The Synthesis of Some Quinazoline Nucleosides[1]

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The synthesis of 1-(β-D-ribofuranosyl)-2,4-quinazolinedione (IV) has been accomplished in above 80% yield by treatment of 2,4-bis(trimethylsilyloxy)quinazoline with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide followed by removal of the benzoyl groups with methanolic ammonia or sodium methoxide. Proof of the β-configuration was obtained by stepwise conversion of IV into O², 5′-anhydro-1-(2′, 3′-O-isopropylidene-β-D-ribofuranosyl)-4-quinazolone (XI). The site of sugar attachment was established as N_1 by methylation of IV followed by acidic hydrolysis of the product to yield 3-methyl-2,4-quinazolinedione. This assignment was confirmed by ultraviolet and infrared absorption data. Treatment of 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione with phosphorus pentasulfide provided the 4-thio derivative (V) which upon reaction with methanolic ammonia at 100° resulted in replacement of the 4-thio group with concomitant debenzoylation to yield 4-amino-1-(β-D-ribofuranosyl)-2-quinazolone (VII). Compounds IV and VII may be regarded as 5,6-benzouridine and 5,6-benzocytidine, respectively, with a fused planar aromatic system. The possible biochemical significance of greater electron interaction in the stacking of heterocyclic bases is discussed. Using similar procedures, 1-(2′-deoxy-β-D-ribofuranosyl)-2,4-quinazolinedione (XIX) and 4-amino-1-(2′-deoxy-β-D-ribofuranosyl)-2-quinazolone (XXI) have also been prepared. 1-(β-D-Ribofuranosyl)-2,4-quinazolinedione (IV) has successfully been converted into O²,2′-anhydro-1-(β-D-arabinofuranosyl)-4-quinazolone (XII) which yielded 1-(β-D-arabinofuranosyl)-2,4-quinazolinedione (XIII) upon ring opening with dilute sodium hydroxide. Acetylation of XIII followed by thiation and treatment with methanolic ammonia gave 4-amino-1-(β-D-arabinofuranosyl)-2-quinazolone (XVI). The reaction procedure for nucleoside formation in such good yields would indicate that this procedure might well be the method of choice for nucleoside synthesis with other unusual heterocyclic systems.

The use of trimethylsilyl derivatives of nitrogen heterocycles in nucleoside synthesis was first introduced by Birkofer, *et al.*,[2] in a novel synthesis of 3-ribofuranosyluric acid. The use of 2,4-bis(trimethylsilyloxy)pyrimidine in a fusion procedure with 2,3,5-tri-O-benzoylribofuranosyl chloride was employed by Nishimura, Shimizu, and Iwai[3] to give uridine in 35% yield. Initial attempts to condense trimethylsilyloxypyrimidines and acetohalo sugars directly in boiling benzene were unsuccessful.[4] The use of polar solvents[5] and milder reaction conditions[5,6] has been more successful. In the present study it was desired to attach the D-ribofuranosyl ring to position 1 of 2,4-quinazolinedione (I). Treatment of I with trimethylchlorosilane in the presence of toluene and triethylamine gave 83% yield of distilled colorless oil, 2,4-bis(trimethylsilyloxy)quinazoline (II) (see Scheme I). Pmr spectra indicated the presence of six methyl groups. Treatment of II with freshly prepared 2,3,5-tri-O-benzoylribofuranosyl bromide in dry acetonitrile for 2 days at room temperature gave 90% yield of crystalline 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione (III). Treatment of III in a refluxing solution of sodium methoxide in methanol removed the benzoyl groups to provide an 88% yield of crystalline 1-(β-D-ribofuranosyl)-2,4-quinazolinedione (IV). Proof of the structural assignment as β was obtained by the stepwise conversion of IV to O²,5′-anhydro-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-quinazolone (XI) (see Scheme II). The nucleoside IV was converted to 1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-2,4-quinazolinedione (IX) and then to the 5′-O-mesyl derivative (X). Treatment of X with sodium iodide gave the 5′-iodo compound which was not isolated but cyclized directly to O²,5′-anhydro-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-quinazolone (XI) with silver acetate in absolute methanol. The cyclonucleoside XI was also prepared directly from X by the action of potassium *t*-butoxide in dimethylformamide at 100° for 1.5 hr.

The fact that XI was actually a cyclonucleoside was established by treatment of XI with liquid ammonia which gave 2-amino-1-(2′,3′-O-isopropylidene β-D-ribofuranosyl)-4-quinazolone. All attempts to remove the isopropylidene group resulted in concomitant hydrolysis of the nucleoside and 2-amino-4-quinazolone was isolated instead of 2-amino-1-(β-D-ribofuranosyl)-4-quinazolone.

The position of attachment of D-ribose was determined in the following manner. A comparison of the ultraviolet absorption spectrum of IV with that of 3-methyl-2,4-quinazolinedione[7] and 1-methyl-2,4-quinazoline,[8] respectively, showed that at a pH of 1 or in neutral solution (see Table I) 1-(β-D-ribofuranosyl)-2,4-quinazolinedione (IV) and the 1-methyl- and 3-methyl-2,4-quinazolinediones possessed very similar spectra with a λ_{max} in the region of 306–312 mμ. However at pH 11, 3-methyl-2,4-quinazolinedione exhibited a bathochromic shift to λ_{max} of 326 mμ while at pH 11, 1-methyl-2,4-quinazolinedione and the nucleoside IV exhibited no such shift (Table I).

Additional evidence for assignment of the sugar moiety to the 1 position was obtained by methylation of IV with methyl iodide in N,N-dimethylformamide in the presence of potassium carbonate. The crude 3-methyl-1-(β-D-ribofuranosyl)-2,4-quinazolinedione was hydrolyzed with 10% hydrochloric acid to yield 3-methyl-2,4-quinazolinedione which was identical with an authentic sample[7] as judged by uv spectra, ir spectra, and mixture melting point data.

Synthesis of the cytidine analog, 4-amino-1-(β-D-

(1) This research was supported by Research Grant GB-5446 from the Molecular Biology Division of the National Science Foundation.

(2) L. Birkofer, A. R. Ritter, H. P. Kulthau, *Angew. Chem.*, **75**, 209 (1963).

(3) T. Nishimura, B. Shimizu, and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **11**, 1470 (1963).

(4) T. Nishimura and I. Iwai, *ibid.*, **12**, 357 (1964).

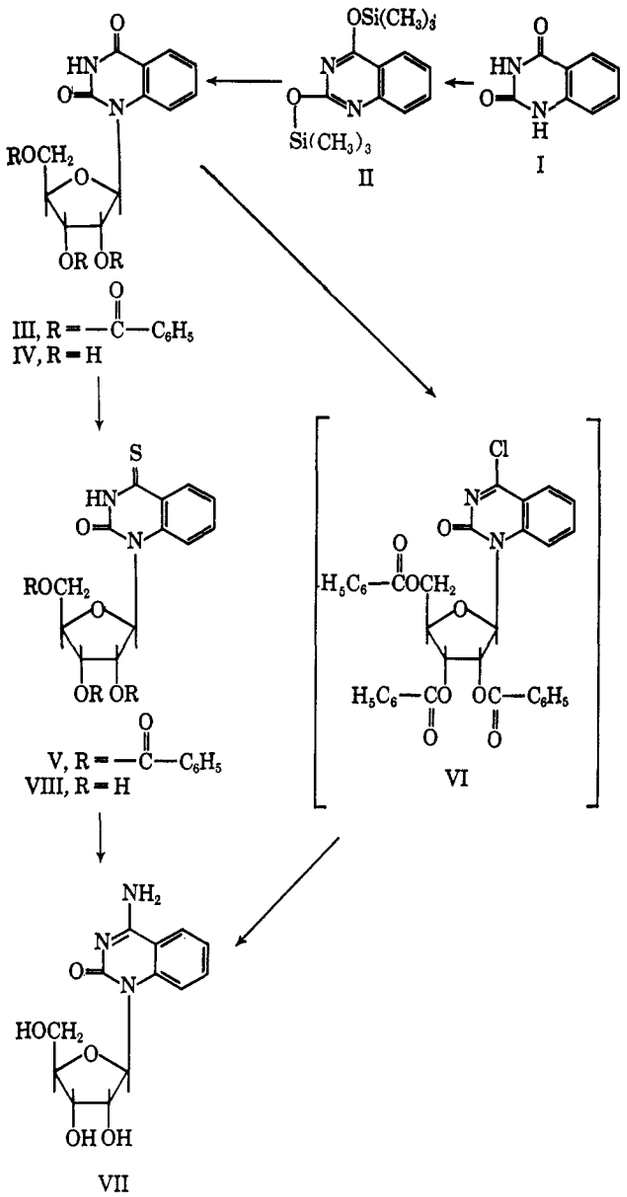
(5) E. Wittenberg, *Z. Chem.*, **4**, 303 (1964).

(6) K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **31**, 1181 (1966).

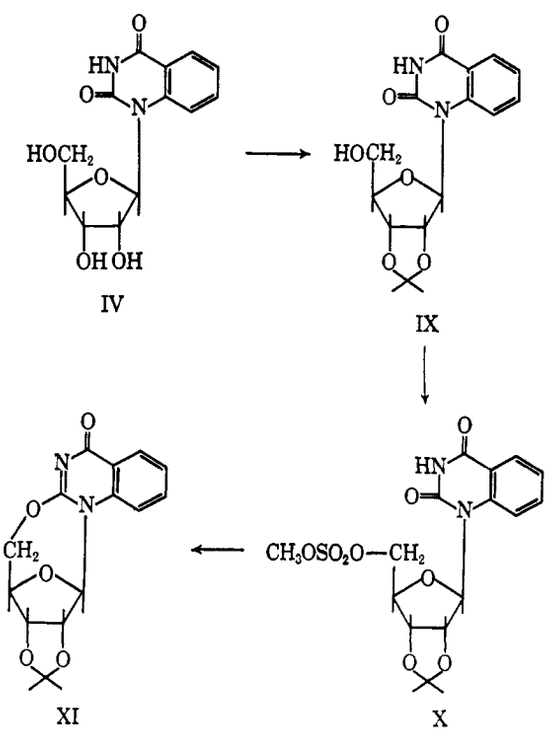
(7) R. P. Steiger and E. C. Wagner, *ibid.*, **18**, 1427 (1953).

(8) A. Chatterjee and S. G. Majumdar, *J. Amer. Chem. Soc.*, **76**, 2459 (1954).

SCHEME I



SCHEME II



ribofuranosyl)-2-quinazolone (VII), was accomplished in two steps by treatment of 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione (III) with phosphorus pentasulfide in pyridine to give a 93% yield of 4-thio-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-2-quinazolone (V), and then reaction of V with methanolic ammonia at 100° gave simultaneous deblocking and replacement of the 4-thio group to provide 4-amino-1-(β-D-ribofuranosyl)-2-quinazolone (VII), in 82% yield. This general method for the introduction of an amino group was first utilized in nucleoside chemistry by Fox, *et al.*[9] A second route to VII involved the treatment of 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione (III) with phosphorus oxychloride and N,N-diethylaniline, to give the presumed 4-chloro intermediate VI which was not isolated but treated directly with methanolic ammonia to give 4-amino-1-(β-D-ribofuranosyl)-2-quinazolone (VII) in 52% yield. This latter procedure, however, gave a mixture of VII contaminated with IV and in general was not as useful as the thiation route.

Preparation of 1-(β-D-arabinofuranosyl)-2,4-quinazolinedione (XIII) was accomplished in two steps from the uridine analog IV (see Scheme III). The method of Nichol and Hampton[10] for inversion of the 2′-hydroxyl was employed. Treatment of IV with diphenyl carbonate and sodium bicarbonate in N,N-dimethylformamide gave O²,2′-anhydro-1-(β-D-arabinofuranosyl)-4-quinazolone (XII) in 90% yield. Treatment of XII with dilute sodium hydroxide on the steam bath gave 1-(β-D-arabinofuranosyl)-2,4-quinazolinedione XIII in quantitative yield. Acid hydrolysis of XIII gave D-arabinose which was characterized by chromatography.

Acetylation of XIII gave 1-(2′,3′,5′-tri-O-acetyl-β-D-arabinofuranosyl)-2,4-quinazolinedione (XIV). Treatment of XIV with phosphorus pentasulfide in pyridine gave 4-thio-1-(2′,3′,5′-tri-O-acetyl-β-D-arabinofuranosyl)-2-quinazolone (XV) which was in turn treated with methanolic ammonia at 100° to give a good yield of 4-amino-1-(β-D-arabinofuranosyl)-2-quinazolone (XVI), the benzo analog of "cytosine arabinoside."

For the synthesis of 1-(2′-deoxy-β-D-ribofuranosyl)-2,4-quinazolinedione (XIX), 2,4-bis(trimethylsilyloxy)quinazoline (II) was treated with 1-chloro-3,5-di-O-*p*-tolyl-2-deoxyribofuranose[11] in acetonitrile (see Scheme IV). An anomeric mixture of nucleosides was obtained which was separated on an alumina column. A 25% yield of pure 1-(3′,5′-di-O-*p*-tolyl-β-D-2′-deoxyribofuranosyl)-2,4-quinazolinedione (XVIII) was obtained plus an additional 35% of mixed anomers. Deblocking XVIII with refluxing sodium methoxide–methanol gave 1-(2′-deoxy-β-D-ribofuranosyl)-2,4-quinazolinedione XIX in 69% yield. Treat-

(9) J. J. Fox, D. Von Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Amer. Chem. Soc.*, **81**, 178 (1959).

(10) A. Hampton and A. W. Nichol, *Biochemistry*, **5**, 2076 (1966).

(11) M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

TABLE I
ULTRAVIOLET ABSORPTION SPECTRA OF VARIOUS QUINAZOLINE NUCLEOSIDES

	λ_{max}^{pH1} mμ	ε	λ_{max}^{pH11} mμ	ε	λ_{max}^{EtOH} mμ	ε
I	309	(3,700)	310	(3,500)	309	(3,800)
	240 (infl)	(9,500)	260 (infl)	(5,300)	241	(9,600)
IV	306	(3,900)	306	(4,200)		
VIII	355	(13,100)	348	(12,300)		
	325 (sh)	(7,300)	274	(5,300)		
	310 (sh)	(6,000)				
	270	(5,400)				
	239	(13,200)				
VII	326	(4,700)	317	(4,500)		
	258	(6,900)				
XIX	307	(4,000)	307	(4,400)		
XXI	326	(4,900)	316	(4,800)		
	258	(6,800)				
XII	309 (infl)	(4,000)	309 (infl)	(4,300)	309	(4,600)
	299.5	(5,000)	300	(5,200)	298	(5,500)
XIII	308	(2,600)	307	(4,300)		
XVI	328	(4,900)	318	(4,900)		
	257	(7,100)				
IX	306	(3,800)	307	(4,000)		
			356 (infl)	(4,800)		
X	305	(3,800)	306	(4,100)		
	240	(8,700)	256	(4,700)		
XI	304	(4,200)	307 (infl)	(5,200)		
			301	(5,600)		
3-N-Methyl-2,4-quinazolinedione	309	(3,700)	326	(3,400)		
	240 (infl)	(9,300)	266	(8,100)		
3-N-Methyl-2,4-quinazolinedione[a]	309	(3,500)	326	(3,300)		
	240 (infl)	(9,000)	266	(7,800)		
1-N-Methyl-2,4-quinazolinedione[b]	312	(4,100)	313	(4,500)		
	242 (infl)	(9,100)				

[a] See ref 7. [b] See ref 8.

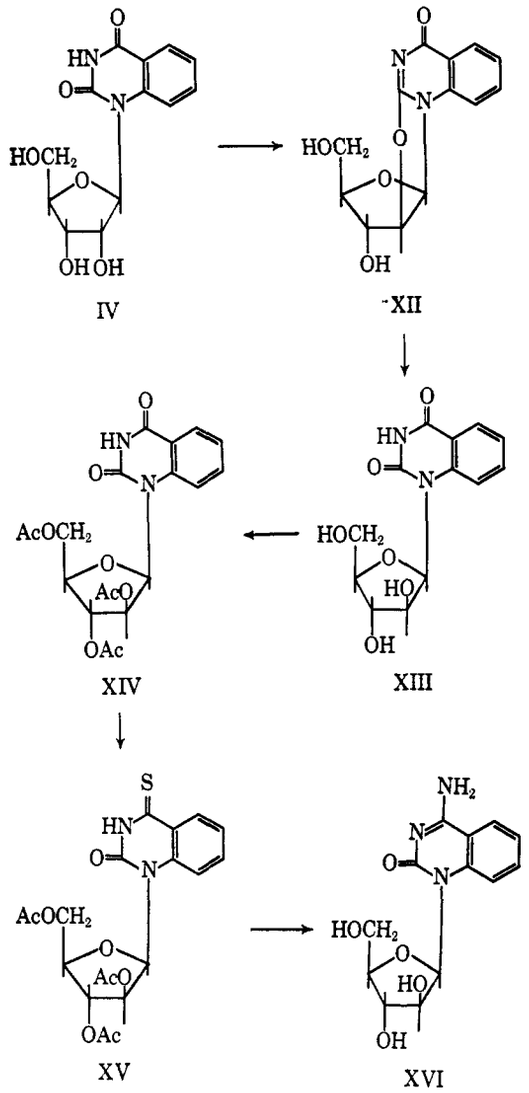
ment of XVIII with phosphorus pentasulfide in pyridine gave XX which in turn gave 4-amino-1-(2′-deoxy-β-D-ribofuranosyl)-2-quinazolone (XXI) with methanolic ammonia. Although no chemical evidence was obtained, the anomeric configuration of XIX (and therefore XXI) was tentatively assigned as β on the basis of the pseudo triplet, $J = 7.5$ cps and peak width was 15.0 cps, due to the anomeric proton.[12] The ultraviolet absorption spectral data for various quinazoline nucleosides are given in Table I.

The spatial limitations for the enzymatic incorporation of a substituted pyrimidine nucleoside into nucleic acid have not been defined although pyrimidines containing groups as large as iodine at the 5 position have been incorporated.[13] In this regard it may be noted that 3-"iso"-adenosine diphosphate is polymerized by azotobacter polynucleotide phosphorylase to yield 3-"iso"-polyadenylic acid.[14] In the present study the fusion of a benzene ring at the pyrimidine positions 5 and 6 results in addition of an aromatic ring in the same plane as the pyrimidine moiety. These derivatives should provide very interesting nucleosides for biochemical study. If the corresponding nucleotides are not incorporated into nucleic acids due to the presence of the benzene ring it is quite possible that these nucleotides may inhibit the polymerase enzymes involved in nucleic acid biosynthesis.

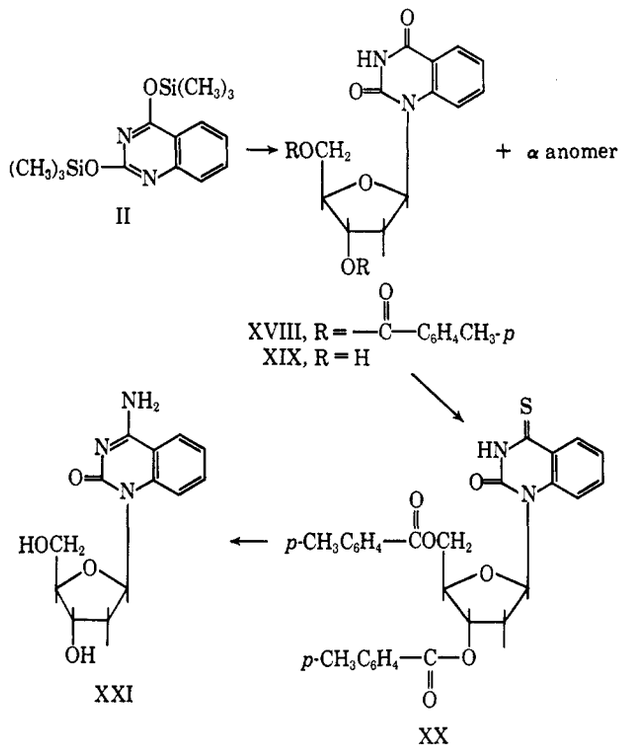
The recent work of Ts'O and coworkers[15-17] has established that vertical stacking of purine with

(12) M. J. Robins and R. K. Robins, *J. Amer. Chem. Soc.*, **87**, 4934 (1965).
(13) W. H. Prusoff, J. J. Jaffe, and H. Gunther, *Biochem. Pharmacol.*, **3**, 110 (1959).
(14) A. M. Michelson, C. Monny, R. A. Laursen, and N. J. Leonard, *Biochim. Biophys. Acta*, **119**, 258 (1966).
(15) P. O. P. Ts'O, I. S. Melvin, and A. C. Olsen, *J. Amer. Chem. Soc.*, **85**, 1289 (1963).
(16) P. O. P. Ts'O and S. I. Chan, *ibid.*, **86**, 4176 (1964).
(17) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'O, *ibid.*, **89**, 3612 (1967).

SCHEME III



SCHEME IV



pyrimidine nucleosides occurs extensively in aqueous solution. It has been recently postulated "that the stacking energy involved in the interaction of the neighboring bases is the major force contributing to the stability of nucleic acid helices." [18] The additional stacking energy due to electron interaction of the benzene ring in the pyrimidine nucleosides described in the present work could contribute significantly to greater stability of a nucleic acid molecule into which these nucleosides are incorporated. Such interactions could well be determined by magnetic resonance spectroscopy. The quinazoline nucleosides described may prove to be important tools for future biochemical studies.

Experimental Section

Melting points were determined with a Thomas–Hoover melting point apparatus and are uncorrected.

2,4-Bis(trimethylsilyloxy)quinazoline (II).—2,4-Quinazolinedione[19] (I) (50 g, 0.31 mole) was azeotropically dried in 2.4 l. of refluxing toluene for 1.5 hr employing a Dean–Stark trap. The solution was cooled to *ca.* 60° and 90 ml of trimethylchlorosilane added. Reflux was again attained and 114 ml of triethylamine dissolved in 400 ml of toluene was added dropwise over a 30-min period. The mixture was refluxed with stirring for an additional 6 hr and left at room temperature overnight. The triethylammonium chloride was removed by filtration and the toluene solution evaporated under reduced pressure. The residue was transferred to a small flask for distillation. The silyl derivative distilled at 93–98° at 0.08 mm and a bath temperature of 178° to yield 78.5 g (83%) of colorless oil.

1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione (III).—2,3,5-Tri-O-benzoyl-D-ribofuranosyl bromide from 25 g (0.05 mole) of 1-O-acetyl-2,3,5-tri-O-benzoylribofuranose was dissolved in 350 ml of dry acetonitrile and 15 g (0.05 mole) of 2,4-bis(trimethylsilyloxy)quinazoline (II) was added. The cloudy solution was stirred for 0.5 hr during which time the solution became clear. The reaction was allowed to proceed at room temperature for 50 hr and the solution then evaporated to dryness under reduced pressure. The resulting foam was dissolved in 180 ml of boiling absolute ethanol and 45 ml of water was added. This solution was stirred and heated on the steam bath for 15 min to achieve crystallization of the product. The mixture was cooled and filtered and the product dried to yield 26.9 g (90%) of III, mp 180–182°. Further recrystallization from 80% ethanol gave fine colorless needles which melted at 180.5–181.5°.

Anal. Calcd for $C_{34}H_{26}N_2O_9$ (mol wt 606.56): C, 67.32; H, 4.32; N, 4.62. Found: C, 67.53; H, 4.40; N, 4.31.

1-(β-D-Ribofuranosyl)-2,4-quinazolinedione (IV). Method A.—1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione (III) (12 g, 0.02 mole) was added to a solution of 1.15 g of sodium in 250 ml of absolute methanol. The solution was refluxed for 0.5 hr then evaporated to dryness under vacuum. The resulting solid was partitioned between 500 ml of water and 150 ml of ether. The aqueous portion was extracted twice more with 100 ml of ether each and the combined ether extracts were washed with 50 ml of water. The aqueous phases were combined and acidified to pH 5 with approximately 60 ml of Dowex 50 × 2 (H^+) resin. The resin was removed by filtration and the aqueous phase concentrated to approximately 40 ml. The cooled solution was filtered and the product recrystallized from a mixture of 700 ml of methanol and 40 ml of water which was concentrated to 150 ml and cooled. The product was filtered and dried to yield 5.08 g (88%) of product, mp 226–227°.

Method B.—1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione (III) (10 g, 16.5 mmole) was heated with 150 ml of methanol (previously saturated with ammonia at

(18) W. M. Huang and P. O. P. Ts'O, *J. Mol. Biol.*, **16**, 523 (1966).

(19) N. A. Lange and F. E. Sheibley, "Organic Synthesis," *Coll. Vol. II*, John Wiley and Sons, Inc., New York, N. Y., 1943, p 79.

0°) in a sealed bomb at 100° for 8 hr. The bomb was cooled overnight, the methanol solution evaporated to dryness and the residue triturated with 400 ml of dichloromethane. The resulting solid was recrystallized from a mixture of 500 ml of methanol and 10 ml of water which was concentrated to 250 ml. The product was removed by filtration and dried to yield 4.43 g (91%) of needles with mp 226–226.5° and $[\alpha]^{28}D$ +16° (*c* 1.0, dimethylformamide). This product was identical with that prepared by method A.

Anal. Calcd for $C_{13}H_{14}N_2O_6$ (mol wt 294.26): C, 53.06; H, 4.80; N, 9.52. Found: C, 53.05; H, 4.96; N, 9.37.

1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-4-thio-2-quinazolone (V).—To a solution of 6.0 g (0.01 mole) of 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione (III) in 100 ml of pyridine was added, with stirring, 8.9 g (0.038 mole) of phosphorus pentasulfide. The solution was refluxed gently (bath temperature 130–140°) for 17 hr. Completion of reaction was determined by tlc on alumina HF 254 plates with methanol–ethyl acetate (1:20) as solvent. The pyridine solution was evaporated to one-third the original volume and poured into 2 l. of water at 60°. The mixture was stirred for 2–3 hr then left at room temperature overnight. The resulting solid was filtered, dissolved in chloroform, which was dried and removed under vacuum. The resulting foam was boiled with 150 ml of methanol to induce crystallization. The solid was filtered and dried to yield 5.75 g (93%) of yellow solid which melted at 180–181°. Tlc on silicAR 7GF with chloroform–ethyl acetate (9:1) showed *ca.* 3–5% unconverted III. The analytical sample was prepared by preparative plate chromatography on silicAR 7GF with the same solvent and V recrystallized from ethanol–benzene (10:1). The analytical sample melted at 179.5–180.5°.

Anal. Calcd for $C_{34}H_{26}N_2O_8S$ (mol wt 622.63): C, 65.58; H, 4.21; N, 4.50. Found: C, 65.81; H, 4.47; N, 4.66.

4-Amino-1-(β-D-ribofuranosyl)-2-quinazolone (VII). Method A.—1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-4-thio-2-quinazolone (V) (2 g) was heated in 150 ml of methanol (previously saturated with ammonia at 0°) in a bomb at 100° for 7 hr and then cooled to room temperature overnight. The resulting solution was concentrated under vacuum. The residue was triturated with 250 ml of dichloromethane and the resultant solid was recrystallized from 180 ml of methanol and 15 ml of water concentrated to *ca.* 25 ml. The product weighed 0.77 g (82%) and melted at 259–260° dec.

Method B.—1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-2,4-quinazolinedione (III) (6 g, 0.01 mole) was refluxed in a solution of 60 ml of phosphorus oxychloride and 3 ml of N,N-diethylaniline for 10 min. The phosphorus oxychloride and N,N-diethylaniline were removed under vacuum and the resulting foam was dissolved in 20 ml of chloroform. This solution was poured slowly into a vigorously stirred ice-cold solution of 2% sodium hydroxide (200 ml). Dilute sodium hydroxide was added as necessary to keep the pH greater than 11 at all times. The basic solution was extracted with two 100-ml and two 50-ml portions of chloroform. The combined chloroform solution was then washed with ice water and saturated salt solution and dried over sodium sulfate containing a little potassium carbonate. The yellow solution was evaporated to dryness; the oil was placed in a sealed bomb with 170 ml of methanolic ammonia and heated at 100° for 7 hr. The cooled methanol solution was evaporated, the residue triturated with dichloromethane, and the solid, mp 140–180°, crystallized from 400 ml of methanol and 50 ml of water which was concentrated to *ca.* 80 ml. This solution was cooled to room temperature and filtered. The product was a mixture of 4-amino-1-(β-D-ribofuranosyl)-2-quinazolone (VII) and 1-(β-D-ribofuranosyl)-2,4-quinazolinedione (IV). Separation of VII from IV was easily achieved by fractional crystallization since VII crystallizes first from a methanol–water mixture. Yield of the cytidine analog VII was 1.52 g (52%) and of the uridine analog IV was 0.90 g (31%). One additional recrystallization of VII from methanol–water gave material which melted at 259–260° dec; $[\alpha]^{28}D$ −3° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{13}H_{15}N_3O_5$ (mol wt 293.27): C, 53.24; H, 5.16; N, 14.33. Found: C, 53.25; H, 5.24; N, 14.33.

1-(β-D-Ribofuranosyl)-4-thio-2-quinazolone (VIII).—A solution of 0.5 g (1.7 mmoles) of 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-4-thio-2-quinazolone (V) and 0.1 g (4.3 g-atoms) of sodium in 15 ml of absolute methanol was refluxed gently for 70 min. The methanol was evaporated and the residue partitioned between 100 ml of water and 25 ml of ether. The aqueous phase was extracted with two 10-ml portions of ether then acidified to pH 6 with 10% hydrochloric acid. The solution was concentrated to *ca.* 5 ml and the solid filtered. This solid was recrystallized twice from ethanol and melted at 179–180°, $[\alpha]^{28}D$ +53° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{13}H_{14}N_2O_5S$ (mol wt 310.32): C, 50.31; H, 4.55; N, 9.03. Found: C, 49.96; H, 4.88; N, 8.69.

3-N-Methyl-2,4-quinazolinedione from Methylation of IV.—1-(β-D-Ribofuranosyl)-2,4-quinazolinedione (IV) (0.29 g, 1 mmole) was stirred in 2 ml of dry dimethylformamide containing 0.1 g of dry potassium carbonate and 0.01 ml of methyl iodide for 20 hr at room temperature. The potassium carbonate was filtered and washed with 1 ml of dimethylformamide. The filtrate was diluted with 25 ml of dichloromethane and the resulting solid removed by filtration. This filtrate was taken to dryness and the residue refluxed in 10 ml of 10% hydrochloric acid for 1 hr. The solution was filtered hot and gave tan needles on cooling. The needles were recrystallized from ethanol–water using charcoal. The resulting needles were sublimed for analysis (bath temperature 175–180°), and melted at 240–241°.

Anal. Calcd for $C_9H_8N_2O_2$ (mol wt 176.17): C, 61.36; H, 4.58; N, 15.90. Found: C, 60.82; H, 4.43; N, 16.10.

This material on mixture melting point determination with authentic material prepared by the method of Steiger and Wagner[7] was undepressed and the ir and uv curves were identical.

1-(2′,3′-O-Isopropylidene-β-D-ribofuranosyl)-2,4-quinazolinedione (IX).—A solution of 450 ml of dry acetone, 1.60 ml of 2,2-dimethoxypropane, and 2.0 ml of 70% perchloric acid was prepared and left at room temperature for 5 min. To this solution was added 1.32 g (4.5 mmoles) of 1-(β-D-ribofuranosyl)-2,4-quinazolinedione (IV) and the mixture was stirred for 0.5 hr. Pyridine (1.6 ml) was added and the solution was concentrated to *ca.* 70 ml where 23 ml of 10% sodium carbonate was added. The solution was again concentrated to remove the remaining acetone. The aqueous solution was then extracted four times with dichloromethane (one 25-ml portion and three 10-ml portions). The combined dichloromethane solution was dried and evaporated. The resulting glass was crystallized by solution in 30 ml of benzene followed by the addition of 20 ml of cyclohexane to yield 1.43 g (95%) of material which melted at 120–123°. Crystallization of IX from benzene–cyclohexane gave pure product which melted at 125–127°.

Anal. Calcd for $C_{16}H_{18}N_2O_6$ (mol wt 334.32): C, 57.48; H, 5.43; N, 8.38. Found: C, 58.05; H, 5.80; N, 8.17.

1-(2′,3′-O-Isopropylidene-5′-methanesulfonyl-β-D-ribofuranosyl)-2,4-quinazolinedione (X).—A solution of 0.85 g of 1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-2,4-quinazolinedione (IX) and 0.21 ml of methanesulfonyl chloride (1.1 moles) in 15 ml of dry pyridine was left in the refrigerator for 15 hr and then at room temperature for an additional 7 hr. Water (0.45 ml, 10 moles) was then added and the solution stirred at room temperature for 45 min. At this time 25 ml of water was added and the solution extracted with three 25-ml portions of chloroform. The chloroform was washed with six 10-ml portions of ice-cold 3 *N* sulfuric acid until the washes were distinctly acidic and then twice with 5% sodium bicarbonate (20 ml and 5 ml) until the washes were basic. The chloroform solution was then dried over sodium sulfate and taken to dryness. The foam was crystallized by solution in 8 ml of benzene followed by the addition of 1 ml of cyclohexane to yield 0.95 g of crystals which melted at 190–192° with some softening *ca.* 125°. The product was recrystallized from benzene–methanol and melted at 190–192°.

Anal. Calcd for $C_{17}H_{20}N_2O_8S$ (mol wt 412.41): C, 49.51; H, 4.89; N, 6.79. Found: C, 49.16; H, 4.99; N, 6.71.

O²,5′-Anhydro-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-quinazolone (XI). Method A.—To a refluxing mixture of 0.4 g (0.97 mmole) of 1-(2′,3′-O-isopropylidene-5′-methanesulfonyl-β-D-ribofuranosyl)-2,4-quinazolinedione and 0.3 g of anhydrous sodium iodide in 6 ml of dioxane was added six drops of water and an additional 6 ml of dioxane. After 1.5 hr refluxing the bumping from precipitated salts was such that six more drops of water and 2 ml of dioxane were added and reflux continued 1 hr. To the cooled solution was added 50 ml of dichloromethane and this solution was extracted with three 10-ml portions of water. The water was back extracted with 5 ml of dichloromethane and the combined organic phases were washed with saturated salt solution and then dried over sodium sulfate.

The dichloromethane was removed under vacuum and the resulting oil plus 0.7 g of silver acetate was refluxed for 15 min in 100 ml of absolute methanol. The precipitated silver iodide was filtered and the filtrate saturated with hydrogen sulfide to remove any residual silver. Again the methanol was filtered and the filtrate concentrated to *ca.* 2 ml. The resulting precipitate was recrystallized from 15 ml of benzene and 0.5 ml of methanol concentrated to 10 ml and then 1 ml of cyclohexane added. The compound began to darken at 270° and melted at 280–285° dec.

Anal. Calcd for $C_{16}H_{16}N_2O_5$ (mol wt 316.30): C, 60.75; H, 5.10; N, 8.86. Found: C, 60.55; H, 5.25; N, 8.88.

Method B.—A solution of 0.81 g (0.002 mole) of 1-(2′,3′-O-isopropylidene-5′-O-methanesulfonyl-β-D-ribofuranosyl)-2,4-quinazolinedione (X) in 30 ml of dry dimethylformamide and 4 ml of 0.5 *M* potassium *t*-butoxide in *t*-butyl alcohol was heated at 100° for 1.5 hr. The gellike solution was taken to dryness and the residue extracted twice with boiling chloroform. The chloroform was taken to dryness and the residue crystallized from 30 ml of benzene and 4 ml of methanol concentrated to 10 ml. This gave 0.43 g of product (90%) which melted at 280° after softening at 245°. This product (XI) was identical with that prepared by method A.

1-(3′,5′-Di-O-*p*-tolyl-β-D-2′-deoxyribofuranosyl)-2,4-quinazolinedione (XVIII).—3,5-Di-O-*p*-tolyl-2-deoxyribofuranosyl chloride[11] (14 g, 0.035 mole) and 15 g (0.05 mole) of 2,4-bis(trimethylsilyloxy)quinazoline (II) were reacted in 300 ml of dry acetonitrile. The solution remained cloudy throughout the reaction period. After 42 hr the reaction mixture was evaporated to dryness. The trimethylsilyl groups were hydrolyzed by dissolving the product in 100 ml of boiling ethanol and adding 20 ml of water. This solution was boiled for several minutes then evaporated to dryness under vacuum. The solid was triturated with 500 ml of dichloromethane, filtered, and evaporated leaving 16.61 g of mixed deoxynucleosides plus some sugar. The α and β anomers could be separated by tlc on silicAR 7GF using chloroform–ethyl acetate (4:1).

Purification was achieved by column chromatography on neutral alumina (Merck). The column was 2 in. in diameter by 22 in. high and contained 800 g of alumina. The column was packed in benzene. The nucleoside mixture was dissolved in benzene and applied to the column. The sugar was washed through in 550 ml of 20% benzene–ethyl acetate and an additional 1100 ml of 20% benzene–ethyl acetate eluted nothing more. The eluent was changed to ethyl acetate and after 1700 ml of ethyl acetate the β anomer began to be eluted. The following 4450 ml contained pure anomer which weighed 4.7 g on evaporation of the solvent. From this point the α anomer was eluted with the β anomer.

Total weight of material eluted from the column was 14.9 g (90%) with 3.8 g of sugar, 4.7 g of pure β anomer (25.4%), 6.2 g of mixed anomers, and 0.2 g of α anomer. The over-all yield of α and β anomers was 60%.

The pure β anomer was recrystallized from benzene–cyclohexane and gave 4.21 g which melted at 126–131°. Further crystallization from the same solvent gave material which melted at 129–134°.

Anal. Calcd for $C_{29}H_{26}N_2O_7$ (mol wt 514.41): C, 67.69; H, 5.09; N, 5.45. Found: C, 67.47; H, 5.12; N, 5.45.

1-(β-D-2′-Deoxyribofuranosyl)-2,4-quinazolinedione (XIX).—1-(3′,5′-Di-O-*p*-tolyl-β-D-2′-deoxyribofuranosyl)-2,4-quinazolinedione (XVIII) was added to a solution of 0.1 g (4.3 g-atoms) of sodium dissolved in 21 ml of absolute methanol and the solution was allowed to reflux gently for 30 min. After evaporation of the solvent the residue was partitioned between 100 ml of water and 25 ml of ether. After two more extractions with ether the organic phases were combined and back extracted with 15 ml of water. The aqueous phases were combined and carefully acidified to pH 5 with 6 ml of Dowex 50 × 2 (H^+) resin. The resin was removed by filtration and the filtrate concentrated to 3 ml, cooled and filtered and the solid dried leaving 0.34 g of colorless needles which melted at 159–164°. This material was recrystallized from benzene–methanol to yield 0.26 g (69%) which melted at 167.5–168°, $[\alpha]^{28}D$ +39° (*c* 1.0 dimethylformamide).

Anal. Calcd for $C_{13}H_{14}N_2O_5$ (mol wt 278.26): C, 56.11; H, 5.07; N, 10.07. Found: C, 56.14; H, 5.22; N, 10.23.

1-(3′,5′-Di-O-*p*-tolyl-β-D-2′-deoxyribofuranosyl)-4-thio-2-quinazolone (XX).—To a solution of 1.57 g (3.05 mmoles) of 1-(3′,5′-di-O-*p*-tolyl-β-D-2′-deoxyribofuranosyl)-2,4-quinazolinedione (XVIII) in 45 ml of pyridine was added 2.1 g (9.4 mmol) of phosphorus pentasulfide and the solution gently refluxed for 13 hr (bath temperature 120–130°). Completion of the reaction was determined by tlc on alumina HF 254 plates using methanol–ethyl acetate (1:9) as solvent. At this point there was some unreacted XVIII but also there was some cleavage of the nucleoside. The solution was reduced by *ca.* two-thirds under vacuum and the remaining pyridine solution poured into 700 ml of water at *ca.* 45°. The solution, after cooling, was extracted seven times with chloroform (one 100-ml and six 50-ml portions). The chloroform extracts were combined and washed with ice water and saturated salt solution and then dried over sodium sulfate. The chloroform was removed under vacuum and the residual foam crystallized by boiling in 50 ml of methanol with vigorous stirring. This yielded 1.22 g (75%) of yellow crystals which melted at 194–196°. Purification was achieved by preparative layer chromatography on silicAR 7GF with ethyl acetate–chloroform (1:20) as solvent and the product was crystallized from benzene–ethanol. The analytical sample melted at 195.5–196°.

Anal. Calcd for $C_{29}H_{26}N_2O_6S$ (mol wt 530.58): C, 65.64; H, 4.94; N, 5.28. Found: C, 65.43; H, 5.07; N, 5.31.

4-Amino-1-(β-D-2′-deoxyribofuranosyl)-2-quinazolone (XXI).—A mixture 0.75 g (1.4 mmoles) of 1-(3′,5′-di-O-*p*-tolyl-β-D-2′-deoxyribofuranosyl)-4-thio-2-quinazolone (XX) in 100 ml of methanolic ammonia (saturated at 0°) was left at room temperature in a pressure bottle for 3 days. The methanolic solution was taken to dryness and the residue triturated with 10 ml of dichloromethane and 50 ml of ether. The solid was filtered and recrystallized from a methanol and water mixture. The deoxycytidine analog XXI melted at 212.5–213°; $[\alpha]^{28}D$ +72° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{13}H_{15}N_3O_4 \cdot {}^1/_2H_2O$ (mol wt 286.28): C, 54.54; H, 5.63; N, 14.68. Found: C, 54.19; H, 5.65; N, 14.70.

O²,2′-Anhydro-1-(β-D-arabinofuranosyl)-4-quinazolone (XII).—A mixture of 3.0 g (1.02 mmoles) of 1-(β-D-ribofuranosyl)-2,4-quinazolinedione (IV), 2.85 g (1.33 mmoles) of diphenyl carbonate, and 0.05 g of sodium bicarbonate in 10 ml of dry dimethylformamide was heated at 150° for 30 min. There was an initial vigorous evolution of gas which subsided after 5 min. Tlc on silicAR 7GF with ethyl acetate–methanol (9:1) as solvent showed very little starting material after 10 min. The refluxing solution was allowed to cool for a few minutes and then poured slowly into 800 ml of vigorously stirred ether. The precipitate was filtered and recrystallized from 200 ml of methanol concentrated to 100 ml. The filtered and dried solid weighed 2.53 g (90%) and melted at 259–259.5°.

Anal. Calcd for $C_{13}H_{12}N_2O_5$ (mol wt 276.24): C, 56.52; H, 4.38; N, 10.14. Found: C, 56.56; H, 4.42; N, 10.12.

1-(β-D-Arabinofuranosyl)-2,4-quinazolinedione (XIII).—O²,2′-Anhydro-1-(β-D-arabinofuranosyl)-4-quinazolone (XII) (1 g) was heated on the steam bath in 50 ml of 0.05 *M* sodium hydroxide for 15 min. Completion of the hydrolysis was determined by tlc on silicAR 7GF with water as developing solvent. The solution was cooled and neutralized to pH 5–6 with Dowex 50 × 2 (H^+). The resin was filtered and the aqueous solution concentrated to dryness. The solid was recrystallized from 50 ml of methanol and 7 ml of water concentrated to 5 ml. The thick colorless rods weighed 1.03 g (98%) and melted at 209–211° but resolidified as it apparently lost the sugar, $[\alpha]^{28}D$ −18° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{13}H_{14}N_2O_6$ (mol wt 294.26): C, 53.06; H, 4.80; N, 9.52. Found: C, 52.99; H, 4.80; N, 9.70.

1-(2′,3′,5′-Tri-O-acetyl-β-D-arabinofuranosyl)-2,4-quinazolinedione (XIV).—A solution of 0.90 g (3.05 mmoles) of 1-(β-D-arabinofuranosyl)-2,4-quinazolinedione (XIII) in a mixture of 10 ml of pyridine and 10 ml of acetic anhydride was left at room temperature overnight. The clear solution was poured into a well-stirred mixture of ice water (200 ml). The mixture was stirred at 0° for 2 hr then filtered. The solid was recrystallized from methanol by the addition of water to give 1.30 g (100%) which melted at 205–206°.

Anal. Calcd for $C_{19}H_{20}N_2O_9$ (mol wt 420.37): C, 54.28; H, 4.80; N, 6.66. Found: C, 54.40; H, 4.85; N, 6.53.

1-(2′,3′,5′-Tri-O-acetyl-β-D-arabinofuranosyl)-4-thio-2-quinazolone (XV).—1-(2′,3′,5′-Tri-O-acetyl-β-D-arabinofuranosyl)-2,4-quinazolinedione (XIV) (1.30 g, 3.1 mmoles) was dissolved in 50 ml of pyridine and 2.9 g (12.5 mmoles) of phosphorus pentasulfide was added. The solution was stirred at reflux for 7 hr (bath temperature 140°). Completion of the reaction

was determined by tlc on alumina HF 254 with methanol–ethyl acetate (1:20) as solvent. One-half to two-thirds of the pyridine was removed under vacuum and the remaining solution was poured into 900 ml of water at 65–70°. This solution was stirred and cooled as the thione crystallized. The yield was 1.02 g (77%) which melted at 149–150°.

4-Amino-1-(β-D-arabinofuranosyl)-2-quinazolone (XVI).—1-(2′,3′,5′-Tri-O-acetyl-β-D-arabinofuranosyl)-4-thio-2-quinazolone (XV) (0.90 g, 2.05 mmoles) was heated in 125 ml of methanolic ammonia at 100° for 6 hr in a bomb. The reaction was cooled and filtered and the filtrate taken to dryness. Crystallization of the residue from 60 ml of methanol and 10 ml of water concentrated to 13 ml gave 0.38 g (63%) of XV. The product was crystallized from methanol–water. The melting point was 217–219° dec; $[\alpha]^{28}D$ −24° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{13}H_{15}N_3O_5 \cdot H_2O$ (mol wt 311.29): C, 50.15; H, 5.50; N, 13.50. Found: C, 50.28; H, 5.43; N, 13.48.

2-Amino-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-quinazolone.—O²,5′-Anhydro-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-quinazolone (XI) (660 mg) was treated with 50 ml of liquid ammonia in a sealed bomb at room temperature for 50 hr. After removal of the ammonia the product was crystallized from benzene–methanol and gave colorless needles which melted at 171–172° after sintering at 168°.

Anal. Calcd for $C_{16}H_{19}N_3O_5$ (mol wt 333.34): C, 57.65; H, 5.75; N, 12.61. Found: C, 58.35; H, 5.92; N, 12.33.

2-Amino-4-quinazolone.—All attempts to remove the isopropylidene group from 2-amino-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-quinazolone proved futile as the sugar linkage was too labile under the conditions necessary to remove the isopropylidene group. The nucleoside (200 mg) was hydrolyzed by heating on the steam bath in 25 ml of 20% formic acid for 10 min. The resulting 2-amino-4-quinazolone which was isolated was identical with the product described by Trattner, *et al.*[20]

Hydrolysis of 1-(β-D-Arabinofuranosyl)-2,4-quinazolinedione (XIII).—A solution of 5–10 mg of XIII in 2 ml of 10% hydrochloric acid was heated on the steam bath for 1 hr. The cooled solution yielded 2,4-quinazolinedione (I) as a precipitate. The filtrate was chromatogrammed against D-ribose, 2-deoxy-D-ribose, D-arabinose, and D-xylose on Whatman No. 1 paper using the ethyl acetate–*n*-propyl alcohol–water (4:1:2) system described by Hall.[21] A second chromatogram was run using butanol saturated with water as solvent. Both chromatograms were run for 40 hr and gave good separation of the known sugars with the unknown sugar at the same R_f as arabinose. The sugars were detected using the aniline hydrogen phthalate spray of Partridge.[22]

Registry No.—I, 86-96-4; II, 15135-19-0; III, 15135-20-3; IV, 15135-21-4; V, 15180-27-5; VII, 15135-22-5; VIII, 15135-23-6; IX, 15185-75-8; X, 15185-76-7; XI, 15135-24-7; XII, 15135-25-8; XIII, 15135-26-9; XIV, 15185-77-0; XV, 15180-28-6; XVI, 15135-27-0; XVIII, 15135-28-1; XIX, 15135-29-2; XX, 15135-30-5; XXI, 15135-31-6; 3-N-methyl-2,4-quinazolinedione, 607-19-2; 2-amino-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-quinazolone, 15135-33-8.

(20) R. B. Trattner, G. B. Elion, G. H. Hitchings, and D. M. Sharefkin, *J. Org. Chem.*, **29**, 2674 (1964).
(21) R. H. Hall, *Anal. Biochem.*, **4**, 395 (1962).
(22) S. M. Partridge, *Nature*, **164**, 443 (1949).

Notes

The Isolation and Structural Elucidation of Voacristine Hydroxyindolenine

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Earlier reports have described the isolation from *Ervatamia dichotoma* (Roxb.) Blatter of coronaridine[4] and heyneanine.[5] We report herewith the isolation and characterization of voacristine hydroxyindolenine and its synthesis from voacristine.

Partition of the ethanol extract of *E. dichotoma* root bark between ether and 4% hydrochloric acid gave a crude alkaloid fraction upon neutralization of the acid and extraction with ether. The ether extract was washed with 1% sodium hydroxide to remove the phenolic bases and the nonphenolic bases were chromatographed on neutral alumina. Elution with benzene–chloroform (1:1) yielded a fraction which was further fractionated by partition chromatography.[6] Six purple bands were visible on the partition column. After elution, the third band yielded heyneanine (I).[5] The fourth band was rechromatographed on a partition column and yielded four bands. Treatment of the third band with benzene–Skellysolve B gave a crystalline material. Recrystallization from the same solvent system yielded colorless crystals, $C_{22}H_{28}N_2O_5$ (by high resolution mass spectrometry), mp 176–179° dec, $[\alpha]^{26}D$ −22° (*c* 0.51, chloroform). The ultraviolet spectrum (λ_{max}^{EtOH} 229.5, 268, 291, 300 (sh), 314; 12,380, 4400, 4780, 4410, 3810) and infrared spectrum suggested an indole-type alkaloid bearing substituents in the aromatic ring. The infrared spectrum showed broad bands at 2.80 (w) and 3.10 μ (m), indicative of the presence of hydroxyl groups, and a band at 5.76 μ (s), indicative of the presence of a carbomethoxy group. The nmr spectrum contained a doublet (1 H) centered at τ 2.66 (J_{ortho} = 8 cps), a doublet of

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(3) University of Wisconsin. The investigation at the University of Wisconsin was supported by Public Health Service Grant No. HE-02952 from the National Heart Institute.
(4) S. M. Kupchan, A. Bright, and E. Macko, *J. Pharm. Sci.*, **52**, 598 (1963).
(5) S. M. Kupchan, J. M. Cassady, and S. A. Telang, *Tetrahedron Lett.*, 1251 (1966).
(6) K. S. Brown, Jr., and S. M. Kupchan, *J. Chromatog.*, **9**, 71 (1962).