1969

The Effects of Two Varying Distributions of Practice Sessions on the Improvement of Long Jump Performance of Women

Lucille Florence Lindskov

PHOSPHATE DERIVATIVES OF 5-FLUOROURACIL

AS ANTICANCER COMPOUNDS

BY

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This thesis is approved as a creditable and independent
investigation by a candidate for the degree, Master of Science,
and is acceptable as meeting the thesis requirements for this
degree, but without implying that the conclusions reached by the
candidate are necessarily the conclusions of the major department.

_____   _____
Thesis Advisor   Date

Head, Pharmaceutical Chemistry   Date
Department

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HL

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INTRODUCTION

Cancer is a disease which has been recognized since ancient times and which in every generation has killed many victims of all ages and of all stations in life. The disease has been fought against from generation to generation with whatever ideas and tools available at that time. Today, although cures for many diseases have been found, cancer is still under investigation and more efforts are demanded to overcome the disease. In cancer chemotherapy, it is hoped that drugs can be found which will have a selectively toxic effect on neoplastic cells.

5-Fluorouracil was synthesized in 1957 by Duochinky, Pleven, and Heidelberger and has been intensively studied. The agent produces a significant incidence of objective responses in patients suffering from advanced solid tumors, particularly in breast and gastrointestinal cancers, and prolongs the life of patients with breast carcinomas, but at the expense of some bone marrow and gastrointestinal toxicities. The toxicities result from the lack of real selectivity against cancer cells by this drug.

The cytotoxic action of nitrogen mustard compounds was first applied to the treatment of malignant neoplasms in the 1940s following the observation of Gilman and Philips that these agents caused regression of certain experimental tumors, Hodgkins' disease, and lymphosarcoma. A nitrogen mustard derivative, cyclophosphamide, was synthesized by Arnold, Bourseaus and Brock in 1958. The

selective activity of cyclophosphamide toward cancer cells in comparison to nitrogen mustards per se is of interest and induced the research objective of synthesizing a similar derivative of 5-fluouracil to decrease the side effects of 5-fluorouracil.

RESEARCH OBJECTIVE

It is believed that a significant percentage of human cancer can be cured or at least controlled by chemotherapy, but the number of 'cures' of neoplastic disease in man that have been attained by the use of drugs is still small. The major stumbling block to successful cancer chemotherapy, besides the emergence of natural or aquired drug-resistance, is a lack of real selectivity against tumor cells. This results from the close metabolic resemblance of cancer cells to the normal cells from which they are derived. Because of the close similarity of normal cells and neoplastic cells, it is often necessary to use drugs close to their toxic dosage range in order to cause anticancer effects. The effectiveness of most anticancer agents, excluding the hormones, is probably related to their ability to inter-fere with cell division and hence the toxicity associated with their use is usually encountered in the parts of the body where rapid cell proliferation takes place, such as in the bone marrow and in the gastrointestinal tract. Early signs of bone marrow toxicity are leukopenia and thrombocytopenia. In addition to bone marrow and gas-trointestinal disturbances, the most frequently observed side reactions are nausea and vomiting which probably result from central nervous system disturbances (1). Other toxic symptoms encountered with a number of drugs are alopecia, anorexia, hepatoxicity, and thrombo-phlebitis (2). If a certain compound has a wide spectrum of toxicity to cells, the anticancer utility of such a compound will be very

narrow and the therapeutic index poor. Because of this, a great amount of effort has been expended in the search for compounds that are selectively toxic to malignant tissue. This search has met with some success, but the compound that will destroy only malignant tissue with little or no effect on normal cells has not, as yet, been found or synthesized.

The discovery that mustard gas (bis-chloroethyl sulfide) resulted in a drastic reduction in white blood cell count led to research relative to aliphatic nitrogen mustards. Nitrogen mustards, as exemplified by mechlorethamine (I), readily cyclize under physiological conditions to the relatively stable ethylene iminium ion (II) then react with available nucleophilic centers resulting in an alkylated (denatured) substrate (III) (Fig. 1) (2).

$$
\begin{array}{ccc}
H_3C-N\begin{array}{l} CH_2CH_2Cl \\ CH_2CH_2Cl \end{array} & \longrightarrow & H_2C-CH_2 \\ & & H_3C \stackrel{N^{\oplus}}{\diagup} CH_2CH_2Cl \qquad Cl^{\ominus} \\
I & & II
\end{array}
$$

$$
\xrightarrow{RAH} \qquad H_3C-N\begin{array}{l} CH_2CH_2AR \\ CH_2CH_2Cl \end{array} \quad + \quad HCl
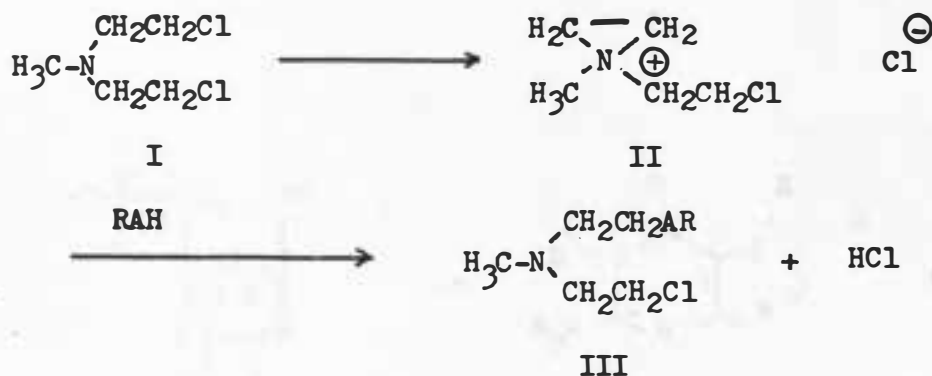$$

III

Fig. 1 Reaction between mechlorethamine and nucleophilic compound (RAH).

When mechlorethamine was incubated with RNA or DNA, the major site of alkylation was shown to be the 7-nitrogen of guanine (IV) (Fig. 2) (3).

in the treatment of cancer than any of its congeners. The beneficial
results of mechlorethamine in Hodgkins' disease and less predictably,
in other lymphomas have been extensively confirmed. Palliative results
also have been observed in chronic leukemias, polycythemia vera, and
carcinomas of the bronchus, ovary, breast, and nasopharynx (6). The
compound, however, cannot be referred to as being selectively toxic
because of the fact that the effective dosage of this agent is often
limited by bone marrow toxicity, severe nausea, and vomiting. For
producing more selectively toxic nitrogen mustards, several modified
forms of nitrogen mustard have been synthesized and clinically investi-
gated, e.g., 1,6-bis(2-chloroethylamino)-1,6-dideoxy-D-mannitol
(degranol, VII), 3-(p- bis(2-chloroethyl)amino phenyl)-L-alanine
(melphalan, VIII), and 2- bis(2-chloroethyl)amino tetrahydro-2H-1,3,
2-oxazaphosphorine-2-oxide (cyclophosphamide, IX).

$$CH_2NHCH_2CH_2Cl$$
$$HOCH$$
$$HOCH$$
$$HCOH$$
$$HCOH$$
$$CH_2NHCH_2CH_2Cl$$

VII

$$HOOCCHCH_2- \bigcirc -N(CH_2CH_2Cl)_2$$
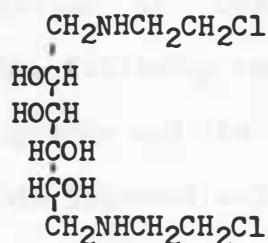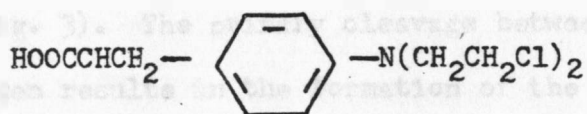
VIII
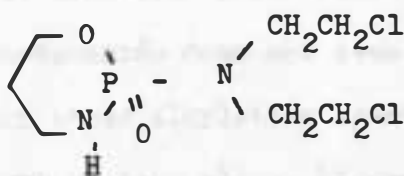
IX

The development of cyclophosphamide resulted in one of the more successful applications of latentation (2). Although the long-term cyclophosphamide treatment has the side effects of leukopenia, thrombopenia and atrophy of epidermis (7), the greater specificity of cyclophosphamide was studied and proved in comparison with other nitrogen mustards (mechlorethamine, normechlorethamine.(XI), etc.) (8). Due to deactivation by the cyclophosphamide moiety, the compound is not toxic to tumor cells in culture even at high concentration (9), but, if it is incubated with liver homogenate prior to cytotoxicity evaluation, it is highly toxic (10). These facts indicate that the chemically inert structure may be cleaved by cellular phosphatases and phosphamidases (11). These enzymes do not occur in circulating plasma and cancerous cells contain greater amounts of phosphamidases (12) and phosphatases (13) than do normal cells. From this reasoning, some selectivity in toxicity could be assumed and apparently has been exhibited (6). Cyclophosphamide is presumed to be activated according to the following reactions (Fig. 3). The primary cleavage between the phosphorus and the ring nitrogen results in the formation of the very stable internal salt (X). Under physiological conditions, hydrolysis at the nitrogen-phosphorus bond of the salt may occur so that the toxic normechlorethamine (XI) is liberated (2). Some dispute has been made that metabolism of cyclophosphamide does not seem to liberate either normechlorethamine or any other alkylating agent (6).

In order for cellular growth to take place, biosynthesis of DNA is essential. If this synthesis is interfered with, cellular growth

$$\longrightarrow \ H_2N(CH_2)_3\text{-}O\text{-}\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}\text{-}N(CH_2CH_2Cl)_2$$

X

$$\longrightarrow \ H_2N(CH_2)_3\text{-}O\text{-}\overset{\overset{\displaystyle O}{\|}}{P}\text{-}(OH)_2 \ + \ HN(CH_2CH_2Cl)_2$$
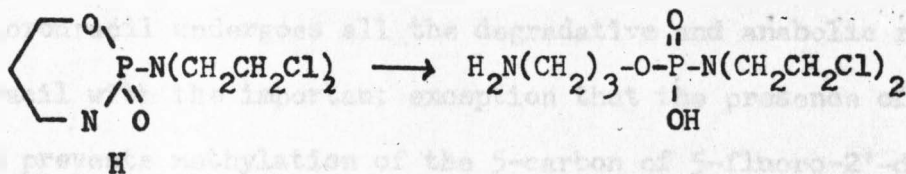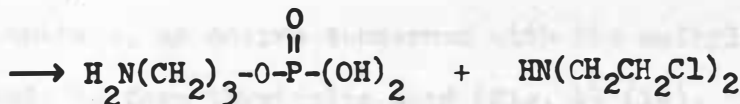
XI

Fig. 3 Activation of cyclophosphamide

is impaired. An important basic component of the DNA helix is thymine (5-methyluracil), linked as the deoxyribonucleotide to other deoxyribo-nucleotides in the helical structure. Since uracil is first incor-porated into 2'-deoxyuridine-5'-phosphate and this is methylated via the thymidylate synthetase system to give the thymine analogue derivative (Fig. 4) (14), a stable group in the 5-position on the uracil molecule would block this methylation. The compound 5-fluorour-acil (XII) has proved this to be true.

XII
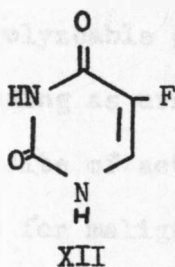
5-Fluorouracil is a potent cytotoxic agent because it is bio-isosteric with uracil and masquerades as uracil up to a biosynthetic point.

5-Fluorouracil undergoes all the degradative and anabolic reactions of uracil with the important exception that the presence of the fluoro group prevents methylation of the 5-carbon of 5-fluoro-2'-deoxyuridine 5'monophosphate (FUdRMP) to form thymidylic acid and inhibits thymidylate synthetase, an enzyme concerned with the methylation of deoxyuridylic acid to form thymidylic acid (Fig. 4) (14). 5-Fluorouracil has had extensive trial against cancer in man. Clinical responses have been reported in a variety of cancers including those of the large bowel, breast, stomach, ovary, thyroid, pancreas, cervix, pharynx and urinary bladder (15). Because of the lack of selective toxicity, tumor regression usually is obtained at the expense of some degree of toxicity which may be severe. Toxicologic effects are observed in the more rapidly proliferating tissues: the bone marrow, the gastrointestinal tract and sometimes, the skin, conjunctiva and vaginal and other mucosal tissues. Severe toxicity is associated with extensive ulceration and hemorrhage of the gastrointestinal tract and aplasia of the marrow (14).

It would seem promising to incorporate onto the molecule of 5-fluorouracil a large, hydrolyzeable group that would interfere with the bio-isosterism (masquerading as uracil) and yet could be removed (hydrolyzed) at the desired site of action. This should result in a compound selectively toxic for malignant tissue if the cleavage took place within the malignant cells.

Considering the discussion of cyclophosphamide, the synthesis of a similar derivative of 5-fluorouracil (XIII) would be desirable.
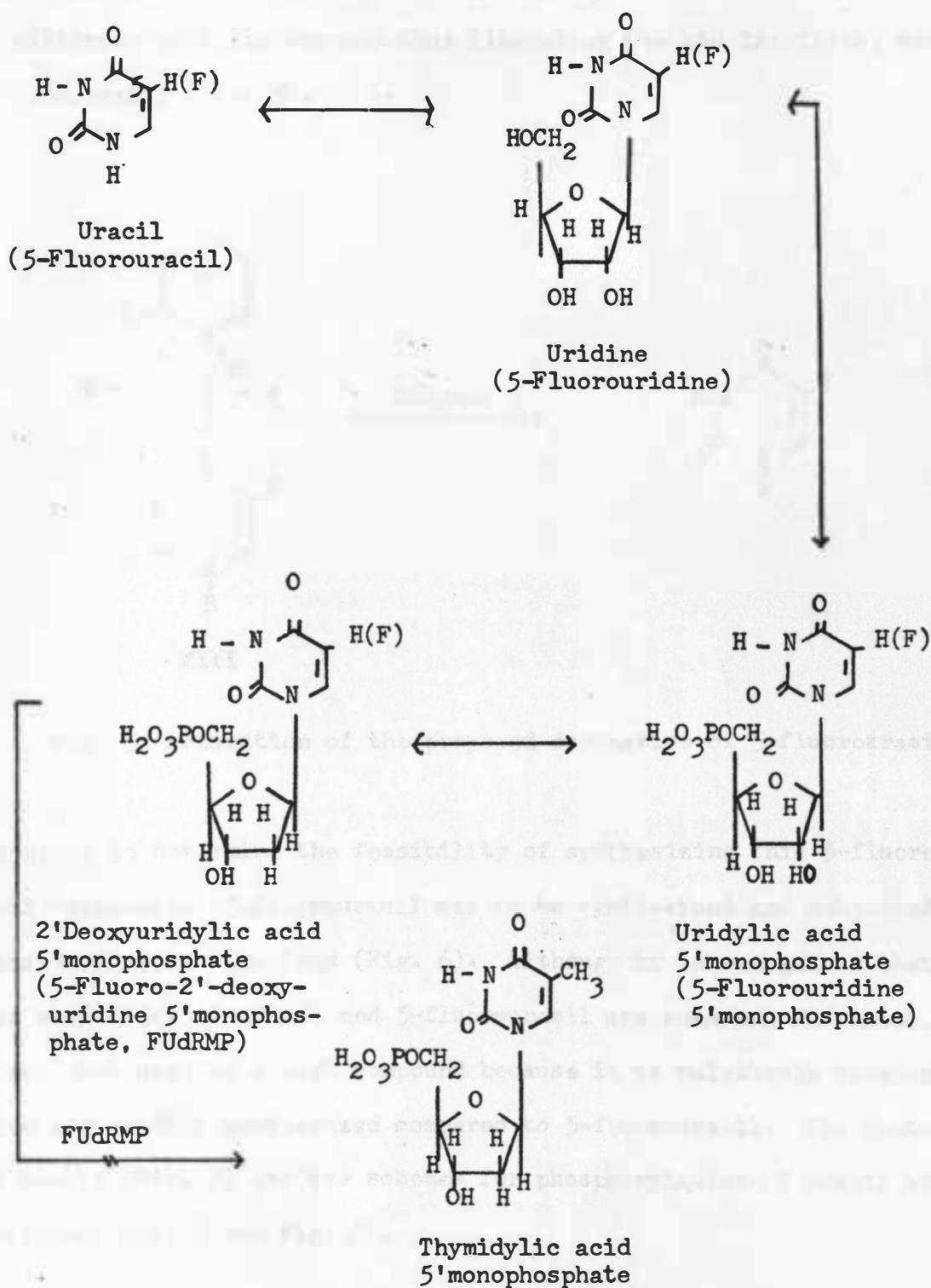
Fig. 4 Synthesis of thymidylic acid 5'monophosphate and
action mechanism of 5-fluorouracil

This compound would hopefully undergo cleavage under the influence
of malignant cellular enzymes thus liberating the bio-isosteric, toxic
5-fluorouracil (Fig. 5).

Enzymes

XIII
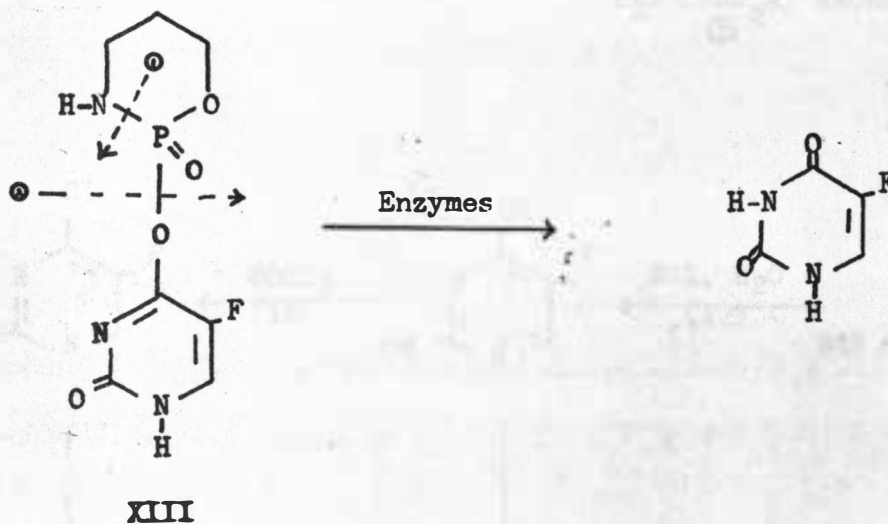
Fig. 5 Activation of the proposed derivative of 5-fluorouracil

In order to determine the feasibility of synthesizing this 5-fluorour-
acil derivative, 5-fluorouracil was to be synthesized and subjected to
phosphorylation reactions (Fig. 6). Although it is recognized that
the activities of uracil and 5-fluorouracil are somewhat different,
uracil was used as a test compound because it is relatively inexpen-
sive and readily synthesized compared to 5-fluorouracil. The synthesis
of uracil (Fig. 7) and the schemes for phosphorylation of uracil are
outlined (Fig. 8 and Fig. 9).

$$F-CH_2Et \ + \ HCO_2Me \ + \ CH_3CH_2OK \longrightarrow \ \underset{H}{\overset{KO}{\diagdown}}C=\underset{CO_2Et}{\overset{F}{\diagup}}$$

(16)

$$\underset{\underset{\oplus}{H_2N-C=NH_2}}{\overset{S-Et}{|}}\overset{\ominus}{X}, \ NaOMe$$

POCl$_3$
(18)

HCl, H$_2$O
(17)

(19) 2 MO-$\overset{O}{\underset{OR}{\overset{||}{P}}}$-OR

(20) 2 K$_2$CO$_3$, 2 Cl-$\overset{O}{\underset{OEt}{\overset{||}{P}}}$-OEt

XIV

XV
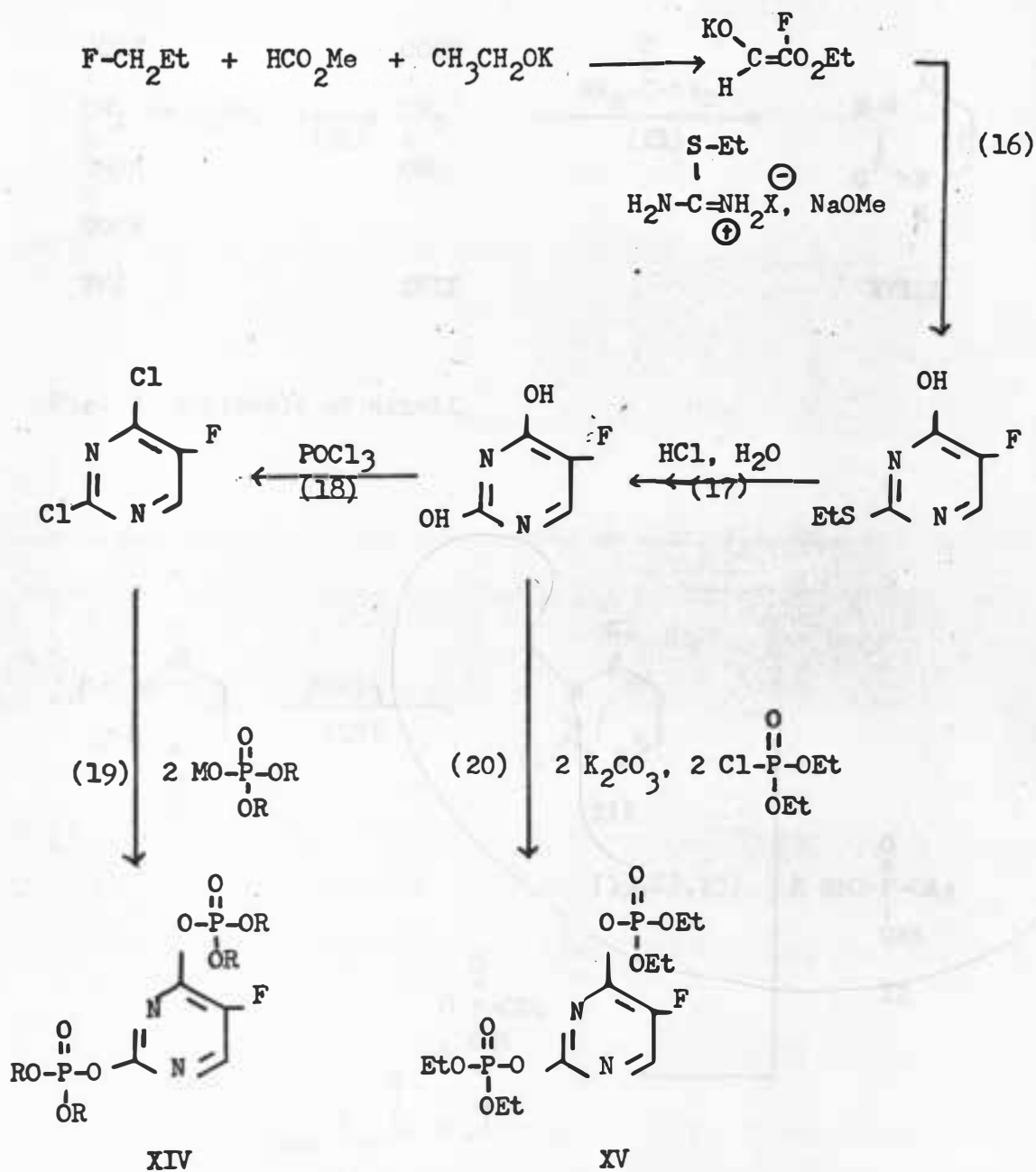
Fig. 6 Schemes for phosphorylation of 5-fluorouracil

Fig. 7 Synthesis of uracil

b.

2 AgO-P-OAg (19, 24)

c.

2 NaO-P-ONa (19)

d.

2 NaI (25)

XXII

(26) 2EtO-P-OEt
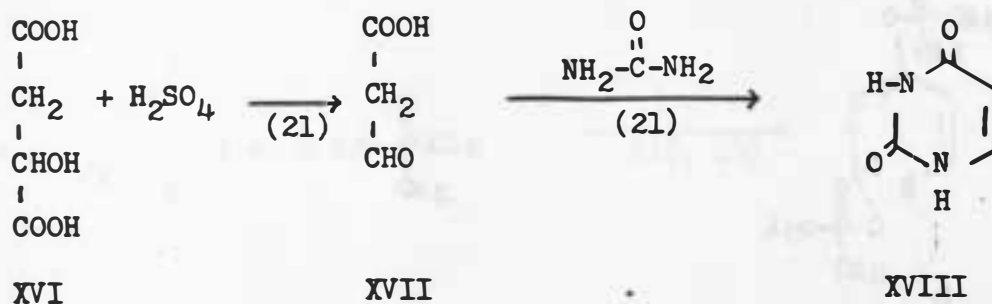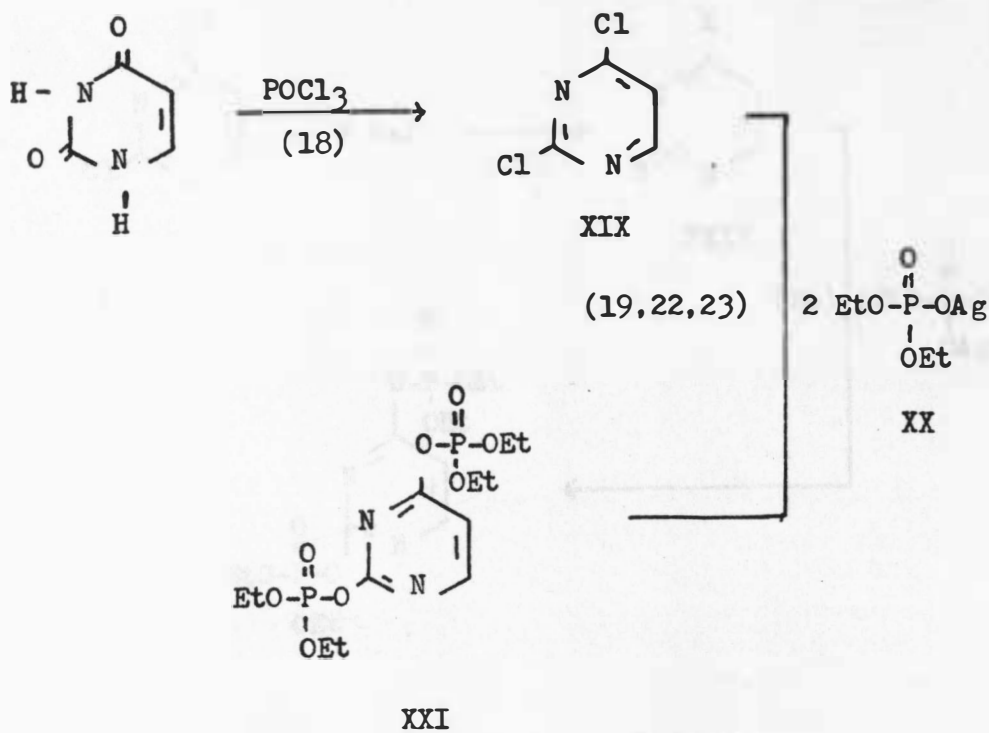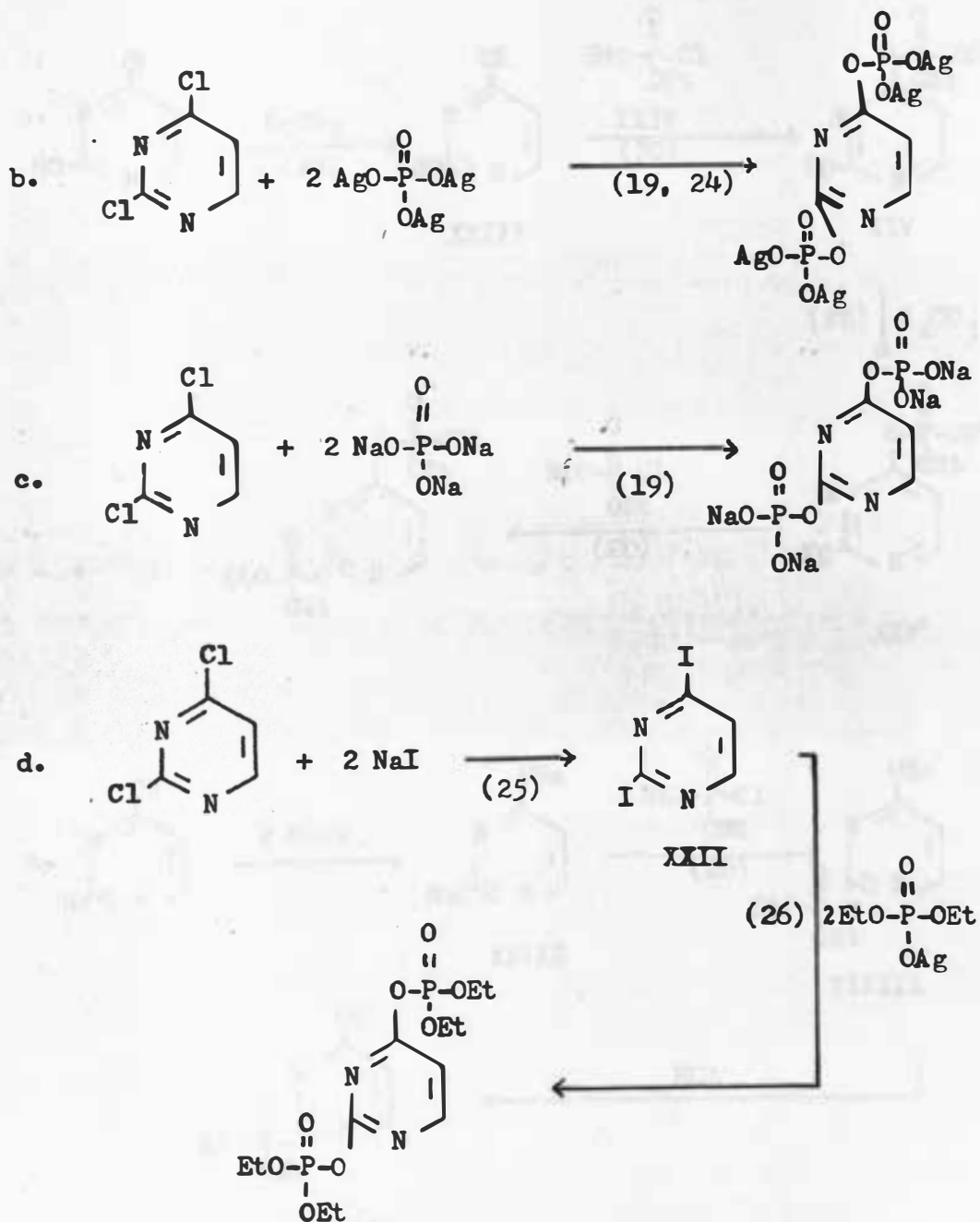
Fig. 8 Scheme I for phosphorylation of uracil
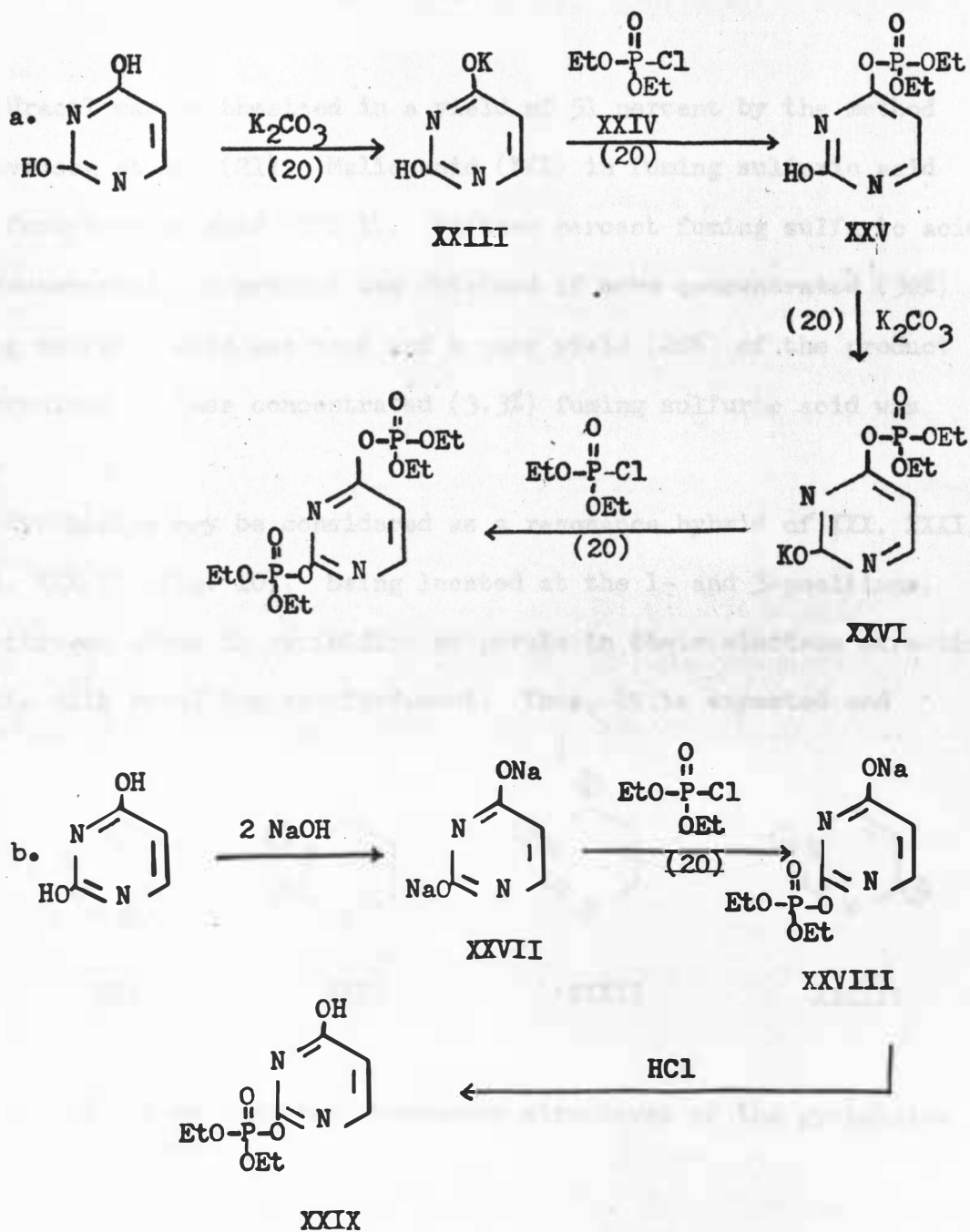
Fig. 9 Scheme II for phosphorylation of uracil

DISCUSSION

Uracil was synthesized in a yield of 51 percent by the method
of Davidson et al. (21). Malic acid (XVI) in fuming sulfuric acid
gave formylacetic acid (XVIII). Fifteen percent fuming sulfuric acid
was recommended; no product was obtained if more concentrated (30%)
fuming sulfuric acid was used and a poor yield (20%) of the product
was obtained if less concentrated (3.3%) fuming sulfuric acid was
used.

Pyrimidine may be considered as a resonance hybrid of XXX, XXXI,
XXXII, XXXIII (Fig. 10). Being located at the 1- and 3-positions,
the nitrogen atoms in pyrimidine cooperate in their electron directive
effects with resulting reinforcement. Thus, it is expected and

XXX   XXXI   XXXII   XXXIII
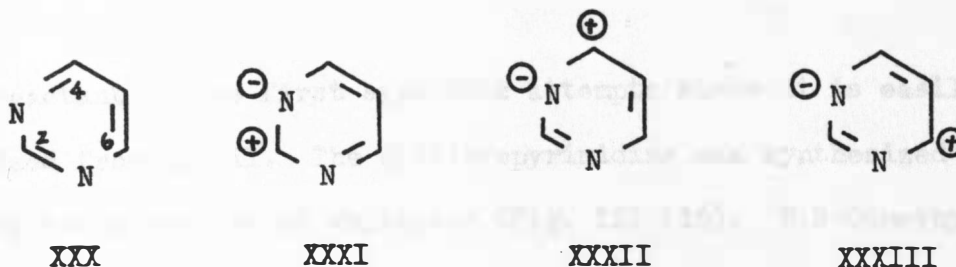
Fig. 10 Some important resonance structures of the pyrimidine
         ring

indeed pyrimidine does possess properties similar to those of pyridine,
but markedly different from benzene (29). The 2-, 4-, and 6-positions
of pyrimidine, because of the powerful electron withdrawing effect of

the neighboring nitrogen atoms, are electron deficient. Halogen atoms at these positions, like those in alkyl halides, are easily substituted by nucleophilic agents (30). Since 2,4-dihalo pyrimidine possesses activity towards substitution reactions similar to alkyl halides, attempted reaction of 2,4-dichloropyrimidine with the silver salt of diethyl phosphate was applied in this research (Fig. 8). Alkyl iodides are popularly used in these reactions because the carbon-iodide bond is less stable and thus, the iodides have higher reactivity than the other halides (Fig. 11) (31). 2,4-Dichloropyrimidine (XIX) was chosen

Iodides $>$ Bromides $>$ Chlorides $>$ Fluorides

$\longleftarrow$ Increasing rate of reaction
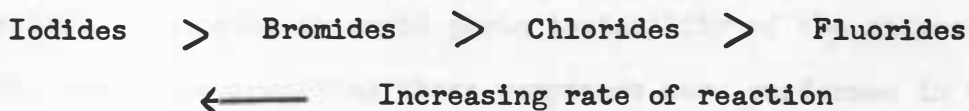
Fig. 11 Reactivity of alkyl halides

as the reactant in the first synthetic attempts since it is easily synthesized from uracil. The dichloropyrimidine was synthesized from uracil by the procedure of Whittaker (Fig. 12) (16). N,N-Dimethyl
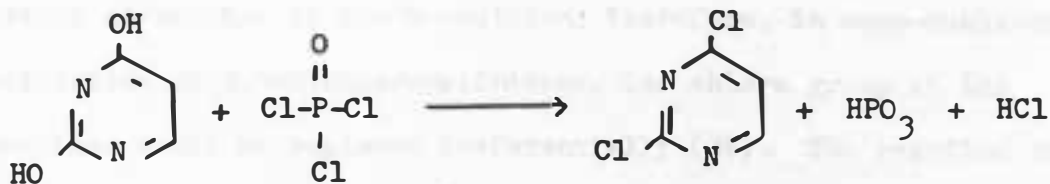
Fig. 12 Reaction between uracil and phosphoryl oxychloride

aniline was added as a proton scavenger thus allowing a faster, more complete reaction. When an electron withdrawing group, e.g., fluoro, is present at the 5-position of uracil, the conversion of hydroxy groups with phosphoryl chloride is also successful. In this case, for effective reaction N,N-diethyl aniline is used (32).

Silver diethyl phosphate (XX) was synthesized from triethyl phosphate using the procedure of Drushel and Felty et al. (23). The only modification was, instead of triethyl phosphate being decomposed by concentrated barium hydroxide solution, a mixture of triethyl phosphate and barium hydroxide in water was refluxed for one and one-half hours (27). In order to avoid photo instability of the silver compounds, reactions involving these compounds were performed in the dark. Silver diethyl phosphate is not soluble in most organic solvents, very soluble in water and soluble in hot dioxane. Since the silver salt is very unstable in hot, commercial grade dioxane, purified dioxane was heated to about 60° for recrystalization of the salt.

The difference of electron distribution in different positions of pyrimidine is indicated (Fig. 13) (33). Electron density at the 2-position is more depleted by the inductive effects of the ring nitrogen atoms than at the 4-position; therefore, in mono-nucleophilic substitution of 2,4-dichloropyrimidine, the chloro group at the 2-position would be replaced preferencially (34). The reaction of 2,4-dichloropyrimidine and silver diethyl phosphate was run according to the procedure of Wolform et al. (19). Benzene, in which the silver salt is apparently insoluble, was used as the solvent. The

$$+.054$$

$$-.0948 \; N \overset{4}{\underset{\underset{N}{\overset{|}{2}}}{\diagdown}} +.007$$

$$+.0746$$
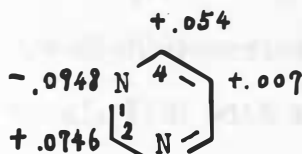
Fig. 13 Electron distribution of pyrimidine

molar amounts of the reactants were such that disubstitution was expected (Fig. 8), but no product, either mono- or di-substituted (XXI), was obtained. A reaction in purified dioxane which dissolves both of the reactants was also run, but the reaction was hindered by the instability of the silver salt in hot dioxane. In order to increase the possibility of interaction between the reactants, the pyrimidine and the silver salt were heated at 135° and also 155° without any solvent, but no product was obtained.

Reaction between alkyl halides and metallic (dialkyl) phosphates takes place at the oxygen atom of the phosphoryl group since the bond of the phosphoryl group is polar, hence the oxygen atom carries an appreciable negative charge and can interact with an electrophilic center (Fig. 14) (35). Increasing the basicity of the phosphate (Fig. 15) would increase the negativity of the oxygen atom and might increase the reaction rate of the nucleophilic substitution (Fig. 8). Silver phosphate was synthesized following the procedure of Sneed et al. (24). To avoid 2, 4-dichloropyrimidine being hydrolized to uracil in the presence of water, the silver phosphate was dried to constant weight and commercial duodecahydrated sodium phosphate was

dried at 105° for 12 hours to the least hydrated form. The dried

inorganic phosphates and 2,4-dichloropyrimidine were heated according

to the method of Wolform et al. (19) with some modifications: the

reaction times were lengthened and the reactions were run in an

atmosphere of nitrogen.

$$R^{\delta+} \quad\quad\quad O^{\delta-}$$
$$X^{\delta-} \quad\quad\quad \| $$
$$\oplus \quad \ominus \quad -P^{\delta+}-OR$$
$$Ag \quad :O \quad \quad |$$
$$\quad\quad\quad OR$$
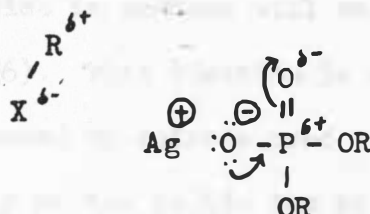
Fig. 14 Mechanism of reaction between alkyl halide and silver
dialkyl phosphate

The reaction was run in the recommended solvent, benzene, and also

at 120° without any solvent. All of the attempts were unsuccessful.

$$\begin{array}{ccc}
O & O & O \\
\| & \| & \| \\
NaO-P-ONa & AgO-P-OAg & AgO-P-OEt \\
| & | & | \\
ONa & OAg & OEt
\end{array}$$

$$\longleftarrow \text{ Increasing basicity}$$

Fig. 15 Basicity of phosphates

The reason that increasing the basicity of the phosphate did not

increase the nucleophilic replacement might be due to the insolubility

of the inorganic phosphates in benzene and also to decreased reactivity

of 2,4-dichloropyrimidine in comparison with alkyl chlorides. Brown
suggests (32) that the chloro groups in 2,4-dichloropyrimidine have
activities similar to those in aliphatic compounds, but more nearly to
that of the chloro group in chloro-2,4-dinitrobenzene.

An attempt was made to synthesize 2,4-diiodopyrimidine because
of expected increased reactivity of the iodo over the chloro analogue
(Fig. 8). Sodium iodide in acetone will react with alkyl chlorides to
give alkyl iodides (36). This reaction is useful since alkyl iodides
cannot always be prepared by methods used for alkyl chlorides and
bromides. The ability of the iodide ion to displace the other halo-
gens is due in part to the fact that sodium iodide is soluble in
acetone while sodium chloride is not. The insolubility of sodium
chloride in acetone helps favor the formation of the alkyl iodide (36).
In an attempt to synthesize diiodopyrimidine (XXII), two methods were
followed: the procedure of Rosenkranz et al. (28) with acetone as
the solvent and the procedure of Kingdon and Wright (25) with 2-
butanone as the solvent. 2-Butanone has a higher boiling point and
thus, higher reaction temperature can be obtained. After refluxing,
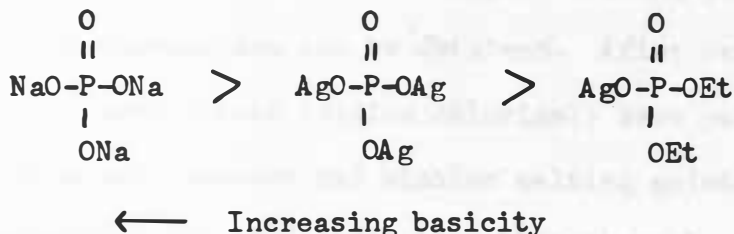only trace amounts of precipitate (sodium chloride?) were noticed
and the products from both methods had similar melting points (60-62°)
as 2,4-dichloropyrimidine (61-62°) indicating that the chloro groups
at the 2- and 4-positions of pyrimidine were not replaced by iodide.

In dialkyl phosphorochloridate, the phosphorus-oxygen double
bond is polar (35), the phosphorus being positive and electrophilic
toward nucleophiles. This results in Sn2 replacement (Fig. 16) (37).

$$R'O^{\ominus} M^{\oplus} + \underset{\underset{OR}{|}}{\overset{\overset{O}{\|}}{RO-P-X}} \longrightarrow \left[\underset{\underset{R'O}{\diagup} \underset{OR}{\diagdown}}{\overset{\overset{O}{\|} \diagup X}{RO-P}} \right] \longrightarrow \underset{\underset{RO}{|}}{\overset{\overset{RO}{\diagdown} \diagup O}{R'O-P}} + M^{\oplus} X^{\ominus}$$
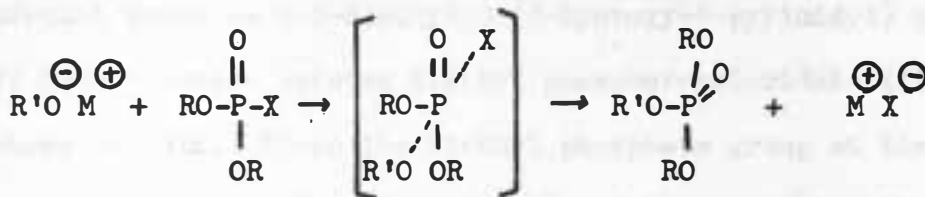
Fig. 16   Reaction between metal alkoxide and dialkyl phosphoro-
chloridate

With this reaction, the insecticidal agent, 0,0-diethyl,0-(2,4-di-
methyl-6-pyrimidyl) phosphate (XXXVI) was synthesized by Geigy (Fig.
17) (29).   An attempt was made to synthesize 0,0,0,0-tetraethyl,0,
0-pyrimidine-2,4-yl diphosphate (XXI) following the method of Geigy
(Fig. 8).
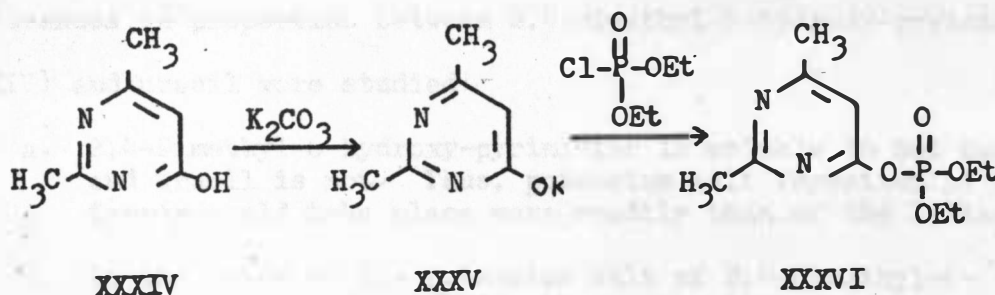
Fig. 17   Synthesis of 0,0-diethyl,0-(2,4-dimethyl-6-pyrimidyl)
phosphate

Uracil is an acid with pKa values of 9.38 and 12 (10) and since the
hydroxylic proton at the 4-position is more ionizeable than the
hydroxylic proton at the 2-position (38), the mono-potassium salt
would be formed at the 4-position (XXIII).   Because of this, the

expected product would be 0,0-diethyl,0-(2-hydroxy-4-pyrimidyl) phos-

phate (XXV) from reaction between diethyl phosphorochloridate (XXIV)

and the potassium salt. Since the diethyl phosphate group at the

4-position would theoretically lower the pKa of the remaining 2-

hydroxy group, 0,0-diethyl,0-(2-hydroxy-4-pyrimidyl) phosphate would

be capable of forming a potassium salt with potassium carbonate at the

2-position (XXVI). Reaction of the potassium salt with diethyl phos-

phorochloridate, again, by nucleophilic substitution, would give 0,0

0,0-tetraethyl,0,0-pyrimidine-2,4-yl diphosphate (XXI). In the first

step, uracil and potassium carbonate were refluxed for three hours in

benzene and water was removed azeotropically. Only a trace amount of

water was collected. No product was obtained in this experiment. The

differences in properties between 2,4-dimethyl-6-hydroxy-pyrimidine

(XXXIV) and uracil were studied:

a. 2,4-Dimethyl-6-hydroxy-pyrimidine is soluble in hot benzene
and uracil is not. Thus, potassium salt formation of the
former would take place more readily than of the latter.

b. In the anion of the potassium salt of 2,4-dimethyl-6-
hydroxy-pyrimidine (XXXV), there are electron releasing
substituents, methyl groups, at the 2- and 4-positions.
The methyl groups would increase the basic strength of
the anion of the salt and increase the nucleophilic dis-
placement reaction. In the anion of the mono-potassium
salt of uracil, there are no electron releasing methyl
groups.

The reason that the method of synthesizing 0,0-diethyl,0-(2,4-di-

methyl-6-pyrimidyl) phosphate (XXXVI) could not be applied to the

synthesis of 0,0,0,0-tetraethyl,0,0-pyrimidine-2,4-yl diphosphate

might be due to these differences. A reaction between the mono-

sodium salt of uracil (XXXVII) and diethyl phosphorochloridate in

benzene has been attempted (39) with no product obtained. This seems
to indicate that the second difference (b.) is more important.
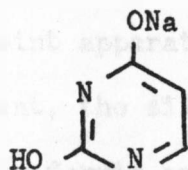
XXXVII

In the di-sodium salt of uracil (XXVII), the anion on the 2-
carbon is more basic and thus would be more readily attacked by the
nucleophilic agent than the anion on the 4-carbon (Fig. 8). In this
experiment, uracil was obtained as the product when treated with water.
To explain this, the assumption was made that the monophosphorylated
compound (XXVIII) was obtained but was not stable in the presence of
water (Fig. 18).
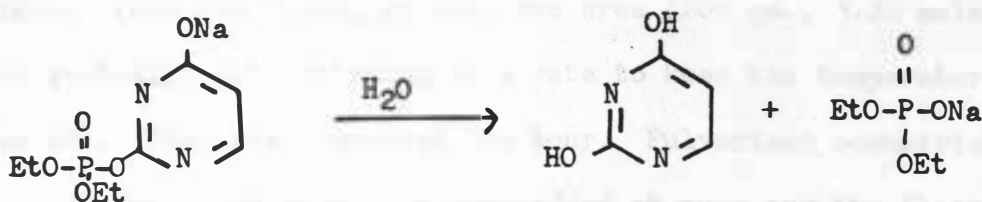
XXVIII

Fig. 18   Hydrolysis of sodium salt of 0,0-diethyl,0-(4-hydroxy-
2-pyrimidyl) phosphate

EXPERIMENTAL

All melting points recorded in this work were taken using a
capillary tube melting point apparatus (Tottoli) and are uncorrected.
To determine silver content, the silver compound was titrated with
ammonium thiocyanate using ferric ammonium sulfate as the indicator.
Nitrogen content was determined by the Kjeldahl semi-micro method.

PREPARATION OF URACIL (21):

Thirty percent fuming sulfuric acid (1080 gm., 600 ml.) was
diluted with 95 percent sulfuric acid (402 gm., 225 ml.) to make 15
percent fuming sulfuric acid (1482 gm., 825 ml.). In a 5000-ml.,
round-bottomed, three-necked flask fitted with an efficient mechanical
stirrer and a thermometer was placed the 15 percent fuming sul-
furic acid (1304 gm., 800 ml.) which was chilled to 0° in a freezing
mixture. Into the flask, crystalline urea (200 gm., 3.20 mole) was
added gradually with stirring at a rate to keep the temperature
below 10°. This step required one hour. Pulverized commercial malic
acid (200 gm., 1.50 mole) was then added at once and the flask was
fitted with a water-cooled condenser and heated on the steam-bath
for one hour. During the first 15 minutes, large volumes of carbon
monoxide, carbon dioxide and sulfur dioxide were evolved. At the end
of the heating period, the flask was cooled and its contents were
poured into ice water (2400 ml.). On cooling, the crude uracil

separated. The yield of the crude product was 123 gm. (65.3%). The uracil was washed with water (suspended in water and again filtered) and recrystallized from boiling water (2500 ml.) with the aid of charcoal (20 gm.). After drying at 100° for four hours, the recrystallized product weighed 96.4 gm., which was 51.2 percent of the theoretical amount and melted at 333-335° (reported: yield, 50-55%, m.p. 335°)

PREPARATION OF 2,4-DICHLOROPYRIMIDINE (18,40):

A mixture of uracil (25.0 gm., 0.445 mole), phosphorus oxychloride (125 ml., 1.40 mole) and N,N-dimethylaniline (31 ml., 0.24 mole) was refluxed for two hours. The dark brown homogeneous solution was cooled and most of the excess phosphorus oxychloride was distilled under reduced pressure. Crushed ice (180 gm.) was then gradually added to the dark-colored viscous residue with vigorous stirring. The temperature of the mixture was kept below 20° during this treatment because of the ease of hydrolysis of the dichloropyrimidine to uracil. After five minutes, ether (250 ml.) was added. The ether layer was washed with four 50-ml. portions of water and dried over anhydrous sodium sulfate. The ether was removed by distillation and the residue was distilled under reduced pressure. The product, 2,4-dichloropyrimidine, was collected as colorless crystals at 88°/17 mm. The yield of the product was 23 gm. (70%), m.p. 60-62° (reported: yield, 87%, m.p. 61-62°, b.p. 100°/22 mm.).

PREPARATION OF SILVER DIETHYL PHOSPHATE (22,23,27,41):

In a 500-ml., round-bottomed flask (with condenser), a mixture
of triethyl phosphate (90 gm., 0.49 mole), barium hydroxide octa-
hydrate, and water (200 ml.) was refluxed for one and one-half hours.
The hot mixture was saturated with carbon dioxide, cooled and filtered.
The filtrate was concentrated to a small volume and diluted with eth-
anol to give barium tetraethyl diphosphate. After filtering and drying,
the crude product weighed 59.4 gm. (57.6%). To obtain a pure product,
the crude barium salt was dissolved in a minimum amount of hot water
and hot ethanol (2000 ml.) was added. The water-ethanol solution was
cooled slowly and the barium salt precipitated out in white, shining
needle-form crystals. The yield of the recrystallized product, which
does not melt over a flame, was 47.7 gm. (46.3%). Twenty ml. of 50
percent silver nitrate solution containing 10 gm. (0.060 mole) and 60
ml. of 3.8 percent sodium hydroxide solution containing 2.3 gm.
(0.060 mole) were prepared with carbon dioxide free water and mixed.
The precipitate of silver oxide (6.9 gm., 9.060 mole) was washed with
carbon dioxide free water by decantation. The recrystallized barium
tetraethyl phosphate (13.2 gm., 0.0298 mole) was decomposed by the
theoretical amount of sulfuric acid (58.30 ml., 1.024 N, 0.02900 mole)
with stirring, care being taken to avoid an excess of acid. After
20 minutes, the suspension was filtered through a Buchner funnel
which was precoated with a bed of Filteraid to remove the barium
sulfate. The filtrate was neutralized by the freshly prepared silver
oxide (6.9 gm., 0.060 mole) in the dark. The neutralized solution

(pH 5) was evaporated _in vacuo_ to give a white waxy product of silver
diethyl phosphate which weighed 16.0 gm. (100%), m.p. 115-150°. The
silver salt was recrystallized from purified dioxane and stored in
the dark. The yield of the recrystallized product (white, shining,
needle-form crystals) was 12.0 gm. (75%), m.p. 150-155°. Silver
content of the salt was 41.08 percent (theoretical: 41.33 percent).

ATTEMPTED PREPARATION OF 0,0,0,0-TETRAETHYL,0,0-PYRIMIDINE-2, 4-YL DIPHOSPHATE (19):

A. Benzene as solvent:

In the dark, 2,4-dichloropyrimidine (3.0 gm., 0.020 mole),
freshly prepared silver diethyl phosphate (15.8 gm., 0.0605 mole),
anhydrous calcium sulfate (5.0 gm.) and benzene (60 ml.) were placed
in a 250-ml., round-bottomed flask with condenser. The mixture was
warmed at 50° for 30 minutes and then heated to reflux for one and
one-half hours. Vigorous stirring was maintained throughout. After
cooling, the reaction mixture was suction-filtered through a Büchner
funnel. The benzene was removed from the filtrate by distillation
on a water bath under water pump pressure. The resulting yellow
syrup (1.5 gm.) gave a positive test for silver ion and turned to
bluish-purple on exposure to light. The pale yellow residue from the
filtration, which also turned bluish-purple on exposure to light, was
extracted with three 50-ml. portions of hot acetone and the extract
was evaporated _in vacuo_ to a small volume.

Skelly B (400 ml.) was added to the syrup to induce crystallization.
No crystalline product was obtained except a trace amount of impurity.
No product could be isolated from the reaction mixture.

B. Dioxane as solvent:

In an Ehrlenmeyer flask (with condenser), silver diethyl
phosphate (1 gm., 0.004 mole) and purified dioxane (100 ml.) were
heated in the dark. After the silver salt dissolved, 2,4-dichloro-
pyrimidine (0.3 gm., 0.002 mole) was added. The colorless dioxane
solution turned yellow with some black precipitate in a few minutes
after reflux started. The same result was obtained in about one hour
if the temperature was kept around 60°. The fact that, in the absence
of 2,4-dichloropyrimidine, a dioxane solution of the silver salt
changed from colorless to light red with a black precipitate after
being heated at 60° for three hours led to the conclusion that the
silver salt was not stable in purified dioxane and dioxane would not
be useful as the solvent in this reaction.

C. Without any solvent:

A mixture of silver diethyl phosphate (1 gm., 0.004 mole) and
2,4-dichloropyrimidine (0.3 gm., 0.002 mole) was heated at 135° on a
wax bath overnight. The soft solid was extracted with purified
dioxane (50 ml.) and filtered. The solvent was removed from the

filtrate under reduced pressure and a small amount of yellow syrup was
left. The syrup did not solidify on cooling and gave a negative test
for silver ion. The same result was observed when the mixture was
heated at 155° for six hours. The two different ways of heating were
also applied to a mixture of silver diethyl phosphate (0.5 gm., 0.002
mole) and 2,4-dichloropyrimidine (0.3 gm., 0.002 mole). The same
results were obtained.

ATTEMPTED PREPARATION OF TETRASILVER,0,0-PYRIMIDINE-2,4-YL
DIPHOSPHATE (19,24):

A. Benzene as solvent:

In the dark, 145 ml. of normal hydrated trisodium orthophos-
phate solution containing 18.0 gm. (0.0421 mole) was poured into 150 ml.
of normal silver nitrate solution containing 25.0 gm. (0.148 mole).
The yellow precipitate of trisilver phosphate was collected by suction
filtration, washed with water, dried in the oven (105°) to constant
weight and stored in the dark. In a 250-ml., round-bottomed flask
fitted with a water-cooled condenser, an efficient mechanical stirrer
and an inlet tube for nitrogen were placed 2,4-dichloropyrimidine
(2.50 gm., 0.0168 mole), the freshly prepared silver phosphate (17.0
gm., 0.0410 mole) and benzene (50 ml.). The mixture was refluxed
with stirring in an atmosphere of nitrogen for 24 hours in the dark.
After being cooled to room temperature, the mixture was filtered.

The yellow residue was not soluble in water but soluble in ammonium hydroxide (silver phosphate?). The benzene was removed from the filtrate. The appearance and the melting point (57-61°) of the yellow solid residue, which weighed 2.6 gm., indicated that the residue was the starting material, 2,4-dichloropyrimidine.

B. Without any solvent:

In the dark, freshly prepared dry silver phosphate (17.0 gm., 0.041 mole) and 2,4-dichloropyrimidine (2.5 gm., 0.0168 mole) were placed in a 50-ml., round-bottomed, two-necked flask fitted with an inlet tube for nitrogen, air-cooled condenser, and a gas outlet and heated at 120° in an atmosphere of nitrogen overnight. This method was not successful apparently because of the sublimation of the dichloropyrimidine onto the cooler upper area of the reaction apparatus at this reaction temperature.

ATTEMPTED PREPARATION OF TETRASODIUM,0,0-PYRIMIDINE-2,4-YL DIPHOSPHATE (19):

Trisodium orthophosphate duodecahydrate (35.7505 gm.) was dried in the oven (105°) to constant weight (18.7545 gm., i.e. 1.592 moles water of hydration/mole). In a 100-ml., round-bottomed flask fitted with condenser were placed the dried sodium phosphate (7.10 gm., 0.0353 mole), 2,4-dichloropyrimidine (2.50 gm., 0.0168 mole) and

redistilled xylene (50 ml.). With stirring, the mixture was heated
to reflux for 12 hours. After cooling, the reaction mixture was
filtered with suction. The residue (7.5 gm.) gave a negative test
for chloride and was extracted with three 50-ml. portions of hot
alcohol. The solvent was removed from the extract and only a trace
amount of yellowish solid impurity was left. The xylene was distilled
under reduced pressure from the filtrate and the appearance and the
melting point (56-62°) of the yellow solid residue, which weighed
2.7 gm., indicated that the residue was the starting material, 2,4-
dichloropyrimidine.

ATTEMPTED PREPARATION OF 2,4-DIIODOPYRIMIDINE:

A. Acetone as solvent (28):

Sodium iodide was dried in the oven (110°) for two hours. A
mixture of anhydrous sodium iodide (9.1 gm., 0.064 mole) and 2,4-di-
chloropyrimidine (1.48 gm., 0.0100 mole) in redistilled acetone
(200 ml.) was refluxed for 48 hours. After cooling, the reaction
mixture with a trace amount of precipitate was filtered. The acetone
solution was evaporated in vacuo and the residue was extracted with
hot anhydrous benzene (100 ml.). The benzene extract was concentrated
at reduced pressure. The product distilling at 85°/15 mm. was col-
lected and weighed 0.5 gm., m.p. 60-62° (reported: 2,4-dichloro-
pyrimidine melts at 61-62°).

B. 2-Butanone as solvent (25,42):

A mixture of anhydrous sodium iodide (8.0 gm., 0.054 mole) and 2-butanone (70 ml.) was heated on a steam bath for one hour with a water-cooled reflux condenser. 2,4-Dichloropyrimidine (2.0 gm., 0.013 mole) was added to the mixture and heating was maintained for an additional 48 hours with occasional shaking. The reaction mixture with a trace amount of precipitate was cooled to room temperature and filtered through a Buchner funnel with suction. The residue was washed with 2-butanone (15 ml.). The butanone solution was evaporated in vacuo and the residue was extracted with hot benzene (100 ml.). The benzene extract was evaporated under reduced pressure and the product boiling at 87°/15 mm. was collected and weighed 0.5 gm., m.p. 60-62° (reported: 2,4-dichloropyrimidine melts at 61-62°).

ATTEMPTED PREPARATION OF 0,0-DIETHYL,0-(2-HYDROXY-4-PYRIMIDYL) PHOSPHATE (20):

In a 100-ml., round-bottomed, three-necked flask fitted with an efficient mechanical stirrer, a reflux condenser and inlet of nitrogen gas were placed uracil (11.2 gm., 0.100 mole), potassium carbonate (14.0 gm., 0.100 mole) and benzene (25 ml.). The mixture was refluxed for three hours with stirring and the small amount of water formed was removed by azeotropic distillation. Diethyl phosphorochloridate (19.0 gm., 0.110 mole) was then added and the mixture was refluxed for eleven

hours. The suspension was allowed to cool to room temperature and filtered with suction. The residue was washed with benzene. The filtrate and the washings were combined and washed with five percent potassium carbonate (20 ml.) and two 20-ml. portions of water and dried over sodium sulfate overnight. The syrup (3 gm.) obtained on solvent removal did not solidify on cooling. The filter cake was extracted with three 50-ml. portions of hot acetone, three 50-ml. portions of hot chloroform, and three 50-ml. portions of hot dioxane. Only trace amounts of impurity (syrup) with strong odor (garlic-like) were obtained after each solvent fraction was evaporated under reduced pressure. The residue was also extracted with three 50-ml. portions of hot alcohol and a trace amount of white crystals precipitated in the alcohol extract on cooling. A Kjeldahl determination indicated that these white crystals contained no nitrogen. No product could be isolated from the reaction mixture.

ATTEMPTED PREPARATION OF O,O-DIETHYL,O-(4-HYDROXY-2-PYRIMIDYL PHOSPHATE (20,42):

Uracil (11.2 gm., 0.100 mole) was added to a solution of sodium hydroxide (187 ml., 1.07 N, 0.200 mole). After heating for one-half hour, the solvent was evaporated under reduced pressure and the residue (disodium salt of uracil) was dried in the vacuum oven for four hours. The dried product weighed 17.9 gm. (115% apparent yield) and did not melt over a flame. In a three-necked, round-bottomed flask fitted with an efficient mechanical stirrer and water-cooled condenser,

the disodium salt of uracil (10.0 gm., 0.0641 mole), diethyl phos-

phorochloridate (11.1 gm., 0.0641 mole), mercurous chloride (0.300 gm.,

0.00110 mole) and benzene (100 ml.) were heated to reflux for 12

hours. The reaction mixture was cooled and filtered. The dried

residue was washed by suspending in water with stirring and then

filtered. The insoluble residue (7.9 gm.) melted at 320-335^0. This

indicated that the residue was the starting material, uracil, with

some impurity.

RESULTS AND CONCLUSIONS

According to the procedure of Davidson and Bandisch, uracil
was synthesized in a yield of 51 percent (reported yield: 50-55
percent); following the method of Whittaker, 2,4-dichloro-pyri-
midine was synthesized with a yield of 70 percent (reported yield:
87 percent). Various methods were applied to the uracil and the
dichloropyrimidine to test the feasibility of phosphate formation of
uracil. All of the attempts were unsuccessful.

The first synthetic route (Fig. 7) was planned according to
the aliphatic property of 2,4-dichloropyrimidine and the negative
results indicated that the chloro groups at the 2- and 4-positions
of the dichloropyrimidine were not reactive enough toward nucleo-
philic substitution reactions in comparison with chloro groups in
alkyl chlorides. The introduction of the fluoro group, a highly
electronegative group, at the 5-position would withdraw electrons
by an inductive mechanism and activate the substituents at the 2- and
4-positions (44). By the activating effect of the fluoro group, the
phosphate formation (XIV) from 2,4-dichloro-5-fluoropyrimidine and
the metal salt of dialkyl phosphoric acid may be possible.

In the second synthetic route (Fig. 8), uracil was used as the
starting material. Although 5-fluorouracil is bio-isosteric to uracil,
the introduction of a fluoro group at the 5-position has a great effect
on the electron distribution in the molecule and thus, the acid
strength of 5-fluorouracil is 30 times that of uracil (2). Although

with the higher acidity, 5-fluorouracil forms a potassium salt more readily than uracil, the fluoro group would delocalize the negative charge on the oxygen of the anion of the potassium salt (XXXVIII) and thus, would stabilize the anion and reduce its basic strength.
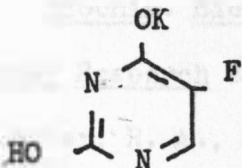
XXXVIII

Since the anion of the mono-potassium salt of uracil (XXII) appeared to be too weakly basic to react with diethyl phosphorochloridate, it seems even less possible to synthesize 0,0,0,0-tetraethyl,0,0-(5-fluoropyrimidine-2,4-yl) diphosphate (XIV) by this method.

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