionization voltage of 70 eV. Chromatographic check of the proceeding of the reactions and the purity of the prepared compounds was carried out by TLC on Silufol UV-254 plates with the systems $CHCl_3$ and benzene–ethanol–ammonia (45:15:1). Spots were visualized in UV light. Characteristics of the prepared compounds are summarized in Table 3.

Data of elemental analyses corresponded with calculated values.

Reaction of α-Cyano-β-dimethylaminocrotonamide (I) with Acetal (II). A. A mixture of 15.3 g (100 mmole) of enamine I and 16.1 g (100 mmole) of acetal II in 150 ml of dry DMF is refluxed for 2 h, cooled, the precipitate is filtered off, and washed with acetone to yield compound IV. The mother liquor is evaporated under vacuum and acetone is added to the residue. The mixture is cooled, the precipitate is filtered off, and washed with acetone to yield compound V. The mother liquor is poured out in water and compound VI is isolated.

B. A mixture of 15.3 g (100 mmole) of enamine I and 24.1 g (150 mmole) of acetal II in 150 ml of dry DMF is refluxed for 2 h, concentrated under vacuum to half of its volume, and cooled. The precipitate is filtered off and washed with acetone to yield compound IV. The mother liquor is evaporated under vacuum, the residue is dissolved in $CHCl_3$, and chromatographed over an SiO_2 column ($CHCl_3$). Compound VI is isolated.

C. A mixture of 15.3 g (100 mmole) of enamine I and 32.2 g (200 mmole) of acetal II in 150 ml of dry DMF is refluxed for 2 h and the solution is evaporated under vacuum. To the residue is added acetone, the mixture is cooled, and the precipitate is filtered off to yield IV. The mother liquor is evaporated under vacuum and the residue is chromatographed over SiO_2 ($CHCl_3$) to isolate compound VI.

2-Ethoxy-3-cyano-4-dimethylamino-6-methylpyridine (VII). A mixture of 0.88 g (5 mmole) of pyridone IV and 1.61 g (10 mmole) of acetal II in 10 ml of dry DMF is refluxed for 2 h, the solution is evaporated under vacuum, and compound VII is isolated.

2-Chloro-3-cyano-4-dimethylamino-6-methylpyridine (VIII). A. A mixture of 0.88 g (5 mmole) of pyridone IV, 0.54 g (7.4 mmole) of dry DMF, and 0.9 g (5.9 mmole) of freshly distilled $POCl_3$ in 10 ml of toluene is refluxed for 6 h. Then 5 ml of a saturated aqueous sodium acetate solution is added and the mixture is stirred for 20 min. The toluene solution is extracted with 10% aqueous Na_2CO_3 solution, dried, evaporated under vacuum, and compound VIII is isolated.

TABLE 2. UV and PMR Spectra of Compunds IV-VII

Compound	UV spectrum (ethanol) λ_{max}, nm log ε	PMR spectrum, chemical shift, δ, ppm (DMSO-d_6)							
		1-NH	5-H	4-N(CH_3)$_2$	6-CH_3	4-CH_3	6-N(CH_3)$_2$	2-OC_2H_5	
								OCH_2	CH_3
IV	234,6(4,49); 295,7(4,15)	11,26 (br.s, 1H)	5,81 (s, 1H)	3,17 (s, 6H)	2,10 (s, 3H)	—	—	—	—
V	233,8(4,04); 260(3,48); 346,5(4,23)	11,0 (br.s, 1H)	5,71 (s 1H)	—	—	2,21 (s, 3H)	3,05 (s, 6H)	—	—
VI	272,5(4,24); 315(4,34)		6,19 (s, 1H)	—	—	2,29 (s, 3H)	3,09 (s 6H)	4,40 (q, 2H)	1,33 (t, 3H)
VII	232,9(4,47); 271,9(3,88); 334,4 (2,76)		6,31 (s, 1H)	3,14 (s, 1H)	2,27 (s, 3H)	—	—	4,32 (q, 2H)	1,30 (t 3H)

TABLE 3. Characteristics of the Prepared Compounds

Compound	Yield, %	Mp. °C	Empirical formula	IR spectrum ν_{max}, cm^{-1}	Values of m/z (relative intensity, %)
IV	A 39,5 B 33,9 C 19,1	300—2 (dec.)	$C_9H_{11}N_3O$	1610 (C=O) 2200 (C≡N)	177(100), 176(52), 162(73), 148(22), 134(24), 133(15), 106(20), 78(10)
V	A 31,4 B 13,4 C —	255—6	$C_9H_{11}N_3O$	1610 (C=O) 2210 (C≡N)	177(100), 162(69), 148(95), 133(25), 106(22), 78(33)
VI	A 5,8 B 12,7 C 18,7	73—5	$C_{11}H_{15}N_3O$	2200 (C≡N)	205(91), 190(21), 176(37), 162(65), 148(100), 134(13), 133(17), 106(13), 78(18)
VII	45	86—7	$C_{11}H_{15}N_3O$	2200 (C≡N)	205(100), 204(77), 190(45), 177(79), 176(66), 162(59), 148(20), 146(34), 45(39), 43(61)
VIII	89,6	146—7	$C_9H_{10}N_3Cl$	2200 (C≡N)	195(61) (^{35}Cl), 194(39) (^{35}Cl), 186(10) (^{35}Cl), 144(33), 90(20)
IX	53	104—6	$C_{11}H_{16}N_4$	2200 (C≡N)	204(90), 203(72), 189(100), 176(41), 161(28), 160(31), 146(40), 119(18), 118(21), 45(43), 43(43)
X	85,3	123—4	$C_{16}H_{18}N_4$	2180 (C≡N) 3380 (NH)	
XI	89,7	84—5	$C_{14}H_{20}N_4$	2200 (C≡N)	
XII	83,6	101—2	$C_{13}H_{18}N_4O$	2200 (C≡N)	
XIII	87,5	280—2 (dec.)	$C_9H_{12}N_4$	2180 (C≡N) 3125, 3305, 3395 (NH_2)	
XIV	57	220—2	$C_8H_{12}N_2O$	1620 (C=O)	152(100), 137(40), 123(18), 109(25), 108(30), 82(10), 81(16)
XV	88,3	100—2	$C_8H_{13}N_3$	3100, 3280, 3365, 3460 (NH_2)	151(100), 150(29), 136(28), 122(28), 108(22), 107(25)

Notes. Crystallization solvents: IV) DMF; V) mixture of MeOH and DMF; VI, XV) mixture of benzene and petroleum ether; VII, IX, XI, XIV) aqueous MeOH; VIII) MeOH; XII, XIII) i-PrOH.

B. A mixture of 13.8 g (78 mmole) of pyridone IV, 115 ml (1260 mmole) of $POCl_3$, and 6.93 ml (55 mmole) of dimethylaniline in 230 ml of dry dioxane is refluxed for 2 h, cooled, and evaporated under vacuum. To the residue is added a saturated sodium acetate solution, the precipitate is filtered off, and washed with water. Compound VIII is obtained.

2,4-Bis(dimethylamino)-3-cyano-6-methylpyridine (IX) is prepared in much the same way as compound VIII by method A from 0.98 g of VIII.

Aminoalkyl Derivatives of 3-Cyano-4-dimethylamino-6-methyl-pyridine (X-XII). A mixture of 1.95 g (10 mmole) of compound VIII and 5 ml of the appropriate amine is refluxed for 4 h, cooled, evaporated under vacuum, and poured out in water. The precipitate is filtered off to yield compounds X-XII.

2-Amino-3-cyano-4-dimethylamino-6-methylpyridine (XIII). In a cylinder, 8 g (40.9 mmole) of VIII in 150 ml of an alcoholic NH_3 solution is heated at 220°C for 9 h, the precipitate is filtered off, and washed with water. Compound XIII is isolated.

4-Dimethylamino-6-methyl-1,2-dihydropyridin-2-one (XIV). A mixture of 0.9 g of XIII and 9 ml of 100% H_3PO_4 is heated at 150-160°C for 1.5 h, cooled, poured out on ice, alkalized with 40% NaOH to pH 10.0, extracted with $CHCl_3$, and evaporated under vacuum. Compound XIV is obtained.

TABLE 4. Acute Toxicity and Analgesic Activity of Derivatives of Pyridine and Pyridone-2 in Mice

Compound	LD_{50} in mice on oral administration, mg/kg	Analgesic activity, change in TPS on oral administration at a dose of 50 mg/kg (% of control)	
		Hot plate	Tail flick
IV	350	+12	—
V	500	+20	—
X	1000	+26*	+10
XI	1000	—	—
XII	1000	+32 *	+20*
XIII	300	+36*	+40*
XIV	1000	+18	—
XV	125	+30*	+25*
Analgin	3000	+50*	+35*

Notes. *) Different from control with $p < 0.05$, −) no effect.

2-Amino-4-dimethylamino-6-methylpyridine (XV). To a solution of PPA, prepared from 60 g of H_3PO_4 and 62.8 g of P_2O_5, is added 4 g of XIII. The mixture is stirred at 180-190°C for 4 h, cooled, 300 ml of water is added, the mixture is alkalized to pH 9.0-10.0 with 8% NaOH, extracted with $CHCl_3$, and the extract is evaporated under vacuum. Compound XV is obtained.

EXPERIMENTAL (BIOLOGICAL)

The biological activities of the derivatives of pyridine and pyridone-2 were studied with respect to analgesic and antihypertensive effects. We also determined the acute toxicity.

The acute toxicity was determined in male mice weighing 18-20 g after oral administration. The LD_{50} was calculated by the method of [10].

The analgesic activity was studied with the model of thermal painful irritation of the mice (tail flick [9] and hot plate [12]).

Experiments were carried out with male mice weighing 20-22 g; each group consisted of 10 animals. The compounds were administered orally at a dose of 50 mg/kg one hour before application of the painful irritation. As reference compound we used analgin at the same dose. Animals in the control group were given orally the corresponding volume of a physiological solution. Changes in the threshold of pain sensitivity (TPS) were determined as a percentage of that of the control group.

In normotensive male rats weighing 250-300 g that had been anesthetized with urethane (1 g/kg intraperitoneally) we studied the effect of the compounds on the arterial pressure (AP) in the case of intravenous administration. The compounds were administered via a catheter in a jugular vein at doses of 1, 2, and 5 mg/kg. The arterial pressure was recorded by a direct method in the common carotid artery with a pressure gauge (Trantec, USA) with registration on a Gemini 7070 (Ugo Basile, Italy) automatic recorder.

In unanesthetized spontaneously hypertensive (SH) male rats of the Okamoto-Aoki strain weighing 250-280 g we studied the effect of the compounds on the AP after oral administration. The AP was recorded by an indirect method in the caudal artery of the animals by a photoelectric sensor by means of a set of instruments of the firm IITC (USA) [8]. The compounds were introduced in the stomach with a probe at a dose of 50 mg/kg. The AP was recorded before and 1, 2, and 3 h after administration of the compound. The effect of each compund was studied in six animals.

Statistical processing of the experimental results was carried out by determination of the averages and their standard deviations; when comparing the averages we used the Student t test.

The experimental data obtained during investigation of the acute toxicity and the analgesic activity of the derivatives of pyridine and pyridone-2 are summarized in Table 4.

From the data given in Table 4 follows that compounds XI, XII, XIV, and X are compounds of low toxicity, and that compounds XIII, XV, IV, and V are moderately toxic according to classification [4].

In experiments with mice it was found that the pyridine derivatives have analgesic activity that is most distinct in compounds XII and XIII. Compound XV is less active. However, comparison of the doses of compound XII that cause toxic and analgesic effects points to the fact that it has a larger therapeutic width than compounds XIII and XV.

Pyridone-2 derivatives XIV, IV, and V do not give an effect on the TPS on thermal painful irritation. With respect to the expression of the analgesic effect the studied compounds do not surpass analgin, which was used as reference.

In experiments with anesthetized and unanesthetized rats it was shown that both in the case of intravenous and in the case of intragastric administration most of the compounds studied do not have an effect on the AP, with the exception of compounds XV and X.

Compound XV caused a drop in the AP in anesthetized rats on intravenous administration starting at a dose of 1 mg/kg (ΔAP = 15 mm Hg, duration of the effect 5-6 min); when the dose was increased to 2 and 5 mg/kg we observed a dose-dependent increase in hypotensive effect (the decrease in the AP was 25 and 35 mm Hg, respectively) and a lengthening of its duration (up to 10-15 min). On oral administration at a dose of 50 mg/kg compound XV caused a lowering of 20 mm Hg in the AP in unanesthetized SH rats for 1 h after administration, however, towards the second hour the AP increased to its starting value.

Compound X caused lowering of the AP in anesthetized rats only beginning at an intravenous dose of 5 mg/kg (ΔAP = 35 mm Hg), but the hypotensive effect lasted longer than with compound XV and went on for 25-30 min. During investigation of the effect of compound X on the AP of unanesthetized SH rats it was shown that the antihypertensive effect of the compound (lowering of the AP by 25-30 mm Hg) was observed for 2 h after administration, that is with respect to the duration of the effect compound X surpasses compound XV.

When analyzing the structure-activity relationship among the studied derivatives of pyridine and pyridone-2 it may be mentioned that introduction of a CN group at position 3 of the pyridine ring leads to lowering of the acute toxicity of the pyridine derivatives and that it increases that parameter for pyridone-2 derivatives. Moreover, change to the pyridone-2 derivative causes loss of antihypertensive and analgesic activities of the compounds.

LITERATURE CITED

1. N. S. Vul'fson, V. G. Zaikin, and A. I. Mikaya, Mass Spectrometry of Organic Compounds [in Russian], Moscow (1986), p. 134.
2. V. G. Granik, A. V. Grigor'ev, and M. K. Polievktov, Khim. Geterotsikl. Soedin., No. 11, 1523-1526 (1977).
3. L. V. Ershov and V. G. Granik, Khim. Geterotsikl. Soedin., No. 5, 646-649 (1985).
4. K. K. Sidorov, Toxicology of Novel Industrial Chemical Compounds [in Russian], Leningrad (1973).
5. N. I. Smetskaya, N. A. Mukhina, and V. G. Granik, Khim. Geterotsikl. Soedin., No. 6, 799-802 (1984).
6. N. Z. Tugusheva, L. V. Ershov, V. G. Granik et al., Khim.-farm. Zh., No. 7, 830-834 (1986).
7. W. C. Bowman, Trends Pharm. Sci., 5, 183-185 (1982).
8. R. D. Bunag and J. Butterfield, Hypertension, 4, No. 6, 898-903 (1982).
9. F. E. D'Amour and D. L. Smith, J. Pharmacol. Exp. Ther., 72, 74 (1941).
10. J. T. Litchfield, and J. R. Wilcoxon, J. Pharmacol. Exp. Ther., 96, 99-113 (1949).
11. K. Loffelboldz and W. Weide, Trends Pharm. Sci., 3, 147-149 (1982).
12. G. Woolfe and A. MacDonald, J. Pharmacol. Exp. Ther., 80, 300-308 (1944).