5-Alkylpyrimidine Nucleosides. Preparation and Properties of 5-Ethyl-2′-deoxycytidine and Related Nucleosides

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The α and β anomers of 5-ethyl-2′-deoxycytidine have been prepared (a) by thiation and amination of the known α,β-3′,5′-di-*O*-(*p*-chlorobenzoyl)-5-ethyl-2′-deoxyuridine and (b) by condensation of O^2,N^4-bis(trimethylsilyl)-5-ethylcytosine with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-*erythro*-pentofuranosyl chloride. The former procedure simultaneously makes available the 4-thiothymidine analogs, α- and β-5-ethyl-4-thio-2′-deoxyuridines, while the latter provides an alternative route to the known antiviral thymidine analog, 5-ethyl-2′-deoxyuridine. Minor improvements have been introduced in the thiation procedure. Furthermore, the relative mobilities on silica gel of the α and β anomers of the blocked thiated nucleosides differed sufficiently to permit their preparative separation by tlc with only a single development; the possible general applicability of this fact to fractionation of anomeric nucleosides is emphasized. Circular dichroism data for all the anomeric nucleoside pairs are reported. The β anomer of 5-ethyl-2′-deoxycytidine exhibited low, but significant, activity against herpes simplex virus, but not vaccinia or vesicular stomatitis viruses, in primary rabbit kidney cell cultures. In combination with 5-fluorouracil, β-5-ethyl-2′-deoxycytidine provoked additional reversible growth inhibition in *Salmonella typhimurium* following its *in vivo* deamination to 5-ethyl-2′-deoxyuridine. The α and β anomers of 5-ethyl-2′-deoxycytidine 5′-phosphate were prepared by standard phosphorylation procedures; the α anomer was dephosphorylated by snake venom 5′-nucleotidase at a rate comparable to that for the β anomer, a result of some interest in relation to the specificity of 5′-nucleotidase.

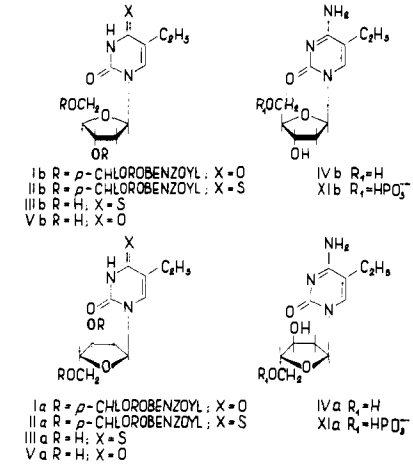
Following earlier reports on antiviral activity of 5-ethyluracil,[1,2] it was established that 5-ethyl-2′-deoxyuridine[3-5] exhibits high activity *vs.* herpes simplex and vaccinia viruses, comparable to that of 5-iodo-2′-deoxyuridine.[4,6-8] This, in turn, pointed to the utility of preparing 5-ethyl-2′-deoxycytidine for biological testing. Additional interest attaches to the preparation of 5-ethyl-2′-deoxycytidine in view of the ability of 5-ethyluracil to undergo incorporation into bacterial DNA,[9] of 5-ethyl-2′-deoxyuridine into phage DNA,[10,11] the apparent absence of mutagenic effects of 5-ethyl-2′-deoxyuridine in a phage system,[4,11] and the recent isolation of a phage DNA in which the 5-methyl of thymine is replaced by a substituted pentyl chain.[12,13] Furthermore, the introduction of 5-ethyl substituents into the residues of polyuridylic[14,15] and polycytidylic[16] acids, or natural nucleic acids,[10,11] leads to destabilization of their helical structures. It is also not without relevance that poly 5-ethyluridylic acid is active as an *in vitro* messenger.†

One pathway to the α and β anomers of 5-ethyl-2′-deoxycytidine (IVa and IVb) is *via* thiation and subsequent amination of the previously reported[4] α,β-1-(3,5-di-*O*-*p*-chlorobenzoyl-2-deoxy-D-ribofuranosyl)-5-ethyluracil (Ia and Ib). Thiation of Ia,b with an excess of P_2S_5 in pyridine[17] gave a yield of about 35%. Thiation in dioxane[18-20] under reflux for only 30–45 min (progress of the reaction being monitored by tlc on silica gel with solvent F) made it possible to reduce the P_2S_5 concentration to 1 mol equiv with no reduction in overall yield of IIa,b and facilitating subsequent preparative tlc separation of IIa and IIb, with overall yields of about 65%.

The difference in mobilities in solvent F between IIa and IIb (much more pronounced than for the corresponding ketonucleosides Ia and Ib, see Table I) was such that a complete separation of a 60-mg mixture was obtained with only one development on a 2-mm layer of silica gel on a single 20 × 20 cm plate. This suggested simplification of the conventional tedious separation of anomers (normally done at the level of protected ketonucleosides, followed by thiation and amination of each anomer), as follows. The mixture of protected anomeric ketonucleosides is thiated and the mixture of 4-thio anomers separated with improved yield (~85%) by tlc as above; the α and β anomers are then deblocked to the corresponding 4-thionucleosides or aminated to give the aminonucleosides.

Removal of protecting groups from IIa and IIb, by treatment with CH_3ONa, gave the corresponding α- and β-5-ethyl-4-thio-2′-deoxyuridines (IIIa and IIIb).

Amination of IIa and IIb with methanolic ammonia at 120° gave the desired α and β anomers of 5-ethyl-2′-deoxycytidine (IVa and IVb), which were deaminated with HNO_2 at room temperature[21] to provide quantitative yields of α- and β-5-ethyldeoxyuridine, with properties identical with those of authentic samples.



A second, larger scale preparation of IVa and IVb was by means of a modification of the Hilbert–Johnson procedure.[21,22] The previously reported 5-ethylcytosine (VI)[23] was silylated with TMCS in anhydrous C_6H_6 in the presence of Et_3N to give O^2,N^4-bis(trimethylsilyl)-5-ethylcytosine (VII). This was condensed with 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl chloride (VIII)[24] in anhydrous acetonitrile in the presence of molecular sieves or mercuric bromide.

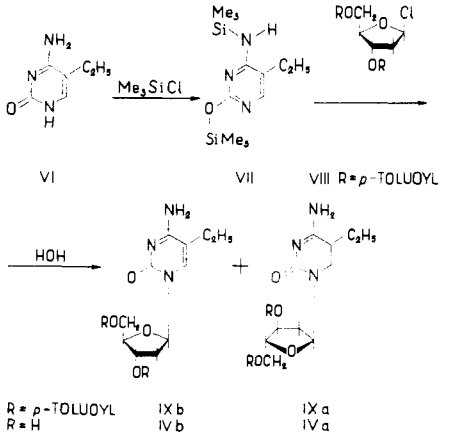
The overall yield of the α and β anomers of 1-(3,5-di-*O*-*p*-toluoyl-2-deoxy-D-ribofuranosyl)-5-ethylcytosine (IXa and IXb) obtained with the use of molecular sieves was 63% but the ratio of IXa to IXb was only 5:1. An improved yield of the biologically important β anomer (IXa:IXb = 5:2) resulted when the molecular sieves were re-

† L. Nowak, unpublished work, Polish Academy of Sciences, 1969.

Table I. Thin-Layer Chromatography of 5-Ethylpyrimidine 2′-Deoxynucleosides[a]

Compound	R_f or ^nR_f values in solvent systems								
	A	B	C	D	E	F	G	H	I
Uracil	0.43	0.53	0.77	0.41					
5-Ethyluracil	0.72	0.82	0.89						
1-(3,5-Di-*O*-*p*-chlorobenzoyl-2-deoxy-α-D-ribofuranosyl)-5-ethyluracil (Ia)						0.26			
1-(3,5-Di-*O*-*p*-chlorobenzoyl-2-deoxy-β-D-ribofuranosyl)-5-ethyluracil (Ib)						0.32			
1-(3,5-Di-*O*-*p*-chlorobenzoyl-2-deoxy-α-D-ribofuranosyl)-5-ethyl-4-thiouracil (IIa)				0.77	0.78	0.49			
1-(3,5-Di-*O*-*p*-chlorobenzoyl-2-deoxy-β-D-ribofuranosyl)-5-ethyl-4-thiouracil (IIb)				0.79	0.81	0.65			
α-5-Ethyl-4-thio-2′-deoxyuridine (IIIa)				0.40	0.26				
β-5-Ethyl-4-thio-2′-deoxyuridine (IIIb)				0.42	0.29				
α-5-Ethyl-2′-deoxycytidine (IVa)	0.68	0.59	0.26					[9]0.72	0.79
β-5-Ethyl-2′-deoxycytidine (IVb)	0.69	0.60	0.30					[9]0.80	0.79
α-5-Ethyl-2′-deoxyuridine (Va)	0.75	0.81	0.86	0.34	0.18				
β-5-Ethyl-2′-deoxyuridine (Vb)	0.73	0.82	0.86	0.33	0.19				
5-Ethylcytosine (VI)	0.62	0.60	0.12						
1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-α-D-ribofuranosyl)-5-ethylcytosine (IXa)							[5]0.34		
1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-ethylcytosine (IXb)							[5]0.46		
α-5-Ethyl-2′-deoxycytidine 5′-phosphate (XIa)	0.08							0.0	0.60
β-5-Ethyl-2′-deoxycytidine 5′-phosphate (XIb)	0.07							0.0	0.61

[a] For solvents and other details see Experimental Section.



placed by $HgBr_2$, but at the expense of the overall yield (42%).

The mixture of blocked anomers was subjected to fractional crystallization from ethanol, yielding most (60–82%) of the α anomer IXa, essentially free of IXb. The mother liquors, containing IXa and IXb in the ratio 1:1, were treated in two different manners. The first of these involved the separation, by preparative chromatography on silica gel HF_{254} with solvent system G, of IXa from IXb, followed by removal of protecting groups with MeONa in MeOH, and column chromatography on Dowex 50W (H^+) to yield purified IVa and IVb.

The second method, more convenient for use on a small (milligram) scale, involved removal of the blocking groups from the mixture of anomers, followed by separation of the free nucleoside anomers IVa and IVb on HF_{254} silica gel with solvent system H. This procedure[25] is widely applicable to anomeric mixtures of various unblocked nucleosides and possesses the additional advantage of rapidity, as well as applicability to the analysis of nucleosides isolated from biological materials for the possible presence of α anomers, bearing in mind that minor quantities of the α anomers of cytidine 2′-phosphate and cytidine 3′-phosphate have been isolated from yeast RNA.[26]

As expected, the purified anomers IVa and IVb exhibit uv spectra which are virtually identical with that for 5-methyl-2′-deoxycytidine (X); both are slightly stronger bases (pK = 4.61 and 4.65) than X (pK = 4.40). The CD spectrum of IVb is also similar to that for X, but with a somewhat higher molar ellipticity, as can be seen from Table II which presents CD data for the other 5-ethyl-2′-deoxynucleosides.

The nmr spectra are consistent with the structures of IVa and IVb. The anomeric $H_{1'}$ protons of IVa and IVb show an appreciable difference in chemical shifts, 5.97 (quartet) and 5.51 ppm (triplet), respectively. The difference in values of $J_{1'-2''}$ is less marked (7.0 Hz for IVa and 6.3 Hz for IVb) than for $J_{1'-2'}$ (2.5 and 6.3 Hz, respectively), while for the β anomer IVb, $J_{1'-2'} = J_{1'-2''}$. The protons of the 5-ethyl substituents of IVa and IVb exhibit an approximately constant difference in chemical shifts of about 1.2 ppm: $\delta(CH_2)$ (quartet) 2.15 and 1.68 ppm, $\delta(CH_3)$ (triplet) 0.94 and 0.43 ppm. For the anomers Ia and Ib the values of $J(CH_2-CH_3)$ are not significantly different, 7.2 and 7.5 Hz, respectively.

Both IVa and IVb were phosphorylated according to the procedure of Tener[27] for unprotected deoxynucleosides to give the α and β anomers of 5-ethyl-2′-deoxycytidine 5′-phosphate (XIa and XIb). The CD spectra of the two anomeric nucleotides exhibited bands with the same signs as the nucleosides, but with small differences in molar ellipticity (Table II). Both were quantitatively hydrolyzed by 5′-nucleotidase to IVa and IVb (see below and Experimental Section).

Biological Activity. The α and β anomers, IVa and IVb, were tested by Dr. E. De Clercq (Rega Institute for Medical Research, Louvain, Belgium) for activity against three viral systems in primary rabbit kidney cell cultures. The cells were inoculated with 60 PFU (vaccinia virus), 200 PFU (herpes simplex virus), or 80 PFU (vesicular stomatitis virus) per 0.2 ml per tube. The virus was allowed to adsorb to the cells for 1 hr at 37° and cell cultures were then incubated with varying concentrations of nucleoside (0.004–200 μg/ml in MEM supplemented with 3% calf serum, 1 ml/tube). Viral pathogenicity was recorded after 1 day (vesicular stomatitis), 2 days (vaccinia), or 3 days (herpes simplex). Controls included 2′-deoxycytidine and the known active Vb.[4] Activity was measured as the minimal concentration giving 50% inhibition of viral pathogenicity. Neither IVa nor IVb showed activity against vacci-

nia or vesicular stomatitis. With herpes, IVa was also inactive, but IVb was active at 40 μg/ml as compared to 2 μg/ml for Vb and >200 μg/ml for 2′-deoxycytidine. It remains to establish whether the low, but significant, activity of IVb (as compared to Vb) is an intrinsic property of the compound or due to its intracellular deamination to the active Vb.[4] These studies are being continued to include also a polyethylenimine salt of XIb, which may result in enhanced transport across cell membranes.[28]

Dr. Estera Krajewska of this Institute has been examining the effects of IVb and Vb on the growth of *Salmonella typhimurium* strain LT-2. On a minimal medium, in combination with the inhibitor 5-fluorouracil or its nucleosides, Vb provoked additional growth inhibition which could be selectively reversed by addition of thymidine, suggesting that Vb blocks *de novo* synthesis of an as yet uncharacterized thymine nucleotide precursor. A quantitatively similar effect was observed with IVb (but not with the α anomer IVa), probably due to its enzymatic deamination to the active Vb, since IVb was inactive against a mutant strain DL-38 deficient in deoxycytidine deaminase. This was further confirmed by showing that IVb was deaminated *in vitro* to Vb by cytosine nucleoside deaminase from the wild strain LT-2.

Both the anomeric nucleotides XIa and XIb were readily dephosphorylated to the corresponding nucleosides by bacterial phosphatase, as well as by *Vipera ammodytes* venom, used as a source of 5′-nucleotidase, the reactions being followed by chromatography (Table I). The rates of dephosphorylation by venom 5′-nucleotidase were about threefold less than for cytidine 5′-phosphate, used as a control; nonspecific phosphatase activity was negligible, since cytidine 2′(3′)-phosphate was unaffected under these conditions. The susceptibility of the α anomer XIa to 5′-nucleotidase is surprising and of interest in relation to the specificity of this enzyme, which we are now subjecting to further study.

Experimental Section‡

Melting points, uncorrected, were measured on a Boetius microscope hot stage. Nmr spectra were run on solutions in D_2O, using a Varian HA-100 MHz instrument, with DSS as internal standard. CD spectra were recorded on solutions in 10-mm cuvettes with the aid of a JASCO UV-ORD-CD Model 5 instrument. Uv absorption spectra were obtained by means of a Zeiss (Jena) VSU 2-P instrument, using 10-mm path length cuvettes. The same instrument was used for spectral titrations and determinations of pK values. Walpole acetate buffers were employed in the pH range 3–4 and Sörensen phosphate buffers in the range 6–8.4. Standard 0.1 N HCl was taken as pH 1, and 0.01, 0.1, and 1 N NaOH as pH 12, 13, and 14, respectively. Measurements of pH were carried out with a Radiometer PHM22 meter, using a semimicro glass electrode. Linde Air Products Co. (USA) molecular sieves type 4A, ⅛-in. pellets, were used in the synthesis of IXa and IXb. Analytical tlc, using 20 × 20 cm plates, made use of cellulose (Merck, Darmstadt, DC-fertigplatten No. 5718/0025 cellulose F) with solvents A and B and silica gel (Eastman, Rochester, Chromatogram Sheet No. 6060) with solvent C. Preparative separations were performed on 20 × 20 cm tlc plates covered with 2-mm layers of silica gel $HF_{254\,+\,366}$ and/or HR (Merck, Darmstadt) (solvents D–H) or Whatman paper No. 3MM (solvent B). Solvent systems were as follows (v/v): (A) *i*-PrOH–concentrated NH_4OH–H_2O, 7:1:2; (B) aqueous *sec*-BuOH (20°), upper layer; (C) Et_2O–DMF, 7:3; (D) $CHCl_3$–MeOH, 85:15; (E) $CHCl_3$–MeOH, 9:1; (F) $CHCl_3$–Et_2O, 9:1; (G) Me_2CO–C_6H_6, 1:3; (H) Et_2O–DMF, 8:2; (I) MeOH–H_2O–concentrated NH_4OH, 6:2:1.

1-(3,5-Di-*O*-*p*-**chlorobenzoyl-2-deoxy-α,β-D-ribofuranosyl)-5-ethyluracil (Ia,b).** This was prepared, and the α and β anomers separated, as previously described:[4] Ia, mp 186.5–187.5°; Ib, mp 195–196°.

‡ Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical value.

Table II. Circular Dichroism Data for 5-Ethyl-2′-deoxynucleosides

Compound	Solvent	λ, nm	$\theta \times 10^{-3}$
α-5-Ethyl-4-thio-2′-deoxyuridine (IIIa)	pH 7	340	−3.1
β-5-Ethyl-4-thio-2′-deoxyuridine (IIIb)	pH 7	273	0.6
		355	1.6
β-4-Thiothymidine[a]	pH 7	272	0.5
		355	1.2
α-5-Ethyl-2′-deoxycytidine (IVa)	pH 7	278	−11.2
β-5-Ethyl-2′-deoxycytidine (IVb)	pH 7	278	6.3
β-5-Methyl-2′-deoxycytidine (X)	pH 7	281	4.7
α-5-Ethyl-2′-deoxyuridine (Va)	pH 7	270	−9.1
β-5-Ethyl-2′-deoxyuridine (Vb)	pH 7	275	5.1
β-Thymidine[b]	Water	272	4.0
α-5-Ethyl-2′-deoxycytidine 5′-phosphate (XIa)	pH 7	280	−12.4
β-5-Ethyl-2′-deoxycytidine 5′-phosphate (XIb)	pH 7	280	4.2

[a] W. Bähr, H. Sommer, and K. H. Scheit, *Biochim. Biophys. Acta*, **287**, 427 (1972). [b] D. W. Miles, M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *J. Amer. Chem. Soc.*, **91**, 831 (1969).

1-(3,5-Di-*O*-*p*-**chlorobenzoyl-2-deoxy-β-D-ribofuranosyl)-5-ethyl-4-thiouracil (IIb).** To 534 mg (1 mmol) of the anomer Ib dissolved in 25 ml of anhydrous dioxane was added 250 mg of P_2S_5 and the mixture heated under reflux for 40 min, following which tlc (solvent C) demonstrated disappearance of starting compound. The reaction mixture was brought to room temperature, filtered, and concentrated under vacuum to a viscous oil. The latter was dissolved in 5 ml of $CHCl_3$ which was deposited on six preparative plates of silica gel $HF_{254\,+\,366}$ and developed with solvent F. The major, yellowish band (R_f 0.65) was eluted with $CHCl_3$, the eluate brought to dryness under reduced pressure, and the residue dissolved in anhydrous EtOH and again brought to dryness. The resultant residue of IIb was recrystallized from anhydrous EtOH to yield 341 mg (62%) of yellow amorphous powder: mp 160–162°; λ_{max} (EtOH anhydrous) 242, 335 nm (ε 35,040, 14,940); λ_{min} 290 nm (ε 2756). *Anal.* ($C_{25}H_{22}N_2O_6Cl_2S$) C, H, N.

1-(3,5-Di-*O*-*p*-**chlorobenzoyl-2-deoxy-α-D-ribofuranosyl)-5-ethyl-4-thiouracil (IIa).** The anomer Ia, 534 mg (1 mmol), was thiated as above and the product IIa isolated in the same manner to yield 358 mg (64%), in the form of yellow needles: mp 165–166°; R_f 0.49 (solvent F); λ_{max} (EtOH anhydrous) 242, 338 nm (ε 34,490, 14,230); λ_{min} 290 nm (ε 1850). *Anal.* ($C_{25}H_{22}N_2O_6Cl_2S$) C, H, N.

Modified Procedure for IIa and IIb. A mixture containing Ia (21.4 mg) and Ib (32 mg) was thiated and chromatographed as for IIb; the two yellow bands (R_f 0.49 and 0.65) corresponding to IIa and IIb eluted with $CHCl_3$ were brought to dryness and crystallized from EtOH. The yield of IIa was 16.2 mg (85%) and IIb, 26.6 mg (83%).

5-Ethyl-4-thio-2′-deoxyuridine (IIIb). To a continuously stirred solution of IIb (475 mg, 0.85 mmol) in 6 ml of anhydrous MeOH was added 3.5 ml of 0.4 M CH_3ONa and the mixture left overnight at room temperature. To this was then added 15 ml of methanol–water (2:1) and sufficient Dowex 50W (H^+) to bring the pH to about 6. The mixture was vigorously stirred for 15 min and filtered. The resin was washed with aqueous MeOH, the combined filtrates were brought to dryness under reduced pressure, and the residue was dissolved in 10 ml of H_2O. The aqueous solution was brought to dryness under reduced pressure at 30°, and this operation was repeated twice more. The final residue was taken up to 1.5 ml of MeOH and deposited on five silica gel preparative plates and developed twice in the same direction with solvent D. The upper, yellowish band (R_f 0.57) was eluted with 1:1 $CHCl_3$–MeOH; the eluate was brought to dryness under reduced pressure and rechromatographed on silica gel HR. The yellow band was eluted with MeOH, the eluate concentrated to dryness *in vacuo*, the residue taken up in hot EtOH, and ether added

to faint turbidity. The desired product precipitated in the form of a chromatographically homogeneous yellow oil (161 mg, 67%): λ_{max} (pH 2–5) 337 nm (ϵ 21,100); λ_{min} (pH 2–5) 280 nm (ϵ 2264); λ_{max} (pH 12) 322 nm (ϵ 18,417); λ_{min} (pH 12) 258 nm (ϵ 2113); λ_{max} (pH 7.2) 388 nm (ϵ 20,680); λ_{min} (pH 7.2) 280 nm (ϵ 2450). *Anal.* ($C_{11}H_{16}N_2O_4S \cdot 0.5H_2O$) C, H, N.

α-5-Ethyl-4-thio-2′-deoxyuridine (IIIa). This was prepared from IIa according to the procedure for IIIb and isolated as a yellow oil, 149 mg (62%), chromatographically homogeneous, uv data as for IIIb. *Anal.* ($C_{11}H_{16}N_2O_4S \cdot 0.5H_2O$) C, H, N.

O^2,N^4-Bis(trimethylsilyl)-5-ethylcytosine (VII). This was prepared according to the procedure of Nishimura and Iwai[29] for cytosine. To a 250-ml flask was added 5.5 g (0.04 mol) of 5-ethylcytosine (VI),[23] 9.5 g (0.09 mol) of Me_3SiCl in 132 ml of anhydrous C_6H_6, and, dropwise and with constant stirring, a solution of 9.73 g of Et_3N in 36 ml of anhydrous C_6H_6. The mixture was left for 4 days at room temperature and filtered, and the clear filtrate was concentrated under reduced pressure to a colorless oil. This was used without further purification in the condensation reaction with VIII.

2-Deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl Chloride (VIII). This was prepared as described by Hoffer;[24] mp 109–110°.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-α-D-ribofuranosyl)-5-ethylcytosine (IXa). Compound VII, above, from 5.5 g (0.04 mol) of VI, 150 ml of anhydrous MeCN, 11.7 g (0.03 mol) of VIII, and 4A molecular sieves (BDH) (or $HgBr_2$, 5.7 g) were stirred for 10 days at room temperature. The reaction mixture was then taken up in dichloromethane and passed through a Celite filter. The filtrate was brought to dryness; the residue was suspended and shaken in a 1:1 mixture of $NaHCO_3$ and EtOH and again brought to dryness. The residue was extracted with $CHCl_3$ and the extract brought to dryness. The final residue was dissolved in boiling EtOH and heated under reflux for 30 min. An aliquot of the ethanolic solution of IXa + IXb was chromatographed on a preparative silica gel plate developed several times in solvent G, the two bands were eluted with 1:1 $CHCl_3$–MeOH, and the OD_{268} was determined for each anomer. This solution was subjected to fractional crystallization from EtOH. Fraction I (4.8 g with the use of molecular sieves and 3.2 g when $HgBr_2$ was employed) consisted exclusively of the α anomer IXa. Fractions II–V (4.4 g with molecular sieves and 2.93 g with $HgBr_2$) were approximately 1:1 mixtures of the anomers IXa and IXb. The total yield of the condensation reaction was 9.2 g (63%) or 6.13 g (42%). The pooled fractions II–V were subjected to chromatographic separation by two different procedures. One of these is detailed, below, as method C for IVb. The other, described in the next paragraph, involved the separation of IXa and IXb on a preparative scale by chromatography on HF_{254} silica gel plates with solvent H.

The plates were activated overnight at 115° and 30 mg of the anomer mixture was deposited as a band on each plate. The plates were developed ten times in the same direction, with drying in a stream of air at 60° between successive developments. This procedure gave two uv-absorbing bands with R_f values of 0.71 and 0.83. The bands were eluted with Me_2CO and the eluates brought to dryness. The band with R_f 0.71, on crystallization from MeOH, yielded 1.6 g of pure α anomer IXa. The total yield of IXa was 6.4 g (44%). An analytical sample was obtained by recrystallization three times from EtOH with active carbon (Carbo Medicinalis, Merck, Darmstadt, GFR): mp 100–101°; λ_{max} (EtOH) 240, 275 nm (ϵ 39,850, 9070); λ_{min} 265 nm (ϵ 8150). *Anal.* ($C_{27}H_{29}N_3O_6 \cdot H_2O$) C, H, N.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl-5-ethylcytosine (IXb). The eluate of the faster migrating band from the former section was brought to dryness and crystallized from EtOH to yield 0.50 g (11%) of the pure β anomer IXb. Recrystallization from MeOH gave an analytical sample: mp 105–106°; λ_{max} (EtOH anhydrous) 242.5, 275 nm (ϵ 42,260, 10,190); λ_{min} 268 nm (ϵ 9690). *Anal.* ($C_{27}H_{29}N_3O_6 \cdot H_2O$) C, H, N.

5-Ethyl-2′-deoxycytidine (IVb). Method A. A 10-ml ampoule of Rasotherm glass containing 246 mg (0.4 mmol) of IIb and 7 ml of methanolic NH_3 (saturated at 0°) was sealed and shaken for 2 hr at 37° and then heated at 120° for 16 hr. Solvent was removed, the residue taken up in water and brought to dryness, and the latter operation repeated once. The residue was dissolved in 10 ml of water and extracted three times with $CHCl_3$. The aqueous phase was concentrated under reduced pressure to about 1 ml, deposited on three sheets of Whatman 3 MM paper (50 × 45 cm), and developed with solvent B. The band with R_f 0.36 was eluted with water; the elute was brought to dryness and codistilled twice from anhydrous EtOH. The final residue was taken up in the minimal volume of ethanol at 0°, anhydrous Et_2O added to pronounced turbidity, and the whole mixture stored overnight in the deep freeze (−30°). The resulting precipitate was collected by centrifugation, washed with cold (−30°) EtOH, and dried under vacuum over P_2O_5 at room temperature: yield 54 mg (50%) of the HCl salt of IVb. Recrystallization from EtOH gave an analytical sample, mp 138–139°. Drying under vacuum over P_2O_5 at 100° led to slight yellowing of the product but with no change in melting point: λ_{max} (pH 1) 228 nm (ϵ 12,340); λ_{min} (pH 1) 246 nm (ϵ 1220); λ_{max} (pH 7) 278 nm (ϵ 8660); λ_{min} (pH 7) 255 nm (ϵ 5170).

Method B. Of the pooled fractions II–V above (remaining following crystallization of IXa and consisting of a 1:1 mixture of IXa and IXb), about 120 mg was taken up to 10 ml of MeOH. To this was added 1 ml of 1.6 *M* MeONa and the solution stirred for 16 hr. The reaction mixture was diluted two times with MeOH and brought to neutrality with Dowex 50W (H^+), the resin removed by filtration, and the filtrate brought to dryness. The residue was dissolved in 100 ml of H_2O, deposited on a 15 × 5 cm column of Dowex 1-X8 (OH^-), and eluted with water. Fractions of 20 ml were collected, monitoring uv absorption at 278 nm. The fractions falling within the main absorption peak were pooled and brought to dryness to give 102 mg of a mixture of the anomers IVa and IVb. This was dissolved in MeOH, deposited on 12 silica gel $HF_{254+366}$ preparative plates (0.5 mg/cm), and developed (solvent C) nine times in the same direction, the plates being dried at 50° each time. The band with higher mobility was eluted with MeOH–$CHCl_3$ (1:1), the eluate brought to dryness, and the residue crystallized from EtOH–Et_2O: mp 194°; yield 25 mg (76%). The R_f values and spectral data were identical with those for IVb obtained by method A above.

Method C. Compound IXb (1.17 g, 2.3 mmol) was taken up in 35 ml of anhydrous MeOH, 17 ml of 0.4 *M* MeONa added, and the solution left overnight at room temperature, filtered, and deposited on a 14 × 2.5 cm Bio-Rad 50–100 mesh AG50W-X8 (H^+) column previously washed with 1 l. of MeOH. The column was eluted with MeOH until the eluate exhibited no uv absorption (3 l.) and then with water (1 l.). Compound IXb was then eluted with 9 *M* NH_4OH, collecting 10-ml fractions. The fractions absorbing at 278 nm (No. 10–60) were pooled and brought to dryness (at 30°), and the residue was taken up in 200 ml of anhydrous EtOH and brought to dryness. The residue was dissolved in boiling EtOH and decolorized with charcoal. The resulting clear solution was warmed to 35°, Et_2O added to turbidity, and crystallization allowed to proceed at −30°: yield of crystals of IVb, 620 mg (80%); mp 193–194°; R_f and spectral data as for the product obtained by method A. *Anal.* ($C_{11}H_{17}N_3O_4$) C, H, N.

1-(2-Deoxy-α-D-ribofuranosyl)-5-ethylcytosine (IVa). Method A. The intermediate IIa was aminated and isolated as in method A for IVb to yield 65 mg (60%) of the HCl salt of IVa, mp 132–134°. Its uv spectrum and pK_a value were practically identical with those for IVb.

Method B. The lower band (R_f 0.72) on the tlc plates used for separation of the anomers of IVa and IVb (method B above) was eluted and crystallized as for IVb: yield 23 mg (75%); mp 201.5°.

Method C. The blocking groups were removed from IXa (1.37 g) and the nucleoside was purified and crystallized as for IVb (method C above) to give 673 mg (35%) of IVa, mp 199–201°. *Anal.* ($C_{11}H_{17}N_3O_4$) C, H, N.

Deamination of IVa and IVb. A small-scale adaptation of the procedure of Winkley and Robins[21] was applied. A Pierce Chemical Co. 300-μl vial with a screw cap and Teflon washer, containing 1 mg of IVa or IVb, 0.2 ml of 2 *N* MeCOOH, and 4 mg of $NaNO_2$, was shaken for 40 hr at room temperature. The vial was opened and solvent removed under vacuum over KOH. The residue was extracted with 200 μl of hot ethanol, which was deposited on Whatman 3 MM paper and developed with solvent B. The spots corresponding to Va and Vb were eluted with water and gave uv and CD spectra identical with those for authentic Va and Vb.

α and β Anomers of 5-Ethyl-2′-deoxycytidine 5′-Phosphate (XIa and XIb). These were prepared by phosphorylation of IVa and IVb as described by Tener[27] for unprotected deoxynucleosides. Both products were dephosphorylated by bacterial alkaline phosphatase (Worthington, BAPC) according to standard conditions[30] to the corresponding nucleosides, shown by chromatography. Each of the products was treated with *Vipera ammodytes* venom (stock solution 5 mg/ml, used as a source of 5′-nucleotidase), the incubation mixture including 1.5 μM nucleotide, 60 μl of 0.5 *M* Tris–HCl buffer pH 8.8, 20 μl of 0.1 *M* $MgCl_2$, 100

μl of water, and 5 μl of venom solution. Cytidine 5′-phosphate was used as a control for 5′-nucleotidase activity and cytidine 2′(3′)-phosphate as a control for possible presence of nonspecific phosphatase. On incubation at 37°, the cytidine 5′-phosphate was quantitatively converted to cytidine in 1 hr, XIa to IVa in 2.5 hr, and XIb to IVb in 3.5 hr, as shown by chromatography (Table I). Cytidine 2′(3′)-phosphate exhibited only trace dephosphorylation after prolonged (24 hr) incubation.

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6-Phenyl-4,5-dihydro-3(2*H*)-pyridazinones. A Series of Hypotensive Agents

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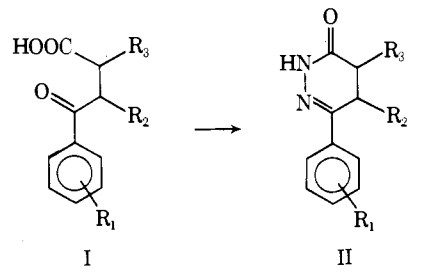
A variety of 6-phenyl-4,5-dihydro-3(2*H*)-pyridazinones (II) have been synthesized and examined for hypotensive activity in the normotensive rat. Considerable activity in this area has been observed for a variety of substituents on the phenyl moiety. The compounds containing acetamido and cyano groups combined with a 5-methyl group (*e.g.*, II, R_1 = $NHCOCH_3$ or CN; R_2 = CH_3; R_3 = H) exhibit particularly potent and long-lasting hypotensive activity.

The finding that 6-phenyl-4,5-dihydro-3(2*H*)-pyridazinones (II) possessed reproducible activity in a rat hypotensive screening program prompted us to synthesize a number of derivatives in this area.

Chemistry. The synthesis of this series of compounds II is readily accomplished in high yields by refluxing the requisite γ-keto acid I with hydrazine hydrate in ethanol. (For reviews on pyridazine chemistry, see ref 1.) Therefore, the two synthetic approaches toward this series of compounds involve preparation of various substituted γ-keto acids I followed by ring closure and modification of the preformed pyridazinones II. Both of these approaches have been utilized. A listing of the compounds prepared is given in Tables I and II.

The standard synthesis of γ-keto acids such as I involves a Friedel–Crafts reaction between an aromatic compound and succinic anhydride in the presence of a Lewis acid such as aluminum chloride. (For comprehensive discussions of this reaction, see ref 2.) This results in para substitution in the case of simple monosubstituted benzene derivatives. A number of γ-keto acids were prepared *via* this route and converted to the desired pyridazinones as indicated in Table I. In those cases which produced new compounds and if there was any doubt as to the substitution pattern, nmr was utilized to establish the structures.



I II