30°. A small amount of insoluble material was removed by centrifugation. The slightly yellow but clear supernatant was decanted and the centrifuge bottle was rinsed with 20 ml. of methanol. The *p*H of the combined supernatants was 9.0–9.5.[10] If the solution has a *p*H lower than 8.5, it should be properly adjusted by the addition of solid sodium methoxide in small portions.

(*b*) *Solution of* IX. All the following steps were conducted under nitrogen. To 18.4 g. (0.20 mole) of freshly distilled thioglycolic acid in 200 ml. of anhydrous methanol was added 23.2 g. (0.43 mole) of sodium methoxide in small portions with stirring. The temperature was kept between 15–20°. The resulting solution was opalescent and slightly pink. It was poured into a separatory funnel which had been deaerated with nitrogen. The flask was rinsed with 30 ml. of methanol. The *p*H of the combined methanol solution was 11.

The solution of V was poured into a 3-necked flask provided with a calcium chloride tube. After flushing the apparatus with nitrogen the solution of IX was added dropwise with vigorous stirring over a period of 1 hr. The temperature of the reaction mixture was kept between 15 and 20° by applying an ice water bath. The reaction mixture was then stirred for 3 hr. and was allowed to stand for 20 hr. at room temperature. It was then centrifuged and the bottle was rinsed with 20 ml. of methanol. The combined clear supernatants gave a negative nitroprusside test and the *p*H was between 10 and 11. The solution was added dropwise to 2.5 l. of pure acetone over a period of 1 hr. The white precipitate was collected on a Buchner funnel. The flask was rinsed twice with a mixture of 50 ml. of methanol and 250 ml. of acetone. The white cake was finally washed with 500 ml. of acetone and sucked dry. It was quickly transferred to a desiccator and the adhering solvents were removed at 25° by applying an oil pump vacuum (0.1 mm.) overnight. The yield of crude X was approximately 87 g. (70–80%). By using pure, recrystallized IV the yield of X could be increased to 86% of a more stable product.

(10) Measured by adding one drop of this solution to pHydrion papers moistened with water.

Recrystallization of X. The 86.6 g. of crude, pulverized X was dissolved in 433 ml. of methanol by gentle heating (50°) on a water bath with stirring. A clear colorless solution resulted. To the warm methanol solution acetone (approximately 1000 ml.) was added in small portions with stirring until the mixture became turbid. The white precipitate was filtered off quickly by gravity and discarded. To the filtrate more acetone was added with stirring until a permanent turbidity occurred and the first crystals were formed. The stoppered Erlenmeyer was allowed to stand at room temperature in the dark for 20 hr. The crystalline precipitate of X was filtered, washed with 600 ml. of acetone, and dried in vacuo (oil pump) overnight, and finally over phosphorus pentoxide at 0.1 mm. yielding approximately 62 g. The compound is extremely hygroscopic. For analysis a sample was dried over phosphorus pentoxide at 0.1 mm. and 80° for 3 hr.

Anal. Calcd. for $C_{16}H_{25}HgNNa_2O_6S$: Hg, 33.1; S, 5.3. Found: Hg, 33.3; S, 5.3.

Stability studies at 50 ± 3°. The test solutions were prepared by dissolving 242 mg. of X in 2 ml. of distilled water. The mercury content of these solutions corresponds to that of the solutions prepared for injection. The *p*H of the solutions was, if necessary, adjusted to 9.0–9.5 with 0.1*N* sodium hydroxide.

In experiments with EDTA the calculated amount of the disodium salt of EDTA (disodium versenate, analytical reagent, Bersworth) was dissolved in water. The *p*H was adjusted to 8 with dilute sodium hydroxide before X was added. The *p*H after the addition of X was 9.0–9.5.

Most of the solutions decomposed by forming a black precipitate of HgS. The supernatant remained clear. In some solutions evidence of deterioration consisted of a yellow precipitate which after some time turned black. The supernatant of the latter samples was turbid.

Acknowledgment. We are indebted to Dr. W. Reiss and his staff for the analyses reported herein.

RADNOR, PA.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF MEDICINE, YALE UNIVERSITY]

Synthesis and Physical Properties of 4-Oxo- and 4-Thio-pyrimido[4,5-*d*]pyrimidine[1]

HENRY G. MAUTNER

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4-Aminopyrimidine-5-carboxamide and 4-aminopyrimidine-5-thiocarboxamide reacted with acetic anhydride and triethyl orthoformate to yield 4-oxo- and 4-thiopyrimido[4,5-*d*]pyrimidine, respectively. These compounds showed greater lack of stability to the removal or replacement of substituents than the isomeric pteridines, and were stronger acids and weaker chelating agents.

The considerable antileukemic and carcinostatic activity of several purine derivatives[2] has led to the synthesis of several related heterocyclic systems, most of which were designed in such a way as to permit ribosidation in the equivalent of the 9-position of purines. This seemed to be important since it was believed that 6-mercaptopurine, the purine with the widest use as an antileukemic agent, had to be converted to the riboside or the ribotide before it could become an active metabolite,[3] a view supported by the observation that mercaptopurine-resistant bacteria were incapable of converting hypoxanthine to purine ribotides. Recently, however, it was found that methylation in

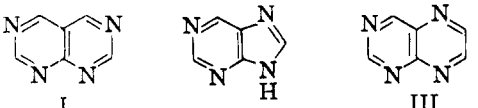
(1) This work was presented before the Medicinal Chemistry Section at the American Chemical Society meeting, New York, N. Y., September 1957, 30–O.

(2) J. H. Burchenal in Current Research in Cancer Chemotherapy, Report No. 4, 11 (1956).

(3) H. E. Skipper, J. R. Thomson, D. J. Hutchison, F. M. Schabel, and J. J. Johnson, *Proc. Soc. Expt. Biol. Med.*, **95**, 135 (1957).

the 9- position of purines[4] and in the analogous position of certain pyrazolo[3,4-*d*]pyrimidines[5] was compatible with antitumor activity, although ribosidation of the 5-membered ring was now impossible, unless prior demethylation occurred. Furthermore, it was observed that ribosidation of 6-mercaptopurine failed to increase its efficacy;[3] although, it is conceivable that this observation reflects the metabolic instability of purine ribosides.

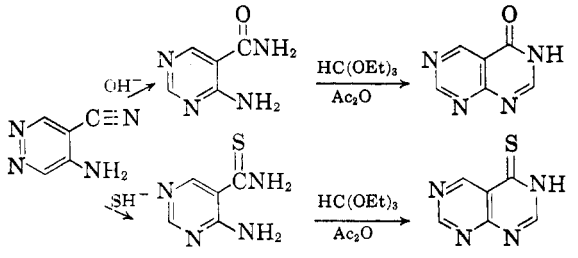
These observations raised the possibility of synthesizing purine antagonists containing a pyrimidine ring in place of the imidazole ring, even though such compounds might form ribosides with more difficulty than the purines. The pyrimido[4,5-d]-pyrimidine ring system (I) was chosen since it not only resembles purine (II) but is isomeric with pteridine (III) and would, therefore, be suitable for the preparation of potential antagonists of either purines or folic acid.



I II III

The pyrimido[4,5-*d*]pyrimidine ring system was first discussed by T. B. Johnson,[6] who also recognized the unsymmetrical [5,4-*d*] system. Only a few of the symmetrical compounds have been synthesized, most of these being related to thiamine and thiochrome.[7] Recently a complex 4-aminopyrimido[4,5-*d*]pyrimidine was prepared by E. C. Taylor *et al.*[8]

The following reaction sequence was utilized for the synthesis of the compounds described here:



4-Amino-5-cyanopyrimidine was synthesized from formamidine hydrochloride and malononitrile by the method of Baddiley, Lythgoe, and Todd[9] and converted in good yield to 4-aminopyrimidine-5-carboxamide[15] and quantitatively to 4-aminopyrimidine-5-thiocarboxamide. The addition of hydrogen selenide to the nitrile yielded a very unstable, orange red crystalline compound, presumably the selenocarboxamide.

Cyclization in the usual fashion[10] of the amide and the thioamide in the presence of acetic anhydride and triethyl orthoformate yielded 4-oxo- and 4-thiopyrimido[4,5-*d*]pyrimidine, respectively.

Attempts to desulfurize 4-thiopyrimidopyrimidine with Raney nickel under many different conditions, or to convert the 4-oxo- into the 4-chloro compound, have so far been unsuccessful. It appears that these pyrimidopyrimidines are even more unstable than their isomers, the pteridines, the latter being a series of compounds in which replacement reactions are notoriously difficult.[11,11a] Attempts to prepare 4-aminopyrimido[4,5-*d*]pyrimidine and the 2-oxo-4-amino and 2-thio-4-amino compounds by the reaction of 4-amino-5-cyano: pyrimidine with formamide, urea, or thiourea respectively, were unsuccessful.

Physical properties. Like the isomeric pteridines, these pyrimidopyrimidines decompose at high temperatures without a sharp melting point.[10] The purity of the compounds was indicated by means of paper chromatography and ultraviolet absorption spectra.

The intrared spectra of the pyrimidopyrimidines are extremely similar to those of the isomeric pteridines. Unfortunately, because of solubility problems the spectra had to be determined in potassium bromide disks, thus making the distinction between shifts due to internal and external hydrogen bonding impossible.

The following table compares the ultraviolet spectra and acid dissociation constants of the related pyrimidopyrimidines and pteridines:

TABLE I

	Pteridines			Pyrimido[4,5-*d*]pyrimidines		
Derivative	pK_a	λ_{max}	log E_{max}	pK_a	λ_{max}	log E_{max}
4-Oxy	7.89	230,265,310	3.98,3.54,3.82	7.20	286,315[a]	3.75,3.54
anion		242,333	4.23,3.79		231,312	4.08,3.81
4-Thio	6.81	256,390	4.13,4.00	6.23	254,382	4.05,3.97
Anion		265,408	4.22,3.93		254,386	4.04,3.94

[a] Inflection.

(4) R. K. Robins and H. H. Li, *J. Am. Chem. Soc.*, **79**, 490 (1957).

(5) H. E. Skipper, R. K. Robins, and J. R. Thomson, *Proc. Soc. Expt. Biol.*, **89**, 594 (1955).

(6) T. B. Johnson and Y. F. Chi, *Rec. trav. chim.*, **49**, 197 (1930).

(7) For instance, T. Matsukawa and T. Iwatsu, *Science*, **115**, 212 (1952).

(8) E. C. Taylor, A. J. Crovetti, and R. J. Knopf, *J. Am. Chem. Soc.*, **80**, 427 (1958).

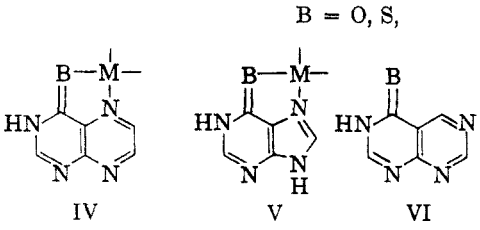
(9) J. Baddiley, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 386 (1943).

(10) A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.*, 474 (1951).

(11) D. J. Brown in "Ciba Foundation Symposium on Chemistry and Biology of Pteridines," Little, Brown, and Co., Boston, 1954, p. 62.

(11a) The relative lack of stability of pyrimido [4,5-d] pyrimidines has since been confirmed and elucidated by E. C. Taylor, Abstracts, American Chemical Society Meeting, San Francisco, Calif., April 1958, 2-M.

It can readily be seen that while 4-oxo- and 4-thiopteridine (IV) and the analogous 6-oxo- and 6-mercaptopurine (V) can form strong internal hydrogen bonds and are powerful chelators[12,13] such hydrogen bonding or chelation is not possible in the 4-substituted pyrimido[4,5-*d*]pyrimidines (VI).



When methanolic cupric chloride was added to 6-mercaptopurine or to 4-thiopteridine solutions immediate color changes followed by precipitation were observed. In contrast 4-thiopyrimido[4,5-*d*]-pyrimidine, appears to be only a poor complexing agent as indicated by the failure of its solutions to change color in the presence of cupric chloride. Chelation would appear to be a convenient method for distinguishing 4-substituted pyrimido[4,5-*d*]-pyrimidines from the isomeric pteridines and pyrimido[5,4-*d*]pyrimidines.

As expected from a consideration of the above mentioned differences in hydrogen bonding and chelating ability, the pyrimido[4,5-*d*]pyrimidines are stronger acids than either the purines or the pteridines.

Biological activity. 4-Thiopyrimido[4,5-*d*]pyrimidine was found to be a weak inhibitor of *L. casei*, this inhibition being strongly reversed by adenine or hypoxanthine, and weakly by cysteine. Against mouse lymphoblasts, L-5178-Y, grown in culture, this compound exerted an action equal to that of 6-mercaptopurine (G. A. Fischer), but it was inactive in inhibiting the growth of the solid mouse tumor L-1210 (J. J. Jaffe).

EXPERIMENTAL

4-Amino-5-cyanopyrimidine. The method of Baddiley, Lythgoe, and Todd[8] was used involving the room temperature reaction of malononitrile and formamidine hydrochloride[14] in ethanol. Attempts to increase the yield by heating the reaction mixture were unsuccessful.

4-Aminopyrimidine-5-carboxamide. 4-Amino-5-cyanopyrimidine was treated with potassium hydroxide and hydrogen peroxide in the usual fashion.[15]

4-Aminopyrimidine-5-thiocarboxamide. In a mixture of 300 cc. of ethyl alcohol and 24 cc. of concentrated ammonium hydroxide were dissolved 2.1 g. (0.0175 mole) of 4-amino-5-cyanopyrimidine. The mixture was heated to 60° until all solid had dissolved. Hydrogen sufide was bubbled through the warmed solution for 3 hr. The hot solution was treated with decolorizing carbon, filtered, and cooled. A yield of 2.6 g. (96.5%) of pale greenish yellow, glittering needles effervescing at 203-205° and melting at 254.5–255°[16] was obtained.

Anal.[17] Calcd. for $C_5H_6N_4S$: C, 38.94; H, 3.92; N, 36.34. Found: C, 39.09; H, 3.84; N, 36.04.

4-Oxopyrimido[4,5-d]pyrimidine. A mixture of 1.2 g. (0.0087 mole) of 4-aminopyrimidine-5-carboxamide, 5 cc. of acetic anhydride, and 5 cc. of ethyl orthoformate was permitted to reflux gently for 1 hr. The clear red reaction mixture was evaporated to dryness on a flash evaporator at 45°. To the residue 10 cc. of ice-cold acetone were added and the mixture stirred and filtered to yield 0.72 g. (56%) of light yellow powder. The material was recrystallized from a small amount of water with the use of decolorizing carbon. Glittering, faintly cream colored crystals decomposing over a range of 220–250° were obtained.

Anal. Calcd. for $C_6H_4N_4O$: C, 48.65; H, 2.72; N, 37.83. Found C, 48.63; H, 2.79; N, 35.87.

4-Thiopyrimido[4,5-d]pyrimidine. In a mixture of 17 cc. of acetic anhydride and 17 cc. of ethyl orthoformate were placed 3.25 g. (0.0211 mole) of 4-aminopyrimidine-5-carboxythioamide. On heating, an orange precipitate separated after a few minutes. After 1 hr. of gentle reflux the mixture was cooled and filtered. The yellowish orange crystalline product was washed with a small quantity of cold acetone and found to weigh 2.2 g. (62%). Recrystallization from boiling water yielded orange, fibrous needles decomposing over a range of 260–275°C.

Anal. Calcd. for $C_6H_4N_4S$: C, 43.89; H, 2.46; N, 34.13. Found: C, 43.85; H, 2.42; N, 33.83.

Ultraviolet spectra. A Beckman model DU spectrophotometer with quartz cells was used. Solutions were made up from weighed quantities of the compounds using *p*H 7 and *p*H 10 phosphate citrate and borate buffer, respectively.

Dissociation constants. Potentiometric determinations were made in duplicate, using a Photovolt model 110 *p*H meter.

Chelate formation. To 0.005 molar solutions of 6-mercaptopurine, 4-thiopteridine, and 4-pyrimido[4,5-*d*]pyrimidine in absolute methanol were added half the equivalent amounts of cupric chloride in methanol. The mercaptopurine solution turned orange immediately with rapid separation of a brown precipitate. The 4-thiopteridine solution became blood red instantaneously; later a small quantity of black material was deposited. The 4-thiopyrimidopyrimidine solution remained unchanged except for a very gradual slight darkening of the yellow color after the cupric chloride was added.

Microbiological assay. The determinations were made using *L. casei*[18] ATCC No. 7469 grown on the medium described by Welch *et al.*,[19] containing 0.046 μg./cc. of folic acid. The growth of the organisms was followed by Klett readings. 4-Thiopyrimidopyrimidine produced 50% inhibition at 412 μg./cc. (2.45 μM/cc.) as compared to a value of 160 μg./cc. (0.94 μM/cc.) for 6-mercaptopurine. Full growth was restored when the organisms were permitted to grow in the presence of inhibitory concentrations of thiopyrimidopyrimidine and equimolar amounts of adenine or hypoxanthine. Even a two-fold excess of glutathione or cysteine produced only partial restoration of growth.

(12) A. Albert, *Biochem. J.*, **54**, 646 (1953).

(13) H. G. Mautner, *J. Am. Chem. Soc.*, **78**, 5292 (1956).

(14) D. J. Brown, *J. Appl. Chem.*, **2**, 202 (1952). Later batches were obtained from Fluka AG., Buchs, Switzerland.

(15) D. J. Brown and L. N. Short, *J. Chem. Soc.*, 331 (1953).

(16) All melting points are uncorrected.

(17) Microanalyses were performed at the Huffman Microanalytical Laboratories, Wheatridge, Colo.

(18) We are indebted to Dr. Gertrude B. Elion of the Wellcome Laboratories for a sample of the *L. casei* strain used.

(19) A. D. Welch, C. A. Nichol, R. M. Anker, and J. W. Boehne, *J. Pharmacol. Expt. Therap.*, **103**, 403 (1951).

Acknowledgments. We are indebted to Professor Adrien Albert of the Australian National University for providing samples of 4-oxo- and 4-thiopteridine, to Miss Eva Schur for determining spectra, acid dissociation constants and performing the microbiological assays, and to Professor Arnold D. Welch for his encouragement during the course of this investigation.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF IRWIN, NEISLER & CO.]

Reductive Alkylation of Indole with Pyridinecarboxaldehydes

ALLAN P. GRAY

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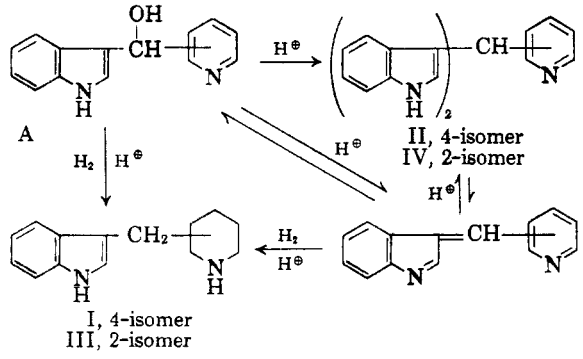
Reductive alkylation of indole has been effected with both 2- and 4-pyridinecarboxaldehyde. Modest yields of the corresponding skatylpiperidines were obtained, accompanied in each case by the unreduced 3,3′-diindolylmethylpyridine. The course of these reactions is discussed.

A recent report[1] from these laboratories described the reaction of indoles with pyridinecarboxaldehydes in glacial acetic acid. The available evidence supported the formulation of the products as 3,3′-diindolylmethylpyridines, impurities resulting from dissociation equilibria and oxidation reactions being considered responsible for coloration[2a] (particularly marked in acid solution).

In this connection it appeared of interest to attempt the reductive alkylation of indole with 2- and 4-pyridinecarboxaldehyde.[2b] When equimolar quantities of indole and 4-pyridinecarboxaldehyde were dissolved in glacial acetic acid and hydrogenated at room temperature over Adams catalyst, there were obtained 29% of 4-skatylpiperidine (I) and 20% of the unreduced 4-(3,3′-diindolylmethyl)pyridine (II). Under the same conditions, 2-pyridinecarboxaldehyde afforded appreciably less 2-skatylpiperidine (III) and correspondingly more of the diindolyl product (IV). The following equilibria would appear to be involved:



Thus, this constitutes a one-step synthesis of the skatylpiperidines, albeit by no means in spectacular yield. (No doubt yields could have been improved considerably by using more than one molar equivalent of aldehyde. Since present interest was focused on the course of the reaction, this was not done.) Although I has not previously been reported, III has been prepared by Akkerman and Veldstra,[3] and, more conveniently, by Bader and Oroshnik.[4] These latter authors condensed 2-pyridyllithium with 3-indolecarboxaldehyde and hydrogenated the resulting, isolated pyridylcarbinol (A) over Adams catalyst in a mixture of acetic acid and ethanol. In addition to 2-skatylpiperidine they obtained 3.5% of what was apparently 3-indolyl-2-piperidinemethanol and 30% of a substance the structure of which was not established, but which, rather implausibly, they considered to be the symmetric ether of the hydroxy compound. Since similar equilibria would be expected to be involved, the apparent absence, here, of products containing oxygen might be attributable to the greater acidity of the medium used.

Bader and Oroshnik's comments,[4] suggesting that hydrogenation of an indolyl-substituted pyridine in glacial acetic acid (in place of the acetic acid in ethanol which they used) will result in reduction of the benzenoid ring of the indole nucleus rather than in saturation of the pyridine ring, require answer. The structures assigned to I and III are in accord with the fact that the compounds absorb as typical indoles in the ultraviolet region (see Table I). Further, the physical properties of III correspond well with those of the 2-skatylpiperidine previously obtained.[3,4] In fact, in our experience[5] the hydrogenation in glacial acetic acid of indolyl substituted pyridine bases [particularly 2- and 4-(3-

(1) A. P. Gray and W. L. Archer, *J. Am. Chem. Soc.*, **79**, 3554 (1957).

(2a) See also M. Strell, A. Zocher and E. Kopp, *Chem. Ber.*, **90**, 1798 (1957).

(2b) In a mechanistic sense, this was expected to parallel the base catalyzed 3-alkylation of indoles by alcohols [see E. F. Pratt and L. W. Botimer, *J. Am. Chem. Soc.*, **79**, 5248 (1957) for leading references].

(3) A. M. Akkerman and H. Veldstra, *Rec. trav. chim.*, **73**, 629 (1954).

(4) H. Bader and W. Oroshnik, *J. Am. Chem. Soc.*, **79**, 5686 (1957).

(5) Unpublished work from these laboratories.