The Synthesis of Some Substituted Pyrimido[4,5-*d*]dihydropyrimidines[1]

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Cyclization of 4-amino-5-carbethoxy-2-mercaptopyrimidine with formamide has been accomplished, giving 4-hydroxy-7-mercaptopyrimido[4,5-*d*]dihydropyrimidine(1*H*,2*H*). In this reaction a critical factor is the temperature: below 110° no reaction takes place; above 140° the material decomposes, forming 4-amino-2-mercaptopyrimidine. As an intermediate step in the cyclization reaction, 120–130°, a Schiff's type base is formed. From the cyclized material the following compounds have been prepared: 4,7-dimercapto-, 4-hydroxy-, 4-chloro-, 4-mercapto-, and 4-aminopyrimido[4,5-*d*]dihydropyrimidine(1*H*,2*H*).

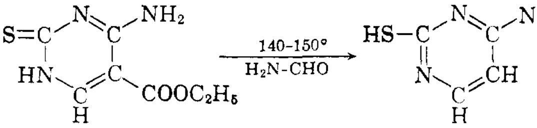
The activity of 6-mercaptopurine[4-9] against transplantable tumors in animals, against adenocarcinoma, sarcoma, and leukemia, stimulated our interest in preparing its analog in the pyrimido[4,5-*d*]pyrimidines, namely, 4-mercaptopyrimido[4,5-*d*]pyrimidine. The work on this subject was initiated early in 1957, but almost at the time of completion, April, 1959, the dissertation abstract of Knopf[10] was published, describing the preparation of 4-hydroxy-, 4-mercapto-, and 4-aminopyrimido[4,5-*d*]pyrimidines, which are compounds with which we also are concerned.

About the same time Chatterji and Anand[11,12] published the syntheses of four 5-aminopyrimido[4,5-*d*]pyrimidines, containing in the 2-position H, CH_3, NH_2, or OH. The 2,5-diaminopyrimidino[4,5-*d*]pyrimidine was found to be a very active diuretic agent orally.

Recently a patent[13] and also an extensive article by Taylor[14] were published, describing the syntheses of a number of pyrimido[4,5-*d*]pyrimidines, substituted mainly in the 2,5- and 2,4,7-positions. In all these syntheses, 4-amino-5-cyano- and 4-amino-5-carbethoxypyrimidine were used as starting materials.

Our starting material, 4-amino-5-carbethoxy-2-thiopyrimidine (I), was prepared by condensation of thiourea with ethyl ethoxymethylenecyanoacetate as described by Johnson and Ambler,[15] with the modification that the condensation was carried out in the presence of sodium ethoxide instead of metallic sodium. This reduced the conversion of I to the sodium carboxylate and resulted in the crude product of greater purity than that reported by the previous workers.

Initially we planned to hydrolyze I to the free acid and to cyclize it with formamide; after several attempts however we conclude that cyclization under this condition was not feasible since the free acid decomposed very easily on heating with formamide. Heating the acid with formamide even at about 95°, for twenty-four hours, caused much decarboxylation, yielding 4-amino-2-mercaptopyrimidine (II).[16] Since an ester stabilizes a carboxylic group, the cyclization with the ester was investigated, whereupon observations have been made that at 110° no reaction takes place; at 120–130° a Schiff base (III) is formed; and above 140° the ester (I) decomposes, forming 4-amino-2-mercaptopyrimidine (II).



I II

By heating I with formamide for several hours at 110–140° a compound was obtained which did not melt below 360°; however, above 330° a gradual decomposition occurred. From the infrared and nuclear magnetic resonance spectra and from the elementary analyses of this product and its derivatives it appeared that the cyclization had been achieved and 4-hydroxy-7-mercaptopyrimido[4,5-*d*]dihydropyrimidine(1*H*,2*H*) (IV) had been obtained. No doubt the hydrogen donor is formamide, which on heating above 130° with suitable hydrogen acceptors undergoes a Leukart type reaction.[17]

In considering the spectra of compound IV it should be mentioned that the infrared spectrum gave two distinct peaks at about 2.90 and 5.90 μ, which are characteristic of hydroxy- and aminopyrimidine, respectively.[18] Such a spectrum may

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[4] D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock, G. B. Elion, and G. H. Hitchings, *Cancer Res.*, **13**, 593 (1953).

[5] H. E. Skipper, *Ann. N. Y. Acad. Sci.*, **60**, 268, 315 (1954).

[6] L. W. Law, *Proc. Soc. Expl. Biol. Med.*, **84**, 409 (1953).

[7] A. Gellhorn and E. Hirschberg, *Cancer Res.*, **3**, 1 (1955).

[8] G. H. Hitchings and C. P. Rhoads, *Ann. N. Y. Acad. Sci.*, **60**, 183 (1954).

[9] J. D. Davidson, *Cancer Res.*, **20**, 227 (1960).

[10] R. J. Knopf, *Dissertation Abstr.*, **19**, No. 8, 1922 (1959).

[11] S. K. Chatterji and N. Anand, *J. Sci., Ind. Res.* (India), **17B**, 63 (1958).

[12] C. K. Chatterji and N. Anand, *ibid.*, **18B**, 272 (1959).

[13] M. L. Hoefle and R. F. Meyers, U.S. Patent 2,949,466 (August 16, 1960).

[14] E. C. Taylor, R. J. Knopf, R. F. Meyer, A. Holmes, and M. L. Hoefle, *J. Am. Chem. Soc.*, **82**, 5711 (1960).

[15] T. B. Johnson and J. A. Ambler, *ibid.*, **33**, 982 (1911).

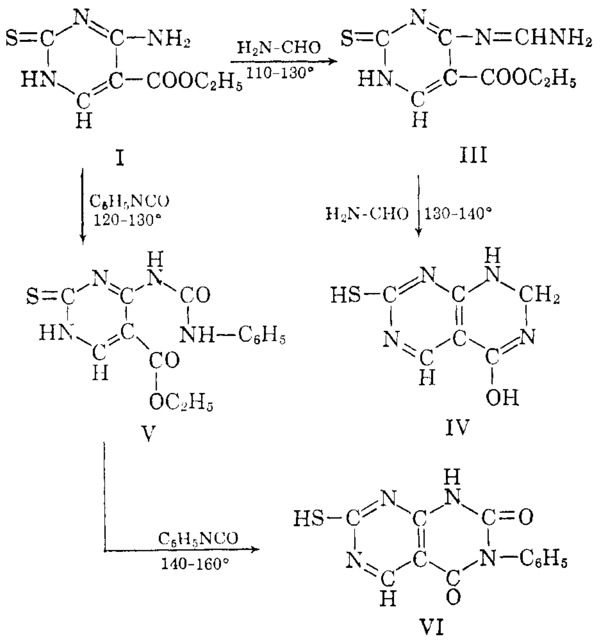
[16] G. H. Hitchings, G. B. Elion, E. A. Falco, and P. B. Russel, *J. Biol. Chem.*, **177**, 347 (1949).

[17] F. S. Crossley and M. L. Moore, *J. Org. Chem.*, **9**, 529 (1944).

[18] T. L. V. Ulbricht, *Naturwissenschaften*, **46**, 416 (1958).

result from keto–enol type of equilibrium, which is concerned with the 3- and 4-positions. This assumption can be supported by the fact that the Schiff base (III) is an intermediate in the cyclization reaction, thus the cyclization itself must take place at the carboxylic group. Further the nuclear magnetic resonance spectrum (n.m.r.) gave a clear absorption peak at 284 c.p.s., characteristic for an isolated methylene, attached to an electronegative group. Such a system could be formed by hydrogenation of the Schiff's base. The spectrum gave also a pronounced absorption peak at 506 c.p.s., characteristic for hydrogens in an aromatic system; thus the ring of the starting material must have retained its aromaticity.

The reactivity and the cyclization of I was also studied by heating of I with phenyl isocyanate. It was interesting to discover that at lower temperatures, 120–130°, a stable phenyl ureidopyrimidine (V) was formed, which on heating at 140–160°, with an excess of phenyl isocyanate, underwent ring closure to give 7-mercapto-3-phenylpyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)dione (VI).



Treating IV with phosphorus pentasulfide in boiling pyridine gave a nearly quantitative yield of 4,7-dimercaptopyrimido[4,5-*d*]dihydropyrimidine-(1*H*,2*H*) (VII). Compounds VI and VII exhibit physical properties similar to those of compound IV. They do not melt below 350°, are readily soluble in alkalies, hot formamide, almost insoluble in water and common organic solvents; in addition they possess considerable toxicity although much less than that of compound IV. Removing the sulfur from the 7-position of IV, using W-6 Raney nickel[19] in an aqueous medium at about pH 7, rendered the compound soluble in water and decreased its stability; 4-hydroxypyrimido[4,5-*d*]-dihydropyrimidine(1*H*,2*H*) (VIII) was thus obtained as a monohydrate; m.p. 252–254°. Desulfurization with W-6 Raney nickel in an alkaline medium removed the sulfur and split the ring, yielding a sodium salt, which on acidification gave 4-amino-5-carboxypyrimidine (IX).

In an attempt to confirm the structure assigned to VIII, I was desulfurized, but no success was achieved in attempting to cyclize the product (X) with formamide to give VIII. X appears to be unstable and on heating with formamide undergoes decomposition, even at 80°.

It is interesting to mention that the compound prepared by Knopf,[10] 4-hydroxypyrimido[4,5-*d*]-pyrimidine, gives an identical infrared absorption spectrum as the compound VIII.

The structure of VIII was also very well supported by the n.m.r. spectrum. Using deuterium oxide as the solvent and the oscillation frequency of 60 Mc., the same experimental conditions which were used for IV, four clearly resolved resonance peaks were obtained. Peaks at 514 and 503 c.p.s. indicate two hydrogens of aromatic character. The peak at 284 c.p.s. shows an isolated methylene group and the peak at 312 c.p.s. can be accounted for —NH—, —OH, and H_2O, since at this frequency the protons of these groups are capable of undergoing a rapid exchange with one another.

The additional support of structure VIII has been obtained from the ultraviolet absorption spectra. Knopf's compound, 4-hydroxypyrimido-[4,5-*d*]pyrimidine and VIII show the same absorption maxima, $\lambda_{\max}$ 260 mμ, but the extinction coefficients are 3775 and 3464, respectively. This indicates that VIII must have a higher degree of saturation. Unfortunately the problem of insolubility did not allow to study the ultraviolet spectra of the parent compounds, 4-hydroxy-7-methylmercaptopyrimido[4,5-*d*]pyrimidine and IV.

It was found that the water of hydration of VIII is very strongly bound. It could not be completely removed by heating the substance for half an hour at 200°/0.3 mm., nor by recrystallization from formamide at 120°. From these observations it may be assumed that the water of hydration of compound VIII is covalently bonded, a phenomenon common in heterocyclic chemistry.[20] By reaction of VIII with phosphorus pentasulfide in boiling pyridine dehydration occurred, giving in about 15% yield 4-mercaptopyrimido[4,5-*d*]dihydropyrimidine(1*H*, 2*H*) (XI), the dihydro derivative of the desired analog of 6-mercaptopurine.

No procedure could be developed to purify XI by recrystallization. A small sample was purified by sublimation at 250°/0.5 mm.

After several attempts a procedure was developed for preparing 4-chloropyrimido[4,5-*d*]dihydropy-

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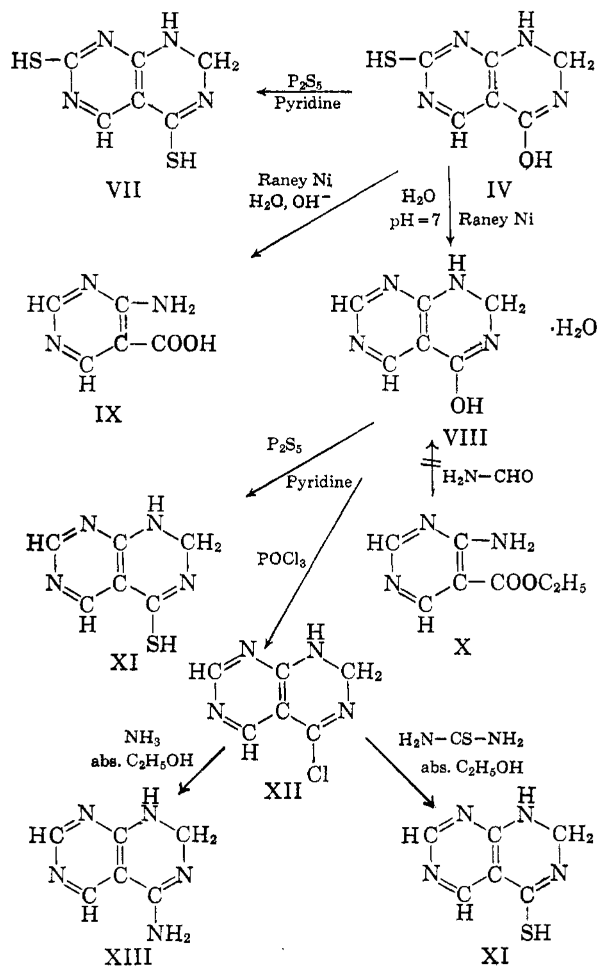
(19) H. R. Billica and H. Adkins, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 176.

(20) D. D. Perrin and Y. Inoue, *Proc. Chem. Soc.*, 342 (1960).



rimidine(1*H*, 2*H*) (XII) by treating VIII with phosphorus oxychloride. The crude product gave satisfactory analyses, but it appeared to be more unstable than XI. No recrystallization procedure could be developed and an attempt to sublime the compound decomposition began at about 230°/0.3 mm. Support for the structure of XII was obtained by preparation of XI and 4-aminopyrimido-[4,5-*d*]dihydropyrimidine(1*H*,2*H*) (XIII). Compound XIII proved more stable than the other dihydropyrimidines (VIII, XI, and XII).

The most toxic of these compounds is IV, with less toxicity observed in compound VII, LD_{50} 17.8 and 178, respectively, orally in rats. No significant useful pharmacological activity was found.

Experimental[21,22]

4-Amino-5-carbethoxy-2-thiopyrimidine (I).—One mole (168 g.) of ethyl ethoxymethylenecyanoacetate,[23,24] 1.1 moles (80 g.) of alcohol-soluble thiourea, and 500 ml. of absolute ethyl alcohol were heated until the material dissolved. The solution was then allowed to cool to room temperature and with stirring, 600 ml. of absolute alcohol containing 1 g.-atom of sodium was added dropwise. After 1 hr. the addition was complete and the reaction mixture was allowed to stand at room temperature for 1 hr. more, whereupon a voluminous yellow solid separated. The temperature of the reaction mixture was raised, and the clear solution was refluxed for about 6 hr. It then stood at room temperature overnight and was acidified with glacial acetic acid. The product obtained weighed 180.5 g. (90.5%), m.p. 272–274°. For analysis a sample was recrystallized from 98% formic acid (15 ml./g.), m.p. 279–280°.

Anal. Calcd. for $C_7H_9O_2N_3S$: C, 42.24; H, 4.50; N, 20.90. Found: C, 41.90; H, 4.60; N, 20.90.

4-Amino-2-mercaptopyrimidine (II).—Five grams of I and 50 ml. of freshly distilled formamide was heated for about 50 hr. at about 150°. After heating for 6 hr. the material dissolved completely. At the end of the heating period the reaction mixture stood at room temperature overnight. The crystals which formed (3.0 g.) were filtered by suction, washed with water and acetone, and dried; m.p. 283–285°, browning at 275°. This solid was then suspended in 50 ml. of water and 1 *N* sodium hydroxide was added until it dissolved, then 0.1 g. of activated carbon was added with stirring, allowed to stay at room temperature for about 30 min., and filtered. The filtrate acidified with acetic acid, gave a precipitate which was filtered, washed with water and acetone, and dried at 110°/0.5 mm., m.p. 283–285°.

Anal. Calcd. for $C_4H_5N_3S$: C, 37.80; H, 3.93; N, 33.00; S, 25.19. Found: C, 37.84; H, 4.07; N, 33.02; S, 25.10.

Ethyl 4-[(aminomethylene)amino]-2-thioxo-5-pyrimidinecarboxylate (the Schiff Base) (III).—Fifty grams of dried I and 350 ml. of freshly distilled formamide were heated at 100–110° for about 24 hr. A small aliquot portion was then taken, filtered, the residual solid was washed with water and acetone, and dried at 140°/0.5 mm.; m.p. 279–280°.

Anal. Calcd. for I, $C_7H_9O_2N_3S$; C, 42.24, H, 4.50; N, 20.90. Found: C, 42.10, H, 4.65; N, 20.90.

The remaining reaction mixture was heated further for 40 hr. and during that time the temperature of the bath was gradually increased to 130°. A sample was then removed, purified as before, and dried; m.p. above 285° with gradual decomposition; at 260–280°/0.5 mm. the material sublimed.

Anal. Calcd. for III, $C_8H_{10}O_2N_4S$: C, 42.47; H, 4.82; N, 24.85. Found: C, 42.31; H, 4.42; N, 24.85.

4-Hydroxy-7-mercaptopyrimido[4,5-*d*]dihydropyrimidine-(1*H*,2*H*) (IV).—The reaction mixture, from which a sample of III was isolated, was heated for about 48 hr. more; during this time the temperature of the silicone bath was gradually increased to 130–140°. It was than allowed to stay at room temperature overnight. The solid which separated was filtered, washed with hot water, alcohol, and acetone, and dried at 110°/0.5 mm. to give about 70% of a dark gray product which gradually decomposed when heated above 330°; however, the compound did not melt even at 350°. The crude material was suspended in water and 2 *N* sodium hydroxide added until solution occurred. The solution was decolorized with activated charcoal, allowed to stay at room temperature for about 1 hr. while stirring, and filtered by gravity. The filtrate was acidified with glacial acetic acid, the precipitated white amorphous solid was filtered by suction, washed with hot water, alcohol, and acetone, and dried at 140°/0.5 mm. The grayish white product did not melt when heated to 350°.

Anal. Calcd. for $C_6H_6ON_4S$: C, 39.56; H, 3.31; N, 30.85. Found: C, 39.46; H, 3.34; N, 30.90.

Direct Synthesis of IV.—Ten grams of I, 100 ml. of freshly distilled formamide, and 100 ml. of *p*-xylene were heated in a silicon bath at 140–145°, for about 50 hr. During heating the white material disappeared and a gray solid formed. This was then filtered hot by suction, washed with water, alcohol, acetone, and ether, and dried in a vacuum pistol for

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[21] All melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected.

[22] Microanalyses by Micro-Tech Laboratories, Skokie, Ill.

[23] DeBellemont, *Compt. rend.* **128**, 1338 (1899); **129**, 5 (1900); *Bull. soc. chim.*, **18**, 28 (1901).

[24] Commercially available from Kay-Fries Chemicals, Inc., 180 Madison Ave., New York 16, N. Y.

2 hr. at 110°/0.5 mm; m.p. >350°; gradual decomposition took place above 300°, yield 6.5 g.

Anal. Calcd. for $C_6H_6ON_3S$: N, 30.85. Found: N, 30.45.

One gram of this product was recrystallized from 125 ml. of formamide by heating at 140-150° until a clear solution was obtained. This was decolorized with charcoal, filtered by gravity, and allowed to stay at room temperature overnight. The precipitate was filtered off, washed with water, alcohol, acetone, and ether, and dried as before, giving 0.85 g. of a grayish white product which did not melt even at 360°, but above 330° started to develop a brown color, and gradually decomposed.

Anal. Calcd. for $C_6H_6ON_4S$: N, 30.85. Found: N, 30.75. This material is very hygroscopic.

Ethyl 1,2-Dihydro-4-(3-phenylureido)-2-thioxo-5-pyrimidinecarboxylate (V).—One gram of I and 15 ml. of phenyl isocyanate were heated for 6 hr. at 120°. After heating for 3 hr., a clear solution was obtained and after further heating for a total of 6 hr. the temperature of the bath was increased to 130° and maintained at this point for 2 hr. The reaction mixture was then filtered with suction and unchanged phenyl isocyanate was removed in a vacuum until a dry residue was obtained. The residue was stirred with 25 ml. of methanol, filtered by suction, and then recrystallized twice from 50 ml. of dioxane, giving 1.3 g. of product, m.p. 182-183°.

Anal. Calcd. for $C_{14}H_{14}O_3N_4S$: C, 52.80; H, 4.80; N, 17.60; S, 10.05. Found: C, 53.28; H, 4.63; N, 17.60; S, 9.78.

7-Mercapto-3-phenylpyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)dione (VI).—Ten grams of I and 100 ml. of phenyl isocyanate were heated in a silicone bath at 120° for 6 hr. The temperature of the bath was then gradually increased to 140-145°, heated in this range for 4 hr., and then allowed to cool to room temperature. The solution was poured, with stirring, into 500 ml. of anhydrous ether. The solid which formed was filtered off with suction and dried at room temperature, giving 15.5 g. of a light yellow product. This material was then suspended in 300 ml. of boiling acetone and filtered. The residue was suspended again in 100 ml. of boiling dioxane, filtered by suction, washed with acetone, and dried at 140°/0.5 mm.; m.p. about 350°, slowly decomposing above 280°.

Anal. Calcd. for $C_{12}H_8O_2N_4S$: C, 52.93; H, 2.96; N, 20.60. Found: C, 52.80; H, 2.80; N, 20.55.

4,7-Dimercaptopyrimido[4,5-*d*]dihydropyrimidine(1*H*,2*H*) (VII).—One gram of IV, 80 ml. of pyridine, and 5 g. of phosphorus pentasulfide were refluxed for 5 hr. The pyridine was then removed in a vacuum, the residue suspended in 100 ml. of boiling water and filtered by suction. The precipitate was dissolved in 50 ml. of 1 *N* sodium hydroxide, decolorized with activated carbon, and filtered by gravity. After acidification of the filtrate with glacial acetic acid the product which separated was filtered off, washed with water and acetone, and dried at 140°/0.5 mm. It did not melt even when heated above 290°.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.35; H, 3.15; N, 28.30; S, 31.90. Found: C, 36.30; H, 2.95; N, 28.22; S, 31.70.

4-Hydroxypyrimido[4,5-*d*]dihydropyrimidine(1*H*,2*H*)-monohydrate (VIII).—One gram of IV, 170 ml. of distilled water, and 3 g. of a freshly prepared Raney nickel were refluxed with stirring for 3 hr. This was then filtered hot by suction, washed with 50 ml. of boiling water, the filtrate and the wash solution combined and evaporated under vacuum to dryness on a water bath, giving 0.80 g. of material which, when recrystallized from 15 ml. of water yielded 0.63 g., m.p. 252-254°.

Anal. Calcd. for $C_6H_8O_2N_4$: C, 42.80; H, 4.78; N, 33.38. Found: C, 42.89; H, 4.70; N, 33.46. λ_{max} 260, ϵ 3464.

4-Amino-5-carboxypyrimidine (IX).—A solution of 1 g. of IV, 170 ml. of distilled water, 30 ml. of 2 *N* sodium hydroxide, and 3 g. of freshly prepared Raney nickel was refluxed with stirring for 3 hr. This was then filtered hot, washed with hot water, the washings and the filtrate were combined, cooled in an ice bath, and acidified with concentrated hydrochloric acid to about pH 4. The material which precipitated was then filtered off by suction, recrystallized from hot water, and dried for 2 hr. at 110°/0.5 mm.; m.p. 287-288°.

Anal. Calcd. for $C_5H_5O_2N_3 \cdot H_2O$: C, 38.05; H, 4.45; N, 26.70. Found: C, 37.85; H, 4.30; N, 26.67.

4-Amino-5-carbethoxypyrimidine (X).—Twenty grams of I, 60 g. of freshly prepared Raney nickel, and 1000 ml. of absolute ethyl alcohol were refluxed with stirring for 3 hr. The reaction mixture was then filtered hot by suction, the residue washed with 100 ml. of boiling ethanol, and the filtrates combined and concentrated to dryness. The residue was extracted with 400 ml. of boiling ether and the ether evaporated to give 9.5 g., 56.5 % yield, of a white amorphous product, m.p. 104-106°. The material was very easily soluble in water and almost all organic solvents. It sublimed readily and quantitatively at 120-130°/0.5 mm., m.p. 106-106.5°.

Anal. Calcd. for $C_7H_9O_2N_3$: C, 50.30; H, 5.37; N, 25.10. Found: C, 50.26; H, 5.56; N, 25.02.

4-Mercaptopyrimido[4,5-*d*]dihydropyrimidine(1*H*,2*H*) (XI).—A solution of 1 g. of VIII, 3.0 g. of phosphorus pentasulfide, and 50 ml. of pyridine was heated on a steam bath for 1 hr. The solvent was then removed in a vacuum, the residue was slurried with 50 ml. of ice-cold water, filtered by suction, washed with acetone and ether, and dried at 110°/0.5 mm. A dark orange material which gradually decomposed when heated above 240° was obtained. No procedure could be developed to purify this material by recrystallization. A small sample was purified by sublimation, at about 250°/0.5 mm., yielding a pure white material; also decomposing above 240°.

Anal. Calcd. for $C_6H_6N_4S$: C, 43.40; H, 3.66; N, 33.70; S, 19.50. Found: C, 43.44; H, 3.89; N, 33.85; S, 19.20.

4-Chlorpyrimido[4,5-*d*]dihydropyrimidine(1*H*,2*H*) (XII).—About 3 g. of VIII was dissolved in 20 ml. of phosphorus oxychloride and the solution refluxed for 1 hr. The excess phosphorus oxychloride was distilled under vacuum, at 50°; to the residue was added 30 g. of crushed ice, and the precipitate was slurried with a spatula and quickly filtered by suction, while cold. The residue was washed with 20 ml. of ice-cold water, then with acetone and ether and dried in a vacuum pistol at 110°/0.5 mm. The light red product (0.8 g.) obtained developed a dark color when heated above 235°; however, it did not melt at 330°.

Anal. Calcd. for $C_6H_5N_4Cl$: C, 42.75; H, 2.98; N, 33.23; Cl, 21.02. Found: C, 41.85; H, 3.05; N, 33.84; Cl, 20.02.

The material decomposed in aqueous solution, was insoluble in common organic solvents and did not sublime on heating under vacuum. No procedure could be developed to prepare this compound in purer form.

4-Aminopyrimido[4,5-*d*]dihydropyrimidine(1*H*,2*H*) (XIII).—Four-tenths of a gram of XII and 50 ml. of absolute ethanol were placed in a 100-ml. three-neck reaction flask, equipped with a magnetic stirrer, condenser, and an inlet tube. The flask was then immersed in a Dry Ice bath, anhydrous ammonia was bubbled through and then the temperature of the bath was gradually increased. This bath was finally replaced by an ice-cold water bath. In about 1 hr. the bath temperature was gradually increased to about 100° where it was held for 1 hr. The reaction mixture was cooled to room temperature and filtered by suction. The residue was washed with 50% ice-cold ethanol, then with acetone and ether and dried at 110°/0.5 mm. About 0.25 g. of a product was obtained which gradually decomposed when heated above 220°. This sample was suspended in 10 ml. of cold water, immersed in an ice bath and 10% hydrochloric acid solution was added dropwise until a clear solution was obtained. The solution was then decolorized with charcoal, filtered by gravity, and neutralized with cold solution of ammonium hydroxide to about pH 8. The suspended yellowish amorphous precipitate was kept in a refrigerator for about 1 hr., filtered by suction, washed with alcohol, acetone, and ether, and dried at 110°/0.5 mm. The sample decomposed gradually when heated above 240°, but did not melt even at 330°.

Anal. Calcd. for $C_6H_7N_5$: N, 46.60. Found: N, 46.21. No procedure could be developed to prepare a sample of higher purity by recrystallization or vacuum sublimation.

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Studies on the C-Methyl-γ-benzopyrone System. Orientation in the Isoflavone Series. II[1]

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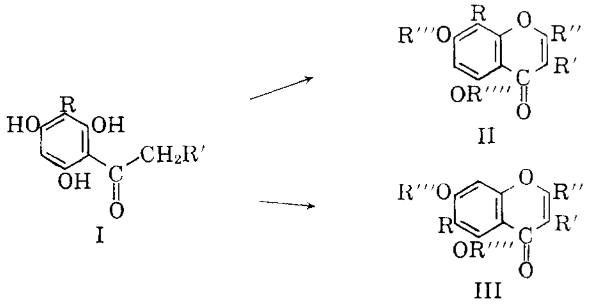
The ethoxalylation of 4′- and 2′-methyl ethers of 2,4,6-trihydroxy-3-methyldeoxybenzoin has resulted in the formation of an isomeric mixture of isoflavones of 5,6,7- and 5,7,8-orientations. The orientation of each isomer has been authenticated by comparison with synthetic standards. The investigation of the condensation of 2,4,6-trihydroxy-3-methyldeoxybenzoin with acetyl chloride and pyridine at 0° (followed by refluxing with aqueous sodium carbonate) and acetic anhydride and sodium acetate at high temperature has also given rise to an isomeric mixture of 2-methylisoflavones of the same orientations. The dual course of very facile Baker–Venkataraman transformation followed by isoflavone ring closure has been suggested for the formation of isomeric mixtures in some cases.

Mukerjee and Seshadri[2] have stated that in the synthesis of C-methyl isoflavones that "8-methyl compounds are generally formed if the temperature of the reaction is high[3a,b] (boiling acetic anhydride) whereas, if conducted at 0°,[3b] the 6-methyl compounds result." The present paper describes the reinvestigation of the work and reports our findings which do not agree with those of the previous workers.[3a,b]

Ethoxalyl Chloride–Pyridine.—Our earlier work[4] on the ethoxalylation of a C-methyl deoxybenzoin (I. R = Me, R′ = C_6H_5) established the formation of a mixture of isoflavones of 5,7,8- and 5,6,7-orientations. An explanation as to the dual course of cyclization involving 2- and 6-hydroxyl groups also was advanced. Mehta and Seshadri,[3b] however, using a deoxybenzoin of the similar type (I. R = Me, R′ = p-$MeOC_6H_4$) reported the exclusive formation of an isoflavone of 5,6,7-orientation. As their findings do not agree with our previous results and ideas the ethoxalylation of 2,4,6-trihydroxy-3-methyl-4′-methoxydeoxybenzoin was reinvestigated.

Mehta and Seshadri[3b] found that the deoxybenzoin (I. R = Me, R′ = p-$MeOC_6H_4$) on ethoxalylation gave a product, m.p. 176–178°, which was assigned the structure of ethyl 5,7-dihydroxy-4′-methoxy-6-methyl isoflavone-2-carboxylate (III. R = Me, R′ = p-$MeOC_6H_4$, R″ = CO_2Et, R‴ = R″″ = H). The carbethoxyisoflavone on hydrolysis yielded the corresponding carboxyisoflavone (melting point not reported) (III. R = Me, R′ = p-$MeOC_6H_4$, R″ = CO_2H, R‴ = R″″ = H). The crude carboxyisoflavone on decarboxylation at 275° gave an isoflavone, m.p. 210–212°. It was characterized by them as 5,7-dihydroxy-4′-methoxy-6-methylisoflavone (III. R = Me, R′ = p-$MeOC_6H_4$, R″ = R‴ = R″″ = H) by partial methylation to 5-hydroxy-7,4′-dimethoxy-6-methylisoflavone (III. R = R‴ = Me, R′ = p-$MeOC_6H_4$, R″ = R″″ = H) and by comparing it with the nuclear methylation product of genistein.[5]



The present authors have now found the product, m.p. 176–178°, to be a mixture of the two isomers. It was, however, resolved by a careful fractional crystallization from benzene–methanol into two distinct products melting at 199–201° and 201–203°. The mixed melting point of the two isomers was found to be 172–173°.

The carbethoxyisoflavone, m.p. 199–201°, on usual hydrolysis gave the corresponding carboxyisoflavone m.p. 280–281°, which on subsequent de-

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