ANALOGS OF PURINE NUCLEOSIDES AND PURINE MONO- AND POLYNUCLEOTIDES

I. SYNTHESIS OF PHOSPHATE ESTERS OF 6-SUBSTITUTED 9-(α,ω-DIHYDROXY-2-ALKYL)PURINES

S. A. Giller, I. N. Goncharova,
I. N. Getsova, L. I. Mironova,
É. I. Bruk, G. F. Nazarova,
and L. N. Petrulyanis

UDC 547.963.32'857.7

The phosphorylation of 6-substituted 9-(α,ω-dihydroxy-2-alkyl) purines with β-cyanoethyl phosphate and polyphosphoric acid was investigated. Preparative methods for the preparation of mono-, di-, and cyclophosphates were developed, and the promising character of the use of polyphosphoric acid for the preparation of phosphate esters in the hypoxanthine and adenine series with a protected exoamino group is shown.

In correspondence with the idea expressed in 1962 [1], the synthesis of model analogs of nucleic acids with retention of the natural pyrimidine and purine bases and the polyelectrolyte character of the macromolecular chain containing conformationally similar α,ω-polymethylenediol substituents in place of a sugar residue seems of definite interest. When such model analogs are associated with nucleic acids, they may affect certain functions of the latter and thereby cause important biological effects. In this connection, we accomplished a series of studies involving the synthesis and investigation of the physical and biochemical properties of model analogs of pyrimidine nucleosides and pyrimidine mono- and polynucleotides [2, 3].

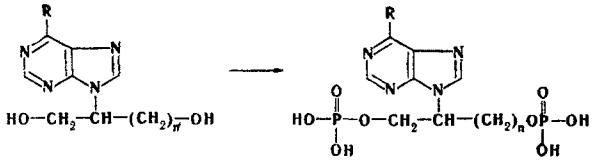
With the present communication we open up a new series of studies with a report of the results of research on the synthesis of model analogs of purine nucleosides and purine mono- and polynucleotides and of a study of their effect on the molecular biological level.

The first paper of this series is devoted to a study of phosphate esters of 6-substituted 9-(α,ω-dihydroxy-2-alkyl)purines. (See scheme on following page.)

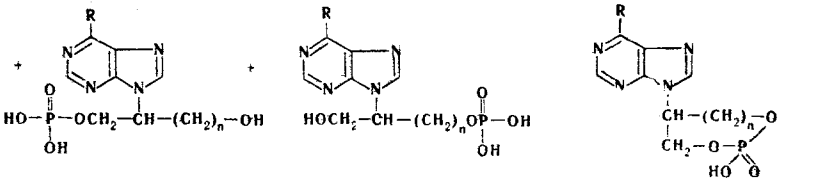
The starting model analogs of adenosine and inosine — L-6-amino-9-(1,4-dihydroxy-2-butyl)- (I), 6-amino-9-(1,3-dihydroxy-2-propyl)- (II), 6-hydroxy-9-(1,4-dihydroxy-2-butyl)- (III), and 6-hydroxy-9-(1,3-dihydroxy-2-propyl)purine (IV) — were synthesized by the method in [4]. β-Cyanoethyl phosphate and polyphosphoric acid (PPA) were used as the phosphorylating agents.

We first studied the phosphorylation of dihydroxybutyl derivative I with β-cyanoethyl phosphate in the presence of dicyclohexylcarbodiimide in anhydrous pyridine. The method used to carry out the phosphorylation of I-IV differs little from the method [5] used in the phosphorylation of adenosine. However, a substantial difference is, of course, observed in the formation of the phosphorylation products. Thus an investigation of an analytical sample with columns filled with DEAE-Sephadex A-25 by stepwise gradient elution with a triethylammonium acetate buffer showed that the reaction mixture after phosphorylation and subsequent hydrolysis contains 55% 6-amino-9-(1,4-dihydroxy-2-butyl)purine 1',4'-diphosphate (V) with R_f 0.25 and 25% monophosphorylated products, of which two separately emerging substances have identical R_f values (0.47) and correspond to 6-amino-9-(1,4-dihydroxy-2-butyl)purine 1'-monophosphate (VI) and 4'-monophosphate (VII), whereas the third compound has R_f 0.65. From the electrophoresis data, in analogy with the arrangement

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 12, pp. 1674-1679, December, 1974. Original article submitted October 17, 1973.



I–IV, XIX, XXIV, XXV V, IX, XII, XVI, XX, XXVI, XXIX



VI, X, XIII, XVII, XXI VII, XIV, XXII VIII, XI, XV, XVIII, XXIII, XXVII

I, II, V–XI R = NH_2; III, IV, XII–XVIII R = OH; XIX–XXIII R = $NHCH_2C_6H_5$; XXIV–XXVII R = $NHCOC_6H_5$

on the chromatogram of spots corresponding to natural nucleotides, the 6-amino-9-(1,4-dihydroxy-2-butyl)-purine 1',4'-cyclophosphate structure (VIII) was assigned to the latter; this was subsequently confirmed by a study of the protolysis constants (VIII is a monobasic acid with pK ~ 1) and by hydrolysis.

We were able to isolate diphosphate V, a mixture of monophosphates VI and VII, and 1',4'-cyclophosphate VIII (Table 1) by chromatography of the reaction mixture with Dowex-50 ion-exchange resin (H^+ form).

The corresponding 1',3'-diphosphate (IX), monophosphate (X), and 1',3'-cyclophosphate (XI) were obtained by phosphorylation of dihydroxypropyl derivative II with β-cyanoethyl phosphate in the presence of dicyclohexylcarbodiimide and subsequent chromatographic separation on Dowex-50 (H^+ form).

In order to find the optimum reaction conditions, we investigated the phosphorylation of 9-(1,3-dihydroxy-2-propyl)adenine (II) at various reagent ratios. The reaction mixture was investigated on QAE-Sephadex with stepwise gradient elution with an aqueous ethanol solution of $(NH_4)_2CO_3$ containing 10% ethanol (its optimum concentration was selected experimentally). The compounds were identified by means of electrophoresis and paper chromatography.

The dependence of the yields of the phosphate esters on the amount of dicyclohexylcarbodiimide for a constant ratio of the phosphorylating agent to the starting dihydroxypropyl compound was investigated, and it was shown that the highest yields of diphosphate IX (~ 70%) and monophospate X (~ 20%) were obtained for a diol–β-cyanoethyl phosphate–dicyclohexylcarbodiimide ratio of 1 : 6 : 6. Under these conditions, the yields of cyclophosphate and pyrophosphates turn out to be quite insignificant. However, the pyrophosphate content increases to 20% when the reaction mixture is treated with water rather than with aqueous pyridine.

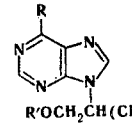
An investigation of the phosphorylation at a constant dicyclohexylcarbodiimide–β-cyanoethyl phosphate ratio and in the presence of variable amounts of β-cyanoethyl phosphate showed that 1',3'-cyclophosphate XI and monophosphate X (in yields of ~ 30 and 20%, respectively) can be obtained at a diol–β-cyanoethyl phosphate–dicyclohexylcarbodiimide ratio of 1 : 1 : 5.

The optimum conditions for the preparation of 6-hydroxy-9-(1,3-dihydroxy-2-propyl)purine monophosphate (XVII) by phosphorylation of diol IV are a diol IV–β-cyanoethyl phosphate–dicyclohexylcarbodiimide ratio of 1 : 2 : 10 and a reaction time of 4 h.

Considering the above-indicated difficulties encountered in the preparation of phosphate esters by phosphorylation of I-IV with β-cyanoethyl phosphate, polyphosphoric acid (PPA) was used in the phosphorylation of hypoxanthine derivatives III and IV. The colorless crystalline substance that was isolated from the reaction mixture proved to be a mixture of several compounds with different R_f values. Analysis of the product of phosphorylation of III on QAE-Sephadex with a solution of ammonium carbonate as the eluent showed that the chief component is 6-hydroxy-9-(1,4-dihydroxy-2-butyl)purine 1',4'-diphosphate (XII). In addition, the mixture also contains 6-hydroxy-9-(1,4-dihydroxy-2-butyl)purine 1'-monophosphate (XIII), the 4'-monophosphate (XIV), cyclophosphate XV, and various pyrophosphates.

We were able to isolate fractions containing diphosphate XII, a mixture of monophosphates XIII and XIV, and cyclophosphate XV by chromatography of the reaction mixture obtained as a result of phosphorylation of diol III and subsequent hydrolysis on an ion-exchange resin.

TABLE 1



Compound	R	R'	R''	n	mp, °C	R_f[a]	Empirical formula	Found, %			Calculated, %			UV spectrum		Yield, %
								C	H	N	C	H	N	λ_{max}, nm	$\varepsilon \cdot 10^{-3}$	
X	NH_2	$PO(OH)_2$	$PO(OH)_2$	2	284—286	0,25	$C_9H_{15}N_5O_8P_2$[b]	28,6	4,2	18,1	28,2	3,9	18,3	261	19,6	54
V	NH_2	H	$PO(OH)_2$	2	170	0,47	$C_9H_{14}N_5O_5P$	35,3	4,9	22,8	35,7	4,7	23,1	262	17	6
VI	NH_2	Cyclophosphate		2	320[c]	0,65	$C_9H_{12}N_5O_4P$	37,5	3,9	23,8	37,9	4,2	24,5	260	12,5	6
VIII	NH_2	$PO(OH)_2$	$PO(OH)_2$	1	272—275	0,21	$C_8H_{13}N_5O_8P_2$	26,7	3,6	18,4	26,0	3,5	18,9	261	14	57
IX	NH_2	H	$PO(OH)_2$	1	136—138	0,42	$C_8H_{12}N_5O_5P$	33,1	4,3	24,5	33,2	4,2	24,2	262	17	17
XI	NH_2	Cyclophosphate		1	300[c]	0,61	$C_8H_{10}N_5O_4P$	35,0	3,6	25,4	35,4	3,7	25,8	260	13,5	10
XII	OH	$PO(OH)_2$	$PO(OH)_2$	2	275	0,25	$C_9H_{14}N_4O_9P_2 \cdot {}^1/_2H_3PO_4$	25,7	3,9	12,4	25,0	3,6	12,9	252	12	63
XIII	OH	H	$PO(OH)_2$	2	227	0,40	$C_9H_{13}N_4O_6P \cdot {}^1/_2H_2O$	34,6	4,0	17,7	34,8	4,4	18,1	252	11,3	11
XV	OH	Cyclophosphate		2	300[c]	0,62	$C_9H_{11}N_4O_5P \cdot H_2O$[d]	35,0	4,0	17,9	35,5	4,3	18,4	250	14,5	10
XVI	OH	$PO(OH)_2$	$PO(OH)_2$	1	230	0,24	$C_8H_{12}N_4O_9P_2 \cdot {}^1/_2H_3PO_4$	23,3	3,6	12,6	22,9	3,3	13,3	250	11	40
XVII	OH	H	$PO(OH)_2$	1	218[c]	0,42	$C_8H_{11}N_4O_6P \cdot H_2O$[e]	31,2	4,0	18,1	31,1	4,2	18,3	252	10	22
XVIII	OH	Cyclophosphate		1	330[c]	0,62	$C_8H_9N_4O_5P \cdot H_2O$	32,6	3,3	18,6	33,1	3,7	19,2	250	12,5	16
XXVI	$NHCOC_6H_5$	$PO(OH)_2$	$PO(OH)_2$	1	227	0,47	$C_{15}H_{17}N_5O_9P_2 \cdot {}^1/_2H_3PO_4$	34,9	3,8	12,7	34,5	3,6	13,4	278	25,5	77
XXIX	$NHCOC_6H_5$	$PO(OH)_2$	$PO(OH)_2$	2	215	0,48	$C_{16}H_{19}N_5O_9P_2 \cdot H_3PO_4$	34,1	4,1	11,9	33,4	3,9	12,2	278	24	44

[a] With a 1 M ammonium acetate–ethanol system (1 : 1) and Whatmann I paper.

[b] Found: P 16.2%. Calculated: P 16.2%.

[c] With decomposition.

[d] Found: P 10.7%. Calculated: P 10.5%.

[e] Found: P 10.2%. Calculated: P 10.3%.

The 1',3'-diphosphate (XVI), 1'-monophosphate (XVII), and 1',3'-cyclophosphate (XVIII) of 6-hydroxy-9-(1,3-dihydroxy-2-propyl)purine were obtained by phosphorylation of dihydroxypropylhypoxanthine IV with PPA and subsequent hydrolysis and separation of the phosphorylation product on an ion-exchange resin.

The phosphate esters isolated in this manner proved to be electrophoretically homogeneous. Monophosphates VI, X, XIII, and XVII and diphosphates V, IX, XII, and XVI are stable in acidic and alkaline media, while cyclophosphates VIII, XI, XV, and XVIII under certain conditions are capable of being hydrolyzed. Thus when they are heated in 1 N solutions of HCl and NaOH at 100° for 3 h and in 0.4 N $Ba(OH)_2$ solution, they are hydrolyzed quantitatively to the corresponding monophosphate esters.

In connection with the task at hand – synthesis of model analogs of poly- or oligonucleotides – it became urgently necessary to obtain mono- and diphosphate esters I and II with protected amino groups.

We accomplished the syntheses of these compounds either by acylation of diphosphates I and II or by phosphorylation of N-acylated diols XIX, XXIV, and XXV. For this, we synthesized and phosphorylated 6-benzylamino-9-(1,4-dihydroxy-2-butyl)purine (XIX). Chromatographic separation with a column filled with DEAE-Sephadex of the mixture of products obtained in the phosphorylation of XIX with β-cyanoethyl phosphate in anhydrous pyridine in the presence of dicyclohexylcarbodiimide showed that 6-benzylamino-9-(1,4-dihydroxy-2-butyl)purine 1',4'-diphosphate (XX) is formed in yields up to 88%, whereas the 6-benzylamino-9-(1,4-dihydroxy-2-butyl)purine 1'-monophosphate (XXI), 4'-monophosphate (XXII), and 1',4'-cyclophosphate (XXIII) constitute only 9% of the yield.

Considering that we were able to obtain the corresponding diphosphates and monophosphates by phosphorylation of hypoxanthinediols III and IV by menas of PPA in sufficiently high yields and having in view the stability of the benzoyl protection of the amino group of purines relative to acid hydrolysis, we synthesized 6-benzamido-9-(1,3-dihydroxy-2-propyl)purine diphosphate and monophosphate (XXIV) and the corresponding butyl derivative (XXV) also with PPA. The chromatographic separation of the mixture of products of phosphorylation of dihydroxypropylpurine XXIV on Dowex-50 resin (H^+ form) made it possible to isolate diphosphate XXVI in 77% yield, as well as fractions containing 6-benzamido-9-(1,3-dihydroxy-2-propyl)purine 1',3'-cyclophosphate (XXVIII) and 1'-monophosphate (XXVII).

Similarly, phosphorylation of XXV gave 6-benzamido-9-(1,4-dihydroxy-2-butyl)purine 1,4-diphosphate (XXIX) in 44% yield.

Thus a comparison of the results of phosphorylation of the analogs of purine nucleosides described in this paper by means of β-cyanoethyl phosphate and PPA enable one to draw the following conclusions: having in view the simplicity and convenience of the synthesis, polyphosphoric acid should be preferred over β-cyanoethyl phosphate for the phosphorylation of 6-hydroxy- (IV) and 6-benzamido-9-(1,3-dihydroxy-2-propyl)purine (XXIV) and their butyl analogs (III and XXV). Insofar as 9-(1,3-dihydroxypropyl)-(II) and 9-(1,4-dihydroxybutyl)adenine (I) are concerned, phosphorylation with β-cyanoethyl phosphate is more efficient.

EXPERIMENTAL

The UV spectra were recorded with a Specord UV-Vis spectrophotometer. A 1 M ammonium acetate–ethanol system (1 : 1) was used for the paper chromatography. The chromatograms were developed in UV light with a UPM apparatus. Electrophoresis on FN-3 paper in a phosphate buffer with pH 7.5 (0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4) was accomplished with a gradient voltage of 700 V.

Phosphorylation of 6-Amino-9-(1,4-dihydroxy-2-butyl)purine (I) with β-Cyanoethyl Phosphate. A 1.3 g (0.0058 mole) sample of I was added to 30 ml of a standard solution of a pyridinium salt containing 5.28 g (0.035 mole) of β-cyanoethyl phosphate in dry pyridine, and the mixture was evaporated to dryness. The residue was dissolved in 180 ml of dry pyridine, a solution of 8.2 g (0.04 mole) of dicyclohexylcarbodiimide in 10 ml of pyridine was added, and the reaction mixture was held at room temperature for 2 days. Water (4 ml) was then added, and the mixture was allowed to stand for 1.5 h. The resulting mass was evaporated to dryness, 80 ml of water was added, and the mixture was re-evaporated. A total of 200 ml of 0.4 N LiOH was added to the residue, and the mixture was refluxed for 45 min. It was then cooled, the precipitate was removed by filtration, and the filtrate was passed through a column containing Dowex-50 (H^+ form). The fractions containing diphosphate V, with R_f 0.25, were evaporated to 50 ml, and the precipitated V was removed by filtration to give 1.2 g (54%) of a colorless crystalline substance with mp 284-286° and R_f 0.25 that was soluble in water and DMSO but insoluble in ethanol, pyridine, and ether.

The fractions with R_f 0.47 corresponding to the monophosphates were evaporated. The oily residue was repeatedly treated with ether and ethanol. Compound VI began to crystallize out of the mixture on prolonged standing. Workup gave 0.1 g (6%) of a product with mp 168-170°.

The fractions with R_f 0.65 corresponding to cyclophosphate VIII were evaporated and worked up similarly to give 0.11 g (6%) of VIII as a colorless crystalline substance with mp > 320°.

Phosphorylation of 6-Amino-9-(1,3-dihydroxy-2-propyl)purine (II) with β-Cyanoethyl Phosphate. The reaction of 1.2 g (0.005 mole) of II, 30 ml of a standard solution of a pyridinium salt containing 5.28 g (0.035 mole) of β-cyanoethyl phosphate, and 6.2 g (0.03 mole) of dicyclohexylcarbodiimide was carried out as in the phosphorylation of dihydroxybutylpurine I. The fractions with R_f 0.21 were evaporated to 50 ml to precipitate colorless crystalline diphosphate IX, which is soluble in water, DMSO, and DMFA and slightly soluble in ethanol, ether, and pyridine. The products [0.97 g (57%)] had mp 272-275° and R_f 0.21. Monophosphate X and 6-amino-9-(1,3-dihydroxy-2-propyl)purine 1',3'-cyclophosphate (XI) were obtained by evaporation of the fractions with Rf 0.42 and 0.61 and prolonged treatment of the oily residues to give 0.22 g (17%) of monophosphate X with mp 136-138° and 0.15 g (10%) of cyclophosphate XI with mp > 300°.

Phosphorylation of 6-Hydroxy-9-(1,4-dihydroxy-2-butyl)purine (III) with Polyphosphoric Acid. A 0.65-g (0.003 mole) sample of III was added with stirring to PPA obtained from 2.3 ml of 85% H_3PO_4 and 3.0 g of P_2O_5, and the mixture was held at room temperature for 30 min and at 40° for 1 h. It was then cooled to 0°, alcohol–ether (1 : 1) was added, and the mixture was stirred for 30 min. The precipitate was treated several times with ether and removed rapidly by filtration. A total of 50 ml of 1 N HCl was added to the residue, and the mixture was heated on a water bath for 1 h and evaporated. The residue was dissolved in 80 ml of water, and the solution was passed through a column containing Dowex-50 resin (H^+ form) and eluted with water. The fractions with R_f 0.25 (165 ml) were evaporated to dryness to isolate 0.7 g (63%) of diphosphate XII with mp 273-275°. The fractions with R_f 0.62 (30 ml) were evaporated and treated repeatedly with ether and ethanol to isolate 0.1 g (10%) of cyclophosphate XV with mp > 330° (dec.). The fractions containing the formylation product with R_f 0.40 (100 ml) were evaporated. Monophosphate XIII began to crystallize to give 0.15 g (11%) of a product with mp 226-227° after prolonged standing of the oily residue and treatment of it with acetone, alcohol, and ether.

Phosphorylation of 6-Hydroxy-9-(1,3-dihydroxy-2-propyl)purine (IV) with Polyphosphoric Acid. The reaction was carried out as in the case of the phosphorylation of dihydroxybutylpurine III. A 0.52-g (0.0025 mole) sample of IV was added to PPA obtained from 2.0 ml of H_3PO_4 and 2.5 g of P_2O_5. The fractions with R_f 0.24 (100 ml) were evaporated, and the residue was treated with acetone and ethanol to give 0.36 g (40%) of diphosphate XVI with mp 228-230°. The fractions with R_f 0.62 (30 ml) were evaporated, and the oily residue of cyclophosphate XVIII began to crystallize on prolonged standing and treatment with acetone and ethanol to give 0.11 g (16%) of a product with mp > 330° (dec.). The fractions with R_f 0.42 (145 ml) were evaporated to give 0.16 g (22%) of monophosphate XVII with mp 216-218°. In addition, mixtures of phosphates XVI and XVIII (0.1 g) and phosphates XVIII and XVII (0.15 g) were isolated from the intermediate fractions. The mixtures were separated on QAE-Sephadex in the bicarbonate form. Monophosphate XVII was eluted with a 0.1 M solution of ammonium bicarbonate containing 10% ethanol, whereas cyclophosphate XVIII and diphosphate XVI were eluted with 0.2 M and 0.3 M solutions of ammonium bicarbonate containing 10% ethanol.

Phosphorylation of 6-Benzylamino-9-(1,4-dihydroxy-2-butyl)purine (XIX) with β-Cyanoethyl Phosphate. A solution of 0.35 g (0.0013 mole) of XIX in 10 ml of pyridine was added to 10 ml of a standard solution of the pyridinium salt of β-cyanoethyl phosphate containing 1.76 g (0.011 mole) of β-cyanoethyl phosphate in dry pyridine, after which the pyridine was removed by vacuum distillation to dryness. Dry pyridine (20 ml) and a solution of 2.8 g (0.014 mole) of dicyclohexylcarbodiimide in 5 ml of pyridine were then added, and the mixture was held at room temperature for 2 days. Water (3 ml) was then added, and the solution was allowed to stand for 1 h, after which it was vacuum evaporated to dryness. The residue was heated on a water bath for 45 min with 50 ml of 0.1 N NH_4OH, after which it was cooled to 0°. The resulting precipitate was removed by filtration, and the filtrate was vacuum evaporated to dryness. The residue was treated twice with 1 N NH_4OH and re-evaporated. According to the results of separation of the residue with a column containing DEAE-Sephadex with elution with ammonium bicarbonate, it was found that the yield of XX, with R_f 0.58, is 88%. The mixture of monophosphorylation products constituted 9% of the overall yield.

Phosphorylation of 6-Benzamido-9-(1,3-dihydroxy-2-propyl)purine (XXIV) with Polyphosphoric Acid. A 0.5-g (0.0016 mole) sample of XXIV was added with stirring to PPA obtained from 2.5 ml of H_3PO_4 and

3.1 g of P_2O_5, and the mixture was heated at 60° for 2 h. It was then cooled and treated with ether–ethanol (1 : 1, three 100-ml portions). The solid material was removed by filtration and dissolved in water, and the solution was passed through Dowex-50 resin (H^+ form) with elution with water to give fractions with R_f 0.47 (50 ml, diphosphate XXVI), R_f 0.75 (100 ml, cyclophosphate XXVII), and R_f 0.63 (80 ml, monophosphate XXVIII). Evaporation of the fraction with R_f 0.47 and treatment of the oily residue with acetone and ethanol gave 0.59 g (77%) of diphosphate XXVI with mp 226-227°. Evaporation of the fractions containing mono- and cyclophosphates gave oily residues, which could not be crystallized.

A similar procedure was used to obtain 6-benzamido-9-(1,4-dihydroxy-2-butyl)purine 1',4'-diphosphate. The product [0.4 g (44%)] had mp 213-215° and R_f 0.48.

LITERATURE CITED

1. S. A. Giller, Status and Aims of Scientific Research on the Synthesis of Physiologically Active Polymeric Substances [in Russian], Riga (1962).
2. S. A. Giller, R. A. Zhuk, M. Yu. Lidak, A. É. Berzinaya, Ya. Ya. Shluke, and I. N. Goncharova, and I. N. Getsova, Summaries of Papers Presented at the Seventh International Symposium on the Chemistry of Natural Compounds [in Russian], Zinatne, Riga (1970), p. 235.
3. S. A. Giller, L. A. Sherin', R. A. Zhuk, and A. É. Berzinya, Khim. Geterotsikl. Soedin., 1671 (1974).
4. M. Yu. Lidak, Ya. Ya. Shluke, B. V. Zarinya, and S. E. Poritere, Khim. Geterotsikl. Soedin., 262 (1971).
5. G. M. Tener, J. Amer. Chem. Soc., 83, 159 (1961).
6. M. Ikehara and E. Ohtsuka, Chem. Pharm. Bull., Tokyo, 9, 27 (1961).