phosphates.[7] The ultraviolet spectra (obtained by eluting the spots with 0.01 *N* hydrochloric acid) of these products were identical with that of guanosine 5′-phosphate. Hydrolysis with 0.1 *N* hydrochloric acid at 100° for 10 minutes gave, in both cases, guanine.

Paper electrophoresis of Product A revealed the presence of six ultraviolet-absorbing components. Three of these components were identified as guanine, guanosine and guanosine 5′-phosphate. The other three spots had mobilities relative to guanosine 5′-phosphate of 0.6, 1.2 and 1.7, the higher mobilities corresponding to those of guanosine 5′-di- and triphosphate,[7] respectively.

(b) **Ion-exchange Analysis.**—The following standard procedure was employed for quantitative analysis of the products obtained above. A 15-mg. sample of the lyophilized material was dissolved and the solution, after adjusting the *p*H to 8.5 with 1 *N* ammonium hydroxide, was applied to the top of a Dowex 2-chloride form (200–325 mesh) column (3 cm. × 0.8 cm. diameter). The column was washed first with water and then with 0.003 *N* hydrochloric acid to remove guanine and guanosine. Elution was then carried out using the linear gradient technique.[7] Five-ml. fractions were collected, using an automatic fraction cutter, and the optical density of the individual fractions at 260 mμ was determined using a Beckman spectrophotometer, model DU. The two peaks which emerged from the column after guanosine 5′-phosphate (GMP) were designated as G-X and G-Y. These two peaks corresponded in their positions to guanosine 5′-di- and triphosphate,[7] respectively. The results of these analyses are shown in Table II.

TABLE II

ION-EXCHANGE ANALYSIS OF PRODUCTS OF PHOSPHORYLATION OF 2′,3′-*O*-ISOPROPYLIDENE GUANOSINE WITH PHOSPHORUS OXYCHLORIDE

Product	% of total topical density at 260 mμ				
	Guanine	Guanosine	Guanosine 5′-phosphate	G-X	G-Y
A	1	49	31	14	5
B	3	61	17	18	1
C	4	55	23	17	1
D	4	52	19	16	9

Isolation of G-X and G-Y.—One gram of the lyophilized solid (Product A, see above) was triturated twice with 15-ml. portions of methyl alcohol to remove most of the inorganic salt (lithium chloride). The residual solid was dissolved in 25 ml. of water, adjusted to *p*H 8.5 with 1 *N* ammonium hydroxide and applied to a column (4 cm. × 2.2 cm. diameter) of Dowex-2 chloride form. The procedure used was as described in the preparation of guanosine 5′-di- and triphosphates.[7] The following fractions were obtained: guanosine 5′-phosphate; with 0.003 *N* HCl + 0.015 *M* NaCl (1,100 ml., 3,077, O.D. units at 260 mμ); G-X, with 0.003 *N* HCl + 0.1 *M* NaCl (1,000 ml., 1,674, O.D. units); G-Y, with 0.003 *N* HCl + 0.2 *M* NaCl (700 ml., 504, O.D. units). G-X was isolated as the barium salt (78 mg.) as described for guanosine 5′-diphosphate.[7] Since this product was found to be contaminated by inorganic pyrophosphate, purification was carried out as follows. Forty milligrams of the barium salt was shaken with Dowex-50-sodium form until it dissolved. The resin was removed by filtration and washed with water. The total solution (10 ml.) of sodium G-X was adjusted to *p*H 2.7 and stirred with 100 mg. of Darco-G-60 charcoal[40] (95% of the optical density was absorbed). The charcoal was washed with 3 portions of 0.003 *N* HCl to remove the last traces of pyrophosphate and then with three 5-ml. portions of ethyl alcohol–ammonia–water[41] to elute G-X. The ammoniacal solution was evaporated under reduced pressure and the product was precipitated as its barium salt at *p*H 7 from its aqueous solution. One reprecipitation was carried out by dissolving the barium salt in 0.5 ml. of 0.5 *N* HCl at 0° and adding ethyl alcohol.

Characterization of G-X.—(1) The above sample was converted to the sodium salt as described above and examined by paper chromatography and paper electrophoresis. Only one ultraviolet absorbing spot, corresponding to guanosine 5′-diphosphate,[7] could be detected. (2) The guanosine (ultraviolet absorption) to labile phosphorus to total phosphorus was found to be 0.9:1.0:2.0. (3) Tris-buffer (*p*H 7.5) and rattlesnake venom[38] were added to a small portion of the sodium salt solution. The mixture was incubated for 6 hours at 37° and then subjected to chromatography in solvent system III. The original spot of G-X (R_f, 0.12) had completely disappeared and a new spot corresponding to an authentic sample of guanosine (R_f, 0.44) had appeared. (4) The periodate–benzidine spray[42] gave a positive test for vicinal hydroxyl groups in both standard guanosine 5′-diphosphate and G-X.

G-Y was isolated by dissolving the solid obtained by lyophilization of the last ion-exchange fraction in 40 ml. of water and absorbing the nucleotide component on 100 mg. of charcoal (Darco-G-60) in two 50-mg. portions. The charcoal was collected by filtration through Celite, washed as above, and the nucleotidic material then eluted with four 5-ml. portions of 5% aqueous pyridine. The barium salt was prepared in the usual manner. Paper chromatography and paper electrophoresis indicated the presence of a major component, resembling guanosine 5′-diphosphate in behavior and a minor component which behaved like guanosine 5′-triphosphate.[7] Phosphorus analysis gave a ratio of labile to total phosphorus of 2:4.6; theory for guanosine 5′-triphosphate, 2:3.

Acknowledgments.—We wish to thank the National Research Council and the National Cancer Institute of Canada for grants in aid of this work.

(40) D. Lipkin, P. T. Talbert and M. Cohn, THIS JOURNAL, **76**, 2871 (1954).

(41) E. Cabib, L. F. Leloir and C. E. Cardini, *J. Biol. Chem.*, **203**, 1055 (1953).

(42) M. Viscontini, D. Hoch and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).

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[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Nucleoside Polyphosphates. V.[1] Syntheses of Guanosine 5′-Di- and Triphosphates

BY ROBERT WARNER CHAMBERS[2] AND H. G. KHORANA

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The syntheses of guanosine 5′-di- and triphosphates by the reaction of guanosine 5′-phosphate and 85% phosphoric acid with dicyclohexyl carbodiimide are reported.

The condensation of an unprotected nucleoside 5′-monophosphate with orthophosphoric acid in the presence of a carbodiimide forms the basis of a simple method for the synthesis of nucleoside 5′-polyphosphates. The application of this method to the preparation of ADP, ATP,[3,4] UDP and

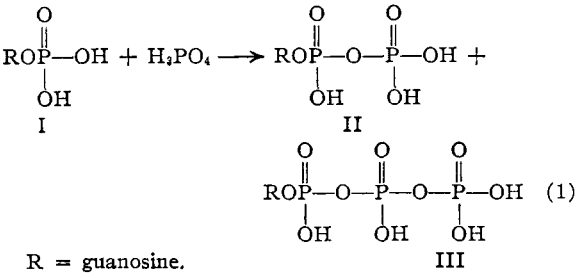
(1) Paper IV. R. W. Chambers, J. G. Moffatt and H. G. Khorana, THIS JOURNAL, **79**, 3747 (1957).

(2) Life Insurance Medical Research Fund Post-doctoral Fellow, 1954–1956.

(3) The following abbreviations are used: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; UDP, uridine 5′-diphosphate; UTP, uridine 5′-triphosphate; GMP, guanosine 5′-monophosphate; GPD, guanosine, 5′-diphosphate; GTP, guanosine 5′-triphosphate; DCC, dicyclohexyl carbodiimide.

(4) H. G. Khorana, THIS JOURNAL, **76**, 3517 (1954).

UTP[3,5] has been recorded previously. In view of the increasing biochemical interest in the naturally occurring ribo- and deoxyribonucleoside 5′-polyphosphates[6] it was considered desirable to test further the generality of the method. Attention was turned, therefore, to the synthesis of guanosine 5′-di- (II) and triphosphate (III) from guanosine 5′-monophosphate[1] (I) (eq. 1)



R = guanosine. (1)

In order to determine the optimum conditions for the formation of GDP[3] and GTP[3] it was necessary to have a convenient and reliable method for analyzing the reaction mixtures. For this purpose, ion-exchange chromatography using a linear gradient elution apparatus, similar to that described by Paar,[7] proved satisfactory. Analytical columns, using Dowex-2 (chloride form) resin, were routinely run overnight and gave well separated sharp peaks of the various components. Use of the more expedient techniques of paper chromatography and paper electrophoresis was investigated in some detail but it was not possible to find a system which would separate GTP from higher guanosine polyphosphates.

The previous work on the condensation of nucleoside monophosphates with phosphoric acid has shown that in aqueous pyridine homogeneous reaction mixtures are not obtained. Since all of the reagent (DCC) must be present in the upper aqueous pyridine phase, this is the reaction phase. The lower "sirupy" phase contains a large proportion of the phosphoric acid as well as varying amounts of the nucleoside monophosphate, depending upon the pyridine–water ratio and the solubility of the nucleotide in pyridine. In this solvent system the rate of condensation was found to be, as expected, a function of the solubility of the nucleotide in the upper phase. Thus, UMP is soluble in anhydrous pyridine and the synthesis of uridine polyphosphates occurs rapidly even when the pyridine–water ratio is as high as fifteen. AMP, on the other hand, has a very low solubility in pyridine and, consequently, the formation of adenosine polyphosphates is much slower even when the pyridine–water ratio is as low as seven.

In the present work, GMP was found to be even less soluble in aqueous pyridine than AMP and, consequently, the rate of polyphosphate formation was very slow. Attempts, therefore were made to find an alternative solvent system. Freshly distilled dimethylformamide was found to give a homogeneous reaction mixture and the condensation was then, as expected, very rapid. Several experiments were performed using varying proportions of GMP, phosphoric acid and DCC. The results obtained in a typical experiment by removing aliquots from the reaction mixture at suitable time intervals are shown in Table I (Experimental Section). These experiments, as may be seen from the data, were disappointing in that GDP and/or GTP could not be obtained as major products of the reaction. Higher guanosine polyphosphates, which have not been investigated further, were formed rapidly after an initial lag period and accounted for more than 50% of the total nucleotidic material.

The lack of specificity in effecting condensation is a disadvantage of the carbodiimide method for the synthesis of nucleoside polyphosphates and this fact necessitates the use of a large excess of orthophosphoric acid. Therefore, large amounts of inorganic polyphosphates are concomitantly formed. As the reaction proceeds the concentration of these substances increases[8] leading to a complicated kinetic situation. Qualitatively, the above results are perhaps not surprising since the condensation of inorganic pyrophosphate, which may form quite rapidly in the homogeneous system, with either GDP or GTP, would lead directly to higher guanosine polyphosphates.

In view of these unsatisfactory results[9] the aqueous pyridine solvent system was investigated in more detail. As in previous work, many experiments were performed in which the proportion of the reactants, pyridine–water ratio and reaction time were varied. The results of three selected experiments are shown in Table I. In no experiment was a completely satisfactory yield of GDP and GTP[10] obtained. The best yield was about 25% each of GDP and GTP, representing a total of about 50% conversion of GMP to the desired products. These conditions were used on a preparative scale.[11]

The main problem in isolating nucleoside di- and triphosphates synthesized by the present method is the separation of the nucleotides from the inorganic polyphosphates. In the adenosine series, ADP and ATP were precipitated as mercury salts with Lohmann reagent. In the uridine series, the removal of the inorganic phosphates was accomplished by repeated precipitation of the free nucleotides from alcohol–ether mixtures. Neither of

(5) R. H. Hall and H. G. Khorana, THIS JOURNAL, **76**, 5056 (1954).

(6) For selected references see ref. 1.

(7) C. W. Paar, *Biochem. J.*, **56**, xxvii (1954).

(8) This will be so, especially, when homogeneous reaction mixtures are employed. In aqueous pyridine, the higher polyphosphates are, probably, removed from the reaction phase (see above) because of their greater insolubility.

(9) Attempts were also made to hydrolyze partially the higher guanosine 5′-polyphosphates to GDP and GTP by heating the reaction mixture at *p*H 4.5 and 100° as described by Lieberman [THIS JOURNAL, **77**, 3373 (1955)]. However, large amounts of GMP were formed and the yields were only slightly better than in the unhydrolyzed reaction mixture. The polyphosphates appeared to be stable on heating in 0.5 *N* sodium hydroxide at 100° for 1 hour.

(10) The optimum reaction time appeared to be 67–72 hours. Long reaction periods were also found necessary by Kennedy for the synthesis of cytidine diphosphate choline and related compounds [E. P. Kennedy, *J. Biol. Chem.*, **222**, 185 (1956)]. This also is due probably to the low solubility of the phosphate esters in the pyridine phase.

(11) The aqueous pyridine system required longer reaction time and much larger quantities of DCC than dimethylformamide reaction mixtures. However, the aqueous pyridine system was selected for the preparative scale since difficulty was experienced in obtaining clean ion-exchange separation of the products from the dimethylformamide runs (see Experimental).

these methods was completely satisfactory, however, since they were attended by large losses of the nucleotides. A mild and general method for the above purpose utilizes the selective adsorption of the nucleotides by charcoal. The use of charcoal for the separation of nucleotides from salts and inorganic phosphates has previously been recorded by a number of workers[12] and the method was used successfully in the present work.[13] However, some irreversible adsorption of the nucleotides occurred and best recoveries, after trials with different kinds of charcoal and eluting agents, were about 65%.

Using charcoal purification in conjunction with ion-exchange chromatography, pure samples of GDP and GTP were obtained in about 10% overall yield, representing a 20% conversion of GMP to the desired compounds. The ultraviolet absorption spectra of the synthetic samples and their behavior on paper chromatography, paper electrophoresis and ion-exchange chromatography were identical with samples of GDP and GTP isolated from yeast.[14] Furthermore, both samples were biologically active in the assay system developed by Ayengar, *et al.*[14] Elemental analysis indicated that GDP, precipitated as the barium salt at an acidic *p*H, was best represented by the formula GDP·$Ba_{1/2}$·$3H_2O$ while dibarium GTP appeared to be the hexahydrate.[15]

While the yields of GDP and GTP were somewhat disappointing this work represents the first chemical synthesis of these newly discovered nucleoside polyphosphates. Furthermore the present experiments together with the independent work reported from other laboratories[16] serve to illustrate the general scope of the carbodiimide method in the synthesis of biologically important "anhydrides." It also seems clear that one limitation of the technique is the contrasting solubility properties of DCC, on the one hand, and the nucleotides and related compounds on the other, in aqueous pyridine. Nevertheless this is the only solvent system which has proved satisfactory so far. It might be expected that the situation would be improved in some cases by using a water-soluble carbodiimide. Experiments on the use of such reagents will be reported in a forthcoming communication.[13b]

Experimental

The Reaction of GMP and Phosphoric Acid with DCC. (a) In Dimethylformamide.—A series of experiments was carried out as follows: GMP (5 mg. of the free acid)[17] was dissolved in 0.4 ml. of freshly distilled dimethylformamide and the solution cooled to 0°. Five microliters (*ca.* 5 equiv.) of 85% phosphoric acid and 58 mg. of DCC were added and the resulting clear solution allowed to stand at 0° for various lengths of time. The reaction was stopped by the addition of water. Dicyclohexylurea was removed by filtration, washed three times with small portions of water and the combined filtrate adjusted to *p*H 8 with ammonium hydroxide. The solution was evaporated under reduced pressure, the residue taken up in water, the solution filtered and re-evaporated. The process was repeated once again to ensure complete removal of dimethylformamide. The clear aqueous solution finally obtained was readjusted to *p*H 8 and diluted to 5 ml. For quantitative analysis, 2 ml. of this solution was allowed to pass slowly through a column (2 cm. long, 0.8 cm. diameter) of Dowex 2 (chloride form) and the column washed with water. Elution of the products was carried out stepwise with the following solutions: GMP with 0.01 *N* HCl + 0.015 *M* NaCl; GDP with 0.01 *N* HCl + 0.1 *M* NaCl; GTP with 0.01 *N* HCl + 0.2 *M* NaCl; higher phosphates with 2 *N* HCl. Five-ml. fractions were collected, maintaining an average flow rate of 0.5 ml./min. The optical density of each fraction was determined at 260 mμ. The results of these experiments are shown in Table I.

TABLE I

REACTION OF GMP AND PHOSPHORIC ACID WITH DCC

	Conditions			Products[a]			
Solvent	H_3PO_4[b]	DCC[b]	Time (hr.)	GMP	GDP	GTP	Higher phosphates
DMF	5	20	0.5	58	18	17	7
DMF	5	20	0.75	13	17	30	42
DMF	5	20	1.0	7	14	23	55
Pyridine–water ratio							
4:1[c]	5	50[e]	24	64	14	13	9
6:1[d]	12	60	49	48	25	17	10
10:1	5	100[f]	67	28	26	25	20

[a] Expressed as percentages of the total optical density recovered from the column. [b] Equivalents based on GMP used. [c] Homogeneous reaction mixture. [d] These conditions are identical to those reported for the preparation of ADP and ATP (experiment 5, Table II[4]). [e] 10 equiv. at first, 10 equiv. each after 2, 4, 7 and 9 hours. [f] 40 equiv. at first, 40 equiv. DCC + 0.1 ml. water after 24 hours; 20 equiv. DCC + 0.1 ml. H_2O after 48 hours.

(b) In Aqueous Pyridine. General Method.—Monopyridinium[18] GMP (63 mg.)[19] was dissolved in an appropriate amount of water and enough pyridine was added to bring the solution to the desired pyridine–water ratio. The final volume of the solvent was 7 ml. in all of the reactions. Orthophosphoric acid (85%) and DCC were added as indicated in Table I and the mixture was shaken vigorously for the specified length of time. The reaction mixture was worked up as described above for the dimethylformamide reactions. Aliquots (0.3 ml.) were analyzed on the same size ion-exchange columns as described above, but using a linear gradient elution technique.[7,20,21] The mixing flask contained 500 ml. of 0.003 *N* hydrochloric acid while the reservoir contained an equal volume of 0.5 *M* sodium chloride solution in 0.003 *N* hydrochloric acid.

The results of the experiments using aqueous pyridine are given in Table I.

Isolation of GDP and GTP.—Monopyridinium GMP (635 mg.) was dissolved in 6 ml. of water and 60 ml. of py-

(12) (a) G. A. LePage and G. C. Mueller, *J. Biol. Chem.*, **180**, 775 (1949); (b) E. Cabib, L. F. Leloir and C. E. Cardini, *ibid.*, **203**, 1055 (1953); (c) R. K. Crane and F. Lipman, *ibid.*, **201**, 235 (1953); (d) D. Lipkin, P. T. Talbert and M. Cohn, THIS JOURNAL, **76**, 2871 (1954).

(13) The method also has been used successfully in subsequent work on the synthesis of nucleoside polyphosphates: (a) A. Kornberg, unpublished work on thymidine polyphosphates; (b) M. Smith and H. G. Khorana, unpublished work on the use of water-soluble carbodiimides in the synthesis of nucleoside polyphosphates.

(14) P. Ayengar, D. M. Gibson, C. H. Lee Peng and D. R. Sanadi, *J. Biol. Chem.*, **218**, 521 (1956). We are grateful to Dr. Sanadi for the enzymatic tests on our synthetic samples and for the gift of a sample of GTP isolated from yeast.

(15) Since GTP was contaminated by a small amount of GDP, the assignment of the exact degree of hydration cannot be conclusive.

(16) (a) E. P. Kennedy (footnote 10); (b) F. M. Huennekens and G. L. Kilgour, THIS JOURNAL, **77**, 6716 (1955); (c) P. T. Talbert and F. M. Huennekens, *ibid.*, **77**, 4671 (1956); (d) P. Berg, unpublished work on the synthesis of α-aminoacyladenylic acids.

(17) Prepared by shaking the barium salt with Dowex-50 (H^+), removing the resin by filtration, lyophilizing to a dry powder and washing the solid three times with anhydrous ether.

(18) Prepared by shaking an aqueous suspension of the barium salt with pyridinium Dowex-50, removing the resin by filtration and lyophilizing to a dry powder.

(19) Small scale reactions such as described using dimethylformamide as the solvent, were not satisfactory because of the difficulty in maintaining vigorous agitation of the heterogeneous reaction mixtures.

(20) See also R. M. Bock and N. Ling, *Anal. Chem.*, **26**, 1543 (1954), for a description of the apparatus.

(21) The gradient elution technique did not give satisfactory results with dimethylformamide reaction mixtures.

ridine was added, followed by phosphoric acid (0.5 ml. of 85%) and DCC (11.6 g.). The two phase reaction mixture was agitated vigorously using a mechanical shaker. After 24 hours, 11.6 g. of DCC and 1 ml. of water were added and the shaking was continued. A final addition of DCC (5.8 g.) and water (1 ml.) was made after 50 hours. After a total reaction time of 72 hours, the mixture was worked up as described above for the small scale experiments except that the filtrate and washings obtained after removal of the dicyclohexylurea were extracted twice with ether to remove a small amount of unchanged DCC. The clear aqueous solution (50 ml.; total optical density in 0.003 *N* hydrochloric acid at 260 mμ, 13,800 units) was adjusted to *p*H 2.8 (*p*H meter) with hydrochloric acid and to it were added five 0.5-g. portions of charcoal,[22] the solution being stirred for three minutes after each addition. After the final addition, the suspension was stirred for ten minutes and the charcoal was then collected by filtration through Celite. The total filtrate (optical density, 2570 units at 260 mμ) was treated in a similar manner with five 0.2-g. portions of charcoal and the charcoal collected again on the first filter bed. The filtrate (1000 optical density units) was retreated with 2 × 0.1 g. of charcoal. The final filtrate contained 200 optical density units and was discarded (total absorption, 97%). The total charcoal was washed free of inorganic phosphates by suspending it three times in 100-ml. portions of 0.003 *N* hydrochloric acid.[23] The total acidic filtrate (1640 optical density units) was treated twice with 0.3-g. portions of charcoal and the filtration and washing repeated. The nucleotidic material was recovered by batchwise elution of the charcoal with an ethyl alcohol–ammonia–water mixture.[12b] The combined eluates were concentrated using a Craig flash evaporator at bath temperature below 30°. The concentrated solution was filtered to remove a small amount of charcoal and some insoluble material. The *p*H of the filtrate (63 ml., containing 8760 optical density units; 64% recovery) was adjusted to 8.5 with ammonium hydroxide and the solution passed through a Dowex-2 (chloride form, 200–325 mesh) column (7 cm. high, 2.2 cm. diameter) at a flow rate of 1 ml./min. The column was washed with water (flow rate 4 ml./min.) until the optical density of the effluent dropped below 0.200 (total water wash, 180 ml. containing 35 optical density units). The nucleotides were eluted in a stepwise manner as follows. One hundred ml. fractions were collected and immediately adjusted to *p*H 7. Eluents were changed when the optical density of the effluent fell below 0.2. GMP with 0.003 *N* HCl + 0.015 *M* sodium chloride (1500 ml. containing 2460 optical density units; 28% of the total optical density applied to the column); GDP with 0.003 *N* HCl + 0.1 *M* sodium chloride (900 ml. containing 2182 optical density units, 25%); GTP with 0.003 *N* HCl + 0.2 *M* sodium chloride (1400 ml. collected, after discarding the first 500 ml., total optical density recovered, 2058 units, 23%); higher phosphates with 2 *N* HCl (800 ml. containing 1649 optical density units, 19%). Recovery of the optical density from the column was quantitative.

GDP and GTP fractions were concentrated to about 30 ml. using a flash evaporator (bath temperature, under 30°), the concentrate readjusted to *p*H 7 and lyophilized.

(22) Nuchar C was treated as described by D. Lipkin, P. T. Talbert and M. Cohn, THIS JOURNAL, **76**, 2871 (1954), for Darco G-60 except that drying was carried out at 60–70° for 48 hours.

(23) Washing with water caused excessive losses of ultraviolet absorbing material.

GDP was isolated as its barium salt by the procedure described by Hall and Khorana[5] for UDP except that 0.5 ml. of 2 *M* barium acetate was used. The product[24] after drying over phosphorus pentoxide *in vacuo* weighed 70 mg. A second crop (11 mg.) was obtained from the mother liquor by the addition of ethyl alcohol. Fifty milligrams of the first crop was dissolved in 0.7 ml. of 0.5 *N* hydrochloric acid at 0°, a small amount of insoluble material was removed by centrifugation and the *mono* barium salt was precipitated by the addition of ethyl alcohol. The product was dried over phosphorus pentoxide *in vacuo* (30 mg.) and then equilibrated with air before analysis. *Anal.* Calcd. for $C_{10}H_{14}N_5O_{11}P_2Ba_{1/2}\cdot 3H_2O$ (564.94): C, 21.26; H, 3.57; P, 10.97. Found: C, 21.30; H, 3.87; P, 11.12. The ratio of guanosine (using 12.4×10^3 as the E_{max} at 257 mμ): labile phosphorus:total phosphorus was 1:1:2. Paper chromatography in the four solvent systems mentioned above, paper electrophoresis (Table II) and ion-exchange chromatography showed a single ultraviolet absorbing component.

TABLE II

CHROMATOGRAPHIC AND ELECTROPHORETIC DATA

Compound[a]	R_f's in solvents III	V	VI	VII	Electrophoretic mobility[b]
GMP	0.24	0.63	0.35	0.28	8.2
GDP	.10	.75	.14	.18	14.9
GTP	.06	.78	.13	.15	16.2

[a] All the data recorded are on sodium salts prepared by shaking the barium salts with Dowex-50 sodium form and spotting the supernatant. [b] Centimeter movement toward anode in 2 hours. (For details see preceding paper.[1])

GTP was isolated as its barium salt, as described by Hall and Khorana[5] for the isolation of barium UTP, and dried over phosphorus pentoxide; 97 mg. *Anal.* Calcd. for $C_{10}H_{12}N_5O_{14}P_3Ba_2\cdot 6H_2O$ (902.02): C, 13.31; H, 2.68; P, 10.30. Found: C, 13.37; H, 3.30; P, 10.47. The ratio of labile phosphorus to total phosphorus was 2:3.

Paper chromatography, paper electrophoresis (Table II) and ion-exchange chromatography showed that the main ultraviolet absorbing component corresponded to GTP isolated from yeast.[14] A trace spot corresponding to GDP also was present. The ultraviolet spectra of synthetic GDP and GTP in 0.01 *N* hydrochloric acid were identical with that of GMP under the same conditions.

Paper Chromatography and Paper Electrophoresis.—Paper chromatography and paper electrophoresis were carried out as described in the preceding paper.[1] The solvent systems designated as III, V, VI and VII proved suitable in the present work. The R_f values and electrophoretic mobilities are listed in Table II.

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(24) Recovery of GDP from the salt cake was only about 60% and the material remaining on the cake could not be freed easily from the salt.