Pyrazolopyrimidine Nucleosides. Part IV.[1] Synthesis and Chemical Reactivity of the *C*-Nucleoside Selenoformycin B and Derivatives

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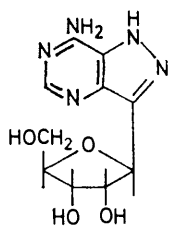
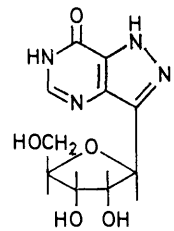
The synthesis of 7-selenoxo-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (2) (selenoformycin B) has been accomplished by a nucleophilic displacement of the chloro-group from 7-chloro-3-(β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine (1) with selenourea in ethanol at reflux temperature. Alkylation of (2) has furnished several 7-alkylseleno-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidines. A study on the relative reactivity of certain 7-substituted 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidines and the corresponding 6-substituted 9-(β-D-ribofuranosyl)purines toward nucleophilic displacement is discussed. 7-Methoxy-3-(β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine (5) and the nebularine derivative 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (3) have also been prepared. The stability of (2) towards basic and approximate physiological pH conditions is discussed.

THE isolation[2,3] and characterization[4,5] of formycin and formycin B as *C*-nucleosides created considerable interest, since they were found to be isomeric with adenosine and inosine, respectively. Additional interest was generated by the finding that the glycosyl bond of these *C*-nucleoside antibiotics possessed an increased stability towards chemical cleavage relative to their naturally occurring counterparts, adenosine and inosine.



Formycin



Formycin B

It was also found that formycin B was not a substrate but in fact actually inhibited erythrocytic purine nucleoside phosphorylase.[6a] The glycosyl bond of the antitumour agent[6b] 6-thioinosine can be cleaved enzymatically by a phosphorylase. 6-Selenoinosine has been reported to be inactive as an antitumour agent, presumably owing to the instability of the selenoxo-group at physiological pH. However, it is tempting to postulate that glycosyl bond cleavage of 6-selenoinosine could be occurring *in vivo*. This prompted us to synthesize the seleno-congener of formycin B which would eliminate the possibility of enzymatic cleavage of the glycosyl bond by a pyrophosphorylase.

Treatment of 7-chloro-3-(β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine[7] (1) with selenourea in ethanol at reflux temperature furnished nucleoside material which was characterized as 7-selenoxo-3-(β-D-ribofuranosyl)-pyrazolo[4,3-*d*]pyrimidine (2) (7-selenoformycin B). The ^{1}H n.m.r. spectrum of (2) revealed a sharp singlet at δ 8·1 (5-H), a doublet at 5·1 for the anomeric proton (H-1′), and a pattern of peaks in the 3·5—4·5 region characteristic of the carbohydrate system.[8] The u.v. spectral data (Table) firmly established the structure

[1] Part III, R. A. Earl, R. P. Panzica, and L. B. Townsend, preceding paper.

[2] M. Hori, E. Ito, T. Takito, G. Koyama, and H. Umezawa, *J. Antibiot.* (*Japan*), 1964, **17A**, 96.

[3] G. Koyama and H. Umezawa, *J. Antibiot.* (*Japan*), 1965, **18A**, 175.

[4] R. K. Robins, L. B. Townsend, F. Cassidy, J. F. Gerster, A. F. Lewis, and R. L. Miller, *J. Heterocyclic Chem.*, 1966, **3**, 110.

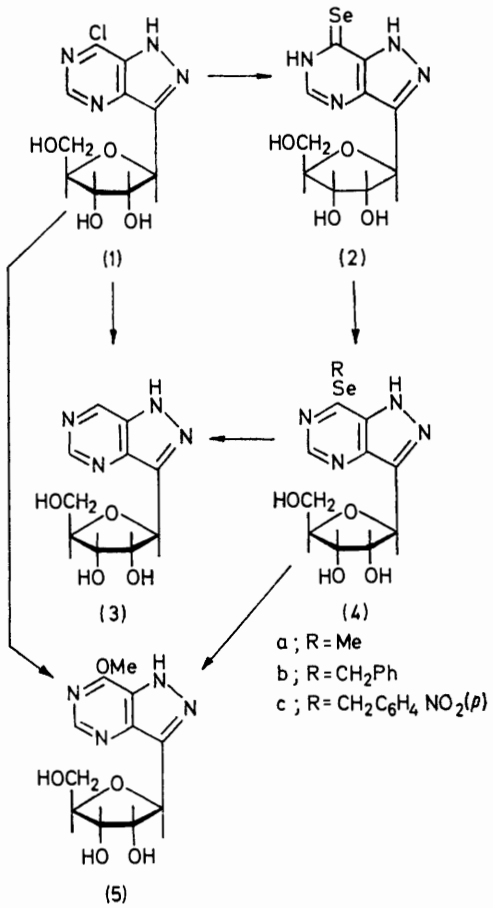
[5] K. Kawamura, S. Tukatsu, M. Murase, G. Koyama, K. Maeda, and H. Umezawa, *J. Antibiot.* (*Japan*), 1966, **19A**, 93; G. Koyama, K. Maeda, H. Umezawa, and Y. Iitake, *Tetrahedron Letters*, 1966, 597.

[6] (*a*) M. R. Sheen, B. Y. Kim, and R. E. Parks, jun., *Mol. Pharmacol.*, 1968, **4**, 293; (*b*) J. A. Montgomery, *Progr. Medicin. Chem.*, 1970, **7**, 69.

[7] R. A. Long, A. F. Lewis, R. K. Robins, and L. B. Townsend, *J. Chem. Soc.* (*C*), 1971, 2443.

[8] This upfield chemical shift for the anomeric proton relative to the chemical shift for the anomeric proton of the corresponding purine nucleoside is characteristic of *C*-nucleosides, see L. B. Townsend in 'Synthetic Procedures in Nucleic Acid Chemistry,' vol. 2, eds, W. W. Zorbach and R. S. Tipson, Interscience, New York, 1972, pp. 267—370.

of the nucleoside as (2). This large bathochromic shift between formycin B and selenoformycin B (86 nm in H_2O) was expected [9] for the conversion of a heterocyclic



ring –NHCO– to –NHCSe– and afforded a ready method for determining the stability of (2) under approximate

U.v. data for certain 7-substituted 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidines [a]

	pH 1	MeOH* or H_2O†	pH 11
Compound	$\lambda_{max.}$/nm ($\varepsilon_{max.}$)	$\lambda_{max.}$/nm ($\varepsilon_{max.}$)	$\lambda_{max.}$/nm ($\varepsilon_{max.}$)
(2)	374 (18,500)	366† (13,720)	353 (15,200)
(4c)	278 (16,520)	320* (18,180)	293 (18,160)
(4a)	338 (12,000)	325† (14,720)	298 (9000)
			332 (9000)
(4b)	338 (7600)	322* (13,720)	300 (8410)
			336 (8840)
(5)	285 (6300)	283† (8450)	300 (6280)
(3)	245 (8810)	257* (3580)	268 (4500)
		300* (4700)	332 (2780)

[a] Spectra were obtained with a Beckman DK-2 u.v. spectrophotometer.

physiological pH and other basic conditions. Treatment of (2) with pH 14, 7·4, and 7 buffer systems revealed essentially no decomposition or degradation as determined by u.v. spectroscopy. This observation was unexpected in view of the lability reported for the exocyclic selenoxo-group of 6-selenoxo-9-(β-D-ribofuranosyl)purine. A plausible explanation for this stability is related to the species present in the mixture (see later). The methylation of (2) with methyl iodide was accomplished in a methanolic sodium methoxide solution to furnish a nucleoside whch was assumed to be 7-methylseleno-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (4a). This tentative structural assignment was based on a pronounced hypsochromic shift (36 nm at pH 1) in the u.v. spectra and a sharp n.m.r. singlet (3H) at δ 2·47 which was assigned to the methyl group of an exocyclic methylseleno-group by analogy with the value reported [10] for an exocyclic methylthio-group. Additional corroboration for the actual site of methylation was obtained by treatment of compound (4a) with Raney nickel, which furnished the nebularine analogue 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (3), although we did encounter more difficulty than expected [11] in removing the methylseleno-group. Removal of the methylseleno-group can be monitored by observing the disappearance of the u.v. peak at *ca.* 330 nm and the appearance of a peak at 257 nm. Further corroboration was obtained by the appearance of a singlet in the ^1H n.m.r. spectrum at δ 9·37 (1H, 7-H) for this nucleoside and in addition there was observed a singlet at δ 9·05 (1H, 5-H), a doublet at 5·15 for the anomeric proton, and a characteristic pattern of peaks in the 3·5—4·5 region for the carbohydrate function. The nucleoside (3) which was obtained from (4a) was also identical with the nucleoside obtained by catalytic removal of the 7-chloro-group from (1). Alkylation of (2) with benzyl bromide and α-bromo-*p*-nitrotoluene under conditions similar to those used for the preparation of (4a) furnished 7-benzylseleno- (4b) and 7-(*p*-nitrobenzylseleno)-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (4c), respectively.

The nucleophilic displacement of an alkylseleno-group was accomplished only after treatment of (4b) with methanolic sodium methoxide at reflux temperature for 24 h. This furnished nucleoside material which was assigned the structure 7-methoxy-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (5) on the basis of a comparison of u.v. spectra, mixed m.p., and t.l.c. in four solvent systems with (5) prepared by treatment of (1) with methanolic sodium methoxide.

The unexpected stability of (2) toward basic conditions and the difficulty encountered in removal of the methylseleno-group from (4a) with Raney nickel prompted us to ascertain the factors involved in this apparently anomalous behaviour. 7-Chloro-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (1) was initially studied. Its u.v. spectral data revealed a significant $\Delta\lambda_{max.}$ [$\lambda_{max.}$ (pH 11) 332 – $\lambda_{max.}$ (pH 6) 300 nm = 32 nm] which was assumed at first to be the result of either a conversion of the exocyclic 7-chloro-group, a simple degradation, or most likely a poly-

[9] The difference between guanosine and 6-selenoguanosine was 106 nm at pH 7, L. B. Townsend and G. H. Milne, *J. Heterocyclic Chem.*, 1970, **7**, 753.

[10] G. R. Revankar and L. B. Townsend, *J. Heterocyclic Chem.*, 1968, **5**, 615.

[11] G. H. Milne and L. B. Townsend, *J. Heterocyclic Chem.*, 1971, **8**, 379.

merization of compound (1) due to intermolecular alkylation. However, all these possibilities were eliminated when a solution (pH 11) of (1) ($\lambda_{max.}$ 332 nm) was adjusted to pH 6 with glacial acetic acid and a u.v. spectrum revealed $\lambda_{max.}$ 255 and 300 nm. T.l.c. of this solution revealed only one component with R_F values identical to those observed for authentic (1). On the basis of these data, it was established that no actual chemical change had occurred at pH 11, which would suggest that the large bathochromic shift observed between pH 6 and 11 must be due to an increase in conjugation at pH 11 by an abstraction of the NH proton from the pyrazole group to furnish the anion [12] of (1). This would explain the unexpected increase in stability of (2) toward basic conditions in comparison to 6-selenoxo-9-(β-D-ribofuranosyl)purine if we can assume that the anion of (2) was present under the reaction conditions. The formation of an anionic species from the alkaline Raney nickel may also explain the unexpected difficulty experienced in a removal of the methylseleno-group from (4a).

It was of considerable interest to determine the relative reactivity of the exocyclic chloro-group towards nucleophilic displacement for the neutral 6-chloro-9-(β-D-ribofuranosyl)purine and 7-chloro-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (1). These compounds were dissolved in absolute methanol and the separate solutions were heated at reflux temperature. The conversion of (1) into (5) was found to occur at a much greater rate than the conversion of 6-chloro-9-(β-D-ribofuranosyl)purine into the 6-methoxy-compound. This ready reaction of (1) with methanol involved neutral (1) rather than the anion since a u.v. absorption peak at >305 nm was not observed at any time during the reaction. Therefore, it would appear that with the neutral compound the exocyclic chloro-group of (1) is more reactive than the chloro-group of the corresponding purine nucleoside and that future investigations with formycin and related *C*-nucleosides must account for the fact that these reactions may be very pH-dependent.

EXPERIMENTAL

M.p.s were taken with a Thomas–Hoover capillary apparatus. U.v. absorption spectra were obtained with a Beckman DK-2 spectrophotometer and ^{1}H n.m.r. spectra on a Varian A-60 instrument using tetramethylsilane as an internal standard. T.l.c. utilizing 0·25 mm thick Baker SilicAR 7 GF plates and the solvent system EtOAc–MeOH (10 : 1) was utilized for chromatographic separations unless otherwise specified. Elemental analyses were performed by Heterocyclic Chemical Corporation, Harrisonville, Mo.

7-*Selenoxo*-3-(β-D-*ribofuranosyl*)*pyrazolo*[4,3-d]*pyrimidine* (2).— 7-Chloro-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (700 mg) was added with stirring to ethanol (25 ml) containing 10 drops of water. Selenourea (500 mg) was then added and the mixture was stirred and heated at reflux temperature for 15 min. The mixture was then kept at 0° for 24 h. The solid was collected by filtration and then added to boiling water (20 ml). Selenium metal was removed from the hot mixture by filtration and the filtrate was cooled to induce crystallization. After 2 h at 0° the solid was collected. The filtrate was evaporated to dryness *in vacuo*, the residue was dissolved in water (*ca.* 15 ml), the selenium metal was removed, and the filtrate was treated as described above. The combined solids were dried in a vacuum desiccator over Drierite at room temperature to furnish 525 mg of a yellow-orange solid, m.p. 70—72°. An analytical *sample* had m.p. 71—72° (resolidifies) (from H_2O) (Found: C, 33·6; H, 4·2; N, 15·7. $C_{10}H_{12}N_4O_4Se,1{\cdot}5H_2O$ requires C, 33·5; H, 4·2; N, 15·65%). The presence of solvate was verified by an n.m.r. spectrum.

3-(β-D-*Ribofuranosyl*)*pyrazolo*[4,3-d]*pyrimidine* (3).— *Method I.* 7-Chloro-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]-pyrimidine (1) (500 mg) was added to water (50 ml) containing conc. NH_4OH (0·5 ml). To this solution was added 10% Pd–C catalyst (150 mg) and the mixture was shaken under a hydrogen atmosphere on a low pressure Parr hydrogenator at 20 lb in^{-2} for 1 h during which time the pressure dropped to 16 lb in^{-2} and then remained constant. The mixture was filtered through Celite and the filtrate lyophilized to afford a white residue. This residue was dissolved in methanol (10 ml) and subjected to prepartive t.l.c. (SilicAR 7 GF; 4 mm thickness; 20 × 40 mm) and developed using the upper phase of an EtOAc–n-propanol–H_2O (4 : 1 : 2, v/v) mixture. The band of R_F 0·5 was eluted using EtOAc–MeOH (4 : 1, v/v) and the eluate was evaporated *in vacuo* to afford a white solid (110 mg), slowly darkens >180°, decomp. >200°, foams >220°. This furnished authentic *material* for a comparison with the nucleoside obtained by Method II (Found: C, 47·6; H, 4·8; N, 22·2. $C_{10}H_{12}N_4O_4$ requires C, 47·6; H, 4·75; N, 22·25%).

Method II. 7-Methylseleno-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (4a) (100 mg) was dissolved in absolute ethanol (25 ml) and to this solution was added W-7 Raney nickel (1 g) [13] which had been previously washed thoroughly with ethanol. The mixture was heated at reflux until the u.v. absorption at 325 nm had completely disappeared (*ca.* 24 h). The Raney nickel was then removed by filtration and washed with boiling ethanol (25 ml) and the combined filtrates were evaporated to dryness *in vacuo*. The residue was dissolved in methanol (5 ml) and applied to a SilicAR 7 GF plate (4 mm thickness; 20 × 40 mm). The plate was developed with the upper phase of an EtOAc–n-propanol–H_2O (4 : 1 : 2, v/v) mixture and the band with R_F *ca.* 0·5 was eluted using EtOAc–MeOH (4 : 1, v/v). The eluate exhibited a u.v. spectrum identical to that observed for the compound prepared by Method I and this was corroborated by t.l.c. in four solvent systems.

7-*Methylseleno*-3-(β-D-*ribofuranosyl*)*pyrazolo*[4,3-d]*pyrimidine* (4a).—To absolute methanol (25 ml) containing methanolic N-sodium methoxide (2·5 ml) was added compound (2) (500 mg). Methyl iodide (225 mg) was then added and the solution was stirred at room temperature for 0·5 h. Selenium metal was removed by filtration and the pH of the solution adjusted to 6 with

[12] The pK values of formycin are 4·4 and 9·17, L. B. Townsend and Roland K. Robins, 'Handbook of Biochemistry,' ed. H. A. Sober, Chemical Rubber Co., Cleveland, 2nd edn. 1970, G-150.

[13] H. R. Billica and H. Adkins, *Org. Synth.*, 1967, Coll. Vol. 3, pp. 176—180.

glacial acetic acid. The solution was evaporated *in vacuo* to afford a white foam which was placed in a Soxhlet apparatus and extracted with 150 ml of diethyl ether at reflux for 40 h. The ether was then evaporated to afford a syrup which was dissolved in EtOAc and this solution was evaporated *in vacuo* to furnish a foam. The foam was suspended in ethyl acetate at reflux and methanol was added to give a clear solution. This solution was added to cyclohexane (100 ml) at reflux and then left at 5° for 24 h. The white solid was collected by filtration and dried *in vacuo* at 100° for 18 h to yield *crystals* (250 mg), softens >155°, foams >180°, decomp. >200° (Found: C, 38·0; H, 4·0; N, 16·4. $C_{11}H_{14}N_4O_4Se$ requires C, 38·25; H, 4·05; N, 16·25%). An alternative procedure was subsequently developed for the purification of compound (2). The filtrate obtained from the removal of selenium metal was stirred with Amberlite IRC-50 resin (5 ml) for 30 min or until the pH of the solution was 6. The resin was removed by filtration and washed with methanol (50 ml). The filtrates were combined and evaporated to dryness *in vacuo* to afford a thick oil to which was added EtOAc (25 ml); the mixture was again evaporated to dryness *in vacuo*. The foam was dissolved in the minimum amount of MeOH–EtOAc (1 : 20, v : v) at reflux and then left at 5° for 18 h. The crystalline solid (550 mg) was collected by filtration and recrystallized as above to yield material (450 mg) identical with the compound prepared by the above method (u.v. spectra, t.l.c., and mixed m.p.).

7-Benzylseleno-3-(β-D-ribofuranosyl)pyrazolo[4,3-d]*pyrimidine* (4b).—7-Selenoxo-3-(β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine (2) (500 mg) was added, with stirring, to a 1N-solution of sodium methoxide (2·5 ml) in absolute methanol (20 ml). Benzyl bromide (240 mg) was added and the solution then stirred at room temperature for 1 h. The pH was adjusted to 6 with glacial acetic acid. The solution was evaporated *in vacuo* to afford a foam which was dissolved in methanol (5 ml) with ice–water (50 ml) then being added. The solid was collected and washed with water (25 ml). The remaining solid was recrystallized to yield *platelets* (400 mg) which were dried *in vacuo* over toluene at reflux, m.p. >160° (from aqueous methanol) (Found: C, 48·45; H, 4·25; N, 13·0. $C_{17}H_{18}N_4O_4Se$ requires C, 48·5; H, 4·3; N, 13·3%).

7-p-Nitrobenzylseleno-3-(β-D-ribofuranosyl)pyrazolo[4,3-d]*pyrimidine* (4c).—7-Selenoxo-3-(β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine (2) (500 mg) was added to absolute methanol (20 ml) containing methanolic N-sodium methoxide (2·5 ml). To this solution was added, with stirring, α-bromo-*p*-nitroluene (325 mg) and the solution was stirred at room temperature for 0·5 h. The pH was adjusted to 6 with glacial acetic acid and the solution was kept at −5° for 18 h. Solid was collected and dried *in vacuo* over Drierite to yield product (500 mg). This was recrystallized from methanol to afford a crystalline *solid* (350 mg) which was dried for 12 h *in vacuo*, softens >130°, decomp. >170° (Found: C, 43·5; H, 3·65; N, 15·05. $C_{17}H_{17}N_5O_6Se$ requires C, 43·85; H, 3·65; N, 15·0%).

7-Methoxy-3-(β-D-ribofuranosyl)pyrazolo[4,3-d]*pyrimidine* (5).—*Method I.* 7-Chloro-3-(β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine (1) (290 mg) was dissolved in absolute methanol (25 ml) containing sodium methoxide (80 mg). The solution was stirred and heated at reflux for 1 h and then Amberlite IRC-50 resin (2·5 ml) was added. The mixture was stirred for an additional 30 min or until the pH of the solution was 6. The resin was collected and washed with hot methanol (50 ml). The filtrates were combined and evaporated *in vacuo* to afford a foam which was recrystallized twice from absolute methanol to yield needles (110 mg). The *product* was dried at 110°, softens 210°, decomp. 220—222° (Found: C, 46·8; H, 4·8; N, 20·2. $C_{11}H_{14}N_4O_5$ requires C, 46·8; H, 4·95; N, 19·9%).

Method II. 7-Benzylseleno-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (4b) (100 mg) was dissolved in absolute methanol (20 ml) containing sodium methoxide (100 mg). The solution was heated at reflux and the reaction was monitored closely by u.v. and t.l.c. for 25 h. It was essentially complete after 24 h. The u.v. absorption peak at *ca.* 336 nm completely disappeared. Amberlite IRC-50 resin (3 ml) was then added and the mixture was stirred at room temperature until the pH was 6—7. The resin was removed by filtration and washed with methanol (50 ml), and the combined filtrates were evaporated *in vacuo* to afford a solid foam. This was recrystallized from methanol (10) to yield a crystalline solid (48 mg) identical to the compound prepared by Method I [u.v. spectra, t.l.c. (four different solvent systems), and mixed m.p.].

Study of the Stability of 7-Selenoxo-3-(β-D-ribofuranosyl)-pyrazolo[4,3-d]*pyrimidine* (2) *towards Different Solutions.*—*Distilled water.* The nucleoside (2) (10 mg) was dissolved in distilled water (20 ml) and stirred at room temperature for 3 days. A frequent check on the solution by u.v. spectroscopy and t.l.c. revealed that only a very small amount of decomposition had occurred during this time. The absorption peak at 384 nm was essentially unchanged.

Aqueous N-sodium hydroxide. The nucleoside (2) (10 mg) was dissolved in N-sodium hydroxide (20 ml) and heated at reflux temperature for 14 h. The absorption peak at 373 nm was essentially unchanged with only a very small amount of selenium metal present. T.l.c. revealed that the major compound in the solution was (2) with only a trace of decomposition products.

pH 7 *Buffer* (*phosphate–citrate*). The nucleoside (2) (10 mg) was dissolved in the buffer (20 ml) [14] and the solution was stirred at room temperature for 3 days. The u.v. absorption peak at 376 nm was essentially unchanged and t.l.c. revealed that only a very small amount of decomposition had occurred.

pH 7·4 *Buffer* (*phosphate-citrate*). The nucleoside (2) (10 mg) was dissolved in the buffer (20 ml) and the solution was stirred at room temperature for 3 days. The u.v. absorption spectrum revealed a peak at 374 nm, but with a more significant decrease in $\varepsilon_{max.}$ than was observed with the pH 7 buffer system. Some selenium metal was observed and t.l.c. revealed the presence of some decomposition products.

Comparative Study of the Nucleophilic Displacement of the Chloro-group from 7-Chloro-3-(β-D-ribofuranosyl)pyrazolo-[4,3-d]*pyrimidine* (1) *and 6-Chloro-9-(β-D-ribofuranosyl)-purine.*—*Methanolic ammonia.* The nucleoside (1) (100 mg) was dissolved in methanolic ammonia (25 ml) (previously saturated at 0°) and then stirred at room temperature. The same procedure was used for 6-chloro-9-(β-D-ribofuranosyl)purine (100 mg) and the separate reactions were monitored by t.l.c. and u.v. for 24 h. The purine was converted entirely into product or products as evidenced by the disappearance of absorption at 263

[14] 'Handbook of Chemistry and Physics,' 49th edn., Chemical Rubber Co., Cleveland, 1968—1969, D-79.

nm for starting material and the appearance of a new peak at 253 nm. T.l.c. demonstrated the total absence of any purine and the presence of a mixture of 6-methoxy-9-(β-D-ribofuranosyl)purine and adenosine by comparison with authentic samples. The nucleoside (1) had not reacted as indicated by essentially no change in the u.v. absorption spectrum and only the presence of starting material on t.l.c.

Comparison of the Reactivities of 7-*Chloro*-3-(β-D-*ribofuranosyl*)*pyrazolo*[4,3-d]*pyrimidine* (1) *and* 6-*Chloro*-9-(β-D-*ribofuranosyl*)*purine towards Methanol at Reflux*.—The nucleoside (1) and 6-chloro-9-(β-D-ribofuranosyl)purine (10 mg of each) were dissolved in absolute methanol (20 ml) and the solutions heated at reflux for 24 h with the reactions being monitored by t.l.c. [EtOAc–MeOH (8 : 1, v/v)] and u.v. spectroscopy. A ready conversion of (1) into another product was observed and the product was identified by u.v. and t.l.c.(four solvent systems) as 7-methoxy-3-(β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine (5) by comparison with authentic (5) (see before). This conversion was much faster than the conversion of 6-chloro-9-(β-D-ribofuranosyl)purine into the 6-methoxy-compound as determined by t.l.c. although there was still some starting material detectable in both reactions after 24 h.

We thank the Drug Research and Development Branch of the National Cancer Institute, National Institutes of Health, Public Health Service, for financial support.

[2/919 *Received*, 25*th April*, 1972]