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Title THE CHLORINATION OF URIC ACID WITH PHOSPHORUS OXYCHLORIDE IN THE PRESENCE OF CERTAIN NITROGENOUS BASES

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In view of the effect N, N-dimethyl- and N, N-diethylaniline have on the course of the phosphorus oxychloride chlorination of uric acid a study of the effect of other nitrogenous bases and other chlorinating agents was undertaken. Phosphorylation side reactions were eliminated by the use of N, N-dimethyl-p-toluidine, N, N-dimethyl-m-toluidine, and N, N-dimethylmesidine; 2, 6, 8-trichloropurine was produced in 12-17% yield. N, N, N', N'-tetraethylphenylenediamine and N, N-dimethyl-p-nitroaniline when used in the chlorination reaction result in only traces of 2, 6, 8-trichloropurine being isolated. When 2, 6-Lutidine was used as the nitrogenous base a 16% yield of 2, 6, 8-trichloropurine was obtained.

Methylphenylphosphoramidic dichloride heated with uric acid in the presence or absence of phosphorus oxychloride gave no yield of

2, 6, 8-trichloropurine. By the use of "pyrophosphoryl chloride" in place of phosphorus oxychloride in the chlorination reaction, with N, N-dimethyl-p-toluidine as the amine, only a trace of 2, 6, 8-trichloropurine was produced, however, when "pyrophosphoryl chloride" was heated with uric acid in a sealed tube, chlorination took place yielding 15% of 2, 6, 8-trichloropurine.

The use of N, N-diethylaniline hydrochloride in place of the free amine in the chlorination reaction proved to be a breakthrough in the study of the chlorination reaction; the reaction was easy to carry out, was free of colored and phosphorylated by-products, and gave a high yield (46%) of 2, 6, 8-trichloropurine.

With N, N-dimethyl-p-toluidine as the amine in the chlorination of uric acid with phosphorus oxychloride two side products were identified to be dichloro-N-methyl-p-toluidinopurine and dichlorohydroxypurine. The substitution of N, N-diethylaniline as the amine gave bis(p-diethylaminophenyl)phosphinic acid as one of the side products.

Known amounts of 2, 6, 8-trichloropurine were isolated by the same method used in the isolation of reaction products, showing that 23-35% of the starting 2, 6, 8-trichloropurine is lost in the isolation procedure. It was shown by ultraviolet spectral analysis that 2, 6, 8-trichloropurine is stable in pH 1 hydrochloric acid.

THE CHLORINATION OF URIC ACID WITH PHOSPHORUS OXYCHLORIDE IN THE PRESENCE OF CERTAIN NITROGENOUS BASES

by

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THE CHLORINATION OF URIC ACID WITH PHOSPHORUS OXYCHLORIDE IN THE PRESENCE OF CERTAIN NITROGENOUS BASES

Chloro substituted nitrogen heterocycles are useful intermediates for synthetic purposes. For this reason much attention has been directed to the problem of devising methods which would lead to the preparation of chloro substituted derivatives in reasonable yields. Although the hydroxy derivitives of nitrogen heterocycles are usually available or reasonably easy to synthesize, the conversion of the hydroxy derivative of the nitrogen heterocycle to the corresponding chloro substituted derivative is a complex reaction often giving low and erratic yields of the desired product. More information regarding the chlorination reaction will have to be uncovered before it will be possible to devise methods leading to improved yields of the chloro substituted product.

The reagent used to convert the hydroxy heterocycle to the corresponding chloro derivitive is phosphorus oxychloride. In the earlier methods the compound to be chlorinated was suspended in refluxing phosphorus oxychloride or heated with the reagent in sealed tubes at elevated temperatures. The excess phosphorus oxychloride was removed and the residual phosphorus halide hydrolyzed in an ice-water mixture from which the chlorinated product was removed by ether extraction.

The earliest chlorinations of hydroxy purines described in the literature were the reactions of methyluric acid by Fischer (13, p. 330-331) in 1884. Ten parts of 9-methyluric acid were mixed with 13 parts of phosphorus pentachloride, and 50 parts of phosphorus oxychloride; heating for 8-9 hours at 130°C yielded 9-methyl - 2, 6-dichloro-8-oxypurine. 9-Methyl-2,6, 8-trichloropurine was prepared from 9-methyl-2, 6-dichloro-8-oxypurine by heating with 1. 25 parts of phosphorus pentachloride and five parts of phosphorus oxychloride in a sealed tube at 160°C for eight hours.

The first chlorination of an unsubstituted hydroxypurine described in the literature was that of uric acid by Fischer (14, p. 2221), and Fischer and Ach (15, p. 2209) in 1897. The potassium salt of uric acid was heated in a sealed tube at 160-170°C for six hours with 1. 2 parts of phosphorus oxychloride; 2, 6-dichloro-8-oxypurine was produced in 40-50% yield. 2, 6, 8-Trichloropurine was prepared by heating the isolated 2, 6-dichloro-8-oxypurine with 70 parts of phosphorus oxychloride in a sealed tube at 150-155°C for four hours. The yield of 2, 6, 8-trichloropurine was 65%, or an overall yield from uric acid of 26-32%.

In 1946 the sealed tube preparation of 2, 6-dichloro-8-oxypurine was repeated by Davoll, Lythgoe, and Todd (9, p. 836) who increased the reaction temperature to 185°C and the reaction time to 19 hours; using an improved isolation procedure

a 53% yield (19, p. 5) was obtained.

Nine years later (in 1955) Boldyrev and Makitra (6, p. 399-404) described a sealed tube chlorination of uric acid yielding 51-53% of 2, 6-dichloro-8-oxypurine; this was obtained by heating a 1:3 ratio of the dipotassium salt of uric acid with phosphorus oxychloride in a sealed tube at 165°C for six hours. By repeating the reaction using 2, 6-dichloro-8-oxypurine in presence of a 100 fold molar ratio of phosphorus oxychloride 2, 6, 8-trichloropurine was produced in 87% yield, or an overall yield from uric acid of 45%. The excess phosphorus oxychloride must be completely removed before hydrolysis of the reaction mass with water to avoid hydrolyzing the 2, 6, 8-trichloropurine.

In 1960 Okumura (28) reduced the sealed tube preparation of 2, 6, 8-trichloropurine to a one step operation. Approximately 10:1 ratios of phosphorus oxychloride and crude uric acid were heated under pressure at 130°C for 15 hours. Hydrolysis of the reaction mixture with ice and extraction with ether yielded 2, 6, 8-trichloropurine in 20-40% yield.

The early chlorinations of hydroxypyrimidines used procedures similar to that of Fischer and Ach for the chlorination of hydroxypurines; the hydroxypyrimidines were likewise heated with phosphorus oxychloride in pressure reactors at elevated temperatures.

Tri- and dichloropyrimidine were prepared in this way by Gabriel in 1900 (16, p. 3667) and 1905 (17, p. 1690). 2, 4, 6-Trichloropyrimidine was obtained in 66% yield by heating barbituric acid with phosphorus oxychloride in a continuously rotating sealed tube for one hour at 140°C. By heating uracil with phosphorus oxychloride in a sealed tube at 140°C, 2, 4-dichloropyrimidine was obtained in 80% yield.

The preparation of 2, 4-dichloropyrimidine was improved by Hilbert and Johnson (20, p. 1155) in 1930. Uracil was suspended in refluxing phosphorus oxychloride for five hours and the 2, 4-dichloropyrimidine isolated in 68% yield. This permitted a much larger operation than the procedure described by Gabriel.

In 1943 a breakthrough in chlorinating technique was made by Kenner et al. (24, p. 575) who discovered that the addition of N, N-dimethylaniline to the reaction mixture aided the chlorination, permitting halogenation to take place at atmospheric pressures. The first use of this procedure was made in the preparation of 4, 6-dichloropyrimidine (24, p. 575). 4, 6-Dihydroxypyrimidine (0. 2 mole), phosphorus oxychloride (2 mole), and N, N-dimethylaniline (0. 1 mole) were refluxed until hydrogen chloride evolution ceased (about three hours); isolation of 4, 6-dichloropyrimidine was achieved in 50% yield.

In 1944 Baddiley and Tophan (1, p. 678-679) extended the use of N, N-dimethylaniline in the chlorination reaction to the preparation of 2, 4,6-trichloropyrimidine and substituted chloropyrimidines. A mixture of barbituric acid, phosphorus oxychloride, and N, N-dimethylaniline was refluxed for five minutes and the reaction product isolated yielding 2, 4, 6-trichloropyrimidine (46%). The procedure appeared to be generally applicable for use with substituted hydroxypyrimidines.

It was shown by Langerman and Banks (26, p. 3011) in 1951 that only catalytic amounts of N, N-dimethylaniline were necessary for the chlorination of barbituric acid; 2, 4, 6-trichloropyrimidine was prepared in 80% yield.

However, King et al. (25, p. 1247-1248) isolated a by-product in a five per cent yield, which was identified as 4, 6-dichloro-2N-methylanilinopyrimidine, from the chlorination products of barbituric acid using the method of Baddiley and Topham. The responsibility for the by-product was traced to N, N-dimethylaniline; the reaction, when repeated using N-methylaniline free reagent, gave the identical side reaction.

The formation of 4, 6-dichloro-2N-methylanilinopyrimidine was thought that of 2, 4, 5-tri-N-methylanilinopyrimidine (23, p. 1247). N, N-Dimethylaniline reacts with 2, 4, 6-trichloropyrimidine to form an intermediate quaternary salt which decomposes to

yield 2, 4, 6-tri-N-methylanilinopyrimidine and methylchloride.

The use of N, N-dimethylaniline in the chlorination of hydroxypurines was first introduced by Davoll, Lythgoe, and Todd (9, p. 836) in the conversion of 2, 6-dichloro-8-oxypurine to 2, 6, 8-trichloropurine. 2, 6-Dichloro-8-oxypurine was heated with N, N-dimethylaniline and phosphorus oxychloride under reflux for 4. 5 hours. Upon isolation of the 2, 6, 8-trichloropurine a 76% yield of product was obtained.

A convenient synthesis of 2, 6, 8-trichloropurine by direct chlorination of uric acid with phosphorus oxychloride in the presence of N, N-dimethylaniline was reported by Davoll and Lowy (8, p. 2936) in 1951. Uric acid was suspended in phosphorus oxychloride, treated with three moles of N, N-dimethylaniline per mole of uric acid, and refluxed for 20 hours; 2, 4, 6-trichloropurine was isolated from the reaction in 16-25% yield.

Robins and Christensen (30, p. 324-326) in 1951 were unable to isolate 2, 6, 8-trichloropurine from the reaction of uric acid, phosphorus oxychloride, and N, N-dimethylaniline, but reported that a reaction had occurred between N, N-dimethylaniline and phosphorus oxychloride. Repeated experiments in the absence of uric acid gave the same product but the yield was much greater even in the presence of only catalytic amounts of uric acid. The product of the reaction between N, N-dimethylaniline and phosphorus oxychloride

was shown to be bis(p-dimethylaminophenyl)phosphinic acid.

N, N-Dimethyl-p-toluidine was used by Greendorfer (19, p. 10-11) in 1962 in place of N, N-dimethylaniline in the uric acid chlorination reaction in an effort to avoid side reactions. The use of this amine increased the yield of 2, 6, 8-trichloropurine only slightly but gave a reaction which was cleaner and free of colored by-products.

Using an improved isolation procedure, Greendorfer (19, p. 13-16) was able to isolate and identify two side products in the chlorination reaction when N, N-dimethyl-p-toluidine was used as the amine. A compound was isolated from two different fractions of the isolation scheme in a combined yield of 13-29%, which was shown by analysis to be an isomer of chloro-N-methyl-p-toluidino-oxypurine. A second compound isolated in 2-12% yield was shown by analysis to be an isomer of dichloro-N-methyl-p-toluidinopurine.

2, 6, 8-Trichloropurine was found to undergo change in the reaction environment used to produce it. 2, 6, 8-Trichloropurine was treated with N, N-dimethyl-p-toluidine in the presence of refluxing phosphorus oxychloride for 20-24 hours. Greendorfer (19, p. 11-12) was only able to isolate 13-16% of the starting 2, 6, 8-trichloropurine. A reaction product was isolated in 29-30% yield and was shown by analysis to be an isomer of dichloro-N-methyl-p-toluidinopurine. Only 45-52% of the added

2, 6, 8-trichloropurine could be accounted for.

Greendorfer (19, p. 16-17) studied the material balance for uric acid chlorinations in the presence of N, N-dimethylaniline and N, N-dimethyl-p-toluidine. Using N, N-dimethylaniline and N, N-dimethyl-p-toluidine as the amines from 44-58% and 65-73% of the starting uric acid could be accounted for respectively. What has happened to the remainder of the uric acid is a problem which has not yet been resolved.

Prolonged reaction times were found by Greendorfer (19, p. 29) to be necessary for the chlorination of uric acid. Reaction times of four hours yielded only a trace of 2, 6, 8-trichloropurine, whereas in 24 hours the yields of 2, 6, 8-trichloropurine rose to 19% (19, p. 29-30).

Robins and Christensen (31, p. 3624) in 1952 used triethylamine in the chlorination of uric acid. In the presence of a limited amount of triethylamine, uric acid was converted with phosphorus oxychloride to 8-chloroxanthine in 30-40% yields. If the monopotassium salt of uric acid was used the yields were consistently above 80%. It should be noted that in the early chlorination procedures it is the eight position on the purine molecule which is the last to be chlorinated. When excess triethylamine was used along with longer reaction times chlorination and amination took place on the purine molecule to produce 2, 8-dichloro-6-diethylaminopurine.

Garkusha (18, p. 1712-1717) prepared 2, 6, 8-trichloropurine from 8-chloroxanthine in 46% yield by heating a mixture of the dry ammonium salt of 8-chloroxanthine with phosphorus oxychloride, and N, N-dimethylaniline at reflux for six hours.

The chlorination of methylated xanthines (11, p. 3508) with phosphorus oxychloride or with mixtures of phosphorus oxychloride and phosphorus pentachloride have been known but chlorinated xanthines are not recovered when xanthine is treated under those conditions (11, p. 3508). The addition of N, N-dimethylaniline to phosphorus oxychloride and xanthine under reflux conditions does not result in the isolation of chlorinated xanthines (11, p. 3508).

Robins and Christensen (31, p. 3624) attempted to chlorinate xanthine with refluxing phosphorus oxychloride in the presence of tertiary aliphatic amines. The use of trimethylamine in the chlorination reaction yielded 2, 6-bis-(dimethylamino)-purine. The reactants were mixed at 0°C and gradually warmed to reflux. The fact that the reaction can be run at atmospheric pressure suggests some type of salt formation in the reaction mixture. Using triethylamine the corresponding compound, 2, 6-bis-(diethylamino)-purine was produced. When tri-N-propylamine was used, 6-di-N-propylamino-2-purinone resulted, and with tri-N-butylamine the corresponding compound, 6-di-N-butylamino-2-purinone, was produced.

Elion and Hitchings (11, p. 3509-3510) in 1956 discovered that "pyrophosphoryl chloride" would chlorinate xanthine in a sealed tube reaction. The "pyrophosphoryl chloride" was prepared by adding a half a molecular equivalent of water to phosphorus oxychloride, heating the mixture until hydrogen chloride evolution ceases, and discarding the thick sirup which forms on the bottom of the reaction flask. When xanthine was mixed with "pyrophosphoryl chloride", and heated in a pressure chamber at 165°C for 19 hours, the reaction product, 2, 6-dichloropurine, was obtained in 43% yield. At lower temperatures a considerable amount of unreacted xanthine was recovered, while at higher temperatures a compound which appeared to be a polymer of 2, 6-dichloropurine was produced.

6, 8-Dihydroxypurine was chlorinated in 38% yield by Robins (29, p. 6671-6675) by heating with phosphorus oxychloride and N, N-diethylaniline at reflux for 3.5 hours.

Early attempts to chlorinate hypoxanthine (10, p. 411) by the usual chlorination procedures were unsatisfactory. Phosphorus oxychloride either in the presence or absence of N, N-dimethaniline did not produce 6-chloropurine. When triethylamine was used in the reaction (3, p. 3624) 6-diethylaminopurine was produced in 66% yield.

In 1954 Bendich, Russell, and Fox (2, p. 6074-6075) discovered the reason for previous failures to isolate 6-chloropurine

from the reaction products of chlorinated hypoxanthine. In the chlorination of hypoxanthine in the presence of N, N-dimethylaniline, a variously colored complex between 6-chloropurine and N, N-dimethylaniline was formed from which 6-chloropurine was not extractable with ether. The complex was easily disintegrated by mixing the concentrated reaction mass with ice and treating it in the cold with strong base. The N, N-dimethylaniline was then removed from the alkaline solution by ether extraction, and the purine isolated in 60-70% yield by a second ether extraction of the acidified solution. Nearly quantitative yields are obtained when the reaction is run on one gram samples of hypoxanthine.

In 1957 (21) and 1958 (22) Hitchings and Elion patented two sealed tube preparations for 6-chloropurine. One method prepared 6-chloropurine by heating hypoxanthine with "pyrophosphoryl chloride" in a pressure chamber and the other by heating hypoxanthine with phosphorus oxychloride in a pressure chamber.

In view of the effect N, N-dimethyl- and N, N-diethylaniline have on the course of the phosphorus oxychloride chlorination of uric acid, a study of the effect of other nitrogenous bases on the yield of 2, 6, 8-trichloropurine was undertaken. The role of the nitrogenous base on the chlorination reaction is not known. Tertiary amines will readily aminate the purine molecule while tertiary aromatic amines will become phosphorylated under the reaction

conditions. Perhaps the amination of the purine indicates that the amine is intimately involved in the process of chlorinating the purine ring, however, it could simply mean that 2, 6, 8-trichloropurine is reactive in the presence of amines and is aminated. If the amine is intimately involved in the chlorination reaction it would seem reasonable that some specific amine might lead to higher yields of chlorinated product. The phosphorylation of the aromatic amine would be a side reaction which has no connection with the chlorination reaction.

When N, N-diethylaniline was used as the amine a 17. 3% yield of 2, 6, 8-trichloropurine was obtained. A large amount of compound 3b (47% yield), presumably chlorohydroxy-N-ethylanilinopurine (as judged by analytical data) was isolated. The separation of products was complicated by the presence of a small amount of an intense blue dye; 74. 3% of the starting uric acid was accounted for.

Since phosphorylation takes place at the para position of the aromatic ring on N, N-dimethylaniline in the chlorination reaction, this position was blocked with a methyl group in an effort to prevent the side reaction. For this reason N, N-dimethyl-p-toluidine was used as the amine in the chlorination reactions; this eliminated the phosphorus containing compound found in fraction 3a. The yield of 2, 6, 8-trichloropurine was 17. 3%, or 21. 2% based on the unrecovered uric acid, with 68. 8% of the starting uric acid accounted for

as unreacted compound or by-products.

The chlorination reaction was repeated using N, N-dimethyl-p-tolutine as the amine allowing the hydrolysis step to warm to 30-40°C. Upon isolating the products only a 6.4% yield, or 6.8% based on unrecovered uric acid, of 2, 6, 8-trichloropurine was obtained. It is thus apparent that it is necessary to keep the hydrolysis reaction cold.

In order to even further reduce the possibility of phosphorylation taking place an experiment with N, N-dimethylmesidine as the amine in the chlorination reaction was undertaken, inasmuch as both the ortho and para positions of the aromatic ring of N, N-dimethylmesidine are blocked by methyl groups. Due to steric hindrance there is no resonance interaction between the dimethylamine group and the phenyl ring, thus, the amine would be slightly more nucleophilic than N, N-dimethyl-p-toluidine. Although no phosphorus containing compound was found in fraction 3, the yield of 2, 6, 8-trichloropurine was reduced to 12%

Inasmuch as blocking the para position on the aromatic ring as in N, N-dimethyl-p-toluidine or the ortho and para positions as in N, N-dimethylmesidine eliminates the phosphorylation reaction the effect of activating the ortho and para positions with a meta methyl group was tested. The chlorination reaction was attempted using N, N-dimethyl-m-toluidine as the amine, again no phosphorus

containing compound, fraction 3a, was isolated; the yield of 2, 6, 8-trichloropurine was 17. 5% or 17. 9% based on unrecovered uric acid. It is interesting that blocking the meta position on the aromatic ring of the amine eliminates the phosphorylation reaction as well as does blocking the para position.

N, N, N', N'-Tetraethyl-p-phenylenediamine was also tested as the amine in the chlorination reaction. In this compound the amino groups being para on the phenyl ring tend to activate each other, they become more nucleophilic. There is no steric hindrance to the resonance interaction of the amino groups and the phenyl ring, and the para position on the phenyl ring is blocked to phosphorylation. It was surprising to find that with this amine in the chlorination reaction only a trace of 2, 6, 8-trichloropurine was produced and the starting uric acid was recovered in 45% yield.

Since activation of the amine group decreases or practically eliminates the yield of 2, 6, 8-trichloropurine, N, N-dimethyl-p-nitroaniline was used as the amine in the chlorination reaction. The para nitro group on the phenyl ring would greatly decrease the nucleophilicity of the amine. Only a trace of 2, 6, 8-trichloropurine was produced, and 91% of the starting uric acid was recovered.

2, 6-Lutidine was also used as the nitrogenous base in the chlorination reaction to determine the reactivity of a pyridine type base. 2, 6, 8-Trichloropurine was produced in 16. 5% yield, or

16. 8% yield based on unrecovered uric acid. Evidently the property which is necessary for a nitrogenous base to be active in the chlorination reaction is present in 2, 6-lutindine as well as in some N, N-dialkylaniline derivitives.

To test the possibility that some combination of amine and phosphorus oxychloride is the active chlorinating agent methylphenylphosphoramidic dichloride was prepared and used in the chlorination reaction. When methylphenylphosphoramidic dichloride was heated with uric acid in the presence or absence of phosphorus oxychloride no 2, 6, 8-trichloropurine was produced. The uric acid was recovered in 91-100% yield. The results of this experiment show that if a combination of amine and phosphorus oxychloride is the active chlorinating agent, the molecule is different than methylphenylphosphoramidic dichloride.

"Pyrophosphoryl chloride" was tried as a chlorinating agent in place of phosphorus oxychloride. When N, N-dimethyl-p-toluidine was used as the amine only a trace of 2, 6, 8-trichloropurine was produced. By heating uric acid with "pyrophosphoryl chloride" in a sealed tube for 19 hours, 2, 6, 8-trichloropurine was produced in 15. 7% yield, or 25. 7% yield based on unrecovered uric acid.

A reaction between phosphorus oxychloride and N, N-diethylaniline was carried out to determine the yield of phosphorylated amine and to determine where the phosphorylated compound would

appear in the isolation procedure. The phosphorylated amine, bis (p-diethylaminophenyl)phosphinic acid (the structure assumed by analogy to the product formed when N, N-dimethylaniline is used as the amine) (30, p. 324-326) appears in fraction 3a in 49% yield.

To determine the losses inherent to the isolation procedure, known amounts of 2, 6, 8-trichloropurine were mixed with phosphorus oxychloride and the 2, 6, 8-trichloropurine isolated by the directions given under isolation procedure. When the 2, 6, 8-trichloropurine was mixed with phosphorus oxychloride and the isolation carried out immediately 77% of the 2, 6, 8-trichloropurine was recovered with 11. 5% more accounted for in other isolation fractions by ultraviolet spectral determination. 2, 6, 8-Trichloropurine was mixed with phosphorus oxychloride and a small amount of sirupy ortho phosphoric acid, and refluxed for two hours; all of the 2, 6, 8-trichloropurine went into solution. The 2, 6, 8-trichloropurine was recovered in 64. 5-74. 5% yield with 13. 6-4. 5% accounted for by ultraviolet spectral determination in fraction one and fraction three.

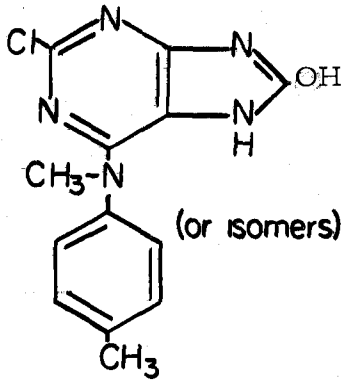
It is now apparent that there are significant losses of 2, 6, 8-trichloropurine inherent to the reaction and isolation procedures used. Greendorfer was able to show that 84-87% of added 2, 6, 8-trichloropurine was lost when it was treated under the chlorination reaction conditions. The isolation studies discussed here indicate

that 23-35% of that loss is inherent to the isolation procedure.

To determine the stability of 2, 6, 8-trichloropurine in acid solution 11. 1 milligrams of 2, 6, 8-trichloropurine was added to one liter of pH 1 hydrochloric acid. The ultraviolet spectra of the solution was obtained several times over a period of one month. There was no significant change in the spectra; thus, 2, 6, 8-trichloropurine is stable in pH 1 hydrochloric acid at room temperature.

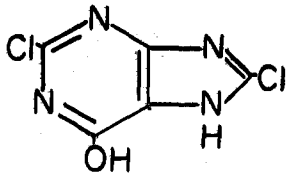
With N, N-dimethyl-p-toluidine as the amine in the chlorination of uric acid with phosphorus oxychloride, the structures of the compounds found in fractions 1c and 1b were postulated to be dichloro-N-methyl-p-toluidino purine and dichlorohydroxypurine respectively (Figure one) on the basis of analytical data (Table III). Greendorfer (19, p. 14-16) previously postulated the indicated structure for fraction 1c. With N, N-diethylaniline as the amine in the reaction, fraction 3a was postulated to be bis(p-diethylaminophenyl)phosphinic acid (Figure one) on the basis of analytical data (Table III) and an analogous structure proven by Robins and Christensen (30, p. 325).

In view of all the accumulated data there appeared to be a possibility that amine hydrochloride might be the active agent in the chlorination reaction. Hydrogen chloride evolved in the chlorination reaction would react with the amine present to form small amounts of amine hydrochloride in the reaction mixture. To test the



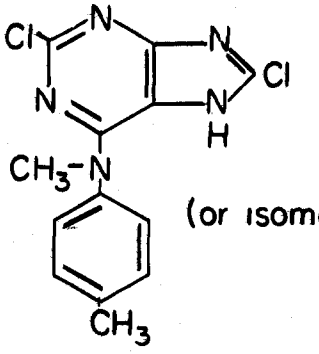
(or isomers)

2a, 3b ①



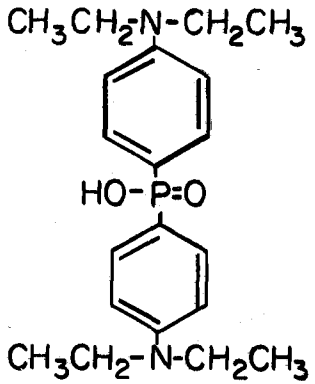
(or isomers)

1b



(or isomers)

1c ①



3a

① structure indicated by Greendorfer (19, p. 14-16)

Figure 1

Possible Compound Structures

hypothesis a reaction was carried out using N, N-diethylaniline hydrochloride in place of the free amine. At the end of the 24-hour reaction period, the reaction mixture was very light tan in color, as opposed to being black when the free amine was used in the reaction. When the reaction mass, after excess phosphorus oxychloride has been removed, was poured over crushed ice an easily hydrolized mixture readily formed, as opposed to a difficultly hydrolyzable thick oil when the free amine was used. No phosphorylated compounds were isolated. 2, 6, 8-Trichloropurine was produced in 46. 2% yield or 56. 7% yield based on unrecovered uric acid. The use of N, N-diethylaniline hydrochloride in the chlorination of uric acid with phosphorus oxychloride gave a reaction which was easy to carry out, was free of colored by-products, and was capable of giving reasonable yields of 2, 6, 8-trichloropurine; the use of N, N-diethylaniline hydrochloride appears to be a breakthrough in the study of this chlorination reaction.

EXPERIMENTAL

Chlorination Procedure

The chlorination procedure used in this investigation was similar to that of Davoll and Lowy as modified by Greendorfer. Phosphorus oxychloride and a nitrogenous base were added to uric acid in the molar ratio of 10:3:1 respectively. The phosphorus oxychloride was freshly distilled, the amine dried over potassium hydroxide, while the uric acid was dried over phosphorus pentoxide prior to use. The reaction mixture was refluxed with the exclusion of moisture and with stirring for 20-24 hours. Distillation of the dark solution under reduced pressure to about half volume yielded a sirup.

Isolation Procedure

The isolation of products was accomplished by the operations shown in Figure two.

The syrupy reaction mixture was poured slowly over crushed ice. Ice was added periodically to the hydrolysis mixture to maintain a few suspended pieces in the solution, thus assuring low temperatures at all times; the tar was triturated frequently to hasten the conversion to a more granular solid. After approximately one

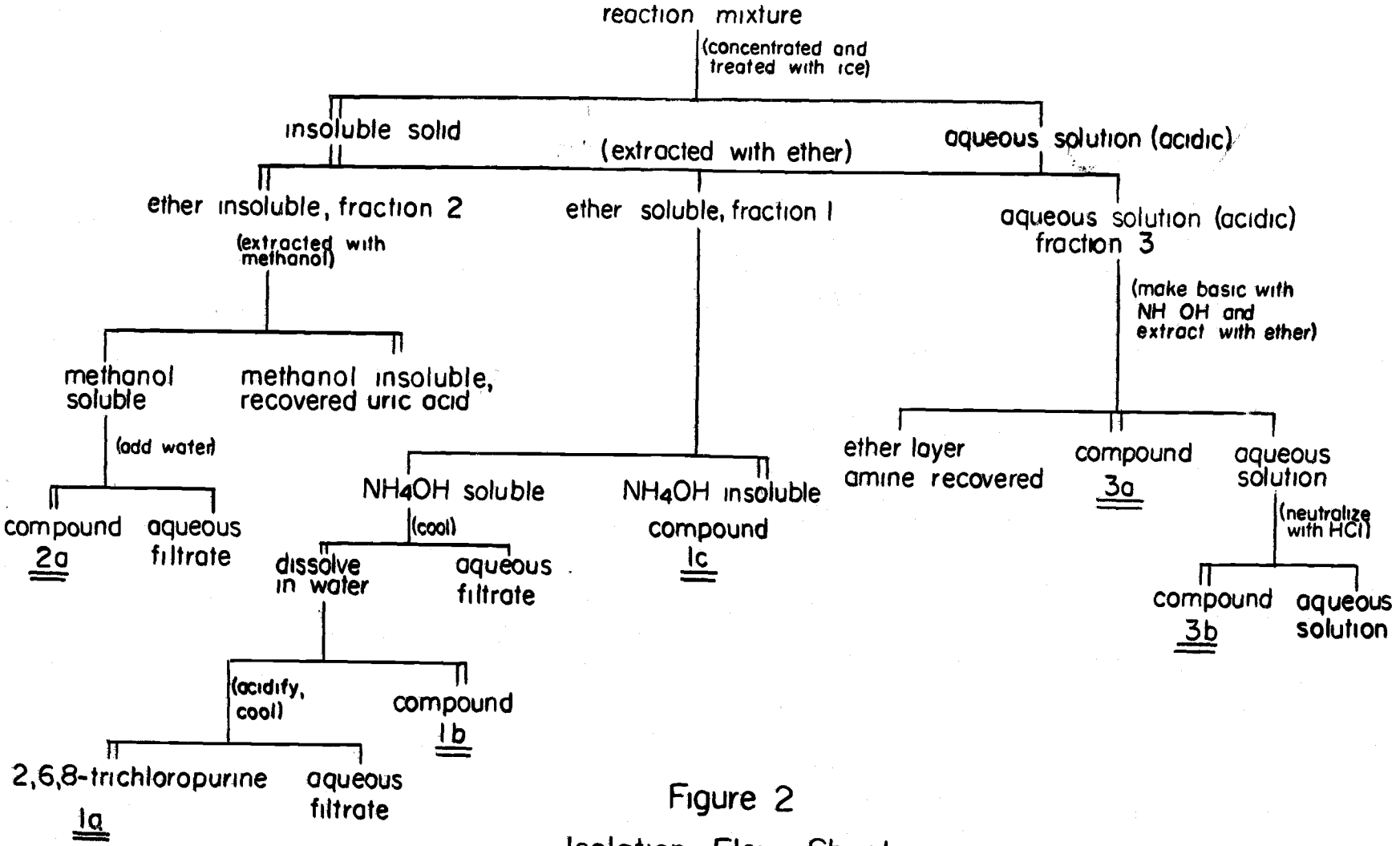


Figure 2

Isolation Flow Sheet

hour the mixture was filtered, and the aqueous filtrate extracted with six portions of ether which were then used to extract the solid.

The ether extracts were combined and evaporated to dryness yielding a solid residue which was extracted with a minimum of boiling 3N ammonium hydroxide and filtered. The insoluble portion was fraction 1c. Upon cooling the filtrate yielded the ammonium salt of 2, 6, 8-trichloropurine as a mass of fine needles. Neutralization of the mother liquor precipitated a small amount of crude 2, 6, 8-trichloropurine.

The ammonium salt of 2, 6, 8-trichloropurine and the crude 2, 6, 8-trichloropurine were dissolved in a minimum of boiling water leaving a water insoluble portion (fraction 1b) which was removed by filtration. The aqueous filtrate was decolorized with Norite and filtered. Acidification of the filtrate with dilute hydrochloric acid and cooling yielded 2, 6, 8-trichloropurine as a mass of fine white needles (fraction 1a).

The aqueous solution remaining from the ether extraction of the hydrolized reaction mass was basified with 28% ammonium hydroxide and the amine removed (42-98% recovery) by ether extraction. A precipitate sometimes formed at this point (fraction 3a) and was removed by filtration. Upon neutralization of the basic aqueous layer with dilute hydrochloric acid a precipitate formed (fraction 3b).

The solid ether insoluble fraction remaining from the ether extraction of the hydrolyzed reaction mass, was extracted with methanol. The methanol insoluble portion, recovered uric acid, was removed by filtration. Addition of water to the methanol solution precipitated a solid (fraction 2a).

The experimental data from the chlorinations of uric acid using the previously mentioned reaction and isolation procedures or modifications of them is given in Table I.

Determination of Losses Inherent to the Isolation Procedure

2, 6, 8-Trichloropurine and phosphorus oxychloride were mixed together and the 2, 6, 8-trichloropurine isolated. The previous combination with a small amount of syrupy orthophosphoric acid added was refluxed for two hours before isolating the 2, 6, 8-trichloropurine. The 2, 6, 8-trichloropurine was isolated by the directions given under isolation procedure; the results of the experiments are shown in Table II.

Stability of 2, 6, 8-Trichloropurine in pH 1 Hydrochloric Acid

1. Eleven milligrams of 2, 6, 8-trichloropurine were added to one liter of pH 1 hydrochloric acid; the solution was mixed well and kept at room temperature. The ultraviolet spectra of the solution was obtained at intervals over a one month period. No significant

Table I, part 1. Data from Chlorination of Uric Acid

Experiment Number	Uric Acid grams	Chlorinating Agent	Nitrogenous Base	Reaction Time, hours	% 2, 6, 8-Trichloropurine
1	20	phosphorous oxychloride (109 ml)	N, N-diethylaniline (57.1 ml)	24[1]	17.3
2	5	" (27.2 ml)	N, N-dimethyl-p-toluidine (12.9 ml)	24	17.3 (21.2[2])
3	10	" (54.5 ml)	"	24	6.4[3] (6.8)
4	2	" (10.9 ml)	N, N-dimethyl-m-toluidine (5.1 ml)	24	17.5 (17.9)
5	1	" (5.4 ml)	N, N-dimethylmesidine (3.18 g)	20	12.0
6	1	" (5.4 ml)	N, N, N', N'-tetraethyl-p-phenylenediamine (29.3 g)	22	trace
7	2	" (21.8 ml)	N, N-dimethyl-p-nitroaniline (59.4 g)	20	trace
8	2	" (10.9 ml)	2, 6-lutidine (4.1 ml)	21	16.5 (16.8)
9	5	phosphorous oxychloride (27.2 ml) +methylphenylphosphoramidic dichloride (20 g)	none	40	none
10	1	methylphenylphosphoramidic dichloride (12 g)	none	24.5[4]	none
11	2.5	"pyrophosphoryl chloride" (37.5 ml)	N, N-dimethyl-p-toluidine (6.5 ml)	21	0.4
12	4.42	" (32 ml)	none	19[5]	15.7 (25.7)
13	5.0	phosphorus oxychloride (27.2 ml)	N, N-diethylaniline hydrochloride (16.6 g)	24	46.2 (56.7)

[1]Reaction not stirred and allowed to stand at room temperature for 10 hours before isolating products.
[2]Percentage based on unrecovered uric acid.
[3]Reaction mass allowed to warm to 30-40°C during hydrolysis step.
[4]Heated at 110°C for 4 hours and then at 155°C for 20.5 hours.
[5]Reaction carried out in a sealed tube at 155°C.

Table I, part 2. Data from Chlorination of Uric Acid

Experiment Number	grams of fraction					% recovered uric acid	% recovered nitrogenous base
	1b	1c	2a	3a	3b		
1		trace	3.9	8.3	15.7	unknown	none
2	0.69	1.10	0.64		0.09	19	62
3	0.3	0.79	3.10	1.80	3.28	6.7	62
4	0.08	0.42	0.44		0.07	2	58
5	trace	trace	0.07		0.67	trace	86
6		trace	0.16	0.05	0.23	45	81
7		trace	unknown			91	42.1
8		0.08	0.41		0.03	2	
9						92	75
10						100	78
11		0.12	0.09		0.56	48	77
12			0.24			40	
13	0.03	0.24	0.33			18.6	98

Table II

DATA FROM 2, 6, 8-TRICHLOROPURINE RECOVERY EXPERIMENTS

Starting 2, 6, 8-trichloropurine	Reflux time	Ortho-phosphoric acid	2, 6, 8-Trichloropurine			
			Recovered in fraction 1	Spectrally determined in fraction 1 filtrates	Spectrally determined in fraction 3	% accounted for
2 grams	none	none	1.54 grams (77.0%)	0.23 gram	not determined	88.5
2 grams	2 hours	1/2 gram	1.49 grams (74.5%)	0.09 gram	not determined	79.0
1 gram	2 hours	1/4 gram	0.64 gram (64.5%)	0.11 gram	0.026 gram	78.1

change in the ultraviolet spectra was observed.

Reaction of Phosphorus Oxychloride and N, N-Diethylaniline

N, N-Diethylaniline, 8.2 ml (0.051 mole) was added to 15.6 ml (0.17 mole) of phosphorus oxychloride and refluxed for 24 hours, allowed to stand at room temperature for four days, and the product isolated according to the directions given under isolation procedure. The yield of fraction 3a (bis(p-diethylaminophenyl)phosphinic acid) was 4.5 grams (49%).

Product Identification

The analytical data for compounds 1b, and 1c when N, N-dimethyl-p-toluidine was used as the base, and for compound 2a when N, N-diethylaniline was used as the base are given in Table III.

The analytical compounds were purified by crystallization. Compound 1b was purified by dissolving in a weakly basic ammonium hydroxide solution, decolorizing with Norite, and making the solution very weakly alkaline by the addition of dilute hydrochloric acid; compound 1b crystallized out. Compound 1c was dissolved in chloroform and filtered. Evaporation of the chloroform precipitated a tan compound which was extracted with a small amount of acetonitrile to remove the brown color. Crystallization from acetonitrile produced white nodules of micro-crystals. Compound

Table III

ANALYTICAL DATA

Compound	Melting point (uncorrected)	Calculated			Found		
		%C	%H	%N	%C	%H	%N
dichloro-n-methyl-p-toluidinopurine experiment 2, fraction 1c	160-163 D	50.8	3.58	22.75	50.2	3.44	22.27
dichlorohydroxypurine experiment 2, fraction 1b		29.3	0.975		28.15	1.23	
bis(p-diethylaminophenyl) phosphinic acid experiment 1 fraction 3a	201-202			7.77			7.88

3a was dissolved in dilute hydrochloric acid, decolorized with Norite, and basified with concentrated ammonium hydroxide until the solution became cloudy. Upon standing crystallization took place. The crystals were further purified by recrystallization from dioxane.

Preparation of N, N-Dimethyl-m-toluidine (3, p. 296-318)

Dimethyl sulfate, 118.5 ml (1.25 mole), was added to 107 ml (1 mole) of redistilled m-toluidine. A rapid exothermic reaction took place, which was cooled in an ice bath, yielding a yellow gel. The reaction was heated at approximately 150°C for three hours, cooled, and concentrated sodium hydroxide added until the mixture was basic. The amine separated, was removed, and dried over anhydrous sodium sulfate.

An equal volume of acetic anhydride was added to the amine and the solution allowed to stand overnight. The mixture was treated with 100 ml of concentrated hydrochloric acid dissolved in 150 ml of water and shaken until the base dissolved. The solution was extracted with three 100 ml portions of ether, which were discarded, and the water layer treated with 25% sodium hydroxide solution to free the base. The amine was separated, dried over solid sodium hydroxide, and distilled. A small fraction boiling between 111-112°C under aspirator vacuum was collected.

Preparation of N, N-Dimethylmesidine (12, p. 972)

Ten grams (0.074 mole) of mesidine, 16.2 grams (0.20 mole) of 37% aqueous formaldehyde, and 120 grams (2.22 mole) of 80% aqueous formic acid were mixed and refluxed for seven hours. Ten ml of concentrated hydrochloric acid was added, and the excess formic acid distilled under aspirator vacuum. The residue was made basic and the crude N, N-dimethylmesidine isolated by steam distillation. The distillate was extracted with three 50 ml portions of ether, the ether removed, and the liquid distilled to yield 7.05 grams (58% yield) of N, N-dimethylmesidine, boiling point range 100-102°C.

Preparation of N, N, N', N'-Tetraethyl-p-phenylenediamine (4, p. 2977-2978)

A mixture of 32.4 grams (0.15 mole) of p-phenylenediamine and 72.8 grams (0.2 mole) (due to an error only 65.5 grams were used) of triethylphosphate (5, p. 109-110) was refluxed for two hours. The mixture was cooled to 50°C, 25 grams of sodium hydroxide dissolved in 100 ml of water added, and the mixture refluxed one hour. Upon cooling the oily layer of amine was removed and the solid sodium phosphate extracted with ether. The combined ether extracts and oil were dried over anhydrous sodium

sulfate.

The ether was removed, the residue treated with an equal volume of acetic anhydride, and allowed to stand overnight. The mixture was treated with 20 ml of concentrated hydrochloric acid dissolved in 30 ml of water, and shaken until the base dissolved. The solution was extracted with three 30 ml portions of ether, which were discarded, and the water layer treated with 25% sodium hydroxide solution to free the base. The oil which formed was removed by ether extraction, dried over anhydrous sodium sulfate, and distilled. An impure mixture distilled over a range of 120-210°C. The product solidified from the distillate and was removed. Upon dissolving the residual oily distillate in 95% alcohol and cooling another crop of crystals was obtained. The yield was 6.3 grams (9.5%) of N, N, N', N'-tetraethyl-p-phenylenediamine. The product was purified by crystallization from 95% alcohol.

Preparation of N, N-Dimethyl-p-nitroaniline (7, p. 740)

To a mixture of 42 grams of 1-bromonitrobenzene, 300 ml of pyridine, and 50 grams of sodium bicarbonate was added 30 grams of dimethylamine hydrochloride dissolved in warm water. The mixture was refluxed for ten hours. At the end of the reflux period the hot solution was filtered free of inorganic salts and the salts extracted with 200 ml of acetone which was added to the pyridine

solution. The pyridine-acetone solution was heated to boiling and water added until the cloud point was reached. On cooling 30 grams (88% yield) of N, N-dimethyl-p-nitroaniline crystallized out as bright yellow needles. The product upon recrystallization from methanol gave a sharp melting point at 164°C, uncorrected.

Preparation of Methylphenylphosphoramidic Dichloride (27, p. 253)

To 66 ml (0.72 mole) of redistilled phosphorus oxychloride was added slowly with cooling and stirring 52 ml (0.48 mole) of N-methylaniline. After the addition of the base the reaction mixture was heated to 150°C until hydrogen chloride evolution ceased. The reaction mass was distilled under aspirator vacuum with the product, 110 grams (93.5%) of methylphenylphosphoramidic dichloride distilling over at 180-184°C as a light yellow liquid.

Preparation of "Pyrophosphoryl Chloride" (11, p. 3509-3510)

To 200 ml of phosphorus oxychloride (2.2 mole) was added 20 ml of water dropwise over a one-half hour period. After the addition of water the mixture was refluxed for 1.5 hours until the hydrogen chloride evolution had practically ceased. The mixture was stirred vigorously during the addition of water and more slowly during the reflux period. The mixture was cooled and the "pyrophosphoryl chloride" decanted from a very thick black sirup.

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