THE REACTION OF PHOSPHORUS OXYCHLORIDE WITH CERTAIN HYDROXYLATED NITROGEN HETEROCYCLES IN THE PRESENCE OF TERTIARY AROMATIC AMINES

by

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Typed by Lilah N. Potter

To my mother and father

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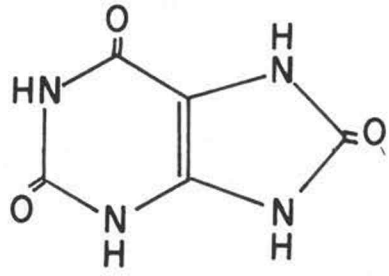
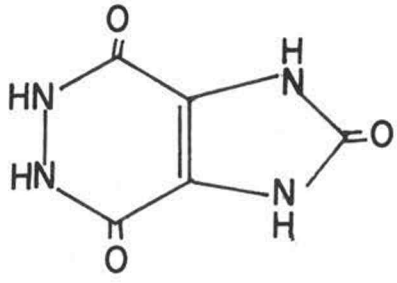
THE REACTION OF PHOSPHORUS OXYCHLORIDE WITH CERTAIN HYDROXYLATED NITROGEN HETEROCYCLES IN THE PRESENCE OF TERTIARY AROMATIC AMINES

In 1959 a method for the preparation of 2,4,7-(1H,3H,5H,6H)imidazo[4,5-d] pyridazinetrione was developed by Paul Laursen (18, p. 31, 33-37) of this laboratory, who in a private communication made his procedure available for further studies. This compound is isomeric with uric acid, differing only in that the nitrogen and carbon atoms in the two and the three positions of uric acid have been reversed:



URIC ACID



2,4,7(1H,3H,5H,6H) IMIDAZO [4,5-d]-PYRIDAZINETRIONE

Because of its close relationship to the purine ring system and since the chemistry of the imidazopyridazines are little known, this compound offers many possibilities for the preparation of potentially interesting purine antagonists as well as an opportunity to study this relatively new ring system.

In order to utilize the imidazopyridazinetrione, it is necessary to convert the compound to a more reactive intermediate such as the trichloro derivative. Initial experiments with the chlorination using the imidazopyridazinetrione prepared by the directions given by Laursen gave rather low yields (12-13%) of the desired product.

The reason for these low yields was thought to stem either from the nature of the chlorination reaction or perhaps from the presence of uncyclized intermediates in the imidazo[4,5-d]pyridazinetrione product. The analysis of the starting material indicated that perhaps some of the problem arose from the presence of uncyclized intermediates.

Imidazo[4,5-d]pyridazinetrione is prepared by the cyclization of the dihydrazide of 2-imidalone-4,5-dicarboxylic acid by heating in 2N hydrochloric acid for 6 hours on the steam bath. Checking back, it was discovered that the dihydrazide had not been completely

cyclized in the last step leading to the imidazo [4,5-d]-pyridazinetrione. In fact some of the samples as judged by the analytical data appeared to be only 50% pure. However, by extending the cyclization period from 6 to 20 hours, it was possible to obtain a cyclized product which consistently was over 90% pure as judged by spectral data. The procedure based on cyclization by boiling hydrazine as described by Jones (12, p. 162) for the preparation of 4,7(1H,5H,6H)imidazo [4,5-d] pyridazinedione was found to be applicable to this problem. In this case the hydrazino salt of the imidazo [4,5-d] pyridazinetrione was obtained from the reaction mixture.

Although the original preparation of the imidazo-[4,5-d] pyridazinetrione was made from the cyclization of the dihydrazide of 2-imidazolone-4,5-dicarboxylic acid, it was discovered that the trione could be prepared directly by the cyclization of the butyl ester of 2-imidazolone-4,5-dicarboxylic acid in refluxing hydrazine hydrate thus materially shortening the route to the trione. Attempts to cyclize the dihydrazide using concentrated sulfuric acid yielded the sulfate salt of the dihydrazide instead of the cyclic product.

Chlorination of the 2,4,7(1H,3H,5H,6H)imidazo-[4,5-d] pyridazinetrione (91% pure) gave improved yields which, however, were low ranging from 19 to 26%. Since

a great deal of work is involved in the preparation of the imidazo [4,5-d] pyridazinetrione, the low yields resulting from the chlorination of this material posed a problem which materially limited its usefulness as a key compound for preparative purposes.

The chloro substituted nitrogen heterocycles are usually excellent intermediates for synthetic purposes particularly with reactions which involve the use of nucleophilic reagents. For the most part these reactions are straightforward and give good yields with no involvement of difficult isolation procedures.

Furthermore, the hydroxy derivative of the corresponding substituted nitrogen heterocycles are usually available or for the most part relatively easy to synthesize. The conversion of the hydroxy derivative of the nitrogen heterocycle on the other hand to the corresponding chloro substituted derivative are for the most part complex reaction giving low yields of the desired product. If more were known about the nature of this reaction (conversion of hydroxy substituted to the chloro substituted nitrogen heterocycle) it might be possible to alter the course of the reaction in such a way as to yield more of the desired product. It was for this reason that this investigation was undertaken.

Phosphorus oxychloride is the reagent which is

most commonly used to convert the hydroxylated heterocycle to the chloro derivative. This is accomplished by suspending the compound in refluxing phosphorus oxychloride for several hours, removing the unreacted phosphorus halides and then isolating the product. Sometimes these reactions are carried out in sealed tubes with or without the addition of phosphorus pentachloride.

One of the earliest applications of these procedures was that of Fischer (7, p. 2208-2210) who reported the conversion of the potassium salt of uric acid to 2,6-dichloro-8-oxypurine by treatment with a 1:2 molar ratio of phosphorus oxychloride in a closed vessel at 160-170°C (yield 40-50%). The trichloropurine (8, p. 2220-2223) was in turn prepared by the action of a large excess of phosphorus oxychloride by repeating the chlorination on the dichloroxypurine in a closed vessel. The yield on the latter reaction was 65% based on 2,6-dichloro-8-oxypurine and 26-32% overall based on the original uric acid.

In 1946 Davoll, Lythgoe and Todd (4, p. 836) prepared 2,6,8-trichloropurine from 2,6-dichloro-8-oxypurine by refluxing a mixture of the suspended purine, dimethylaniline, and phosphorus oxychloride in a 91% yield. Their preparation of the 2,6-dichloro-8-oxypurine by the same procedure gave a 53% yield or an overall yield (from

uric acid) of 48%.

Davoll and Lowy (5, p. 2936) reported a new synthesis of trichloropurine starting from commercial uric acid. These workers refluxed a molar ratio of 1:10:3 of uric acid, phosphorus oxychloride, and dimethylaniline. The trichloropurine was isolated as the ammonium salt in a 16-25% yield. This procedure had the advantage of reducing the reaction to a one-step operation while eliminating the need for sealed tube techniques.

The use of tertiary amines in these chlorination operations was first introduced by Kenner, Lythgoe, Todd and Topham (14, p. 575) in 1943 in the preparation of 4,6-dichloropyrimidine from the corresponding hydroxy derivative. In 1944 Baddiley and Topham (1, p. 678-679) applied this technique to the chlorination of barbituric acid. Langerman and Banks (16, p. 3011-3012) found that even catalytic amounts of dimethylaniline made the technique of using sealed tubes for the chlorination of barbituric acid unnecessary. Robins and Christensen (20, p. 3624-3627) studied the chlorination of uric acid with phosphorus oxychloride in the presence of various aliphatic tertiary amines as well as dimethylaniline. They were unsuccessful in their attempts to isolate trichloropurine from the reaction products of a refluxing mixture of phosphorus oxychloride, uric acid and dimethylaniline.

Furthermore, they reported (19, p. 324-327) the phosphorylation of dimethylaniline in 17% yield by refluxing with oxychloride for a period of 36 hours.

These workers attempted the chlorination of uric acid in the presence of tertiary aliphatic amines. Using small concentrations of trimethylamine resulted in the chlorination of the 8-position to yield unexpected 8-chloroxanthine. Using larger quantities of aliphatic tertiary amines led to the amination of the purine ring in the 6-position to give a 2,8-dichloro-6-diethylaminopurine in a 28% yield.

Furthermore, the substitution of xanthine for uric acid gave a diaminated product in the 2- and 6-positions with both dimethyl- and triethylamine. Hypoxanthine gave 6-diethylaminopurine when treated under similar conditions.

Boldyrev and Makitra (3, p. 399-404) modified the old Fischer procedure for the preparation of trichloropurine by using a molar ratio of 1:3 of the dipotassium salt of uric acid and phosphorus oxychloride and a temperature of 165°. These workers obtained a 53% yield for the first step, the conversion to 2,6-dichloro-8-oxypurine, and an 87% yield for the conversion to trichloropurine (overall 44.5%). They were very careful to remove the excess phosphorus oxychloride as completely as

possible prior to treatment with water to isolate the product to avoid hydrolysis of the trichloropurine to the 2,6-dichloro-8-oxypurine by the acidic media.

Garkusha (10, p. 2869f) prepared trichloropurine by another route; he started with the ammonium salt of 8-chloroxanthine and treated it with a mixture of dimethylaniline and phosphorus oxychloride. A 47% yield of trichloropurine was obtained.

Although uric acid was chlorinated as early as the turn of the century it was not until 1956 (6, p. 3508-3510) that the first successful chlorination of xanthine was reported. This was accomplished by using pyrophosphoryl chloride in place of phosphorus oxychloride in sealed tube reactions at 165° for 19 hours with 43% yield. It is quite probable that xanthine is chlorinated by phosphorus oxychloride but that the problem of isolation has not been resolved. Judging by the work of Robins and Christensen, in which xanthine was aminated in the presence of refluxing the triethylamine and phosphorus oxychloride, lends support to this hypothesis.

On the other hand Bendich, Russell and Fox (2, p. 6073-6076) found that hypoxanthine could be chlorinated by phosphorus oxychloride in a 60-70% yield in the presence of dimethylaniline.

Uracil was chlorinated by Gabriel (9, p. 1690)

in 1905 using phosphorus oxychloride sealed tube reactions with 80% yields. Hilbert and Johnson (11, p. 1154-1155) found that treatment of uracil with an excess of phosphorus oxychloride under reflux gave 2,6-dichloropyrimidine in a 68% yield.

A number of interesting facets of the problem of chlorinating uric acid are now apparent. It would appear that the course of the reaction in the presence of tertiary amines is different, yielding an 8-chloroxanthine, while 2,6-dichloro-8-oxypurine is obtained in the absence of the amine. Furthermore, the tertiary amines either aryl-alkyl or alkyl will aminate uric acid in the presence of refluxing phosphorus oxychloride. An analogous byproduct has been reported by King, King and Spensley (15, p. 1247-1248) in the preparation of trichloropyrimidine from barbituric acid. These investigators found that under the conditions necessary for the chlorination of the hydroxylated compound with phosphorus oxychloride in the presence of N,N-dimethylaniline, the 4,6-dichloro-2-N-methylanilinopyrimidine was formed. Kawai and Takashige (13, p. 20-23) have shown that 2,4,6-trichloropyrimidine was triaminated when it was refluxed in the presence of dimethylaniline. This type of reaction most likely is also involved in the chlorination of purine. Similarly Robins and Christensen

(20, p. 3626) obtained 2,8-dichloro-6-diethylaminopurine when trichloropurine was refluxed with diethylamine either with or without phosphorus oxychloride. Furthermore, dimethylaniline may aminate the purine on the one hand and be phosphorylated by the phosphorus oxychloride on the other. It would appear that failure to obtain a pure chlorinated product with phosphorus oxychloride may stem in part from isolation problems as well as for other reasons.

In the current work the chlorination of 2,4,7-(1H,3H,5H,6H)imidazo[4,5-d]pyridazinetrione by the procedure as outlined by Paul Laursen (see procedure A) gave yields of only 12-13% for the preparation of 2,4,7-trichloroimidazo[4,5-d]pyridazine. By modifying this procedure, at the suggestion of Laursen, in which the reaction time was extended and diethylaniline was substituted for the dimethylaniline (see procedure B), improved yields of 19-26% were obtained. Extension of the reaction time from 20 hours to 5 days, however, did not improve the yield materially (26%).

Since dimethylaniline is phosphorylated under the conditions required for chlorination with phosphorus oxychloride, thus complicating the isolation problem, experiments were undertaken in which a less reactive tertiary amine was used in place of dimethylaniline.

N,N-Dimethyl-p-toluidine was selected for these experiments. The use of this amine did improve the yield of the desired product, giving 33-44% conversion; furthermore, the reaction mixture appeared to be much cleaner and free of colored by-products.

Tne N,N-dimethyl-p-toluidine used in subsequent experiments was pretreated to insure a N-methyl-p-toluidine and toluidine free product. The use of this material did not improve the yields significantly, indicating that the N,N-dimethyl-p-toluidine obtained from the Eastman Kodak Company was relatively free of these impurities. 2,4,7(1H,3H,5H,6H)Imidazo[4,5-d]pyridazinetrione appears to react much more readily with phosphorus oxychloride in the presence of tertiary amines than does uric acid. For this reason an attempt was made to chlorinate the 2,4,7(1H,3H,5H,6H) imidazo[4,5-d]pyridazinetrione in the absence of the tertiary amine, but it was unsuccessful. In fact the imidazo[4,5-d]pyridazinetrione was found to be completely inert to phosphorus oxychloride at reflux temperatures.

Since it has been observed that uric acid will yield aminated products when refluxed in the presence of aliphatic tertiary amines and phosphorus oxychloride, two experiments were undertaken in which trichloropurine was treated with N,N-dimethyl-p-toluidine in the presence

of refluxing phosphorus oxychloride. Only 13-16% of the starting trichloropurine was recovered from these experiments indicating that trichloropurine undergoes change in this environment. In fact a compound was isolated which was identical to the product isolated from the reaction mixtures of phosphorus oxychloride, N,N-dimethyl-p-toluidine, and uric acid. This compound appears to be an isomer of dichloro-N-methyl-p-toluidinopurine as judged by analysis.

In the case of the imidazo[4,5-d]pyridazinetrione no analogous compound was found. It, therefore, appears that trichloropurine is more reactive towards the tertiary aromatic amines than is its imidazopyridazine analog.

In view of the low yield of uric acid chlorinations, as well as the recovery of the starting materials a series of experiments were undertaken in an attempt to isolate other products from this complex reaction mixture. The procedure which was finally adopted for the separation of the products of this reaction is shown schematically in Figure 1.

Uric Acid Experiments

With N,N-dimethylaniline. - Following the procedure shown schematically in Figure 1, compounds I, II, III (trace), and IV-B were isolated. Compound I

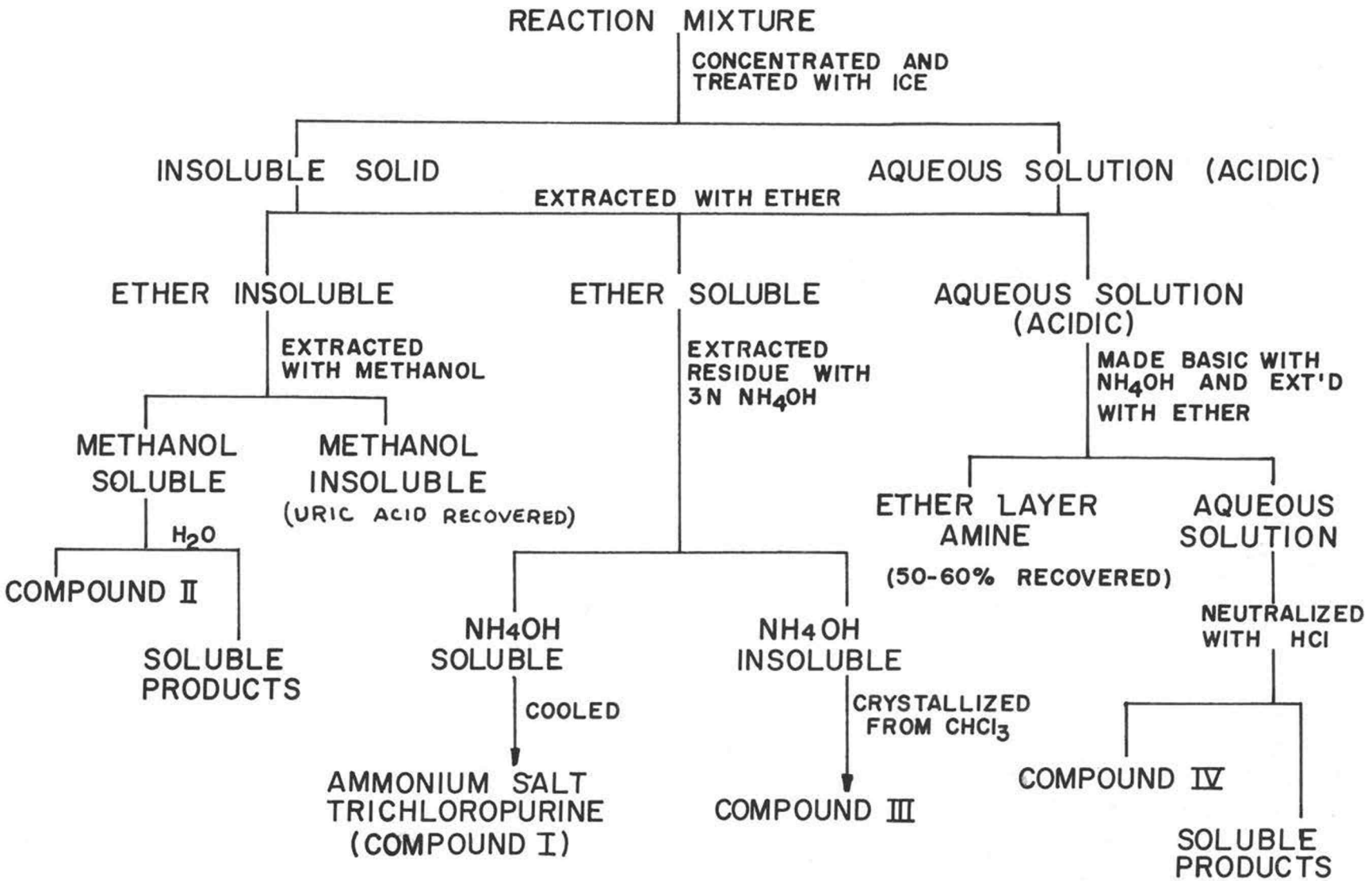

REACTION MIXTURE
CONCENTRATED AND TREATED WITH ICE
INSOLUBLE SOLID
AQUEOUS SOLUTION (ACIDIC)
EXTRACTED WITH ETHER
ETHER INSOLUBLE
ETHER SOLUBLE
AQUEOUS SOLUTION (ACIDIC)
EXTRACTED WITH METHANOL
EXTRACTED RESIDUE WITH 3N NH4OH
MADE BASIC WITH NH4OH AND EXT'D WITH ETHER
METHANOL SOLUBLE
METHANOL INSOLUBLE (URIC ACID RECOVERED)
ETHER LAYER AMINE (50-60% RECOVERED)
AQUEOUS SOLUTION
H2O
COMPOUND II
SOLUBLE PRODUCTS
NH4OH SOLUBLE
NH4OH INSOLUBLE
NEUTRALIZED WITH HCl
COOLED
CRYSTALLIZED FROM CHCl3
AMMONIUM SALT TRICHLOROPURINE (COMPOUND I)
COMPOUND III
COMPOUND IV
SOLUBLE PRODUCTS

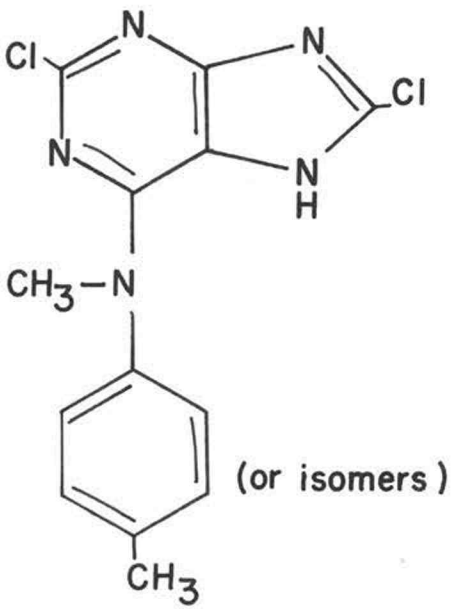
FIGURE I

(trichloropurine) was obtained pure in 15-26% yield. Compound II was so impure that it could not be characterized; however, it was present in quantities of less than 10% by weight. Compound III was present in trace quantities and was not characterized. Compound IV-B was found to be bis(p-dimethylaminophenyl)phosphinic acid as judged by melting point and analytical data. The yield was 10% based on the amount of starting dimethylaniline.

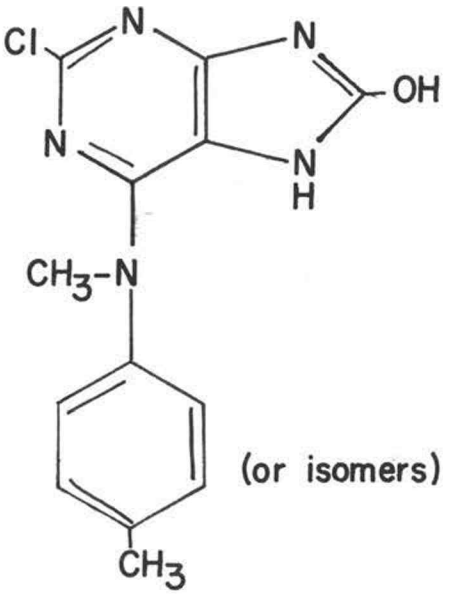
With N,N-dimethyl-p-toluidine. - Compound I (trichloropurine) was obtained pure in 13-25% yield. Compound II appeared to be an isomer of monoaminated-monochlorooxypurine. Analysis showed it to contain 55% carbon and 4.4% hydrogen (53.9% carbon and 4.18% hydrogen are theoretical for a compound of this structure) which supports this viewpoint. The ultraviolet spectrum was not sharp but characteristic of an impure material.

Compound III was one of the isomers of dichloro-N-methyl-p-toluidinopurine as judged by analytical data. This compound gave a sharp ultraviolet spectrum with λ_{max} = 287 mu, $\epsilon = 1.75 \times 10^4$.

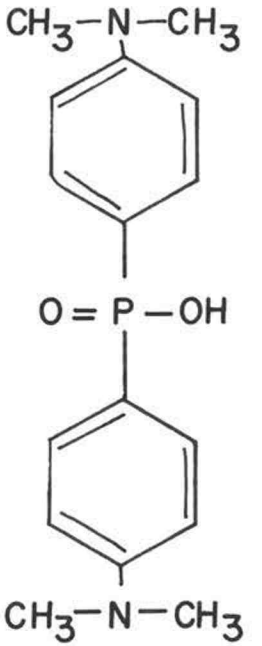
Compound IV-A in this series was entirely different from that obtained with dimethylaniline. It



III



IV-A (and II)



IV-B

FIGURE 2

POSSIBLE STRUCTURES OF COMPOUNDS II, III, IV-A, and IV-B

appeared to be the hydrolysis product of III (i.e. a monochloro-monohydroxy-N-methyl-p-toluidinopurine).

2,4,7(1H,3H,5H,6H)Imidazo[4,5-d]pyridazinetrione Experiments

With N,N-dimethyl-p-toluidine. - Compound I (trichloroimidazo[4,5-d]pyridazine) was obtained in a 33-44% yield. Compound II was isolated in 10-16% yield based on a dichloro-monoaminated compound. Compound III was not found in these reactions and compound IV was isolated only in small amounts.

Material Balance

The material balance for uric acid (see Table I) chlorinations in the presence of dimethylaniline range from 44-58%; in the presence of N,N-dimethyl-p-toluidine the range is from 65-73% (i.e. 65-73% of the uric acid can be accounted for). The only possible way to account for the lost uric acid must be that it is in the aqueous phase as soluble, unextracted derivatives. Since the aqueous solution was neutral the most plausible way to account for water soluble, non-ether extractable derivatives of uric acid must be that they form some sort of a phosphorylated compound. The experiments with 2,4,7-(1H,3H,5H,6H)imidazo[4,5-d]pyridazinetrione indicate

TABLE I

Material Balance

Starting Material	Amine	Trichloro* (NH_4 salt)	Unreacted Starting Material	Comp II	Comp III	Comp IV	Starting Material Accounted for
		%	%	%	%	%	%
Uric acid	Dimethyl-aniline	7.7	48.0	1.9			57.6
"	"	21.4	16.6	6.1			44.1
"	Dimethyl-p-toluidine	12.8	15.0	9.9	12.0	19.2	68.9
"	"	15.7	34.7	4.1	1.6	8.8	64.9
"	"	19.1	27.2	14.5	7.7	4.7	73.2
2,4,7(1H-3H,5H,6H-imidazo-[4,5-d]-pyridazine-trione 91% (pure)	"	37.5		15.5		trace (0.06 g)	53.0
"	"	42.6		10.6		trace (0.05 g)	53.2

*All % yields are based on total amount of starting material

that the formation of such a derivative in this instance is even greater. The search for such a compound or compounds offers an interesting problem for future study.

EXPERIMENTAL

Chlorination Procedures

(A) The procedure of Davoll and Lowy (5, p. 2936) for the chlorination of uric acid as originally modified by Laursen (18, p. 35-37) was used for the preparation of the 2,4,7-trichloroimidazo [4,5-d] pyridazine from the corresponding 2,4,7(1H,3H,5H,6H)imidazo [4,5-d] pyridazinetrione. Phosphorus oxychloride and tertiary aromatic amine were added to the trione compound in the molar ratio of 10:3:1, respectively. The phosphorus oxychloride was freshly distilled in all cases and the various amines were dried over potassium hydroxide prior to use. The 2,4,7(1H,3H,5H,6H)imidazo [4,5-d] pyridazinetrione was pulverized with a mortar and pestle and dried over phosphorus pentoxide. The reaction mixture was refluxed at 110-115°C with the exclusion of moisture. At the end of the reflux period of 6-12 hours (which varied, cf. experiments below) the resulting dark solution was concentrated *in vacuo* to about one-half of the original volume. The syrup was then poured over crushed ice. Enough ice was added to maintain at all times a few suspended pieces in the solution. The time required for the complete hydrolysis of the syrup varied with the amount of syrup and the temperature of the aqueous solution. When too large an

excess of ice was used, the syrup congealed and a hardened tar resulted. This made the further hydrolysis slow. The conversion of the tar to a granular solid was facilitated if not too great an excess of ice were present. The solid mass that formed together with the aqueous solution was extracted continuously for 2-3 days with ether. The ether solution was evaporated to dryness and the residue extracted with a minimum of boiling 3N ammonium hydroxide. The solution was filtered and on cooling the filtrate, the ammonium salt of 2,4,7-trichloroimidazo [4,5-d] pyridazine was deposited as a mass of fine yellow needles which was removed and air dried. Neutralization of the mother liquor with glacial acetic acid recovered a small amount of the crude 2,4,7-trichloroimidazo [4,5-d] pyridazine precipitate which was removed and air dried.

The ammonium salt was dissolved in a minimum of boiling water which was then acidified with dilute sulfuric acid. The crude 2,4,7-trichloroimidazo [4,5-d] - pyridazine obtained from neutralizing the mother liquor was then dissolved in the hot solution, which was decolorized with a small amount of Norite. Filtration of the hot solution followed by cooling yielded a mass of fine yellow needles; this was collected and dried over phosphorus pentoxide.

(B) A private communication from Laursen (17) revealed that certain modifications improved the yield of the desired product. The first of these modifications was the extention of the reflux time to 15 hours with an 8 hour period during which the reaction mixture was allowed to stand at room temperature under moisture free conditions. The second change in procedure dealt with the extraction of the solid and aqueous layer which resulted from the reaction of the concentrated reaction mixture with crushed ice. The solid residue was removed by filtration and extracted with 100-ml portions of ether which were then re-used to extract the aqueous layer. The addition of the ether to the solid makes the solid very tacky and it was found that the extraction could be facilitated if the solid were refiltered after the first few extractions. Very little of the desired product was extracted after about 6 to 8 of these operations. The ether extracts were combined and evaporated to dryness. The residue was dissolved in a minimum of boiling 3N ammonium hydroxide and then treated as described in Procedure A.

(C) The modification in this procedure was the further extention of the reflux time to 20-24 hours with the elimination of the period of standing at room temperature. Furthermore, the solid residue, remaining from

the ether extraction of the solid residue resulting from the reaction of the reaction mixture with crushed ice, was treated as follows. It was extracted in a minimum of methanol and the insoluble portion was removed. The methanol soluble portion could be isolated with difficulty by evaporating the alcohol to very small volumes or more easily by the addition of water to the methanol solution, whereupon a tan compound (II) precipitated.

When the solid resulting from the evaporation of the ether extract of the solid and aqueous layer was re-extracted with the boiling 3N ammonium hydroxide, a residue (compound III) remained, which upon recrystallization from chloroform gave an analytically pure compound.

The original acidic aqueous layer, which had been extracted with ether, was made basic in the cold with concentrated ammonium hydroxide and the amine removed (50-60% recovery) by extraction with ether. Upon neutralization of the basic aqueous layer in the cold with dilute hydrochloric acid a precipitate formed. This compound is designated as compound IV-A when it was obtained from the chlorination of uric acid in the presence of dimethyl-p-toluidine and as compound IV-B when obtained from the chlorination of uric acid in the presence

of dimethylaniline.

The analytical data for compounds II, III, IV-A, IV-B are given in Table III.

The data obtained from the chlorination of uric acid and 2,4,7(1H,3H,5H,6H)imidazo [4,5-d] pyridazinetrione using the forementioned procedures are given in Table IV.

The specific details for each of the chlorinations are listed below.

Chlorinations of Uric Acid in the Presence of Tertiary Aromatic Amines

N,N-Dimethylaniline (mono free)

1. Ten grams (0.06 mole) of uric acid, 55.5 ml (0.61 mole) of phosphorus oxychloride, and 22.7 ml (0.18 mole) of N,N-dimethylaniline were refluxed for 23 hours. The yield of the ammonium salt of 2,6,8-trichloropurine was 1.1 grams (14.8%). The method of isolation employed is described under Procedure (C).

2. Five grams (0.03 mole) of uric acid, 27.5 ml (0.28 mole) of phosphorus oxychloride, and 11.35 ml (0.11 mole) of dimethylaniline were refluxed for 24 hours and subsequently isolated by procedure (C). The yield of the desired 2,6,8-trichloropurine was 1.42 grams (25.5%).

TABLE II

Ultraviolet Spectral Data

Compound	Solvent					
	pH 1.3 (H_2SO_4)		pH 11.9 (NaOH)		Absolute Alcohol	
	λ_{max}	Log ϵ	λ_{max}	Log ϵ	λ_{max}	Log ϵ
2,4,7(1H,3H,5H,6H)imidazo-[4,5-d] pyridazinetrione	279	3.732	288	3.849		
2-imidazolone-4,5-dihydrazide	302	3.900	321	3.982		
2-imidazolone-4,5-dihydrazide sulfate salt (monohydrate)	302	3.911	321	3.980		
compound II (with dimethyl-p-toluidine as amine)					286-9	
compound III (from uric acid chlorination in presence of dimethyl-p-toluidine)					287	4.243
compound III (from trichloro-purine reaction in presence of dimethyl-p-toluidine)					287	4.233
compound IV-A	302		300			
bis(p-dimethylaminophenyl)-phosphinic acid (IV-B)					281	4.502

TABLE III

Analytical Data

Name of Compound	Empirical Formula	Melting Point	Calculated %C	Calculated %H	Found %C	Found %H	Melting Point
Purines							
Dichloro-N-methyl-p-toluidino (III)	$C_{13}H_{11}N_5Cl_2$		50.6	3.60	50.7	3.86	273-274°
Chloro-hydroxy-N-methyl-p-toluidino (II)	$C_{13}H_{12}N_5OCl$		53.9	4.18	54.7	4.39	300
(IV-A)					53.8	4.44	300
Phosphinic Acid							
Bis(p-methylamino-phenyl) (IV-B)	$C_{16}H_{21}N_2O_2P$	208-210°	63.2	6.96	63.0	7.23	207-208°

TABLE IV

Data from the Chlorination of Uric Acid, Imidazo [4,5-d] pyridazinetrione and of Trichloropurine

Compound	Amine	Isolation Procedure	Trichloro[1] Derivative	Compound II	Compound III	Compound IV	Recovered Starting Material
						IV-B	
Uric acid							
10 g	DMA[2]	C	1.1 g[5] (14.8%)	0.33 g	trace	5.5 g	4.8 g
5 g	DMA	C	1.42 g (25.5%)	0.525	trace	0.37	0.83
3 g	DMpT[3]	B	0.7 g[5] (16.3%)				
1 g	DMpT	B (4 hrs)	trace			IV-A	
10 g	DMpT	C	1.7 g (15.1%)	1.7	2.2	3.3	1.5
10 g	DMpT	C	2.23 g[5] (24.1%)	0.7	0.3	1.52	3.47
10 g	DMpT	C	2.72 g[5] (25%)	2.5	1.4	0.81	2.72

TABLE IV - Cont.

Compound	Amine	Isolation Procedure	Trichloro Derivative	Compound II	Compound III	Compound IV
2,4,7(1H,3H,5H,6H)-imidazo [4,5-d] - pyridazinetrione						
16 g	DMA	A	2.7 g (12.7%)			
20.7 g	DMA	A	3.36 g (12.2%)			
3.5 g (91% pure)	DEA[4]	B	1.1 g (26.1%)			
16.4 g (91%)	DEA	B	3.7 g (18.7%)			
1.0 g (91%)	DEA	B (5 days)	0.31 g (25.6%)			
1.0 g (91%)	DMpT	B	0.4 g (33.1%)			
3.0 g (91%)	DMpT	B	1.6 g (44.25%)			
5.0 g (91%)	DMpT (mono free)	C	2.43 g[5] (37.5%)	1.22 g		0.06 g
3.0 g (91%)	DMpT (mono free)	C	1.66 g[5]	0.5		0.05

TABLE IV - Cont.

Compound	Amine	Isolation Procedure	Trichloro Derivative	Compound II	Compound III	Compound II
Trichloropurine						
1.5 g	DMpT	C	0.2 g (13.3%)		0.8 g 38.6 %	
2.0	DMpT	C	0.32 g (16%)		0.8 29 %	

[1]All % yields are on the basis of unrecovered starting material (except in the case of trichloropurine)

[2]DMA - dimethylaniline

[3]DMpT - dimethyl-p-toluidine

[4]DEA - diethylaniline

[5]Ammonium salt of trichloro derivative

N,N-Dimethyl-p-toluidine

1. Three grams (0.018 mole) of uric acid, 18.0 ml (0.2 mole) of phosphorus oxychloride, and 10.0 ml (0.07 mole) of dimethyl-p-toluidine were refluxed for 20 hours and the products isolated according to the directions of procedure (B). The yield of the ammonium salt of trichloropurine was 0.7 gram (16.3%).

2. One gram of uric acid, 5.5 ml of phosphorus oxychloride, and 2.6 ml (0.018 mole) of dimethyl-p-toluidine were refluxed for 4 hours. The reaction mixture was treated according to directions of procedure (B). Yield of the desired product was only in trace quantities.

3. Ten grams of uric acid, 55.0 ml of phosphorus oxychloride, and 26.0 ml of dimethyl-p-toluidine were refluxed for 20 hours. Procedure (C) was employed for the isolation work of this reaction. The yield of trichloropurine was 1.7 grams (15.1%).

4. Ten grams uric acid, 55.0 ml phosphorus oxychloride, and 26.0 ml of dimethyl-p-toluidine (mono free) were refluxed for 24 hours and products isolated by procedure (C). The yield of the ammonium salt of the trichloropurine was 2.23 grams (24.1%).

5. Ten grams of uric acid, 55.0 ml of phosphorus oxychloride, and 26.0 ml of dimethyl-p-toluidine

(mono free) were refluxed for 24 hours. Procedure (C) was used in the isolation of products. The yield of the ammonium salt of trichloropurine was 2.72 grams (25.0%).

Chlorinations of 2,4,7(1H,3H,5H,6H)Imidazo[4,5-d]-pyridazinetrione with Tertiary Aromatic Amines

N,N-Dimethylaniline (mono free)

1. 2,4,7-Imidazo[4,5-d]pyridazinetrione (16.0 grams, 0.095 mole), 90.0 ml (0.98 mole) of phosphorus oxychloride, and 39.0 ml (0.31 mole) of dimethylaniline were reacted and the product isolated using procedure (A). The time of reflux was 6 hours; the yield of 2,4,7-trichloroimidazo[4,5-d]pyridazine was 2.7 grams (12.7%).

2. 2,4,7-Imidazo[4,5-d]pyridazinetrione (20.7 grams, 0.12 mole), 115 ml (1.23 moles) of phosphorus oxychloride, and 47.0 ml (0.37 mole) of dimethylaniline were refluxed for 12 hours and then allowed to stand at room temperature for an additional 14 hours. The products were isolated according to the directions of procedure (A). Before the continuous extraction was started, however, the aqueous layer stood for 24 hours at room temperature. The yield was 3.36 grams (12.2%) of the desired product.

N,N-Diethylaniline (mono free)

1. Three and one-half grams (91% purity, 0.019 mole) of the imidazo [4,5-d] pyridazinetrione, 20.7 ml (0.023 mole) of phosphorus oxychloride, and 10.8 ml (0.068 mole) of diethylaniline were refluxed for 15 hours and then allowed to stand at room temperature for 8 hours. The reaction mixture was then isolated according to the directions of procedure (B). The yield of the 2,4,7-trichloroimidazo [4,5-d] pyridazine- was 1.1 grams (23.7%).

2. 2,4,7-Imidazo [4,5-d] pyridazinetrione (16.4 grams, 91% purity, 0.089 mole), 89.0 ml (0.97 mole) of phosphorus oxychloride, and 47.0 ml (0.29 mole) of diethylaniline were reacted and the products isolated using procedure (B). The reaction yielded 3.7 grams (18.7%) of the trichlorinated compound.

3. One gram (91% purity, 0.0055 mole) of the imiazo [4,5-d] pyridazinetrione, 5.5 ml (0.06 mole) of phosphorus oxychloride, and 2.7 ml (0.017 mole) of diethylaniline were refluxed for 5 days. The reaction mixture was then isolated into its components according to procedure (B). This reaction yielded 0.31 gram (25.6%) of the desired product.

N,N-Dimethyl-p-toluidine

1. One gram of 2,4,7-imidazo [4,5-d] pyridazinetrione (91% pure), 5.5 ml of phosphorus oxychloride, and 2.6 ml of dimethyl-p-toluidine were refluxed for 20 hours. The resulting 2,4,7-trichloroimidazo [4,5-d] - pyridazine was isolated by the directions of procedure (B). The yield amounted to 0.4 gram (33.1%).

2. Three grams (91% pure, 0.016 mole) of the trihydroxylated compound, 16.5 ml (0.18 mole) of phosphorus oxychloride, and 9.0 ml (0.062 mole) of dimethyl-p-toluidine were refluxed for 18 hours. The isolation of the products was accomplished by procedure (B); yield was 1.6 grams (44.25%) of the desired trichlorinated product.

3. Five grams (91% pure, 0.027 mole) of 2,4,7-imidazo [4,5-d] pyridazinetrione, 29.0 ml (0.32 mole) of phosphorus oxychloride, and 14.0 ml (0.1 mole) of dimethyl-p-toluidine (mono free) were refluxed for 24 hours and were subsequently separated into its components by procedure (C). The reaction yielded 2.43 grams (37.5%) of the ammonium salt of the desired product.

4. Three grams of the imidazo [4,5-d] pyridazinetrione, 16.5 ml (0.18 mole) of phosphorus oxychloride, and 7.8 ml (0.05 mole) of dimethyl-p-toluidine were refluxed for 24 hours and the products isolated by

procedure (C). The yield of the desired trichloro derivative was 1.66 grams (42.5%).

The Reaction of 2,6,8-trichloropurine with Phosphorus Oxychloride and N,N-Dimethyl-p-toluidine

1. One-half gram (0.006 mole) of trichloropurine, 8.25 ml (0.09 mole) of phosphorus oxychloride, and 3.87 ml (0.027 mole) of dimethyl-p-toluidine were refluxed for 20 hours and the products isolated by procedure (C). Only 0.2 gram (13.3%) of the trichloropurine was recovered.

2. Two grams (0.009 mole) of trichloropurine, 11.0 ml (0.12 mole) of phosphorus oxychloride, and 5.2 ml (0.036 mole) of dimethyl-p-toluidine (mono free) were refluxed for 24 hours and then the products isolated by procedure (C). The yield of recovered trichloropurine was 0.32 gram (16.0%).

Hydrazino Salt of 2,4,7(1H,3H,5H,6H)Imidazo[4,5-d]-pyridazinetrione

One gram of dihydrazide was placed in 5 ml of hydrazine hydrate (99-100%). On warming to 110°C it all dissolved to give a clear solution. After 10-15 minutes a yellow precipitate formed. The mixture was refluxed for 7½ hours. The yield of solid material which was separated by filtration was 0.7 grams. The

hydrazide hydrate yielded 0.12 gram of the same material when it was evaporated to dryness *in vacuo*. The sample was purified by recrystallizing from concentrated sulfuric acid.

Anal. Calc'd for $C_5H_8N_6O_3$: C, 30.0; H, 4.00.
Found: C, 30.6; H, 3.78.

Reaction of 2,4,7(1H,3H,5H,6H)Imidazo[4,5-d]pyridazinetrione with Phosphorus Oxychloride

One gram of the imidazo[4,5-d]pyridazine was refluxed with 5.5 ml of phosphorus oxychloride for 12 hours. The solid did not dissolve during this time and 0.86 gram of the starting material was recovered after distillation of the reaction mixture to dryness *in vacuo*.

2,4,7(1H,3H,5H,6H)Imidazo[4,5-d]pyridazinetrione

Two grams of the dibutyl ester of 2-imidazolone-4,5-dicarboxylic acid was placed in 10 ml of hydrazine hydrate (99-100%). An exothermic reaction took place and a white precipitate formed. Upon warming the precipitate dissolved. After 10 minutes of refluxing another precipitate formed. The reaction mixture was refluxed for 6 hours and then evaporated to dryness *in vacuo*. The solid residue was suspended in 40 ml of water and concentrated sulfuric acid were added until a pH of 1

was obtained. The precipitate was stirred well and filtered; the yield was 1.1 grams (93%). Analytical samples were recrystallized from concentrated sulfuric acid. The ultraviolet spectral data in pH 11.9 (NaOH) for the above compound were $\lambda_{max} = 288$, $\epsilon = 6.9 \times 10^3$; for a pure sample of the trione $\lambda_{max} = 288$, $\epsilon = 7.06 \times 10^3$.

Sulfate Salt of 2-Imidazolone-4,5-dihydrazide

One and four-tenths of a gram of 2-imidazolone-4,5-dihydrazide was dissolved in 40 ml of concentrated sulfuric acid and filtered with use of a sintered glass funnel. Upon pouring the filtrate onto crushed ice a white precipitate formed, which was filtered and washed well with water, dried, and analyzed.

Anal. Calc'd for $C_5H_{12}N_6O_8S$: C, 19.0; H, 3.83.
Found: C, 18.9; H, 3.98.

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