# SYNTHESIS OF 2-SUBSTITUTED-5,8-DIHYDROXYPYRIMIDO (4,5-d) PYRIDAZINES

by

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# SYNTHESIS OF 2-SUBSTITUTED-5,8-DIHYDROXYPYRIMIDO (4,5-d) PYRIDAZINES

## INTRODUCTION

The discovery that many organic compounds are of therapeutic value in the treatment of tumors has stimulated much interest in certain compounds occuring in the human body together with those which resemble them in structure. Many of these substances incorporate pyrimidine or purine structures in the molecule and hence these heterocycles have received much attention. In fact the search for biologically active compounds has even extended to compounds derived from other related ring systems. Since only one modified purine compound is known in which the imidazole ring of the purine molecule has been replaced by the pyridazine ring, it was only a natural desire to examine other new molecules of this type, especially since these compounds are analogs of the pterins which include 2-amino-4-oxypteridine, a constituent of the folic acid molecule.

This thesis describes the synthesis of several potential purine antagonists, the diethyl 2-substituted-4,5-pyrimidinedicarboxylates and the corresponding 5,8-dihydroxypyrimido (4,5-d) pyridazines formed by their reaction with hydrazine hydrate.

Chlorination or thionation of the hydroxylated pyrimido (4,5-d) pyridazines is of special interest since

intermediates may be synthesized which may then react with various amines and hydrazine derivatives to produce a large number of new and interesting drugs. Furthermore, the unsubstituted nucleus, pyrimido (4,5-d) pyridazine might also be obtained by nuclear dehalogenation of the appropriate chloro derivative.

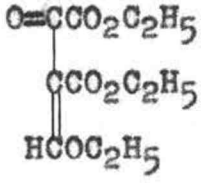
Claisen (7) was the first to couple the ethoxymethylene group with compounds containing an active methylene group by reactions with ethyl orthoformate and acetic anhydride.

Jones (13) has investigated the mechanism and determined the optimum conditions for the formation of ethyl ethoxymethylene oxalacetate, an intermediate needed for the synthesis of the proposed pyrimido (4,5-d) pyridazines. He found that the moles of acetic anhydride employed in this condensation should be equal to, or greater than, the total moles of ethyl orthoformate and ethyl oxalacetate used, in order to obtain maximum yields.

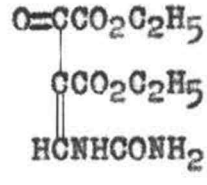
Jones and Whitehead (14) prepared two vic-dicarboxylic acid esters of pyrimidine through the reaction of urea or free guanidine with ethyl ethoxymethylene oxalacetate (I) yielding diethyl 2-hydroxy-4,5-pyrimidinedicarboxylate (III) and diethyl 2-amino-4,5-pyrimidinedicarboxylate (IV), useful intermediates for the proposed studies.

The latter compound was produced directly in

fifty-seven percent yield on addition of (I) to a cold, alcoholic solution containing guanidine obtained by the neutralization of guanidine hydrochloride with sodium ethoxide. In this study it was discovered that heating urea with (I) until an exothermic reaction took place gave the intermediate, diethyl ureidomethylene oxalacetate (II) in ninety-three percent yield. The ureide was also obtained by refluxing diethyl oxalacetate, ethyl orthoformate and urea in ethanol. This ureide could be cyclized by (1) melting II to yield III (75 percent) or (2) by dehydration of II in boiling xylene (88 percent). The ureide could not be cyclized by the use of either sodium ethoxide or hydrochloric acid. Furthermore, no pure product was isolated from the reaction of (I) with thiourea substituted for urea.



I



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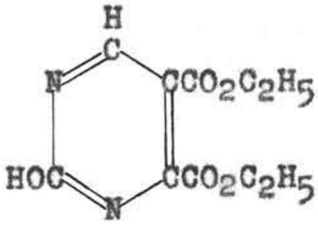

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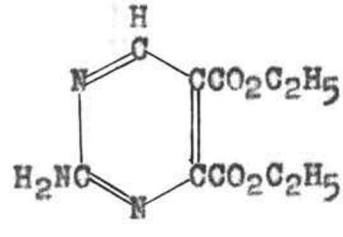


IV

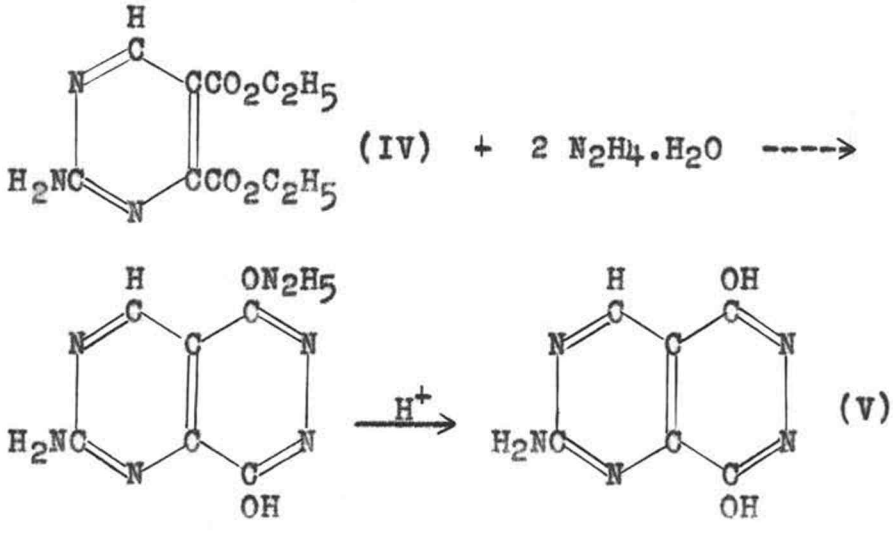
Gabriel and Colman (9, p. 3647) obtained 4,5-pyrimidinedicarboxylic acid by alkaline oxidation of quinazoline with potassium permanganate, but the esters of this compound have not been described in the literature.

Several heterocyclic carboxylic acid esters or anhydrides have been treated with hydrazine by previous investigators to yield corresponding hydrazides or pyridazines. For instance, Meyer and Mally (15) found that pyrolysis of 3-carboxyisonicotinic acid hydrazide or its salt at 370°produced pyrido (3,4-d) pyridazine-1,4-diol. Yale, et al. (18, p. 1937) obtained the same compound by refluxing dimethyl cinchomeronate and hydrazine hydrate in methanol.

Gheorgiu (10) refluxed 2,3-pyridinedicarboxylic anhydride, acetic acid and hydrazine to obtain 5,8-dihydroxypyrido (2,3-d) pyridazine in one hundred percent yield; using ethanol as solvent in place of acetic acid gave only a fifty percent yield. Gleu and Wackernagel (11) using "quinolinyl anhydride" in dilute aqueous hydrazine reported a "quinolinyl hydrazide" which was the same compound that Gheorgiu prepared.

Jones (12) treated diethyl 2-amino-4,5-pyrimidine-dicarboxylate (IV) and various other vic-dicarboxylic acid esters of pyridine, pyrazine, pyridazine, furan, imidazole, thiophene and other ring systems with a methanolic solution of hydrazine hydrate either at room

temperature or with warming for a short time. The first compound yielded 2-amino-5,8-dihydroxypyrimido (4,5-d) pyridazine (V). All of the six-membered ring compounds tested, formed the corresponding dihydroxy bicyclic compounds under these mild conditions while all five-membered ring systems, except thiophene and compounds sterically hindered by a bulky ortho substituent, formed dihydrazides. Jones found that the crystalline products of the six-membered ring systems were soluble in water. They were characterized as salts of hydrazine which became quite insoluble in water when converted to the free dihydroxypyridazines by means of hydrochloric acid.




The only derivatives of the pyrimido (4,5-d) pyridazine ring system which were prepared by Jones were (V) in ninety-three percent yield and its ammonium salt which decomposes at 200° with loss of ammonia. Jones called (V),

2-amino-5,8-dihydroxy-1,3,6,7-tetrazanaphthalene and found that its melting point exceeded 400°.

A chlorination procedure which might be applied to the hydroxylated pyrimido (4,5-d) pyridazines was developed by Davoll and Lowy (8). These workers were able to chlorinate uric acid by refluxing the acid with phosphoryl chloride in the presence of dimethyl aniline for twenty hours. The excess phosphoryl chloride was removed by distillation at reduced pressure and the residue then poured on ice. The solution was extracted with ether, and the ethereal extract was then evaporated to dryness. The solid obtained was treated with dilute ammonium hydroxide, yielding sixteen to twenty-five percent of the ammonium salt of 2,6,8-trichloropurine. This was converted to trichloropurine pentahydrate by acidifying with sulfuric acid. The product was dried at 110° yielding anhydrous 2,6,8-trichloropurine.

Likewise thionation might be accomplished by adapting the method of Castle and Seese (6). These workers, after refluxing 4,7-dihydroxyimidazo (4,5-d) pyridazine and phosphorus pentasulfide in pyridine for ten hours, obtained seventy-nine percent yield of 4,7-dimercaptoimidazo (4,5-d) pyridazine; m.p. 307-311°. The product was repurified by dissolving it in ten percent sodium hydroxide followed by reprecipitation

with glacial acetic acid to give a compound melting at 309-311°.

## EXPERIMENTAL DISCUSSION

Several methods were tried in this Laboratory in attempts to prepare diethyl 2-thio-4,5-pyrimidinedicarboxylate: (a) refluxing diethyl oxalacetate, ethyl orthoformate and thiourea, (b) heating ethyl ethoxymethylene oxalacetate and thiourea and (c) keeping the latter two ingredients at room temperature and under vacuum for several days. All these procedures yielded an orange viscous liquid and an almost quantitative recovery of thiourea. However, a light yellow solid melting at 128-131° was isolated when thiourea dissolved in warm absolute alcohol was mixed with ethyl ethoxymethylene oxalacetate. The product was obtained in 0.8 percent yield only after the viscous liquid had remained evacuated in the freezer for a period of about two months. The analysis on this compound indicated either a monohydrate or diethyl thioureidomethylene oxalacetate.

Attempts to remove water from this compound by refluxing with xylene produced a material which could not be recrystallized. Treatment of another portion with sodium ethoxide gave a product with a melting point in excess of 300° which retained the sodium ion even after treatment with glacial acetic acid.

Boarland and McOmie (2 and 3) determined a large number of ultraviolet absorption spectra for mono- and polysubstituted pyrimidines. These workers observed characteristic bathochromic shifts for each additional substituent in a given position on the pyrimidine ring at a given pH. Applying their findings to the basic, non-tautomeric structure of the pyrimidines being studied in this Laboratory, namely, diethyl 4,5-pyrimidinedicarboxylate which has a maxima at 2670 Å with a log E of 3.62 and then adding the 430 Å shift characteristic of the sulfhydryl group, a maxima of 3100 Å was predicted. The actual value found for the 2-thio-derivative was 3100 Å with log E of 3.97 indicating that the desired pyrimidine may have been produced. Since it required two months to prepare the small amount available and this was depleted in attempts to determine its structure, further experiments with this intermediate were not attempted.

Diethyl 2-methyl-4,5-pyrimidinedicarboxylate was obtained in thirty-four percent yield when (I) was added to free acetamidine base obtained by treatment of the hydrochloride salt with a cold ethanolic solution containing an equivalent quantity of sodium ethoxide. The product was liquid; boiling at 187-191° at 16 mm.

Recently a simple method for making amidine acetates

was described by Taylor and Ehrhart (17). These workers reported that refluxing ethyl orthoformate and ammonium acetate for two hours is sufficient to produce formamidine acetate in seventy percent yield. Formamidine acetate has the advantage that it is not hygroscopic like the hydrochloride and since acetic acid is a weak acid, the compound may often be used directly in condensations without prior liberation of free formamidine. Formamidine acetate heated gently with (I) yielded diethyl 4,5-pyrimidinedicarboxylate boiling at 113-115° at 0.65 mm. in forty-one to forty-five percent yield.

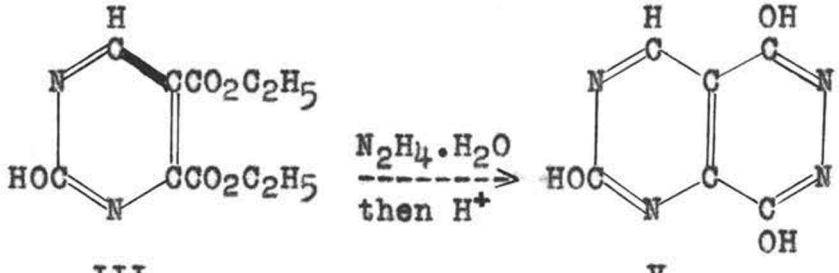
Diethyl 2-phenyl-4,5-pyrimidinedicarboxylate was prepared by essentially the same procedure. A mixture consisting of (I), benzamidine hydrochloride and anhydrous sodium acetate was heated to fusion. The product was extracted from the fusion mixture as a white solid in thirty-one percent yield; m.p. 80.5-81°.

A similar experiment using methyl isothiourea sulfate in place of benzamidine hydrochloride produced a substance melting at 122-124°. This material formed a picrate melting at 222-224° which corresponds to methyl isothiourea picrate. This indicates the formation of methyl isothiourea acetate, a compound not recorded in the literature. Probably the reactivity of this compound is similar to thiourea and a longer reaction time would be required to

produce a significant yield of diethyl 2-methylmercapto-4,5-pyrimidinedicarboxylate.

Methyl isothiourea appears to decompose rapidly when it is liberated from the sulfate with sodium ethoxide as evidenced by the foul smelling mercaptans liberated. No pyrimidine was recovered when diethyl oxalacetate and ethyl orthoformate were added to the above mixture which was then kept at room temperature for three hours and then refluxed for one hour.

Although diethyl 2-hydroxy-4,5-pyrimidinedicarboxylate (III) was synthesized by Jones and Whitehead (14) apparently no efforts were made to synthesize 2,5,8-trihydroxypyrimido (4,5-d) pyridazine (VI) which formed immediately on addition of hydrazine hydrate to a methanolic solution of the ester in this Laboratory. This compound and the other compounds of similar structure produced in these studies were highly hydrated and difficult if not impossible to dry.




III V

After refluxing with xylene, followed by a drying period in the Abderhalden apparatus, the product still contained one and one-half moles of water of hydration.

Similarly 2-methyl-5,8-dihydroxypyrimido (4,5-d) pyridazine was formed by adding hydrazine hydrate to a methanolic solution of diethyl 2-methyl-4,5-pyrimidine-dicarboxylate. This yellow powder which was produced in fifty-six percent yield had a melting point of about 320-321° estimated on a 300° thermometer and did not require refluxing with xylene to produce a dry compound.

Reaction of diethyl 4,5-pyrimidinedicarboxylate with hydrazine hydrate produced a yellow compound, soluble in dilute base and extremely insoluble in acid solution which is characteristic of the cyclized 5,8-dihydroxy-pyrimido (4,5-d) pyridazines. Three different methods were used to produce this compound: (1) reaction as above, (2) refluxing the product from (1) with 2 N hydrochloric acid and (3) refluxing the pyrimidine with an excess of hydrazine hydrate. In all cases after twelve hours drying in the Abderhalden apparatus at 80° and 0.1 mm. the compound gave analytical data indicating a product containing one and three-fourths molecules of water per mole. Refluxing with xylene or heating to 250° under 1 mm. of pressure for one hour removed one half mole of water leaving one and a quarter moles of water per mole of compound

The diethyl 2-phenyl-4,5-pyrimidinedicarboxylate was converted to 2-phenyl-5,8-dihydroxypyrimido (4,5-d) pyridazine which retained two-thirds of a molecule of water on drying. Most of this water was removed by distillation with xylene.

Efforts to thionate 2,5,8-trihydroxypyrimido (4,5-d) pyridazine with phosphorus pentasulfide in pyridine (6) gave a compound in five percent yield which appears to be quite acidic since glacial acetic acid failed to remove the sodium ion. Re-solution in dilute ammonium hydroxide instead of sodium hydroxide and re-precipitating with hydrochloric acid eliminated this difficulty. The analysis of this compound indicates that it is 2,5,8-trimercaptopyrimido (4,5-d) pyridazine tetrahydrate.

Chlorination of 2,5,8-trihydroxypyrimido (4,5-d) pyridazine with phosphorus oxychloride in the presence of dimethyl aniline produced a tarry material in low yield which melted from 50-60°. It could not be purified sufficiently to characterize due to the small quantity, high solubility and difficulty in the crystallization process.

# EXPERIMENTAL

Ethyl ethoxymethylene oxalacetate (13).

A 500 ml. separatory funnel containing 105.5 g. (0.5 mole) of the sodium salt of diethyl oxalacetate, 50 ml. (0.6 mole) of concentrated hydrochloric acid, 50 ml. of water and 100 ml. of ether was shaken until the tan powder went into solution. Additional water was then added to dissolve the sodium chloride precipitate. The ether layer was separated and washed with two-25 ml. portions of water. The solution was then distilled to remove the ether and then fractionated under vacuum to recover pure diethyl oxalacetate; yield 87.6 g. (94 %), b.p. 124-130° at 16-21 mm.

This ester (0.47 mole) was mixed with 121 g. (0.82 mole) of ethyl orthoformate and 135 g. (1.33 moles) of acetic anhydride and the resultant solution was placed in a 500 ml. flask equipped with a hot water condenser. The mixture was heated to 120° for one hour and to 140° for two hours by means of an oil bath. The volatile materials were allowed to escape. The product was distilled at 16 mm. to remove the remaining low boiling constituents. Then 114 g. (69 %) of ethyl ethoxymethylene oxalacetate was collected at 155-160° and 0.65-1.10 mm.

Diethyl ureidomethylene oxalacetate (14).

A mixture of 17.2 g. (0.284 mole) of finely ground urea and 69.2 g. (0.284 mole) of ethyl ethoxymethylene oxalacetate was stirred at room temperature until the clear color became red. Then it was slowly heated with stirring until an exothermic reaction took place which yielded a fluffy white powder. This powder, after washing with two-50 ml. portions of ether, was then dissolved in 100 ml. of hot ethanol. The crystals forming in the cool solution were filtered and dried in the oven at 110° for four hours; yield 52 g. (71 %), m.p. 169-172°.

Diethyl 2-hydroxy-4,5-pyrimidinedicarboxylate (14).

Diethyl ureidomethylene oxalacetate (52 g.) was heated in an oil bath at 175° with constant stirring until the crystals melted. The temperature was then reduced to 160° and heating continued for fifteen minutes. Upon cooling 75 ml. of ethyl acetate was added and the mixture reheated until the dark substance had dissolved. The solution was then refrigerated at 0° overnight and the solid then removed by filtration. The product was redissolved in hot ethyl acetate, treated with norite, filtered and cooled to obtain white crystals. The crystals then were redissolved in a minimum of hot isobutyl alcohol and stirred rapidly while cooling. This process yielded an easily filterable product which upon recrystallizing again from ethyl acetate gave 12.0 g.

(25 %) of white crystals, m.p. 158-160°.

Diethyl 2-amino-4,5-pyrimidinedicarboxylate (14).

A solution consisting of 8.25 g. (0.36 mole) of sodium in 175 ml. of absolute ethanol was placed in a 500 ml. three-necked flask which was equipped with a stirrer and an ice bath. A warm solution of 62.0 g. (0.38 mole) of guanidine hydrochloride in 175 ml. of absolute alcohol was added slowly with stirring. Then 78.0 g. (0.32 mole) of ethyl ethoxymethylene oxalacetate was added to the cold mixture over a fifteen minute interval. Stirring was continued for half an hour with the formation of a yellow precipitate. The solid was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The combined solids were suspended in 150 ml. of water and filtered. The precipitate was resuspended three times with fresh portions of water to wash out sodium chloride and other impurities. The solid was then suspended in 50 ml. of ethanol, filtered and washed with three-10 ml. portions of ethanol; yield 25.4 g. (33.2 %) of white crystals, m.p. 167-168°. Recrystallize from water; m.p. 157-158°.

Diethyl 2-thio-4,5-pyrimidinedicarboxylate.

A. Diethyl oxalacetate was liberated by shaking a mixture of 100 ml. of ether, 50 ml. of concentrated hydrochloric acid and 50 ml. of water with 88.0 g. (0.42 mole) of the sodio salt of diethyl oxalacetate. Additional

water was added until the layers were easy to separate. The ether layer was washed with 50 ml. of water and evaporated under vacuum to give 73 g. (0.39 mole) of diethyl oxalacetate. This was mixed with 58 g. (0.39 mole) of ethyl orthoformate and 30 g. (0.39 mole) of thiourea and the mixture was then refluxed for two hours. The dark orange solution yielded a solid on cooling. Recrystallization from ethanol gave light, fluffy, white crystals; m.p. 170-182° which appeared to be thiourea. The solution did not yield any other material which could be separated.

B. An equimolar mixture of 2.4 g. (0.01 mole) of ethyl ethoxymethylene oxalacetate and 0.8 g. (0.01 mole) of thiourea was thoroughly mixed and gently heated with a flame until the solid was all dissolved. The solution was warmed for half an hour with stirring and then cooled yielding a solid which upon recrystallization from alcohol melted at 168-178°. Thus thiourea appeared to be the only material which was separated from the viscous liquid.

C. The experiment (as described under B) was repeated omitting the heat treatment. The mixture was thoroughly stirred three or four times a day for one week. During this time the mixture was kept in a vacuum desiccator at 0.4 mm. pressure to remove any alcohol or water formed by the reaction. This mixture also turned orange as in previous experiments, while thiourea was the

only material isolated.

D. Ethyl ethoxymethylene oxalacetate (48.8 g., 0.20 mole) was added slowly with stirring to a warm solution of 18.3 g. (0.24 mole) of thiourea in 200 ml. of absolute ethanol. The solution became orange and after one hour it was cooled with an ice bath and 18 g. of crystals were recovered. Following the evaporation of the solvent, 51 g. of oily liquid remained. The crystals consisted principally of thiourea.

Numerous solvent systems were tried with the oily liquid in order to isolate any solid product. The material was stored in the freezer for several weeks both as solvent-free liquid and as an ethanol-ethereal solution; only slight crystallization took place in the viscous system. After another month of storage in the vacuum desicator a little more deposit was obtained on these nuclei. The oily liquid was decanted and the crystals were purified by dissolving in ethanol and reprecipitating several times with cold petroleum ether; yield 0.4 g. (0.8 %) of light-yellow product, m.p. 128-131°.

Anal. Calcd. for $C_{10}H_{12}N_2O_4S \cdot H_2O$: C, 43.79; H, 5.14. Found: C, 43.46; H, 5.31.

Attempts to dehydrate the product by heating 0.25 g. with refluxing xylene were unsuccessful. The sticky solid could not be crystallized from any solvent systems tried.

Diethyl 2-methyl-4,5-pyrimidinedicarboxylate.

A solution containing 8.25 g. (0.36 mole) of sodium dissolved in 175 ml. of absolute ethanol was prepared. The solution was cooled with an ice bath and stirred while a warm solution of acetamidine hydrochloride (36 g., 0.38 mole) in 175 ml. of ethanol was added. Then 78 g. (0.32 mole) of ethyl ethoxymethylene oxalacetate was added with stirring to the ice cold mixture over a fifteen minute interval. During the half hour of stirring, the solution became orange and milky. The ethanol was evaporated under reduced pressure and the viscous liquid was decanted from the solid tar. The liquid was distilled at a pressure of 2.5 mm. White star-shaped crystals formed in the column at 45° and distillation began at 137° with a rapid rise to 150°. Refractionation at 187-191° at 16 mm. yielded 26 g. (34 %) of clear liquid. The refractive index at 22.5° was 1.4876 as compared to 1.4775 for ethyl ethoxymethylene oxalacetate.

Anal. Calcd. for $C_{11}H_{14}N_2O_4$: C, 55.45; H, 5.92. Found: C, 55.00; H, 5.91.

Formamidine acetate (17).

A mixture of 30.0 g. (0.20 mole) of ethyl orthoformate and 31.2 g. (0.41 mole) of dry ammonium acetate was heated on an oil bath at 130-135° for two and a half hours. The remaining liquid was then removed under reduced pressure. Ethanol was added to the solid to

extract the soluble ammonium acetate leaving the product. The filtrate was cooled to obtain a second crop of white formamidine acetate crystals. Combined yield 13.2 g. (63 %), m.p. 154-155°.

Diethyl 4,5-pyrimidinedicarboxylate.

Formamidine acetate (4.2 g., 0.04 mole) and 4.8 g. (0.04 mole) of ethyl ethoxymethylene oxalacetate were mixed at room temperature with the clear liquid changing to orange color. Gentle heating dissolved any remaining solid. Ten ml. of a crude low boiling product was removed at 115-136° and 2.6 mm. pressure. Redistilling at 0.1 mm. gave a forerun at 35-40° which left white crystals in the column. The main product collected was 3.7 g. (41 %) of colorless liquid, b.p. 113-115° at 0.65 mm.

Anal. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.56; H, 5.40. Found: C, 53.38; H, 5.59.

Diethyl 2-phenyl-4,5-pyrimidinedicarboxylate.

A mixture consisting of 7.6 g. (0.031 mole) of ethyl ethoxymethylene oxalacetate, 4.9 g. (0.031 mole) of benzamidine hydrochloride and 2.6 g. (0.031 mole) of fused sodium acetate was heated on the steam bath for one and a half hours. Then 10 ml. of hot ethanol was added to dissolve everything, but sodium chloride. The mixture was filtered and the solid washed again with hot ethanol. After cooling in the freezer, the product was removed by filtration. Then 10 ml. of ether was added to the

filtrate which was cooled to recover more crystals. The combined crystals were then dissolved in a minimum of ethanol and filtered while still hot. Upon cooling, the crystals were removed by filteration and recrystallized from 10 ml. of 75 percent ethanol; yield 2.9 g. (31 %) of white crystals, m.p. 80.5-81°.

Anal. Calcd. for $C_{16}H_{16}N_2O_4$: C, 64.1; H, 5.33. Found: C, 64.14; H, 5.22.

Methyl isothiourea acetate.

In attempting to form diethyl 2-methylmercapto-4,5-pyrimidinedicarboxylate; 2.44 g. (0.01 mole) of ethyl ethoxymethylene oxalacetate, 1.39 g. (0.005 mole) of methyl isothiourea sulfate and 0.82 g. (0.01 mole) of fused sodium acetate were heated gently with stirring for one hour. The viscous liquid was dissolved in absolute ethanol and the insoluble sodium sulfate was removed by filteration. Ether was added to the filtrate giving a flocculent precipitate which was filtered. Recrystallization from ethanol and ether solution gave a small amount of white product melting at 122-124°. Heating this material with picric acid in methanol, cooling, filtering and washing with 50 percent methanol gave a compound melting at 222-224°. This corresponds to methyl isothiourea picrate so the compound isolated must have been methyl isothiourea acetate which is not reported in the literature.

2,5,8-Trihydroxypyrimido (4,5-d) pyridazine.

Six grams of 99-100 percent hydrazine hydrate (0.12 mole) was added dropwise with stirring to 10 g. (0.042 mole) of diethyl 2-hydroxy-4,5-pyrimidinedicarboxylate in 50 ml. of methanol. A white paste formed immediately which was heated on the steam bath with frequent stirring until dry (about 30 minutes). Two hundred ml. of 2 N sodium hydroxide was added and the mixture was heated until the bulk of the material had dissolved. Any insoluble material was removed from the hot solution by filtration. Concentrated hydrochloric acid was then added until the solution was quite acidic, yielding a heavy precipitate. The precipitate was allowed to settle, the water was removed by decantation and the remaining material filtered. The solid was washed with a large quantity of cold water, 100 ml. of ethanol and 50 ml. of ether; yield 8 g. (92 %), m.p. $> 300^{\circ}$. An analytical sample was recrystallized from hot dilute ammonium hydroxide, then filtered, washed with water, ethanol and ether respectively. This repurification was repeated twice. The product was dried by refluxing with xylene, washing with ether and heating at $80^{\circ}$ and 1 mm. for twelve hours in the Abderhalden apparatus.

Anal. Calcd. for $C_6H_4N_4O_3 \cdot 1\frac{1}{2}H_2O$: C, 34.89; H, 3.38. Found: C, 35.19; H,3.20.

2-Amino-5,8-dihydroxypyrimido (4,5-d) pyridazine (12).

Six grams (0.12 mole) of hydrazine hydrate was added to a methanolic solution containing 10.0 g. (0.042 mole) of diethyl 2-amino-4,5-pyrimidinedicarboxylate. The mixture was evaporated to dryness on the steam bath (about 45 minutes) with formation of a precipitate during the first fifteen minutes. The bright yellow powder was dissolved in 500 ml. of water containing 5 ml. of concentrated ammonium hydroxide. Glacial acetic acid was added to reprecipitate the product as a white material. The product was collected, washed with cold water, ethanol and ether; then dried in the desiccator. The yield was 7.4 g. (100 %), m.p.> 300°.

2-Methyl-5,8-dihydroxypyrimido (4,5-d) pyridazine.

A solution of 9.6 g. (0.04 mole) of diethyl 2-methyl-4,5-pyrimidinedicarboxylate and 6.0 g. (0.12 mole) of hydrazine hydrate in 25 ml. of methanol was heated on the steam bath. After three minutes, a yellow precipitate began to form. Heating was continued for half an hour, whereupon the product was removed by filtration. The solid was dissolved in 20 ml. of water containing 5 ml. of concentrated ammonium hydroxide, then acidified with concentrated hydrochloric acid to reprecipitate the product which was filtered and washed with water, 5 ml. of methanol and 5 ml. of ether respectively. The product was repurified twice more, then dried in the Abderhalden

apparatus at 80° under vacuum; m.p. 320-321° (estimated).

Anal. Calcd. for $C_7H_6N_4O_2$: C, 47.18; H, 3.40. Found: C, 47.03; H, 3.65.

5,8-Dihydroxypyrimido (4,5-d) pyridazine.

A. An exothermic reaction took place when 2.2 g. (0.01 mole) of diethyl 4,5-pyrimidinedicarboxylate and 1.5 g. (0.03 mole) of hydrazine hydrate were mixed in 10 ml. of methanol. Purify as in the previous experiment, m.p. > 320°.

Anal. Calcd. for $C_6H_4N_4O_2 \cdot 1\text{-}3/4\ H_2O$: C, 36.82; H, 3.86. Found: C, 36.89; H, 3.66.

B. Refluxing 1.0 g. of diethyl 4,5-pyrimidine-dicarboxylate (0.0045 mole) and 2.0 g. (0.040 mole) of hydrazine hydrate in 25 ml. of methanol for twelve hours produced a similar hydrated product which was purified by the same procedure.

Anal. Found: C, 36.65; H, 3.64.

The compound was heated with 2 N hydrochloric acid for two hours; repurify as before. The compound is very insoluble in acid and soluble in dilute base which is characteristic of these compounds.

Anal. Found: C, 37.15; H, 3.77.

Refluxing the compound with 100 ml. of xylene for one hour, distilling off the xylene, washing with ether and drying in an Abderhalden at 80° and 1 mm. pressure

did not remove all water of hydration.

Anal. Calcd. for $C_6H_4N_4O_2 \cdot 1\frac{1}{4}H_2O$: C, 38.60; H, 3.48. Found: C, 38.64; H, 3.5.

The compound was heated to 250° for one hour under 1 mm. pressure without losing appreciable water.

Anal. Found: C, 38.57; H, 3.74.

2-Phenyl-5,8-dihydroxypyrimido (4,5-d) pyridazine.

Heating a mixture composed of 1.1 g. (0.0037 mole) of diethyl 2-phenyl-4,5-pyrimidinedicarboxylate and 0.55 g. (0.011 mole) of hydrazine hydrate in 25 ml. of methanol for one hour, resulted in the precipitation of a yellow solid. The precipitate was removed by filtration, dissolved in 20 ml. of hot 2 N ammonium hydroxide, filtered while hot and then an excess of hydrochloric acid was added to the filtrate. The crystalline product was filtered, washed with water, ethanol and ether; then repurified twice more in this manner. The compound was dried in an Abderhalden apparatus; m.p. 335° (estimated).

Anal. Calcd. for $C_{12}H_8N_4O_2$: C, 59.9; H,3.33. Found: C, 57.2; H, 3.77.

Refluxing the hydrated product with 100 ml. of xylene and then removing the xylene by distillation gave an anhydrous product.

Anal. Found: C, 59.4; H, 3.37.

2,5,8-Trimercaptopyrimido (4,5-d) pyridazine tetrahydrate.

A solution of 300 ml. of freshly distilled, dry

pyridine containing 3.6 g. (0.020 mole) of 2,5,8-trihydroxypyrimido (4,5-d) pyridazine and 42 g. (0.19 mole) of finely ground phosphorus pentasulfide was refluxed for ten hours. Initial addition of the phosphorus pentasulfide caused a slightly exothermic reaction and a light-yellow, flocculent precipitate formed. As the heating proceeded, small specks of red material rose to the surface and dissolved. The pyridine was removed at reduced pressure and the residue was shaken with 500 g. of ice until the mass dissolved in the melt. The liquid was heated on the steam bath for eight hours, filtered while hot and precipitated with concentrated hydrochloric acid. The brown solid was dissolved in 10 percent sodium hydroxide and precipitated with glacial acetic acid. The process was repeated using ammonium hydroxide and hydrochloric acid. After washing the precipitate with a small amount of water, methanol and ether; the product was dried in an Abderhalden, yield 0.2 g. (3.3 %); m.p. $>300^{\circ}$.

Anal. Calcd. for $C_6H_4N_4S_3 \cdot 4H_2O$: C, 24.1; H, 4.05. Found: C, 23.8; H, 4.3.

Chlorination of 2,5,8-trihydroxypyrimido (4,5-d) pyridazine.

A suspension of 8.0 g. (0.045 mole) of 2,5,8-trihydroxypyrimido (4,5-d) pyridazine in 40.5 ml. (0.45 mole) of phosphorus oxychloride containing 17 ml. (0.22 mole) of dimethyl aniline was refluxed for twelve hours and

allowed to remain at room temperature for twelve hours. The volume was then reduced to one-half by distilling the mixture at reduced pressure. The viscous material was then poured with stirring on 200 g. of ice, allowed to sit for one hour in the cold and then filtered.

The precipitate was washed with three-50 ml. portions of ether and the aqueous solution was then extracted with this ether using continuous liquid-liquid extraction apparatus. The ether was evaporated, 25 ml. of boiling 3 N ammonium hydroxide was added and the liquid was filtered. The liquid was evaporated at room temperature and the tarry material was extracted with hot ethyl acetate, leaving a small amount of insoluble material which was removed by filtration. The liquid was evaporated to dryness and the small amount of solid obtained was recrystallized from ethanol. It was difficult to crystallize, requiring considerable rubbing of the beaker to produce star-shaped crystals resembling beef fat crystals; m.p. 50-60°. Attempts to repurify this 50 mg. of material resulted in loss of the remainder which could not be recovered.

Spectral data. (pH 1.0 unless otherwise noted.)

| Compound | λmax. | log E | λmin. |
|---|---|---|---|
| Diethyl 4,5-pyrimidine-dicarboxylate | 2670 Å | 3.62 | 2430 Å<br>2800<br>3400 |
| 2-hydroxy derivative | 2460 | 4.11 | 2730<br>3100 |
| 2-amino derivative | 2550 | 4.01 | |
| 2-thio derivative | 3100 | 3.97 | 2450 |
| 2-methyl (in ethanol) | 2550 | 3.57 | 2470 |
| 2-phenyl (in ethanol) | 2830 | 4.38 | 2400 |
| 5,8-dihydroxypyrimido (4,5-d) pyridazine | 2260<br>3100 | 4.28<br>3.97 | 2580 |
| 2-methyl " | 2300<br>2750<br>3200 | 3.62 (shoulder)<br>3.40<br>3.36 | <br>2500<br>3000 |
| 2-phenyl " | 2800 | 4.34 | 2350 |
| 2-amino " | 2200<br>2570<br>3050 | 4.12<br>3.90<br>3.55 (shoulder) | 2370 |
| 2,5,8-trihydroxypyrimido (4,5-d) pyridazine | 2200<br>2570<br>3300-3350 | 4.17<br>3.78<br>2.90 (shoulder) | 2380 |
| pteridine (1, p. 3833) | 2300<br>2980<br>3090 | 3.65<br>3.87<br>3.83 | |
| 2-methylpteridine (1, p. 3833) | 2300<br>3050<br>3170 | 3.68<br>3.92<br>3.90 | |

# SUMMARY

Reaction of formamidine acetate, thiourea, acetamidine, benzamidine acetate and urea with ethyl ethoxymethylene oxalacetate produced four new pyrimidines: diethyl 4,5-pyrimidinedicarboxylate, and the 2-substituted thio, methyl and phenyl derivatives as well as the previously known 2-hydroxy derivative. These compounds with the exception of the 2-thio derivative were heated with hydrazine hydrate in methanol to produce 5,8-dihydroxypyrimido (4,5-d) pyridazine, the 2-methyl and 2-phenyl derivatives and 2,5,8-trihydroxypyrimido (4,5-d) pyridazine. These latter compounds hold water tenaciously and tend to be hygroscopic. The diethyl 2-thio-4,5-pyrimidinedicarboxylate compound reacted with hydrazine hydrate to form a precipitate, but due to the small quantity of this pyrimidine available there was insufficient product formed to purify and characterize.

The 2,5,8-trihydroxypyrimido (4,5-d) pyridazine compound reacted with phosphorus pentasulfide in pyridine to yield 2,5,8-trimercaptopyrimido (4,5-d) pyridazine tetrahydrate.

Reaction of the trihydroxy derivative with phosphorus oxychloride in the presence of dimethyl aniline produced a material which was difficult to purify, hence it was not analyzed. Ultraviolet absorption spectra were determined.

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