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Potential Purine Antagonists. V. Synthesis of Some 3-Methyl-5,7-substituted Pyrazolo[4,3-*d*]pyrimidines[1,2]

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The synthesis of 3-methyl-7-hydroxypyrazolo[4,3-*d*]pyrimidine (III) has been accomplished from 3-methyl-4-aminopyrazole-5-carboxylic acid (II) and from 3-methyl-4-aminopyrazole-5-carboxamide (VII) by the reaction of II and VII, respectively, with boiling formamide. 3-Methyl-7-hydroxypyrazolo[4,3-*d*]pyrimidine (III) has been converted to 3-methyl-7-chloropyrazolo[4,3-*d*]pyrimidine (IX) by treatment with phosphorus oxychloride and dimethylaniline. Ammonia and various primary and secondary amines were reacted with IX to yield the corresponding amino and substituted amino-derivatives.

Recent work[3] in this laboratory has resulted in a new route to the synthesis of the pyrazolo[4,3-*d*]-pyrimidine ring system from a pyrazole intermediate. The present study is an extension of this general method to include the preparation of some 3-methylpyrazolo[4,3-*d*]pyrimidines. In this investigation it was felt that an electron-supplying methyl group in position "3" might make up in part the electron deficiency of the pyrazole ring and thus give rise to pyrazolo[4,3-*d*]pyrimidine derivatives which would more closely approximate the electron distribution of the purine ring. What could be gained in this fashion might be lost due to the geometrical dissimilarity. However, it remains to be seen, with regard to purine antagonists, as to which factor might be the most important in biological systems.

Rose[4] treated 4,6-diethyl-2,5-diaminopyrimidine with nitrous acid to form the 5-diazo-derivative which then was cyclized to give 5-amino-3-methyl-7-ethylpyrazolo[4,3-*d*]pyrimidine (referred to by Rose as 5-amino-7-ethyl-3-methyl-1:2:4:6 tetraazaindene). Rose also reports the synthesis of 5-amino - 3,7 - dimethylpyrazolo[4,3 - *d*]pyrimidine which was prepared in a similar manner from 2,5-diamino-4-ethyl-6-methylpyrimidine. However, as might be expected, in this latter instance the isomeric 5 - amino - 7 - ethylpyrazolo[4,3 - *d*]pyrimidine was also formed.

Prior to the present work, these two derivatives were the only 3-methyl-substituted pyrazolo[4,3-*d*]-pyrimidines reported in the literature.

Previous success in this laboratory[3] in the synthesis of derivatives of the pyrazolo[4,3-*d*]pyrimidine ring system suggested that 3-methyl-4-aminopyrazole-5-carboxylic acid (II) might serve as an important intermediate which could be readily converted into various desired 3-methylpyrazolo[4,3-*d*]-pyrimidines. This synthesis has now been realized and represents a new approach to the 3-methylpyrazolo[4,3-*d*]pyrimidines which have previously been synthesized only by ring closure of a pyrimidine intermediate.

3-Methyl-4-nitropyrazole-5-carboxylic acid (I) was prepared by the method of Musante[5] and reduced in aqueous solution with sodium hydrosulfite to give 3-methyl-4-animopyrazole-5-carboxylic acid

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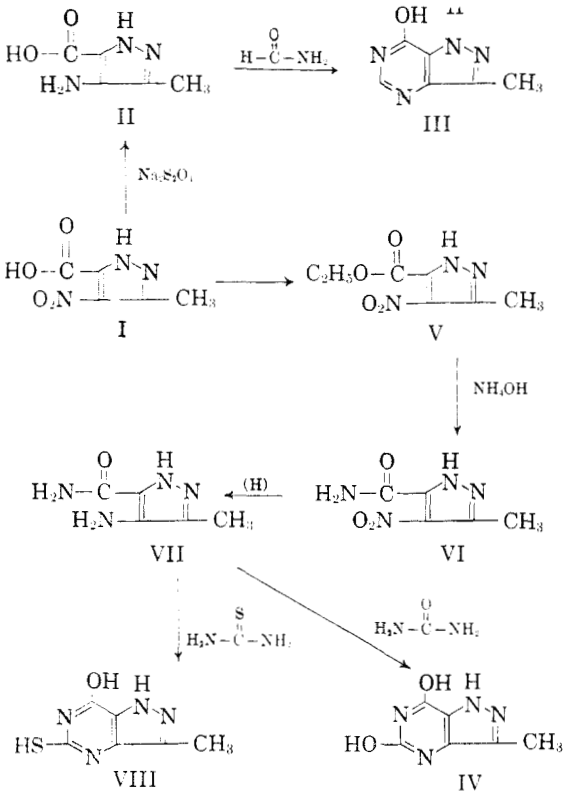
(2) Presented before the Division of Medicinal Chemistry at the 129th Meeting of the American Chemical Society, April 8–13, at Dallas, Texas.

(3) Roland K. Robins, Frederick W. Furcht, Alan D. Grauer, and Jesse W. Jones, *Potential Purine Antagonists. II. Synthesis of Some 5- and 5,7-Substituted Pyrazolo[4,3-d]-pyrimidines*, *J. Am. Chem. Soc.*, **78**, 2418 (1956).

(4) Rose, *J. Chem. Soc.*, 3446 (1952).

(5) Musante, *Gazz. chem. ital.*, **75**, 121 (1945).



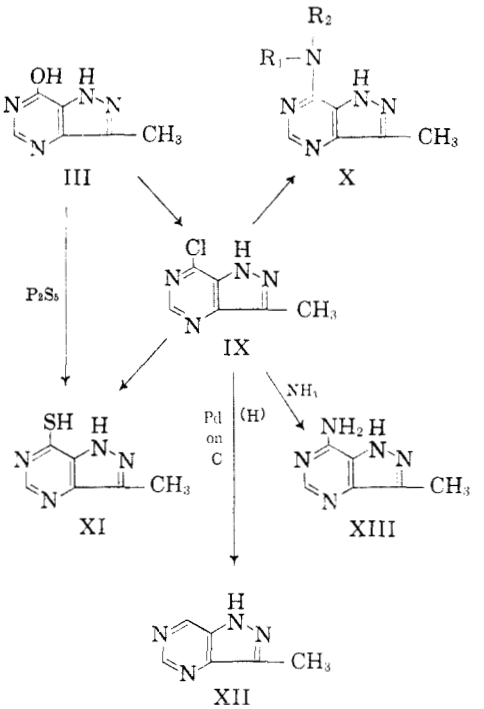
REACTION SCHEME I

(II) in approximately 60% yield. II was cyclized with boiling formamide in typical fashion to yield 3 - methyl - 7 - hydroxypyrazolo[4,3 - *d*]pyrimidine (III). When III was heated with phosphorus oxychloride in the presence of dimethylaniline, 3-methyl - 7 - chloropyrazolo[4,3 - *d*]pyrimidine (IX) was prepared in good yield. Treatment of IX with thiourea in boiling ethanol yielded 3-methyl-7-mercaptopyrazolo[4,3-*d*]pyrimidine (XI). 3-Methyl-7 - hydroxypyrazolo[4,3 - *d*]pyrimidine (III) and phosphorus pentasulfide in boiling pyridine also gave 3-methyl-7-mercaptopyrazolo[4,3-*d*]pyrimidine (XI) in good yield.

Alcoholic ammonia at 150° converted 3-methyl-7 - chloropyrazolo[4,3 - *d*]pyrimidine (IX) to 3-methyl-7-aminopyrazolo[4,3-*d*]pyrimidine (XIII). When IX was heated with various primary and secondary amines in alcoholic solution on the steam-bath, the corresponding 3-methyl-7-substituted aminopyrazolo[4,3-*d*]pyrimidines were prepared. These derivatives are listed in Table I.

Catalytic reduction of 3-methyl-7-chloropyrazolo[4,3-*d*]pyrimidine (IX) with a palladium on charcoal catalyst yielded 3-methylpyrazolo[4,3-*d*]-pyrimidine (XII).

3-Methyl-4-nitropyrazole-5-carboxylic acid (I) was esterified to yield ethyl 3-methyl 4-nitropyrazole-5-carboxylate (V) which was treated with ammonium hydroxide to yield 3-methyl-4-nitropyrazole-5-carboxamide (VI). Catalytic reduction of VI yielded 3-methyl-4-aminopyrazole-5-carboxamide (VII). Boiling formamide and VII gave a second route to the synthesis of 3-methyl-7-hydroxypyrazolo[4,3-*d*]pyrimidine. Fusion of 3-methyl-4-aminopyrazole-5-carboxamide (VII) with



REACTION SCHEME II

urea at 180–200° gave 3-methyl-5,7-dihydroxypyrazolo[4,3-*d*]pyrimidine (IV). Thiourea and VII heated at 190–210° yielded 3-methyl-5-mercapto-7-hydroxypyrazolo[4,3-*d*]pyrimidine (VIII).

Several unsuccessful attempts were made to prepare IV and VIII from 3-methyl-4-aminopyrazole-5-carboxylic acid (II) by urea and thiourea fusion.

The ultraviolet absorption maxima of the various substituted 3-methylpyrazolo[4,3-*d*]pyrimidines are recorded in Table II. Comparison of the ultraviolet absorption spectra of a number of 7 and 5,7-substituted pyrazolo[4,3-*d*]pyrimidines[3] with the spectra of the corresponding 3-methyl-7 and 5,7-substituted pyrazolo[4,3-*d*]pyramidines reveals a great deal of similarity as might be expected. There is noted, however, a small but definite bathochromic shift of the absorption maxima due to the methyl group in position "3."

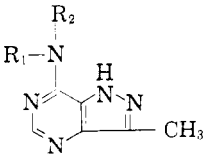
Biological testing of these derivatives is now in progress and will be reported elsewhere at a later date.

EXPERIMENTAL[6]

3-Methyl-4-aminopyrazole-5-carboxylic acid (I). 3-Methyl-4-nitropyrazole-5-carboxylic acid[4] (50 g.) was added to 450 ml. of boiling water. All the acid dissolved and the solu-

(6) All melting points are uncorrected and were taken on a Fisher-Johns melting point block unless otherwise stated.

TABLE I



3-METHYL-7-SUBSTITUTED AMINOPYRAZOLO[4,3-*d*]PYRIMIDINES

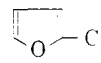
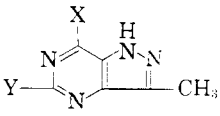
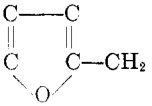
R_1	R_2	Method of Prep.	Yield, %	Analyses Calc'd C	Calc'd H	Calc'd N	Found C	Found H	Found N	M.P., °C.	Recrystallization Solvent
H		B	32	57.4	5.2	30.7	57.6	5.0	30.6	233–234	Water
CH_3	CH_3	B	50	54.2	6.2	39.6	54.1	6.6	39.7	296–297	2-Ethoxyethanol
H	NH_2	A	43	43.9	4.9	51.2	43.9	5.1	51.2	208–210	Water-ethanol
H	C_6H_5	A	55	64.0	4.9	31.1	64.2	4.9	31.0	289–290	2-Ethoxyethanol-water
H	$C_6H_5CH_2$	B	39	65.3	5.4	29.3	65.3	6.0	29.1	220–221	2-Ethoxyethanol

TABLE II



ULTRAVIOLET ABSORPTION SPECTRA OF SOME 3-METHYLPYRAZOLO[4,3-*d*]PYRIMIDINES

X	Y	$\lambda_{max.}$ *p*H 1	$\lambda_{max.}$ *p*H 11	$\lambda_{max.}$ absolute ethanol
NH_2	H	296	303	
OH	H	280	290	
SH	H	340	333	
$N—NH_2$	H	300	295	
OH	OH	292	305	
OH	SH	250, 275	260	
C_6H_5NH	H			260, 320
$C_6H_5CH_2NH$	H			297
Cl	H			256, 308
$(CH_3)_2N$	H	250, 317	248, 310	
H	H		266, 305	
	H	255, 311	240, 297	

tion was allowed to cool to 75°. To the solution then was added, with stirring, 175 g. of sodium hydrosulfite in small portions so that the temperature of the solution remained between 75–80°.

After the addition of the sodium hydrosulfite was complete, the solution was allowed to stand three hours at room temperature and then was placed overnight in the refrigerator. The solid was collected and washed with a small amount of ice-water. The yield was 23.0 g. of a crude product which decomposed at about 180°. Recrystallization from boiling water gave an analytically pure compound which decomposed from 185–190°.

Anal. Calc'd for $C_5H_7N_3O_2$: C, 42.5; H, 5.0; N, 29.8. Found: C, 42.5; H, 5.1; N, 30.3.

Ethyl 3-methyl-4-nitropyrazole-5-carboxylate (V). 3-Methyl-4-nitropyrazole-5-carboxylic acid[4] (40 g.) was placed in a round bottom flask to which had been added 65 ml. of absolute ethanol, 105 ml. of dry benzene, and 44 ml. of concentrated sulfuric acid. The solution was refluxed on the steam-bath for 24 hours. The cooled solution was shaken with 2 × 300 ml. of ether and the combined ethereal solution was washed with water, sodium bicarbonate solution, and again with water. The ethereal solution was dried over sodium sulfate and finally was distilled from a steam-bath. The light gray residue which remained (42.0 g.) melted at 60–65°. Recrystallization from a mixture of benzene and petroleum ether gave a colorless product, m.p. 75–76°.

Anal. Calc'd for $C_7H_9N_3O_4$: C, 42.3; H, 4.5; N, 21.1. Found: C, 42.6; H, 4.6; N, 21.5.

3-Methyl-4-nitropyrazole-5-carboxamide (VI). Ethyl 3-methyl-4-nitropyrazole-5-carboxylate (V) (20 g.) was dissolved in 300 ml. of concentrated ammonium hydroxide. The solution was stirred and heated on the steam-bath for two hours then was treated with charcoal and filtered. The filtrate then was evaporated on the steam-bath to 40 ml. and allowed to cool. The cooled solution was filtered to yield 10.2 g. of product, m.p. 245–250° dec. Recrystallization from boiling water raised the m.p. to 254–255°.

Anal. Calc'd for $C_5H_6N_4O_3$: N, 32.9. Found: N, 32.6.

3-Methyl-4-aminopyrazole-5-carboxamide (VII). Methyl-3-4-nitropyrazole-5-carboxamide (VI) (6 g.) was dissolved in 125 ml. of 95% ethanol. The alcoholic solution was cooled to room temperature, and 0.3 g. of a 10% palladium on charcoal catalyst was added and the mixture was shaken on a

low pressure hydrogenation apparatus at 15 lbs/sq.in. pressure until the absorption of hydrogen had ceased. The catalyst was filtered and the ethanol was evaporated to dryness under reduced pressure. Recrystallization of the residue from water gave 3.5 g. of crystals, m.p. 209–211°.

Anal. Calc'd for $C_5H_8N_4O$: N, 40.0. Found: N, 40.0.

3-Methyl-7-hydroxypyrazolo[4,3-d]pyrimidine (III). *Method* (1). 3-Methyl-4-aminopyrazole-5-carboxylic acid (I) (5 g.) was boiled gently in an open flask with 30 ml. of c.p. formamide for three hours. To the warm solution was added 90 ml. of water, and the solution was placed in the refrigerator overnight. The crude precipitate was filtered and recrystallized from water to yield 2.2 g. of white crystals which decomposed above 330°.

Anal. Calc'd for $C_6H_6N_4O$: C, 48.0; H, 4.0; N, 37.4. Found: C, 48.0; H, 4.2; N, 38.0.

Method (2). 3-Methyl-4-aminopyrazole-5-carboxamide (VII) (5 g.) was boiled with 30 ml. of c.p. formamide for three hours and the product was isolated as in method (1). The yield was 3.1 g. of 3-methyl-7-hydroxypyrazolo[4,3-*d*]-pyrimidine (III) which decomposed above 330°.

Anal. Calc'd for $C_6H_6N_4O$: C, 48.0; H, 4.0; N, 37.4. Found: C, 47.6; H, 4.1; N, 37.8.

The product III prepared by methods (1) and (2) showed identical ultraviolet absorption spectra at *p*H 1 and at *p*H 11.

3-Methyl-5,7-dihydroxypyrazolo[4,3-d]pyrimidine (IV). To a small casserole was added 0.5 g. of 3-methyl-4-aminopyrazole-5-carboxamide (VII) and 1.5 g. of urea. The mixture was heated at 180–200° for one-half hour until the liquid melt solidified. The cooled solid was dissolved in hot 2 *N* sodium hydroxide and the solution was acidified while hot with acetic acid. The crude product was purified by reprecipitation twice from a hot basic solution to give 0.2 g. of an analytically pure sample.

Anal. Calc'd for $C_6H_6N_4O_2$: C, 43.3; H, 3.6; N, 33.7. Found: C, 42.9; H, 3.8; N, 33.8.

3-Methyl-5-mercapto-7-hydroxypyrazolo[4,3-d]pyrimidine (VIII). Thiourea (10 g.) and 4.5 g. of 3-methyl-4-aminopyrazole-5-carboxamide (VII) were thoroughly mixed and heated carefully at 190–210° for one-half hour, after which time the liquid melt solidified. The solid was dissolved in hot 2 *N* sodium hydroxide and the solution was heated with charcoal and filtered. The boiling filtrate was carefully acidified with glacial acetic acid, and the solution was filtered while hot to yield 3.5 g. of crude product. This material was reprecipitated twice for analysis.

Anal. Calc'd for $C_6H_6N_4OS$: C, 39.6; H, 3.3; N, 30.8. Found: C, 39.3; H, 3.4; N, 30.8.

3-Methyl-7-chloropyrazolo[4,3-d]pyrimidine (IX). 3-Methyl-7-hydroxypyrazolo[4,3-*d*]pyrimidine (III) (25 g.), 50 ml. of dimethylaniline (mono-free), and 600 ml. of phosphorus oxychloride were placed in a one-liter round bottom flask. The mixture was refluxed for 2 hours and the excess phosphorus oxychloride was removed under reduced pressure using a steam-bath as a source of heat. The residual red, syrupy liquid was poured, with stirring, on 300 g. of crushed ice, and the cold, aqueous solution was extracted with ether (8 × 500 ml.). The combined ethereal extract was washed twice with 300 ml. of ice water each time. The ether then was dried and distilled to leave 20 g. of crude 3-methyl-7-chloropyrazolo[4,3-*d*]pyrimidine (IX), m.p. 150° dec. Recrystallization from a mixture of benzene and hexane gave colorless needles, m.p. 153–155° dec.

Anal. Calc'd for $C_6H_5ClN_4$: C, 42.7; H, 3.0; N, 33.3. Found: C, 42.7; H, 3.2; N, 33.3.

3-Methyl-7-aminopyrazolo[4,3-d]pyrimidine (XIII). 3-Methyl-7-chloropyrazolo[4,3-*d*]pyrimidine (IX) (4 g.), and 50 ml. of alcoholic ammonia (absolute ethanol saturated with ammonia at 0°) were heated for 12 hours at 150° (inside temperature) in a high pressure bomb. The alcoholic solution was evaporated to dryness and the residue was dissolved in hot water. The solution was boiled with charcoal and filtered, and the hot filtrate was adjusted to *p*H 9 with ammonium hydroxide. The cooled solution yielded 2.0 g. of white crystals, m.p. 315–317° (copper block).

Anal. Calc'd for $C_6H_7N_5$: C, 48.3; H, 4.7; N, 46.8. Found: C, 48.2; H, 5.3; N, 46.2.

3-Methyl-7-mercaptopyrazolo[4,3-d]pyrimidine (XI). *Method* (1). 3-Methyl-7-hydroxypyrazolo[4,3-*d*]pyrimidine (III) (10 g.) and 50 g. of phosphorus pentasulfide were added to 250 ml. of c.p. dry pyridine. This mixture was refluxed for 2 hours and the excess pyridine was distilled off under reduced pressure using a steam-bath as a source of heat. To the residue was added 200 ml. of water, and the solution was allowed to stand at room temperature for 12 hours and finally was heated for 4 hours on the steam-bath. The solution was adjusted to *p*H 3 with glacial acetic acid and was allowed to remain in the refrigerator overnight. The solid was collected and washed with water. The crude product, 6.1 g., was further purified by reprecipitation from a hot basic solution. An analytically pure sample was obtained by recrystallization of a small amount of the purified product from a large volume of water.

Anal. Calc'd for $C_6H_6N_4S$: C, 43.4; H, 3.6; N, 33.7. Found: C, 43.6; H, 3.7; N, 33.6.

Method (2). 3-Methyl-7-chloropyrazolo[4,3-*d*]pyrimidine (IX) (5 g.) and 2.5 g. of thiourea were added to 70 ml. of absolute ethanol. The resulting solution was refluxed for three hours and then was cooled and filtered. The product was dissolved in hot dilute sodium hydroxide and the solution was acidified with acetic acid while hot. The product obtained was identical to that obtained by method (1) as shown by identical ultraviolet absorption curves at *p*H 1 and at *p*H 11.

3-Methylpyrazolo[4,3-d]pyrimidine (XII). 3-Methyl-7-chloropyrazolo[4,3-*d*]pyrimidine (IX) (5 g.) was added to 150 ml. of water and 4 ml. of concentrated ammonium hydroxide. Approximately 1.5 g. of a 5% palladium on charcoal catalyst was added and the mixture was shaken on a low pressure hydrogenator at 20 lbs/sq.in. pressure for 6 hours.

The solution was filtered and the filtrate was evaporated to dryness on the steam-bath. The residue was placed in the thimble of a Soxhlet extractor and was extracted with boiling toluene for 18 hours. The cooled toluene was filtered to give 0.8 g. of product which was further purified by sublimation under reduced pressure to give white crystals, m.p. 233–235°.

Anal. Calc'd for $C_6H_6N_4$: C, 53.7; H, 4.5; N, 41.8. Found: C, 53.9; H, 5.5; N, 41.3.

General method of synthesis of 3-methyl-7-substituted aminopyrazolo[4,3-d]pyrimidines (X) *listed in Table I.* Approximately 0.035 mole of 3-methyl-7-chloropyrazolo[4,3-*d*]-pyrimidine (IX) and 0.1 mole of the primary or secondary amine were added to 150 ml. of absolute ethanol and the solution was heated until the volume was reduced to approximately 25 ml. on steam-bath.

Method (A). If a precipitate appeared upon cooling this solution, the product was filtered, added to 25 ml. of water, and the solution was warmed gently on the steam-bath and adjusted to *p*H 8 with aqueous potassium hydroxide. The solution was cooled, and the resulting solid was collected, washed, and recrystallized from the indicated solvents.

Method (B). If no product crystallized from the alcoholic solution, the solution was evaporated to dryness on the steam-bath and the solid residue was treated with dilute potassium hydroxide as in method (A) and the solid thus obtained was recrystallized from the indicated solvents.

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