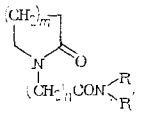
PHARMACOLOGICAL ACTIVITY OF ANALOGS AND CYCLOANALOGS OF PYRACETAM

R. Ya. Popova, T. A. Gudasheva,
S. S. Trofimov, R. U. Ostrovskaya,
and A. P. Skoldinov

UDC 615.214.3:547.745].7

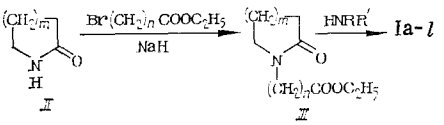
N-Carbamoylmethylbutyrolactam (pyracetam [1], nootropil [2]) is used in medicine as a nootropic agent, improving educability and increasing the resistance of the body to various types of severe stress. In order to arrive at an understanding of the mechanism of the nootropic activity of pyracetam, it was of interest to refine current ideas concerning structure–activity relationships in compounds related to pyracetam. In order to carry out comparative pharmacological studies, several analogs and cycloanalogs of pyracetam (Ia-*l*) have been obtained, differing in the size of the lactam ring, the length of the aliphatic chain, and the substituents on the nitrogen atom and in the side chain, including the corresponding hydrazides (Table 1).



I a: $m=2$, $n=1$, $R=R'=H$; b: $m=3$, $n=1$, $R=R'=H$; c: $m=4$, $n=1$, $R=$ $=R'=H$; d: $m=1$, $n=2$, $R=R'=H$; e: $m=2$, $h=2$, $R=R'=H$; f: $m=3$, $n=2$, $R=R'=H$; g: $m=n=1$, $R=R'=C_2H_5$; h: $m=n=1$, $R+R'=(CH_2)_5$; i: $m=n=1$, $R=H$, $R'=C_6H_5$; j: $m=n=1$, $R=H$, $R'=NH_2$; k: $m=1$, $n=2$, $R=H$, $R'=NH_2$; *l*: $m=3$, $n=2$, $R=H$, $R'=NH_2$.

Pyracetam contains an α-pyrrolidone moiety which is a cyclic form of GABA, which mediates inhibition of the central nervous system. In addition, the role of GABA-ergic mechanisms in the activity of pyracetam has recently been questioned [3] in view of the fact that it has no inhibitory or antispasmodic effects. The pyracetam molecule also contains a fragment of another neuromediator, glycine (as its amide). One aim of the present investigation was to obtain data enabling the extent of the participation of GABA-ergic and glycinergic mechanisms in the manifestation of nootropic activity in pyracetam to be assessed. For comparison, the pharmacological activity of (IIId, g) was also studied, these containing an ethoxycarbonyl group in the side chain in place of the amide group.

Compounds (Ia-*l*) were obtained as follows:



II a: $m=2$; IIb: $m=3$; IIc: $m=4$; IId: $m=1$; IIIa: $m=2$, $n=1$; IIIb: $m=3$, $n=1$; IIIc: $m=4$, $n=1$; IIId: $m=1$, $n=2$; IIIe: $m=n=2$; IIIf: $m=3$, $n=2$; IIIg: $m=n=1$.

Lactams (IIa-d) were converted into their sodio-derivatives by treatment with sodium hydride in benzene, completion of reaction being established by TLC. Alkylation with ethyl bromoacetate (n = 1) proceeded to completion even at room temperature, whereas completion of

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 12, pp. 1439-1445, December, 1983. Original article submitted December 23, 1982.

 0091-150X/83/1712-0858$07.50

TABLE 1. N-Carbamoylalkyllactams (Ia-f)

Compound	Yield, %	mp, °C[a]	R_f[b]	Found, %			Empirical formula	Calculated, %			IR spectrum, cm^{-1}			Mass spectrum
				C	H	N		C	H	N	ν_{CO} lactam	ν_{CO} amide	ν_{NH_2}	M^+
Ia	94	148—9	0,30	54,2	7,8	18,2	$C_7H_{12}N_2O_2$	53,9	7,7	17,9	1690	1615	3315, 3150	156
Ib	93	144—5	0,40	56,4	8,3	16,3	$C_8H_{14}N_2O_2$	56,4	8,2	16,4	1687	1615	3200, 3150	170
Ic	97	119—20	0,30	58,9	8,7	15,3	$C_9H_{16}N_2O_2$	58,7	8,7	15,2	1680	1615	3380, 3180	184
Id	77	142—3[d]	....	....	...	17,9	$C_7H_{12}N_2O_2$	...	...	17,9	...	...	... ...	...
Ie	70	168—70	0,28	56,2	8,3	16,2	$C_8H_{14}N_2O_2$	56,4	8,2	16,4	1678	1628	3357, 3166	...
If	64	140—1	0,30	58,6	8,7	15,3	$C_9H_{16}N_2O_2$	58,7	8,7	15,2	1689	1614	3323, 3170	...

[a]From isopropanol
[b]Grade II alumina, benzene—ethyl acetate—methanol, 4:4:1.
[c]In vaseline oil
[d]Literature value [7], mp 142-143°C.

TABLE 2. N-Ethoxycarbonylalkyllactams (III)

Compound	Yield, %	bp, °C (mm)	R_f[a]	Found, %			Empirical formula	Calculated, %		
				C	H	N		C	H	N
IIIa	68	124—6 (2—3)[b]	0,60	58,5	7,9	7,5	$C_9H_{15}NO_3$	58,4	8,1	7,6
IIIb	62	149—50 (5)[c]	0,65	60,7	8,4	7,1	$C_{10}H_{17}NO_3$	60,3	8,5	7,0
IIIc	70	132—3 (1—2)	0,70	62,0	9,3	6,8	$C_{11}H_{19}NO_3$	61,9	8,9	6,6
IIIe	50	118—20 (3)	0,60	60,1	8,8	7,0	$C_{10}H_{17}NO_3$	60,3	8,5	7,0
IIIf	34	133—5 (2—3)	0,80	62,0	8,7	6,6	$C_{11}H_{19}NO_3$	61,9	8,9	6,6

[a]On grade II alumina, benzene–ethyl acetate–methanol, 4:4:1.
[b]Literature bp [4] 126°C (2 mm).
[c]Literature bp [4] 125-126°C (1 mm).

the reaction with ethyl β-bromopropionate (n = 2) required heating on the water bath. The alkoxycarbonyl derivatives (IIIa-g) (Table 2) were isolated by vacuum distillation, or by chromatography on Al_2O_3. Aminolysis of the esters (IIIa-g) was effected by treating their solutions in methanol with gaseous ammonia, the highest yields (greater than 90%) being obtained when the reaction mixture was kept for 2-3 days at room temperature. Heating accelerated the reaction, but the yield then fell to 20-25%. The IR spectra of the 1-carbamoylalkyllactams contained carbonyl stretching absorption at 1678-1690 cm^{-1} (lactam C = O) and 1614-1625 cm^{-1} (amide I for the carbamoylalkyl group), together with two bands for stretching vibrations of the amino-group (Table 1). Mass-spectrometric examination of (Ia-c) showed that the mode of breakdown of the lactam ring was independent of its size, but on passing from the six-membered to the seven-membered lactam ring the stability of the molecular ion decreased, and the rate of breakdown of the molecules under electron impact increased (Table 3).

EXPERIMENTAL CHEMISTRY

IR spectra were obtained on a Perkin-Elmer 580 instrument (Sweden), in vaseline oil. Mass spectra were recorded on a Varian MAT-112 GC-MS (USA), source temperature T = 100-120°C, ionization energy 70 eV.

N-(ω-Ethoxycarbonylalkyl)lactams (IIIa-c) (General Method). To a suspension of 0.1 mole of sodium hydride in 100 ml of dry benzene was added a solution of 0.1 mole of the lactam in 100 ml of benzene, and the mixture was stirred for 2 h at ambient temperature. The mixture was cooled to 0°C, and 0.1 mole of the ω-bromoalkanecarboxylate ester was added slowly, dropwise, and the mixture stirred for 3 h at ambient temperature. The solvent was removed, and the residue distilled *in vacuo* or chromatographed on a column of alumina (eluent, benzene–ethyl acetate, 1:1). The yields and constants of the esters obtained are given in Table 2.

N-(2-Ethoxycarbonylethyl)butyrolactam (IIId). This was obtained similarly, bp 139-140°C (1 mm Hg); n_D^{20} 1.4700, yield 70%. Found %: C 58.41; H 8.38; N 7.74, $C_9H_{15}NO_3$. Calculated, %: C 58.38; H 8.10; N 7.56. Literature bp [5], 130-131°C (3 mm).

N-Ethoxycarbonylmethylbutyrolactam (IIIg). This was obtained as described in [6], bp 136-139°C (3 mm Hg); n_D^{20} 1.4660.

N-(ω-Carbamoylalkyl)lactams (I) (General Method). A solution of 0.03 mole of the N-(ω-ethoxycarbonylalkyl)lactam in 100 ml of methanol was saturated with gaseous ammonia, and kept for 2-3 days at ambient temperature. The sorbent was then removed and the residue recrystallized from isopropanol. The yields and properties of the resulting 1-carbamoylalkylactams are given in Table 1.

N-Piperidinocarbonylmethylbutyrolactam (Ih). To 0.125 mole of freshly-distilled piperidine was added 0.125 g of finely-divided metallic sodium, followed by the addition under nitrogen of 0.0375 mole of (IIIg). The temperature was then slowly raised until the sodium dissolved completely, then further raised to 120°C, kept at this temperature for 3 h, cooled, diluted with 35 ml of dry toluene, and hexane added until a solid separated which was filtered off to give 42% of (Ih), mp 94-95°C. Found, %: C 62,6; H 8.6; N 13.4. $C_{11}H_{18}N_2O_3$. Calculated, %: C 62.9; H 8.6; N 13.3.

TABLE 3. Relative Intensities of Fragment Ions in the Mass Spectra of N-(Carbamoylmethyllactams (Ia-c)*

m/e for ion i	I_i/I_{M^+}		
	Ia	Ib	Ic
$[M-OH]^+$	2,9	1,6	3,4
$[M-44]^+$	3,4	9,0	94
$[M-58]^+$	4,0	7,0	28
$[M-72]^+$	4,4	10,0	560

*The ratios of the molecular ion current to the total ion current $\frac{IM^+ \cdot 100}{\Sigma I}$ were, for (Ia), 6%, (Ib), 3%, and (Ic) 1%.

(Ig) was obtained as described in [6], yield 35%, bp 179-181°C (3-5 mm). Found, %: N 14.29. $C_{10}H_{10}N_2O_2$. Calculated, %: N 14.14.

N-Hydrazinocarbonylmethylcaprolactam (Iℓ). To a solution of 0.12 mole of hydrazine hydrate in 20 ml of isopropanol was added dropwise at 50°C 0.1 mole of (IIIf), the mixture boiled for 4 h, the solvent distilled off, and the residue treated with 20 ml of isopropanol After distillation of the solvent *in vacuo*, the residue was washed with ether to give (Iℓ) in 85% yield, mp 134-135°C (isopropanol). Found, %: C 54.2; H 8.6; N 21.2. $C_9H_{17}N_3O_2$. Calculated, %: C 54.3; H 8.6; N 21.1.

(Ij) and (Ik). These were obtained similarly. Yield of (Ij) 70%, mp 88-89°C (from isopropanol). Found, %: C 45.6; H 7.0; N 26.6. $C_6H_{11}N_3O_2$. Calculated, %: C 45.8; H 7.0; N 26.7. Literature mp [8], 58°C (isopropanol). The yield of (Ik) was 85%, mp 105-106°C (isopropanol) (literature value [9], 106-107°C).

EXPERIMENTAL PHARMACOLOGY

The compounds were initially studied using standard neurotropic screening tests for overall activity, effect on motor activity (recorded on an Optovarimex multichannel autograph) which was both spontaneous and amphetamine-stimulated (5 mg/kg), either suppressed by nembutal (30 mg/kg) or ethanol (2000 mg/kg), and changes in the hypnotic effect of thiopental-sodium (30 mg/kg). The effects on the following convulsive agents were examined: bicucullin (3 mg/kg), corazole (110 mg/kg), and strychnine (2.5 mg/kg). These compounds, together with nembutal or ethanol, were administered intraperitoneally, thiopental-sodium intravenously, and the amphetamine and convulsive agents subcutaneously. Based on the results obtained in a study of pyracetam [2, 10], the nootropic activity of its derivatives was assessed from two main criteria, namely, effect on resistance to oxygen starvation and on the training process disturbed by amnesia-producing treatment. In experiments on mice, the effects on the duration of life in a hermetically sealed chamber were determined (initial oxygen concentration, 8 vol. %). The effects of the test compounds on retrograde amnesia induced by an electroconvulsive shock (ES) were studied using a passive avoidance model in rats, utilizing the innate burrowing reflex of these animals [11]. The experimental chamber consisted of two sections, a larger one which was illuminated, and a smaller one which was darkened. In the darkened, usually preferred compartment, the animal received a painful electrical stimulus, thus setting up a passive avoidance conditioned reflex in a single training session. ES was applied immediately following training, followed by the administration of the test compound or saline solution. The difference in the time spent by the animal in the darkened compartment before training, and one day subsequent thereto (Δt) was measured. The amnesia-producing effect of ES resulted in the animal being unafraid of entering the dark chamber, resulting in a decrease in the value of Δt, and the anti-amnesia activity of the drug was expressed as an increase in Δt. The experiments utilized 155 rats. The statistical significance of the differences between the control and experimental groups was calculated by the Wilcoxon–Mann–Whitney method [12].

TABLE 4. Principal Features of the Neurotropic Activity of the Test Compounds

$OC(CH_2)_mCH_2N(CH_2)_nCOX$

Compound	m	n	X	Antihypoxemic effect[a]	Effect on spontaneous motor activity[b]	Effect on the convulsive effect of bicuculline[b]	Effect on barbiturate activity[b]	Change in background EEG[c]	Toxicity, g/kg
Pyracetam	1	1	NH_2	25/0,3	O	O	O	O	8,0
Ig	1	1	$N(C_2H_5)_2$	27/0,15	O	O	O		1,5
Ih	1	1	$N(CH_2)_5$	26/0,15	O	O	O		5,0
Ij	1	1	$NHNH_2$	28/0,1	↑	↓	↓	D	6,0
Ii	1	1	NHC_6H_5	25/0,1	↓	↓	↑	S	3,5
IIg	1	1	OC_2H_5	25/0,5			O		6,0
Id	1	2	NH_2	28/0,15	O	O	O		6,0
Ik	1	2	$NHNH_2$	27/0,15	N	↑	↓	D	6,0
IId	1	2	OC_2H_5	21/0,5			O		4,0
Ia	2	1	NH_2	25/0,3	O	O	O		6,0
Ie	2	2	NH_2	24/0,3	O		O	O	
Ib	3	1	NH_2	28/0,3	↓	↓	↑	S	4,5
If	3	2	NH_2	21/0,3	↓		↑	S	4,5
In	3	2	$NHNH_2$	22/0,3	↓			S	4,0
Ic	4	1	NH_2	32/1,0	↓	↓	↑	S	3,5

[a]The numerator denotes the lifespan in a hermetically sealed chamber (O_2 = 8 vol. %) (in min), and the denominator, the threshold dose for this effect (in g/kg). Control values, 20/1.2.

[b]For this and the remaining tests (other than EEG and toxicity), the results are given for a dose of 300 mg/kg. An arrow pointing upwards denotes an enhancement of the effect, downwards, a diminution, O no significant effect, and N, without stimulating spontaneous motor activity, these compounds reduced the depressant effect of nembutal on motor activity.

[c]D) Desynchronization of the EEG, S) synchronization, and O) no change.

In long-term experiments on rabbits with electrodes implanted in the sensomotor, parietal, and occipital regions of the cerebral cortex, the effects of the compounds on the background electrical activity of the brain and the arousal reaction induced by a sound stimulus were examined. Compounds containing the hydrazide group (Ij-m), with possible stimulant activity, were examined for their effects on depression in the EEG induced by nembutal (40 mg/kg by the intravenous route). Pyracetam was used as the standard drug in all the experiments. In agreement with the literature [2, 13], in these experiments pyracetam displayed no activity in the standard tests screening for neurotropic activity. It did not affect motor activity or the orientational reaction, nor did it influence the effects of depressant or stimulant drugs, or convulsive agents (Table 4). At the same time, it had a definite protective effect in hypoxemia, increasing the length of survival of animals in a hermetically sealed chamber. The threshold dose of pyracetam in this test was 300 mg/kg.

Examination of the properties of the cyclic homologs of pyracetam (Ia-c) revealed following facts: The six-membered homolog, like pyracetam itself, was inactive in the neurotropic screening tests, but had a definite antihypoxemic effect.

In the compounds with seven- and eight-membered rings, there were signs of depressant activity, since they substantially reduced motor activity, potentiated barbiturate activity, had moderate anticorazole properties, and induced synchronization of the EEG. The observed capacity of these compounds to increase resistance to oxygen deprivation appeared to be a consequence of their general depressant activity, with an accompanying reduction in energy requirements. These cyclic homologs of pyracetam had none of the anti-amnesia activity possessed by pyracetam itself. Thus, for a value of Δt in the controls of 43 sec, a dose of 200 mg/kg of pyracetam increased this index to 64.7 sec, whereas administration of (Ib) gave a value of 35 sec only.

Having established the importance of the pyrrolidine moiety in the neurotropic effect of pyracetam, we then attempted to determine how the activity was affected by modification of the side chain. A study of analogs of pyracetam with modified side chains (Id, Ig-k) showed them to retain nootropic activity, together with, in several cases, certain types of neurotropic activity. To judge from the threshold doses at which these compounds commenced to display antihypoxemic activity (Table 4), activity was greater in (Ij), and substantially so in the corresponding hydrazides (Ig-k). The hydrazides (Ig) and (Ik) were superior to pyracetam in anti-amnesia activity: In a dose of 200 mg/kg, pyracetam increased Δt to 64.7 sec (43 sec in the controls, $P < 0.05$), whereas after administration of (Ik) in a dose of 200 mg/kg the value of Δt increased to 75.1 sec ($P < 0.001$), and in a dose of (Ig) of 100 mg/kg, it rose to 95.1 sec ($P < 0.010$). The incorporation of the hydrazine moiety in the side chain also resulted in the appearance of clear signs of stimulant activity, manifested as a increase in spontaneous motor activity, marked antagonism to barbiturates (from the effects on the EEG), and enhancement of the effects of convulsive agents. Compound (Ii), which carries a phenyl substituent on nitrogen in the side chain, displayed, in addition to a clear nootropic effect, depressant activity, since it potentiated barbiturate activity, caused synchronization of the EEG, and had anticonvulsive properties. Replacement of the amide group in pyracetam and its homologs (IIId, g) resulted in a diminution in antihypoxemic activity.

The results of earlier electrophysiological experiments showed that pyracetam enhances GABA-ergic inhibition in the cerebral cortex [14]. In this connection, pyracetam was found to have a protective effect against cortical convulsive activity induced by bicucullin, a GABA-receptor blocker (local application to the surface of the cerebral cortex), but not against that induced by strychnine, a blocker of glycinergic receptors. These observations, which demonstrate the more important role of the GABA-ergic component in the activity of pyracetam as compared with the glycinergic component, are in accordance with the present findings that nootropic activity is lost in compounds in which the side chain is the same, but differ from pyracetam in the structure of the cyclic moiety. This is also shown, it would appear, by the results of our comparison of the effects of α-pyrrolidone as an analog of the cyclic portion of the pyracetam molecule, and glycinamide, which is a fragment of the side chain. It was found that in a dose of 120 mg/kg, equimolar to a dose of 200 mg/kg of pyracetam, α-pyrrolidone had no antihypoxic activity, and did not decrease the amnesia-producing effects of ES, although if the dose of pyrrolidone was increased by a factor of two or three it showed antihypoxic effects (the lifespan of the animals in the hermetically-sealed chamber was increased by 25-30%). In a dose of 360 mg/kg, α-pyrrolidone displayed definite anti-amnesia activity, increasing Δt to 60.3 sec as compared with 43 sec in the controls. In a dose equimolar to 200 mg/kg of pyracetam, and in a dose three times greater, glycinamide displayed neither antihypoxic nor anti-amnesia properties (Δt 24.1 and 33.7 sec, respectively).

The conclusion may therefore be drawn that the pyrrolidone moiety plays a predominant part in the occurrence of nootropic activity in pyracetam. Activity is retained in the six-membered ring homolog, but further enlargement of the ring results in the disappearance of selective nootropic activity. When the side chain of pyracetam is modified (chain length increased by one methylene group, substituents on nitrogen), antihypoxic and anti-amnesia activity is retained, and in some instances increased. In addition, depending on the structure of the substituent, additional neurotropic properties are found which are absent in pyracetam (a depressant effect in the N-phenyl derivative, stimulant effects in the hydrazides, etc.).

LITERATURE CITED

1. M. D. Mashkovskii, L. F. Roshchina, A. I. Polezhaeva, et al., Khim.-farm. Zh., No. 8, 132-139 (1977).
2. C. Giurgea, Act. Pharmacol., 25, 115-156 (1972); J. Pharmacol. (Paris), 3, 17-30 (1972).
3. C. Giurgea, in: Medical Chemistry Y. Amsterdam (1977), pp. 195-203.
4. Jpn. Pat. No. 76, 110,031; Chem. Abstr., 86, 66840f (1977).
5. Akira Yokoo and Shiro Morosawa, Nippon Kagaku Zasshi, 77, 599-602 (1956).
6. W. Reppe, Ann., 596, 80-158 (1955).
7. British Pat. No. 1.039 113. Chem. Abstr., 65, 1280a (1966).
8. French Pat. No. 2,361,882; Chem. Abstr., 90, 22796z (1979).
9. West German Pat. No. 2,745,907; Chem. Abstr., 91, 56814g (1979).
10. L. F. Roshchina and R. U. Ostrovskaya, Farmakol. Toksikol., No. 2, 210-213 (1981).
11. J. Bures and O. Buresova, J. Comp. Physiol. Psychol., 56, 268-277 (1963).

12. E. V. Gubler, Numerical Methods of Analysis and the Diagnosis of Pathological Processes [in Russian], Leningrad (1978).
13. M. D. Mashkovskii, L. F. Roshchina, and A. I. Polezhaeva, Farmakol. Toksikol., No. 6, 676-683 (1977).
14. R. U. Ostrovskaya, G. M. Molodavkin, and G. I. Kovalev, Byull. Eksp. Biol., No. 4, 62-64 (1982).

1,6-BIS-[2-CHLOROETHYLAMINO]-1,6-DIDESOXY-D-MANNITOL SALT OF ADENOSINE-5'-TRIPHOSPHATE. SYNTHESIS AND ANTITUMOR ACTIVITY

I. L. Isakov, I. I. Mircheva, and T. P. Pantev

UDC 615.277.3:547.963.32].012.1

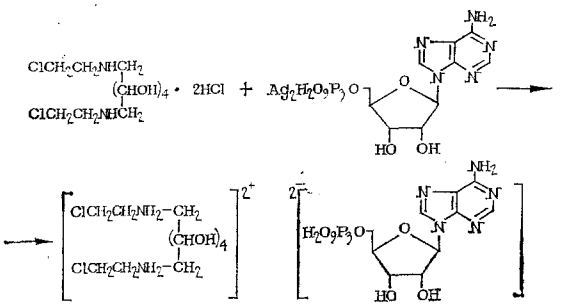
It has been shown in several investigations [1-3] that the use of adenosine-5'-triphosphate (ATP) as the anionic component of basic drugs reduces toxicity, and frequently improves their pharmacological activity.

1,6-Bis-(2-chloroethylamino)-1,6-didesoxy-D-mannitol hydrochloride (degranol, BMC) is known to possess high antitumor activity [4, 5]. Clinically, good therapeutic results have been obtained in the treatment of patients with chronic myeloid and lymphoid leukemia, lymphogranuloematosis, and lymphosarcomas [6].

As with other cytostatic agents which are alkylating agents, the high toxicity of degranol is an obstacle to its more widespread use. On the assumption that the cytostatic activity of bis-(2-chloroethylamines) is due to their alkylating properties [7], modification of the degranol molecule in order to reduce its toxicity is of considerable interest.

We here describe the synthesis of the adenosine-5'-triphosphate salt of 1,6-bis-(2-chloroethylamino)-1,6-didesoxy-D-mannitol, and the cytostatic properties of this drug (degratef) in mice with transplanted tumors (leukemia L-1210 and Lewis lung tumor), in comparison with the corresponding doses of degranol.

The synthesis of degratef was accomplished by reacting the silver salt of ATP with 1,6-bis-(2-chloroethylamino)-1,6-didesoxy-D-mannitol dihydrochloride (DHC), as follows:



An aqueous solution of the silver salt of ATP was mixed with DHC with vigorous stirring and cooling. The resulting compound was isolated by precipitation with cold methanol, followed by filtration, washing with acetone and ether, and drying over P_2O_5 *in vacuo*.

Scientific Institute for Roentgenology and Radiobiology, and the Scientific Institute for Oncology, Academy of Medicine, Sofia, People's Republic of Bulgaria. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 12, pp. 1446-1448, December, 1983. Original article received December 14, 1982.

 0091-150X/83/1712-0864$07.50