[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF BUFFALO]

Pyrazolono(3,4-d)pyrimidines. I. The Preparation of 6-Methylthiopyrazolono-(3,4-d)pyrimidines[1,2,3]

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The synthesis of 6-methylthiopyrazolono(3,4-d)pyrimidines from 2-methylthio-4-chloro-5-carbethoxypyrimidine and hydrazines is described. When hydrazine, methylhydrazine, *p*-nitrophenylhydrazine, and 2,5-dichlorophenylhydrazine were used, intermediate 4-hydrazino-5-carbethoxypyrimidines were isolated. With 1,2-dimethylhydrazine, isopropylhydrazine, phenylhydrazine, *p*-tolylhydrazine, *p*-halophenylhydrazines, and *p*-carboxyphenylhydrazine, the intermediate hydrazinopyrimidines were not isolated.

Systems composed of fused pyrazole and pyrimidine rings recently have received considerable attention as potential antagonists to natural purines. Robins[5] reported the preparation of a number of 1,4-disubstituted and 1,4,6-trisubstituted pyrazolo(3,4-d)pyrimidines by the action of formamide or ureas on 1-substituted 4-cyano-5-aminopyrazoles. Falco and Hitchings[6] synthesized 4,6-dihydroxypyrazolo(3,4-d)pyrimidine by treating 4,5-pyrazoledicarboxamide with sodium hypochlorite. Schmidt and Druey[7] also prepared a number of 1,4,6-trisubstituted pyrazolo(3,4-d)-pyrimidines both by treating 2-substituted 3-amino-4-carbethoxypyrazoles with urea and substituted ureas and by the cyclization of 4-hydrazino-5-pyrimidinecarbonitriles.

Relatively little work has been reported on systems with the pyrazolone and pyrimidine rings fused. Siewert[8] described the synthesis of 2-phenyl-5,7-dihydroxypyrazolono(4,3-d)pyrimidine by the action of potassium cyanate on 1-phenyl-3-carbethoxy-4-amino-5-pyrazolone. Taylor[9] reported the formation of 6-aminopyrazolono(3,4-d)pyrimidine along with 2-amino-8-hydroxypurine when 2,4-diamino-5-pyrimidinecarboxamide was treated with sodium hypobromite.

The 3-oxo group makes the 6-substituted pyrazolono(3,4-d)pyrimidines attractive intermediates for the synthesis of 3,6-disubstituted pyrazolo(3,4-d)pyrimidines which bear some structural relationship to the natural purines. This paper describes the preparation of 2-methylthio-4-hydrazino-5-carbethoxypyrimidines (I–V) and 6-methylthiopyrazolono(3,4-d)pyrimidines (VI–XVII). The synthetic route employed was quite similar to that of Michaelis[10] who prepared pyrazolonolutidines and to that of Kenner[11] who reported several indazolones. Michaelis treated 4-chloro-5-carbethoxylutidine with hydrazine and several arylhydrazines. With the former, he isolated pyrazolono(4,3-c)lutidine, and with the latter, the corresponding 4-arylhydrazino-5-carbethoxylutidines. These hydrazinolutidines on heating cyclized to 2-substituted pyrazolono(4,3-c)lutidines.

When Kenner treated methyl 2-chloro-3,5-dinitrobenzoate with phenylhydrazine, he obtained 5,7-dinitro-2-phenyl-3-indazolone. With hydrazine, 5,7-dinitro-3-indazolone was the product. When ethyl 2-chloro-5-nitrobenzoate underwent the same reaction, the uncyclized products, ethyl 2-(2-phenylhydrazino)-5-nitrobenzoate and ethyl 2-hydrazino-5-nitrobenzoate, were isolated. These both readily underwent base-catalyzed cyclization to the corresponding indazolones. The position of the phenyl group in the phenylhydrazinobenzoate was established by mercuric oxide oxidation to 4-nitro-2-carbethoxyazobenzene.

In the present investigation, when hydrazines were treated with 2-methylthio-4-chloro-5-carbethoxypyrimidine (XVIII),[12] 4-hydrazinopyrimidines or pyrazolono(3,4-d)pyrimidines were formed, depending on the hydrazines used. Hydrazine, methylhydrazine, *p*-nitrophenylhydrazine, and 2,5-dichlorophenylhydrazine formed the corresponding hydrazinopyrimidines (I–IV, Table I).

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(2) In part from a thesis submitted by Martin Hauser to the Graduate School of Arts and Sciences, The University of Buffalo, in partial fulfillment of the requirement for the degree of Master of Arts.

(3) Presented at the 137th Meeting of the American Chemical Society in Cleveland, Ohio, April, 1960.

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(5) H. C. Koppel, E. O'Brien, and R. K. Robins, *J. Org. Chem.*, **24**, 259 (1959), and preceding papers.

(6) E. A. Falco and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 3142 (1956).

(7) P. Schmidt, K. Eichenberger, E. Wilhelm, and J. Druey, *Helv. Chim. Acta*, **42**, 763 (1959), and preceding papers.

(8) G. Siewert, *Arch. Pharm.*, **278**, 327 (1940).

(9) *Cf.* discussion of E. C. Taylor in *The Chemistry and Biology of Purines*, a Ciba Foundation Symposium (Wolstenholme and O'Connor, editors), Little, Brown and Company, Boston, (1957), p. 36.

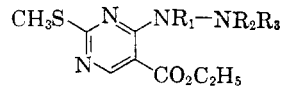
(10) A. Michaelis, *Ann.*, **366**, 324 (1909).

(11) J. Kenner, *J. Chem. Soc.*, 2732 (1914). J. Kenner and E. Witham, *J. Chem. Soc.*, 1053 (1921).

(12) E. Peters, J. F. Holland, B. Bryant, H. J. Minnemeyer, C. Hohenstein, and H. Tieckelmann, *Cancer Research*, **19**, 729 (1959).

TABLE I

2-METHYLTHIO-4-HYDRAZINO-5-CARBETHOXYPYRIMIDINE

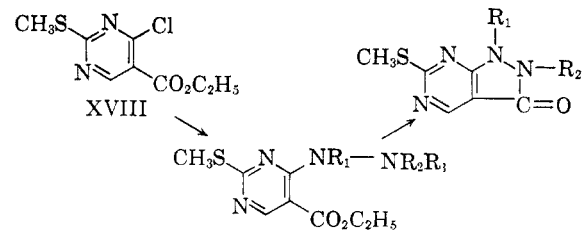


Compound	R_1	R_2	R_3	M.P.,[19]	Yield, %	Formula	Calcd. / Found C	H	N
I	H	H	H	100–101	79	$C_8H_{12}N_4O_2S$	42.09	5.30	24.54
							42.57	5.39	24.32
II	CH_3	H	H	107–109	85	$C_9H_{14}N_4O_2S$	44.60	5.82	23.12
							44.31	5.66	23.51
III	H	H	*p*-$NO_2C_6H_4$	171–173	70	$C_{14}H_{15}N_5O_4S$	48.13	4.33	20.05
							48.20	4.28	20.00
IV	H	H	2,5-$Cl_2C_6H_3$	146–148	88	$C_{14}H_{14}Cl_2N_4O_2S$	45.05	3.78	15.01
							45.20	4.02	14.95
V	H	C_6H_5	$COCH_3$	137–138	27	$C_{16}H_{18}N_4O_3S$	55.47	5.24	16.17
							55.32	5.22	16.18

In the conversion of halogenated pyrimidines to hydrazinopyrimidines with alkyl and arylhydrazines, it is usually assumed that reaction takes place at the nitrogen not bonded to the substituent.[13] Hinman[14] has pointed out that this assumption is not justified in many reactions of hydrazines. Schmidt and Druey[7] treated isopropylhydrazine with 2-dimethylamino-4-chloro-5-pyrimidinecarbonitrile and obtained 1-isopropyl-3-amino-6-dimethylaminopyrazolo(3,4-d)pyrimidine. The identical compound was obtained by hydrolysis of 2-dimethylamino-4-(1-isopropyl-2-acetylhydrazino)-5-pyrimidinecarbonitrile, confirming the position of the isopropyl group. We have found evidence to indicate that in the reactions of XVIII with hydrazines reaction occurs at the α-nitrogen of the alkylhydrazines and the β-nitrogen of the arylhydrazines.

A red to violet color developed when I and II were treated with sodium pentacyanoamineferroate. The test was negative with III and IV. Hydrazines of the type RNH—NH_2 and R_2N—NH_2 are reported to give a red to violet color with this reagent.[15] The infrared spectra of I and II show a medium peak at 1650–1625 cm.$^{-1}$ ascribed to the free amino group at the end of the hydrazine group.[16] This peak is absent in the spectra of III and IV. Although II formed an oil when treated with acetone, it reacted with propionaldehyde to give a solid hydrazone. It was not possible to heat II with these reagents; cyclization occurred to form VII. I formed solid hydrazones with acetone and benzaldehyde. III and IV did not form hydrazones.

Cyclization of I–IV to the corresponding pyrazolonopyrimidines, (VI, VII, XVI, and XVII, Table II) was effected by heating briefly in base.



Treatment of XVIII with isopropyl, 1,2-dimethyl-, phenyl-, *p*-tolyl-, *p*-bromophenyl-, *p*-chlorophenyl-, *p*-fluorophenyl-, and *p*-carboxyphenylhydrazine gave the pyrazolonopyrimidines, (VIII–XV, Table II) directly. In these cases, under the conditions used, the intermediate hydrazinopyrimidines were not detected.

Based on the data for hydrazinopyrimidines, it is probable that VII and VIII are 1-substituted pyrazolono(3,4-d)pyrimidines, while X–XV are 2-substituted. In addition, hydrolysis of 2-methylthio-4-(2-acetyl-2-phenylhydrazino)-5-carbethoxypyrimidine (V), prepared from XVIII and 1-acetyl-1-phenylhydrazine,[17] gave a compound identical with X.

In the aromatic series, of phenylhydrazine and the six *para*-substituted phenylhydrazines treated with XVIII, only *p*-nitrophenylhydrazine did not form a pyrazolonopyrimidine directly. This suggests that the high electronegativity of the nitro group sufficiently reduced the nucleophilicity of the 2′-nitrogen to prevent immediate cyclization. The weaker electron-attracting groups (*p*-bromo, *p*-chloro, *p*-fluoro, and *p*-carboxy) and the electron-releasing *p*-methyl group allow cyclization. The

(13) P. A. Levene, *J. Biol. Chem.*, **63**, 653 (1925). R. Andrisano, *Boll. sci. facoltà chim. ind. univ. Bologna*, **5**, 45 (1944–1947); *Chem. Abstr.*, **44**, 3904e (1950); R. Andrisano and G. Modena, *Gazz. chim. ital.*, **81**, 393 (1951); K. Shirakawa, *J. Pharm. Soc. Japan*, **73**, 635,640 (1953).

(14) R. L. Hinman, *J. Org. Chem.*, **23**, 1587 (1958).

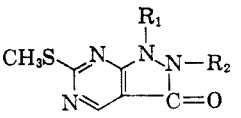
(15) F. Feigl, V. Anger, and O. V. Frehden, *Mikrochemie*, **15**, 184 (1934). F. Feigl, *Spot Tests in Organic Analysis*, Elsevier Publishing Co., New York (1956), p. 292.

(16) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, John Wiley and Sons, Inc., New York City, (1958), pp. 255–256.

(17) H. Behrend and W. Reinsberg, *Ann.*, **377**, 189 (1910).

TABLE II

6-METHYLTHIOPYRAZOLONO(3,4-D)PYRIMIDINES



Compound	R_1	R_2	M.P.,[19]	Yield, %	Formula	Calcd. / Found C	H	N
VI	H	H	340 dec.	75	$C_6H_6N_4OS$	39.55	3.32	30.75
						39.22	3.38	31.01
VII	CH_3	H	273–275	97	$C_7H_8N_4OS$	42.84	4.11	28.55
						42.70	4.24	28.43
VIII	$(CH_3)_2CH$	H	209–210	67	$C_9H_{12}N_4OS$	48.22	5.39	24.98
						48.31	5.15	25.10
IX	CH_3	CH_3	173–175	68	$C_8H_{10}N_4OS$	45.69	4.79	26.65
						45.55	4.70	26.38
X	H	C_6H_5	275–277	83	$C_{12}H_{10}N_4OS$	55.80	3.90	21.69
						55.79	3.89	21.65
XI	H	*p*-$CH_3C_6H_4$	300–302	88	$C_{13}H_{12}N_4OS$	57.33	4.44	20.57
						57.17	4.53	20.79
XII	H	*p*-BrC_6H_4	330 dec.	64	$C_{12}H_9BrN_4OS$	42.74	2.69	16.62
						42.70	2.75	16.64
XIII	H	*p*-ClC_6H_4	340 dec.	62	$C_{12}H_9ClN_4OS$	49.23	3.10	19.14
						49.21	3.48	19.53
XIV	H	*p*-FC_6H_4	310 dec.	65	$C_{12}H_9FN_4OS$	52.16	3.28	20.28
						52.04	3.57	20.29
XV	H	*p*-$HOOCC_6H_4$	360 dec.	77	$C_{13}H_{10}N_4O_3S$	51.65	3.33	18.53
						51.50	3.87	18.33
XVI	H	*p*-$NO_2C_6H_4$	315 dec.	82	$C_{12}H_9N_5O_3S$	47.52	2.99	23.09
						47.35	3.18	23.03
XVII	H	2,5-$Cl_2C_6H_3$	300 dec.	57	$C_{12}H_8Cl_2N_4OS$	44.05	2.46	17.13
						43.96	3.10	17.19

failure of IV to cyclize is also explained on the basis of low nucleophilicity of the 2′-nitrogen.

In the series of aliphatic-substituted hydrazinopyrimidines, the 1′-substituent does not greatly increase the nucleophilicity of the 2′-nitrogen. Further, there is a possible steric effect. Construction of the 5-carbethoxy-4-hydrazinopyrimidines with Courtauld Atomic Models[18] indicates that the preferred conformation, in those cases where a bulky group is located on the 2′-nitrogen, is that which places this nitrogen in a position favorable for nucleophilic attack on the carbonyl carbon. With the unsubstituted I and 1′-substituted II this conformation is not necessarily preferred. However, in VIII, the substituent, although on the 1′-position, is sufficiently bulky to promote cyclization when combined with the electron-release properties of the isopropyl group.

EXPERIMENTAL[19]

2-Methylthio-4-hydrazino-5-carbethoxypyrimidine (I). A solution of 2.0 g. (0.088 mole) of hydrazine in 25 ml. of absolute alcohol was added, with stirring and cooling, to a solution of 10 g. (0.043 mole) of XVIII[12] in 150 ml. of absolute alcohol at such a rate that the temperature did not rise above 25°. After standing for 2 hr. at room temperature, the solution was diluted with an equal volume of water. 1,2-Bis(2-methylthio-5-carbethoxy-4-pyrimidyl)hydrazine precipitated. This was immediately removed by filtration and treated as described below. The solid obtained by evaporation of the filtrate was washed with water and recrystallized from benzene-ligroin (b.p. 30–60°).

1,2-Bis(2-methylthio-5-carbethoxy-4-pyrimidyl)hydrazine. This material, a by-product in the preparation of I, was recrystallized from ethylbenzene to give 1.0 g. of yellow solid, m.p. 240–242°.

Anal. Calcd. for $C_{16}H_{20}N_6O_4S_2$: C, 45.27; H, 4.75; N, 19.80. Found: C, 45.94; H, 4.76; N, 19.67.

2-Methylthio-4-isopropylidenehydrazino-5-carbethoxypyrimidine. A solution of 2.0 g. (0.0088 mole) of I in 50 ml. of acetone was refluxed for 90 min. and the acetone evaporated at room temperature. The residue was recrystallized from benzene-ligroin (b.p. 30–60°) to give 2.3 g. (96%) of white solid; m.p. 164–166°.

Anal. Calcd. for $C_{11}H_{16}N_4O_2S$: C, 49.23; H, 6.01; N, 20.88. Found: C, 49.49; H, 5.75; N, 20.41.

2-Methylthio-4-benzylidenehydrazino-5-carbethoxypyrimidine. A solution of 1.5 g. (0.0066 mole) of I and 0.75 g. (0.0071 mole) of benzaldehyde in 50 ml. of absolute alcohol was refluxed for 2 hr. The hydrazone precipitated after cooling to room temperature and diluting with an equal volume of water. It was recrystallized from alcohol-water to give 1.9 g. (92%) of white solid; m.p. 148–149°.

Anal. Calcd. for $C_{15}H_{16}N_4O_2S$: C, 56.93; H, 5.10; N, 17.71. Found: C, 56.43; H, 4.94; N, 17.77.

2-Methylthio-4-(1-methylhydrazino)-5-carbethoxypyrimidine (II). A solution of 4.1 g. (0.087 mole) of methylhydrazine in 25 ml. of absolute alcohol was added slowly to a solu-

(18) The Ealing Corporation, Natick, Mass.

(19) Melting points are uncorrected. Analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.; Geller Microanalytical Laboratories, Bardonia, N. Y.; and Schwartzkopf Microanalytical Laboratory, Woodside, N. Y.

tion of 10 g. (0.043 mole) of XVIII in 100 ml. of absolute alcohol cooled to 15° at such a rate that the temperature remained at about 15°. After standing for 1 hr. at 15°, the precipitate was filtered, washed thoroughly with water, and recrystallized from benzene-ligroin (b.p. 30–60°).

2-Methylthio-4-(1-methyl-2-propylidenehydrazino)-5-carbethoxypyrimidine. A solution of 0.50 g. (0.0021 mole) of 2-methylthio-4-(1-methylhydrazino)-5-carbethoxypyrimidine and 0.50 g. (0.0086 mole) of propionaldehyde in 25 ml. of absolute alcohol was allowed to stand overnight at room temperature. The solution was then evaporated to dryness in a stream of dry air and the residue recrystallized from ligroin (b.p. 30–60°) to give 0.37 g. (62%) of white solid; m.p. 66–67°.

Anal. Calcd. for $C_{12}H_{18}N_4O_2S$: C, 51.04; H, 6.43; N, 19.85. Found: C, 50.99; H, 6.42; N, 19.38.

2-Methylthio-4-(2-arylhydrazino)-5-carbethoxypyrimidines (III–IV). *General method.* A solution of 10 g. (0.043 mole) of XVIII in 150 ml. of absolute alcohol was added to a solution of 0.088 mole of the arylhydrazine in the minimum amount of absolute alcohol. The resulting solution was heated at 50° for 30 min., then allowed to stand 4 hr. at room temperature. It was next diluted with twice its volume of water and refrigerated overnight at 1°. The precipitate was filtered, triturated with 300 ml. of 5% hydrochloric acid, washed thoroughly with water, and recrystallized from alcohol-water.

2-Methylthio-4-(2-acetyl-2-phenylhydrazino)-5-carbethoxypyrimidine (V). To a solution of 2.3 g. (0.015 mole) of 1-acetyl-1-phenylhydrazine[16] in 55 ml. of absolute alcohol was added a solution of 1.8 g. (0.0075 mole) of XVIII in 25 ml. of absolute alcohol. The resulting solution was allowed to stand 4 hr., diluted with an equal volume of water, and refrigerated overnight at 1°. The precipitate of 2-phenyl-6-methylthiopyrazolono(3,4-d)pyrimidine (X) was removed and 150 ml. of water was added to the solution. After several hours of refrigeration, the solution was filtered and the precipitate recrystallized from water.

Cyclization of hydrazinopyrimidine (I–IV) *to pyrazolono (3,4 d)pyrimidines* (VI, VII, XVI, and XVII). *General method.* A solution of 1 g. of the hydrazinopyrimidine in 10 ml. of 10% potassium hydroxide was boiled for 15 min., cooled, and acidified with 25% acetic acid. The precipitate was washed with water and recrystallized from *n*-butyl alcohol.

1-Isopropyl-6-methylthiopyrazolono(3,4-d)pyrimidine (VIII). A solution of 3.3 g. (0.020 mole) of isopropylhydrazine oxalate, 35 ml. of water, and 2.8 g. of potassium hydroxide was diluted with 350 ml. of alcohol and the precipitate of potassium oxalate removed by filtration. To the filtrate was added a solution of 2.3 g. (0.010 mole) of XVIII in 50 ml. of alcohol and the solution warmed at 60° for 30 min. After cooling, the solution was diluted with 500 ml. of water and placed in the refrigerator at 1°. The precipitate was filtered and recrystallized from *n*-butyl alcohol.

1,2-Dimethyl-6-methylthiopyrazolono(3,4-d)pyrimidine (IX). A solution of 5.3 g. (0.088 mole) of 1,2-dimethylhydrazine in 25 ml. of absolute alcohol was added slowly, with stirring, to 10 g. (0.043 mole) of XVIII dissolved in 125 ml. of absolute alcohol. The temperature was maintained at 25° by cooling. When the reaction subsided, the solution was allowed to stand for 4 hr. and diluted with three volumes of water. After overnight refrigeration at 1°, the precipitate was filtered, washed thoroughly with water, and recrystallized from benzene-ligroin (b.p. 30–60).

2-Phenyl-6-methylthiopyrazolono(3,4-d)pyrimidine (X). *From* XVIII. A solution of 9.5 g. (0.088 mole) of phenylhydrazine in 25 ml. of absolute alcohol was added slowly, with stirring, to 10 g. (0.043 mole) of XVIII dissolved in 200 ml. of absolute alcohol. After standing 2 hr., the solution was poured into three times its volume of 5% hydrochloric acid and stirred for 1 hr. The precipitate was filtered, washed with water, and recrystallized from alcohol.

From V. A solution of 0.5 g. (0.0015 mole) of V in 30 ml. of 2% hydrochloric acid was refluxed for 30 min., cooled, and made just basic to litmus with 10% sodium hydroxide. After 1 hr. in the refrigerator at 1°, the precipitate was filtered, washed with water, and recrystallized from alcohol to give 0.21 g. (56%) of white solid; m.p. 276–278°. Mixed melting points, infrared and ultraviolet spectra showed this material to be identical with that from the preceding preparation.

2-Aryl-6-methylthiopyrazolono(3,4-d)pyrimidines (XI–XV). *General method.* A solution of 2.3 g. (0.01 mole) of XVIII in 25 ml. of absolute alcohol was added to a solution of 0.02 mole of the arylhydrazine in the minimum amount of absolute alcohol. The resulting solution was warmed at 50–60° for 30 min. and allowed to stand for 4 hr., diluted with three times its volume of water and refrigerated overnight at 1°. The precipitate was filtered, washed with water, and recrystallized from *n*-butyl alcohol.

BUFFALO 14, N. Y.

[CONTRIBUTION FROM THE KETTERING-MEYER LABORATORY,[1] SOUTHERN RESEARCH INSTITUTE]

Synthesis of Potential Anticancer Agents. XXV. Preparation of 6-Alkoxy-2-aminopurines[2]

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Some 6-alkoxy-2-aminopurines have been prepared from 2-amino-6-chloropurine.

As a part of our general program to investigate purines in search of more effective anticancer agents, a number of 6-alkoxy-2-aminopurines (I) (Table I) have been prepared. Although they are closely related to guanine(2-amino-6-purinol) (II), they cannot be prepared from this substance directly. Traube and Dudley[3] found that treatment of guanine in aqueous-alcoholic sodium hydroxide solution with iodomethane gave 7-methyl- and 1,7-dimethylguanine.

(1) Affiliated with Sloan-Kettering Institute.

(2) This work was supported by funds from the National Institutes of Health, Contract No. SA-43-ph-1740, and from the C. F. Kettering Foundation. For paper XXIV of this series see J. A. Montgomery and C. T. Temple Jr., *J. Am. Chem. Soc.*, in press.

(3) W. Traube and H. W. Dudley, *Ber.*, **46**, 3844 (1913).