A New Synthetic Method for Some Pyrazolo[4,3-*d*]pyrimidines[1]

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(Received July 20, 1978)

3,7-Dihydroxy- and 3-substituted 7-hydroxypyrazolo[4,3-*d*]pyrimidines were synthesized from diethyl oxaloacetate and ethyl acylpyruvates *via* ethyl 4-amino-5-oxo-2-pyrazoline-3-carboxylate hydrochloride and ethyl 5-substituted 4-amino-3-pyrazolecarboxylates, respectively.

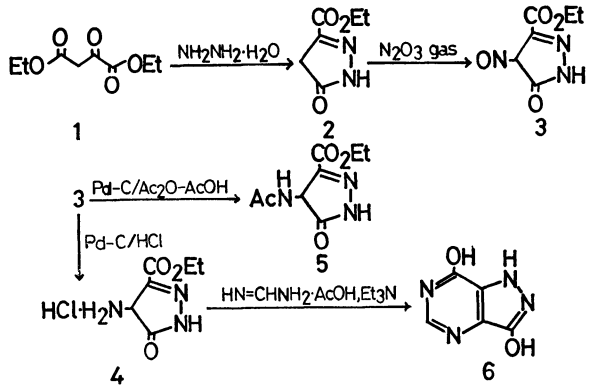
Purine antagonists have been shown to be chemotherapeutic agents against various tumors.[2] Some of pyrazolo[4,3-*d*]pyrimidine derivatives, which are purine analogue, have been also found to have this antagonism.[3] It thus seemed desirable to investigate the synthesis of the pyrazolo[4,3-*d*]pyrimidine ring system.

Recently, some carbon linked nucleoside antibiotics have been found in nature. Among these, formycin and formycin B[4] were characterized as 7-amino- and 7-hydroxy-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine, respectively. The structural characterization of the antibiotics has also regenerated a great interest in the synthesis of the pyrazolo[4,3-*d*]pyrimidine ring system.

The first reported sytnthesis of the ring system was that of Behrend,[5] who utilized 5-amino-6-methyluracil for the preparation of 5,7-dihydroxypyrazolo-[4,3-*d*]pyrimidine. Rose[6] has accomplished the synthesis of the ring system by diazotization of 4-alkyl-5-amino-6-methylpyrimidine followed by an intramolecular coupling. This method is quite satisfactory but definitely restricted to the substituent at position 7 of pyrazolo[4,3-*d*]pyrimidine, since 5-amino-6-methylpyrimidine substituted at position 4 with a hydroxyl, mercapto, or amino group upon diazotization couples to give the oxadiazole, thiadiazole, or triazole ring. Robins and his coworkers[7] synthesized the ring system from 4-nitro-3-pyrazolecarboxylic acid, and Townsend and his coworkers[8] have also synthesized 5,7-disubstituted 3-methylpyrazolo[4,3-*d*]pyrimidines from ethyl 5-methyl-4-nitro-3-pyrazolecarboxylate. But these nitropyrazole methods do not seem to be applicable for the synthesis of sugar derivatives, because nitration was performed in fuming sulfuric acid. The investigation of the synthesis of oxoformycin has appeared in the literature.[9] Formycin B was totally synthesized by Acton and his coworkers[10] in 1971 from 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyldiazomethane as a key intermediate. Arabinosyl analogue was also synthesized by a similar method.[11]

In this paper, we describe a convenient synthesis of 3,7-dihydroxy- and 3-substituted 7-hydroxypyrazolo-[4,3-*d*]pyrimidines starting from diethyl oxaloacetate and ethyl acylpyruvates, respectively, under mild conditions in high yields.

Treatment of diethyl oxaloacetate (**1**) with hydrazine hydrate (1 equiv) in ethanol at room temperature gave ethyl 5-oxo-2-pyrazoline-3-carboxylate (**2**) in 88% yield. Introduction of N_2O_3 gas into an ethanol solution of **2** resulted in the formation of ethyl 4-nitroso-5-oxo-2-pyrazoline-3-carboxylate (**3**) in 95% yield. Catalytic hydrogenation of this nitroso compound **3** over 20% Pd–C in ethanol in the presence of hydrochloric acid at room temperature afforded ethyl 4-amino-5-oxo-2-pyrazoline-3-carboxylate hydrochloride (**4**) in 88% yield. When **3** was hydrogenated in acetic acid in the presence of acetic anhydride at room temperature, ethyl 4-acetamido-5-oxo-2-pyrazoline-3-carboxylate (**5**) was obtained in 75% yield. Treatment of the hydrochloride **4** with formamidine acetate (3 equiv) and triethylamine (5 equiv) in boiling 2-ethoxyethanol gave 3,7-dihydroxypyrazolo[4,3-*d*]pyrimidine (**6**) in 92% yield. The structure was confirmed by spectral data and elemental analysis.

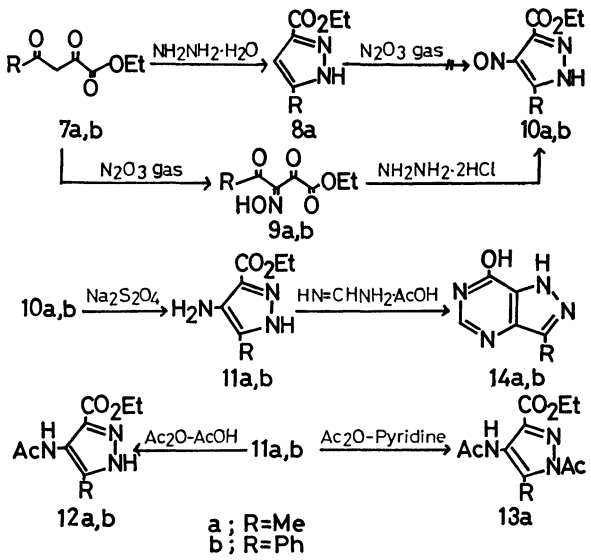


Scheme 1.

Ethyl 5-methyl-3-pyrazolecarboxylate (**8a**)[12] could not be nitrosated at position 4 of the pyrazole ring by the introduction of N_2O_3 gas. Therefore it was decided to introduce the nitroso group prior to the ring closure. Ethyl acetopyruvate (**7a**) was nitrosated to the corresponding hydroxyimino derivative (**9a**) with N_2O_3 gas in ethanol at room temperature. The reaction of this hydroxyimino derivative **9a** with hydrazine dihydrochloride (1 equiv) in water at 0 °C gave blue crystalline ethyl 5-methyl-4-nitroso-3-pyrazolecarboxylate (**10a**). By treatment with excess sodium dithionite in ethyl acetate and water at room temperature, this nitroso compound **10a** was readily reduced to ethyl 4-amino-5-methyl-3-pyrazolecarboxylate (**11a**) in 47% yield, based on ethyl acetopyruvate **7a**. Acetylation of this amino compound **11a** with acetic anhydride in pyridine gave ethyl 4-acetamido-1-acetyl-5-methyl-3-pyrazolecarboxylate (**13a**) in a quantitative yield. On the other hand, when acetylation was carried out in acetic acid, ethyl 4-acetamido-5-methyl-3-pyrazolecarboxylate (**12a**) was obtained in a quantitative yield. The reaction of the amino compound **11a** with formamidine acetate (3 equiv) in boiling 2-

ethoxyethanol afforded a quantitative yield of 7-hydroxy-3-methylpyrazolo[4,3-*d*]pyrimidine (**14a**).

Similarly, 7-hydroxy-3-phenylpyrazolo[4,3-*d*]pyrimidine (**14b**) was obtained as follows. Ethyl benzopyruvate (**7b**) was nitrosated to its hydroxyimino derivative (**9b**). This hydroxyimino derivative **9b** was converted to greenish blue crystalline ethyl 4-nitroso-5-phenyl-3-pyrazolecarboxylate (**10b**) by the reaction with hydrazine dihydrochloride (1 equiv). Reduction of the nitroso compound **10b** by excess sodium dithionite gave ethyl 4-amino-5-phenyl-3-pyrazolecarboxylate (**11b**) in 46% yield, based on ethyl benzopyruvate **7b**. Acetylation of **11b** with acetic anhydride in pyridine gave a complex result. When acetylation was carried out in acetic acid, however, ethyl 4-acetamido-5-phenyl-3-pyrazolecarboxylate (**12b**) was obtained in a quantitative yield. The reaction of the amino compound **11b** with formamidine acetate (3 equiv) in boiling 2-ethoxyethanol gave 7-hydroxy-3-phenylpyrazolo[4,3-*d*]pyrimidine (**14b**) in a quantitative yield. The structure was confirmed by spectral data and elemental analysis.



Scheme 2.

Table 1. UV absorption data for certain substituted pyrazoles and pyrazolo[4,3-*d*]pyrimidines

Compound	pH 1(HCl)		95% EtOH		pH 11(NaOH)	
	λ_{max}(nm)	ε_{max}	λ_{max}(nm)	ε_{max}	λ_{max}(nm)	ε_{max}
2	211	8910	223	10080	225	11580
	256	4020	260	2920	295	2460
3	261	9460	261	8590	300	10400
	357	3660	357	4130	369	5190
4	281	8100	281	7920	253	7600
					288[a]	5460
5	245	6450	245	6580	234	5710
	288	4480	290	4550	302	2950
6	230	10830	231[b]	10530[b]	238	10360
	271	3910	288[b]	4680[b]	285[a]	3800
	288	4600	332[b]	6200[b]	315	5120
	332	4080			332	7040
10a	273[a]	6130	212	8370	329	14740
	303	10020	275[a]	6420	333	15950
			303	10110		
10b	237	12450	237	12490	229	13860
	332	4060	332	4340	341	8010
11a	218	6540	208	5720	236	6380
			296	4900		
11b	223	18980	228	20680	255	22690
			309	9430	288[a]	10340
12a	217	8140	217	7900	230	9200
	259[a]	4620	254[a]	4240	253	13000
12b	225	21790	225	21630	247	24380
13a	221	8040	221	8740	257	8290
	265	3720	265	3830		
14a	281	6470	226	6450	226	9000
					281	8560
					288	8690
					300[a]	5600
14b	232	11590	214[a]	10760	248	13380
			231	12040		

a) Shoulder. b) These values were measured in water.

TABLE 2. ELEMENTAL ANALYSES OF CERTAIN SUBSTITUTED PYRAZOLES AND PYRAZOLO[4,3-*d*]PYRIMIDINES

	Compound	Found (%)			Calcd (%)		
		C	H	N	C	H	N
2	$C_6H_8O_3N_2$	46.36	5.18	18.08	46.15	5.16	17.94
3	$C_6H_7O_4N_2$	39.14	3.85	22.96	38.92	3.81	22.70
4	$C_6H_{10}O_3N_3Cl$[a)]	35.17	5.14	21.01	34.71	4.85	20.24
5	$C_8H_{11}O_4N_3$	44.93	5.21	19.60	45.07	5.20	19.71
6	$C_5H_4O_2N_4$	39.61	2.64	37.04	39.48	2.65	36.84
10a	$C_7H_9O_3N_3$	45.84	4.76	23.22	45.90	4.95	22.94
10b	$C_{12}H_{11}O_3N_3$[a)]	59.90	4.67	17.95	58.77	4.52	17.14
11a	$C_7H_{11}O_2N_2$	49.62	6.61	25.09	49.69	6.55	24.84
11b	$C_{12}H_{13}O_2N_3$	62.36	5.69	18.32	62.32	5.67	18.17
12a	$C_9H_{13}O_3N_3$	51.27	6.22	19.86	51.17	6.20	19.90
12b	$C_{14}H_{15}O_3N_3$	61.62	5.61	15.65	61.53	5.53	15.38
13a	$C_{11}H_{15}O_4N_3$	52.05	6.04	16.80	52.15	5.97	16.59
14a	$C_6H_6ON_4$	47.76	4.05	37.54	48.00	4.03	37.32
14b	$C_{11}H_8ON$	62.14	3.84	26.61	62.25	3.80	26.40

a) These compounds were unstable.

UV absorption data and elemental analyses of these pyrazoles and pyrazolo[4,3-*d*]pyrimidines are listed in Tables 1 and 2.

Experimental

All the melting points are uncorrected.

*Ethyl 5-Oxo-2-pyrazoline-3-carboxylate (**2**).* To a solution of diethyl oxaloacetate (**1**) (19.8 g, 0.1 mol) in 50 ml of ethanol, 80% hydrazine hydrate (6.25 g, 0.1 mol) in 50 ml of ethanol was added dropwise at room temperature. After the solution was stirred for 2 h, the solvent was removed under diminished pressure. The residual solid was recrystallized from ethyl acetate to yield 13.7 g (88%) of **2**. An analytical sample was obtained by an additional recrystallization from ethyl acetate, mp 183—184 °C.

*Ethyl 4-Nitroso-5-oxo-2-pyrazoline-3-carboxylate (**3**).* Into a solution of **2** (15.6 g, 0.1 mol) in 100 ml of ethanol, N_2O_3 gas generated by dropping concd hydrochloric acid to sodium nitrite was introduced at room temperature until the disappearance of **2** was confirmed by TLC. Then the solvent was removed under diminished pressure, and the residual yellow crystals of **3** were recrystallized from ethyl acetate, yield 17.6 g (95%). An analytical sample was obtained by two additional recrystallizations from ethyl acetate, mp 170—172 °C (dec).

*Ethyl 4-Amino-5-oxo-2-pyrazoline-3-carboxylate Hydrochloride (**4**).* The nitroso compound **3** (1.85 g, 0.01 mol) was dissolved in 50 ml of ethanol containing 2 ml of concd hydrochloric acid. A small amount of 20% Pd-C was added and stirred under hydrogen atmosphere. After 450 ml of hydrogen was absorbed, the solution was filtered and evaporated *in vacuo*. The residue was dissoved in a small amount of ethanol and 1.83 g (88%) of **4** was precipitated by the addition of ether. An analytical sample was obtained by an additional reprecipitation from ethanol and ether, mp 160 °C (dec).

*Ethyl 4-Acetamido-5-oxo-2-pyrazoline-3-carboxylate (**5**).* The nitroso compound **3** (3.70 g, 0.02 mol) was dissolved in 150 ml of acetic acid and 6 ml of acetic anhydride. A small amount of 20% Pd-C was added and stirred under hydrogen atmosphere. After 900 ml of hydrogen was absorbed, the solution was filtered and evaporated *in vacuo*. When ether was added to the residue, white crystals of **5** were precipitated, 3.20 g (75%). An analytical sample was obtained by recrystallization from water, mp 189.5—190 °C.

*3,7-Dihydroxypyrazolo[4,3-d]pyrimidine (**6**).* A mixture of **4** (519 mg, 2.5 mmol), formamidine acetate (780 mg, 7.5 mmol), and triethylamine (1.26 g, 12.5 mmol) in 10 ml of 2-ethoxyethanol was refluxed for 1 h under argon atmosphere. Crystals were precipitated when the solution was allowed to stand at room temperature. The precipitate was filtered and recrystallized from water to yield 350 mg (92%) of **6**. An analytical sample was obtained by an additional recrystallization from water, mp>300 °C.

*Ethyl α-(Hydroxyimino)acetopyruvate (**9a**) and Ethyl α-(Hydroxyimino)benzopyruvate (**9b**).* Into a solution of **7a** (15.8 g, 0.1 mol) in 100 ml of ethanol, N_2O_3 gas was introduced at room temperature until the disappearance of **7a** was confirmed by TLC. Then the solvent was removed under diminished pressure and water was added. The aqueous solution was extracted three times with ether and the combined organic layer was dried over anhydrous sodium sulfate. Evaporation of ether *in vacuo* gave a yellow oil of almost pure **9a**. The oily **9a** was used for the next step without further purification. The benzoyl derivative **9b** was obtained as an oil from ethyl benzopyruvate **7b** in a similar manner.

*Ethyl 5-Methyl-4-nitroso-3-pyrazolecarboxylate (**10a**).* To a solution of **9a** obtained from **7a** (3.16 g, 0.02 mol) in 50 ml of water, hydrazine dihydrochloride (2.08 g, 0.02 mol) in 50 ml of water was added dropwise and stirred at 0 °C until the disappearance of **9a** was confirmed by TLC. Then the solution was extracted with ether and the organic layer was dried over anhydrous sodium sulfate. When the solvent was removed under diminished pressure below 40 °C, blue crystals of **10a** were obtained. An analytical sample was obtained by using silica-gel column chromatography eluted with benzene–ethyl acetate (9 : 1), mp 95—98 °C (dec).

*Ethyl 4-Nitroso-5-phenyl-3-pyrazolecarboxylate (**10b**).* Greenish blue crystals of **10b** were obtained from **9b** in a manner similar to that described for **10a** when the removal of the solvent was carried out under an ice-cooled bath. An analytical sample was obtained by using silica-gel column chromatography eluted with benzene–ethyl acetate (5 : 1), mp 26 °C (dec).

*Ethyl 4-Amino-5-methyl-3-pyrazolecarboxylate (**11a**).* To a mixture of **10a** obtained from **7a** (3.16 g, 0.02 mol) in

50 ml of ethyl acetate and 50 ml of water, sodium dithionite was added to the mixture until the disappearance of **10a** was confirmed by TLC. Then the organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was dried over anhydrous sodium sulfate and the solvent was removed under diminished pressure. The crude product was recrystallized from benzene to yield 1.60 g (47% from **7a**) of **11a**. An analytical sample was obtained by an additional recrystallization from benzene, mp 96—96.5 °C.

*Ethyl 4-Amino-5-phenyl-3-pyrazolecarboxylate (**11b**).* In a manner similar to that described for **11a**, **11b** was obtained from **7b** (2.20 g, 0.01 mol) and recrystallized from benzene to yield 1.06 g (46% from **7b**). An analytical sample was obtained by an additional recrystallization from benzene, mp 145—146 °C.

*Ethyl 4-Acetamido-5-methyl-3-pyrazolecarboxylate (**12a**).* A solution of **11a** (85 mg, 0.5 mmol) in acetic acid (5 ml) and acetic anhydride (26 mg, 2.5 mmol) was stirred at room temperature for 30 min. The solution was neutralized with aqueous sodium hydrogencarbonate solution and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield 106 mg (quantitative) of **12a**. An analytical sample was obtained by recrystallization from ethyl acetate, mp 176—177 °C.

*Ethyl 4-Acetamido-5-phenyl-3-pyrazolecarboxylate (**12b**).* In a similar manner, **12b** was obtained in a quantitative yield (137 mg) from **11b** (116 mg, 0.5 mmol). An analytical sample was obtained by recrystallization from ethyl acetate, mp 182.5—183 °C.

*Ethyl 4-Acetamido-1-acetyl-5-methyl-3-pyrazolecarboxylate (**13a**).* A solution of **11a** (169 mg, 1 mmol) in pyridine (5 ml) and acetic anhydride (510 mg, 5 mmol) was stirred at room temperature for 2 days and then the solvent was removed under diminished pressure. The crude product was recrystallized from benzene–hexane to yield 253 mg (quantitative) of **13a**, mp 152—153 °C.

*7-Hydroxy-3-methylpyrazolo[4,3-d]pyrimidine (**14a**).* A mixture of **11a** (338 mg, 2 mmol) and formamidine acetate (624 mg, 6 mmol) in 10 ml of 2-ethoxyethanol was refluxed for 1 h under argon atmosphere. Then the solvnet was removed under diminished pressure and the residue was recrystallized from water to yield 300 mg (quantitative) of **14a**. An analytical sample was obtained by an additional recrystallization from water, mp>300 °C (lit,[7] >300 °C).

*7-Hydroxy-3-phenylpyrazolo[4,3-d]pyrimidine (**14b**).* Crude **14b** was obtained from **11b** (231 mg, 1 mmol) in a similar manner. Recrystallization of crude **14b** from *N*,*N*-dimethylformamide gave 211 mg (quantitative) of **14b**. An analytical sample was obtained by an additional recrystallization from *N*,*N*-dimethylformamide, mp>300 °C.

This research was supported in part by a Kurata Research Grant.

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