Some Dihydro-cytidines and -isocytidines

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Syntheses of 5,6-dihydrocytidine (IV), 5,6-dihydroisocytidine (V), and the 2-thioanalogue (VII) of the former are described. U.v. spectroscopic data for (V) and its 2-*N*-methyl and 2,2-di-*N*-methyl derivatives (IX) and (X) in alcoholic solution reveal amino rather than imino tautomeric states. The n.m.r. spectra of the dihydrocytidines and their derivatives are reported.

UNCOMMON nucleotides which may be incorporated into soluble ribonucleic acid (tRNA) and which are capable of controlling DNA and protein synthesis may prove useful in biological studies and in virus and cancer chemotherapy.[1] 5,6-Dihydrocytidylic acid, which has been isolated from liver,[2] is presumably formed by phosphorylation of dihydrocytidine. The possibility of its replacement by cytidylic or uridylic acids as substrate in an RNA polymerase system, and the alteration of some cytosines in tRNA chains to 5,6-dihydrouracil [3,4] indicate a biosynthetic relationship between cytidines and uridines. Photochemical studies of cytidines [5] and the observation of specific exchange with deuterium ion at C-5 of 5,6-dihydrocytosine derivatives [6] have provided information about their reactivities.

In order to understand the consequences of the above mentioned modification of cytosine derivatives, which may be relevant to mutagenic processes, a synthesis of oligonucleotides containing 5,6-dihydrocytidylic acid seems essential. Previous reports have shown that the amino-group of partially, but not selectively, hydrogenated cytosines [7-9] is readily displaced by water (as nucleophile), yielding the corresponding 5,6-dihydrouracils. It has recently been reported, however, that 5,6-dihydrocytosines can be prepared readily by ring closure of 2-cyanoethylureas [10] and by amination of 5,6-dihydro-4-thiouracil derivatives.[6]

The present paper deals with 5,6-dihydrocytidine and its derivatives, most of which have not been found in natural products. We first showed that 2',3',5'-tri-*O*-acetyl-5,6-dihydro-4-thiouridine (II) † could be aminated to give 2',3',5'-tri-*O*-acetyl-5,6-dihydrocytidine (III) in excellent yield. Thus, the unambiguous dihydrogenation of 2',3',5'-tri-*O*-acetyluridine [11] over rhodium–carbon to 2',3',5'-tri-*O*-acetyl-5,6-dihydrouridine [12] (I)

† Substituted pyrimidines are named here without regard to keto-enol or amino-imino tautomerism, *i.e.* the nomenclature does not reflect the actual state of the molecule.

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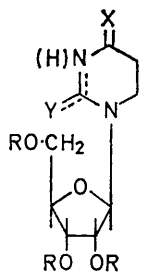
in quantitative yield, followed by selective monothiation,[13,14] proved to be the most convenient method for the preparation of the 4-thio-analogue (II) and thence [15] the 4-amino-compound (III). This selective approach to dihydrocytidine derivatives under moderate conditions suggested a route to oligonucleotides containing dihydrocytidine units. The selective amination of 5,6-dihydro-4-thiouridine in di- and tri-nucleotides will be described in a later paper.

As expected, solvents have a considerable effect on the course of amination of the 4-thio-compounds. Thus, amination of (II) in dioxan simply yielded the amino-derivative (III), whereas in methanol concomitant deacetylation occurred to give the unprotected 5,6-dihydrocytidine (IV), which was stable enough for physico-chemical investigation.

In contrast to the results of earlier investigations, our experience with the isolation of 5,6-dihydrocytidine (IV) showed that hydrogenation of cytidines could also be used as a route to pure 5,6-dihydrocytidines.

Dihydrocytidine in deuterium oxide undergoes deuteriation at position 5 as in the case of dihydrocytosine.[6] The same effect was found with the 5,6-photohydration products of cytidine [16,17] in acid or basic buffered solutions. Possible mechanisms and reaction rates for the deuteriation of cytosines at position 5 have been reported by several authors.[18,19] On exposure of 5,6-dihydro-1-methylcytosine, 5,6-dihydrocytidine, and 5,6-dihydrouridine to unbuffered D_2O solutions, n.m.r. spectroscopy revealed slow C-5 deuteriation of the dihydro-cytosine and -cytidine. With dihydrouridine exchange took place only when ammonia was added (to pH *ca.* 10). In 8 days, 66% of dihydrocytidine (1% solution in D_2O), $[\alpha]_D^{24}$ −27·4° (*c* 2 in MeOH) was converted into 5,6-dihydrouridine, $[\alpha]_D^{24}$ −35·3° (*c* 2 in

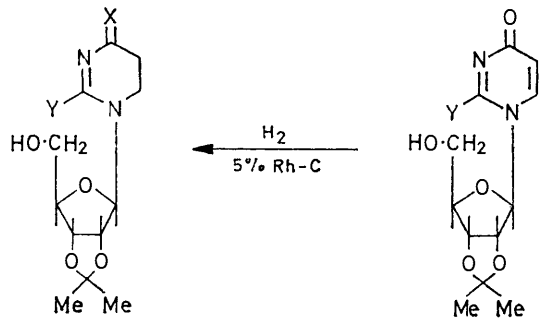


(I) R = Ac, X = Y = O
(II) R = Ac, X = S, Y = O
(III) R = Ac, X = NH, Y = O
(IV) R = H, X = NH, Y = O
(V) R = H, X = O, Y = NH_2
(VI) R = H, X = O, Y = NHMe
(VII) R = H, X = NH, Y = S

MeOH), having quantitatively incorporated deuterium at position 5.

We recently described the synthesis of 2,5′-anhydro-2′,3′-*O*-isopropylidene-5,6-dihydrouridine [14] and its significance in the preparation of 5,6-dihydroisocytidine. We have now prepared 2′,3′-*O*-isopropylidene-5,6-dihydroisocytidine (VIII) by use of ammonia as nucleophile for the ring opening of the anhydro-nucleoside; [14] hydrolysis of the isopropylidene derivative (VIII) to 5,6-dihydroisocytidine (V) was effected with methanolic hydrochloric acid.

In attempting to establish the tautomeric states of dihydroisocytidine, its 2-*N*-methyl and 2,2-di-*N*-methyl derivatives were prepared. Unfortunately ring opening of the foregoing dihydro-anhydro-nucleoside occurred when it was treated with methylamine or dimethylamine. However, treatment of 2,5′-anhydro-2′,3′-*O*-isopropylideneuridine [14] with methylamine in anhydrous methanol or with liquid dimethylamine, produced the corresponding 2-*N*-methyl- (XI) and 2,2-di-*N*-methyl- (XII) 2′,3′-*O*-isopropylideneisocytidines, which, when hydrogenated over rhodium–carbon, yielded the 5,6-dihydro-derivatives (IX) and (X).



(VIII) X = O, Y = NH_2
(IX) X = O, Y = NHMe
(X) X = O, Y = NMe_2
(XI) Y = NHMe
(XII) Y = NMe_2

The isopropylidene group of the mono-*N*-methylisocytidine (XI) could be removed by formic acid, yielding the hitherto unknown 2-*N*-methylisocytidine. Hydrogenation of this unprotected nucleoside over rhodium–carbon yielded 2-*N*-methyl-5,6-dihydroisocytidine (VI).

5,6-Dihydro-2-thiocytidine (VII) was synthesized by amination of 5,6-dihydro-2,4-dithiouridine [14] in dioxan. 2-Thiocytidine has been isolated from a hydrolysate of *E. coli* tRNA.[20]

The u.v. spectroscopic data of the dihydroisocytidines in alcoholic solution are consistent with the proposed structures and with the amino rather than the imino tautomeric state. The n.m.r. data (see Table) are comparable with those reported earlier for dihydrouridines and their thio-analogues.[14]

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were obtained for potassium bromide pellets (liquids as films) with a Perkin-Elmer 137 or 257 spectrophotometer. U.v. spectra were taken for solutions in ethanol (unless otherwise stated) with a Beckman DU-2 or

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a Perkin-Elmer double-beam spectrophotometer model 124. N.m.r. spectra were measured for solutions in deuteriochloroform (unless otherwise stated) on a Varian A60 spectrometer with tetramethylsilane as internal standard for organic solutions [5] and sodium 3-(trimethylsilyl)propane-1-sulphonate for aqueous solutions. The silica gel (Merck HF_{254}, type 60) which was used for column chromatography and for t.l.c. was activated at 110° for 60 min. The products were located by exposure to iodine vapour and by u.v. illumination.

N.m.r. spectra [a,b] (τ values)

Compound	H–1′(d)	H–2′	H–3′	H–4′	H_2–5′	H–6	H–5	CH_3–CO(s)	$(CH_3)_2C$ (s)
(I)	3·95 ($J_{1',2'}$ 6·0)	4·55—(m)[c]—4·74		—5·73— (broad)		6·48(t) ($J_{6,5}$ 6·5)	7·32(t) ($J_{5,6}$ 6·5)	7·86 7·89 7·92	
(II)	3·97 ($J_{1',2'}$ 6·5)	4·55—(m)—4·73		—5·72— (broad)		6·50(t) ($J_{6,5}$ 6·0)	6·92(t) ($J_{5,6}$ 6·0)	7·86 7·89 7·92	
(III)	4·03 ($J_{1',2'}$ 5·5)	4·51—(m)—4·70		—5·75— (broad)		6·57(t) ($J_{6,5}$ 6·0)	7·32(t) ($J_{5,6}$ 6·0)	7·92 (intense) 7·95	
(IV) [d]	4·12 ($J_{1',2'}$ 5·8)	5·67—(m)—6·13			6·21—(m)—6·34	6·57(t) ($J_{6,5}$ 6·5)	7·35(t) ($J_{5,6}$ 6·5)		
(VIII) [e]	4·56 ($J_{1',2'}$ 3·4)	5·07(q) ($J_{2',1'}$ 3·4) ($J_{2',3'}$ 6·5)	5·23(q) ($J_{3',2'}$ 6·5)	5·91(q) ($J_{4',5'}$ 3·0)	6·25(d) ($J_{5',4'}$ 3·0)	6·44(t) ($J_{6,5}$ 7·0)	7·59(t) ($J_{5,6}$ 7·0)		8·46 8·66
(V) [d]	4·57 ($J_{1',2'}$ 6·4)	5·61(q) ($J_{2',1'}$ 6·4)	5·76—(7)[f]—6·03		6·21—(m)—6·28	6·41(t) ($J_{6,5}$ 7·3)	7·44(t) ($J_{5,6}$ 7·3)		
(VII) [d]	3·12 ($J_{1',2'}$ 5·5)	5·60—(m)—6·30				6·48(t) ($J_{6,5}$ 7·0)	7·29(t) ($J_{5,6}$ 7·0)	N–CH_3 (s)	
(XI) [e]	4·45 ($J_{1',2'}$ 4·3)	5·01(q) ($J_{2',1'}$ 4·3) ($J_{2',3'}$ 6·5)	5·13(q) ($J_{3',2'}$ 6·5)	5·65—(4)—5·75	6·19(d) ($J_{5',4'}$ 2·1)	2·38(d) ($J_{6,5}$ 7·5)	4·21(d) ($J_{5,6}$ 7·5)	7·13	8·43 8·65
2-*N*-Methyl iso-cytidine [e]	4·58 ($J_{1',2'}$ 6·8)	5·58(q) ($J_{2',1'}$ 6·8) ($J_{2',3'}$ 5·5)	5·75—(5)—5·95		6·20(d) ($J_{5',4'}$ 2·0)	2·34(d) ($J_{6,5}$ 7·6)	4·21(d) ($J_{5,6}$ 7·6)	7·12	
(XII)	4·25 ($J_{1',2'}$ 4·0)	4·98(q)—(ca.)—5·24(q)	5·67—(m)—5·79		6·09(s) (broad)	2·05(d) ($J_{6,5}$ 7·7)	4·01(d) ($J_{5,6}$ 7·7)	7·05	8·43 8·65
(IX)	ca. 4·96—(m)—5·12—(m)—5·44 (intense)		5·69—(m)—5·83 ca.		6·10(d) ($J_{5',4'}$ 2·0)	6·43(t) ($J_{6,5}$ 6·6)	7·46(t) ($J_{5,6}$ 6·6)	7·17	8·47 8·67
(X)	4·78 ($J_{1',2'}$ 3·8)	5·08—(m)—5·40	5·89—(m)—6·03		6·18(d) ($J_{5',4'}$ 3·0)	6·35—(m)—6·77	7·29—(5)—7·64	6·96	8·45 8·65
(VI) [e]	4·81 ($J_{1',2'}$ 6·6)	5·72(q) ($J_{2',1'}$ 6·6) ($J_{2',3'}$ 5·5)	5·85—(6)—6·16		6·28(d) ($J_{5',4'}$ 3·0)	6·42(t) ($J_{6,5}$ 7·0)	7·50(t) ($J_{5,6}$ 7·0)	7·15	

[a] See introduction to experimental section. [b] Values for doublets (d), triplets (t), and quartet (q) refer to multiplet centres; coupling constants (*J*) are given in Hz. [c] Unresolved multiplet (m). [d] Solution in deuterium oxide. [e] Solution in CD_3OD. [f] Figures in parentheses denote the numbers of lines in multiplets. ca. refers to estimated positions when resonance is obscured by those of other protons.

2′,3′,5′-*Tri*-O-*acetyl*-5,6-*dihydrouridine* [12] (I).—2′,3′,5′-Tri-*O*-acetyluridine [11] $[\alpha]_D^{27}$ +14·6° (*c* 1·2 in MeOH), in anhydrous methanol was hydrogenated at 50 lb in^{-2} over rhodium–carbon for 2 h. Work-up as for 2′,3′-*O*-isopropylidene-5,6-dihydrouridine [14] gave a foamy product in quantitative yield, $[\alpha]_D^{18}$ −26·7° (*c* 1·2 in MeOH).

2′,3′,5′-*Tri*-O-*acetyl*-5,6-*dihydro*-4-*thiouridine* (II).—2′,3′,5′-Tri-*O*-acetyl-5,6-dihydrouridine [12] (I) (2 g, 5·38 mmol) in hot pyridine (10 ml) was treated with phosphorus pentasulphide (1·26 g, 5·58 mmol) in pyridine (25 ml). The mixture was stirred and refluxed for 30 min, diluted with dioxan (25 ml), and evaporated to an oil. This was dissolved in methylene chloride and the solution was filtered. The filtrate was chromatographed on a silica gel column (15 g). Ethyl acetate (50 ml) eluted a pure oily, yellow fraction (1·04 g, 50·6%), R_F 0·65 (EtOAc–Et_2O, 1 : 1). Preparative t.l.c. (silica gel) with ethyl acetate as eluant yielded a yellow *oil*, $[\alpha]_D^{29}$ −44·5° (*c* 1·5 in CH_2Cl_2) (Found: C, 46·45; H, 5·4; N, 7·15; S, 8·45. $C_{15}H_{20}N_2O_8S$ requires C, 46·4; H, 5·2; N, 7·2; S, 8·25%), $\lambda_{max.}$ 279 nm (log ε 4·22), $\lambda_{min.}$ 222 nm (log ε 3·08), $\nu_{max.}$ 3268, 2941, 1761, 1718, and 1232br cm^{-1}.

2′,3′,5′-*Tri*-O-*acetyl*-5,6-*dihydrocytidine* (III).—Into a solution of 2′,3′,5′-tri-*O*-acetyl-5,6-dihydro-4-thiouridine (II) (1·55 g, 4 mmol) in dioxan (60 ml) anhydrous ammonia was bubbled for 90 min. The solution was evaporated to dryness; the residue was dissolved in methylene chloride and chromatographed on a silica gel column (30 g). The starting material was eluted with methylene chloride–ethyl acetate (1 : 1; 100 ml); ethanol (300 ml) then eluted a foam (1·2 g, 81%), R_F 0·73 (CH_2Cl_2–MeOH, 1 : 1). Rechromatography on a silica gel column gave the *product*, which was dried at 20° and 10^{-5} mmHg; $[\alpha]_D^{23}$ −7·6° (*c* 1 in CH_2Cl_2) (Found: C, 48·7; H, 5·9; N, 11·3. $C_{15}H_{21}N_3O_8$

requires C, 48·5; H, 5·7; N, 11·3%), $\lambda_{max.}$ 216 and 240 nm (log ε 3·98 and 4·00), $\lambda_{min.}$ 227 nm (log ε 3·94), $\nu_{max.}$ 3300, 2933sh, 1754, 1645, 1572, and 1244br cm^{-1}.

5,6-*Dihydrocytidine* (IV).—(*a*) Into a solution of 2′,3′,5′-tri-*O*-acetyl-5,6-dihydro-4-thiocytidine (II) (870 mg, 2·35 mmol) in anhydrous methanol (30 ml) anhydrous ammonia was bubbled for 90 min. The solution was then concentrated to 2—3 ml. Silica gel (6 g) was added and the suspension obtained was dried *in vacuo* and transferred in methylene chloride to a silica gel column (20 g). Methylene chloride–methanol (1 : 2; 100 ml) first eluted acetamide and then a foam (330 mg, 57%), R_F 0·33 (MeOH). Rechromatography on a silica gel column gave the *product*, which was dried at 60° and 10^{-5} mmHg; $[\alpha]_D^{23}$ −27·0° (*c* 0·5 in H_2O) (Found: C, 43·85; H, 6·45; N, 17·35. $C_9H_{15}N_3O_5$ requires C, 44·1; H, 6·15; N, 17·15%), $\lambda_{max.}$ 216 and 241 nm (log ε 3·94 and 3·88), $\lambda_{min.}$ 232 nm (log ε 3·85), $\nu_{max.}$ 3333, 2915, 1667, 1631, and 1570 cm^{-1}.

(*b*) A solution of cytidine (486 mg, 2 mmol) in water (15 ml) containing 5% rhodium–carbon (300 mg) was stirred in hydrogen under ambient conditions until 2 mol. equiv. of hydrogen had been absorbed (*ca.* 3 h). The solution was filtered and lyophilized; the foamy residue was dissolved in methylene chloride–methanol (1 : 1) and chromatographed on a silica gel column. The product was worked up as described under (*a*). Elution with methylene chloride–methanol (1 : 10) gave the starting material (70 mg) and then a foam (314 mg, 75%), R_F 0·33 (MeOH), $[\alpha]_D^{24}$ −27·4° (*c* 1 in H_2O), identical (i.r., u.v., and n.m.r. spectra) with the compound obtained in (*a*).

Stability of 5,6-*Dihydrocytidine* (IV) *in Water*.—Freshly chromatographed 5,6-dihydrocytidine (IV) (50 mg), $[\alpha]_D^{21}$ −27·4° (*c* 1·9 in MeOH), was dissolved in distilled water (5 ml) and kept at room temperature. Optical rotations ($[\alpha]_D^{24}$) of this solution after 0, 1, 2, and 24 h and 2, 6, and 8 days were 28·0, 28·8, 29·0, 30·0, 30·5, 32·0, and 32·5° respectively; the pH changed concurrently from 7·5 to 10·0. After 8 days the mixture was lyophilized and the residue chromatographed on a silica gel plate, which was then developed three times with methylene chloride–methanol (1 : 1). The fraction with R_F *ca.* 0·1 was eluted with methanol, yielding 5,6-dihydrocytidine (33%); the fraction with R_F *ca.* 0·7 (66%) showed the characteristics (u.v., i.r., mixed m.p.) of 5,6-dihydrouridine,[12] $[\alpha]_D^{24}$ −35·6° (*c* 2 in H_2O) and −35·3° (*c* 2 in MeOH).

2′,3′-O-*Isopropylidene*-5,6-*dihydroisocytidine* (VIII).—Anhydrous ammonia was bubbled to saturation into a solution of 2,5′-anhydro-2′,3′-*O*-isopropylidene-5,6-dihydrouridine [14] (268 mg, 1 mmol) in anhydrous methanol (50 ml); the solution was left under anhydrous conditions at room temperature for 48 h, then evaporated. The residue yielded *needles* (240 mg, 84%), m.p. 184—186° (from acetone–hexane), $[\alpha]_D^{29}$ −30° (*c* 1 in MeOH) (Found: C, 50·8; H, 6·8; N, 15·0. $C_{12}H_{19}N_3O_5$ requires C, 50·5; H, 6·7; N, 14·75%), $\lambda_{max.}$ 211 and 242 nm (log ε 4·08 and 4·27), $\lambda_{min.}$ 224 nm (log ε 3·95), $\lambda_{max.}$ (3% HCl–EtOH) 205 nm (log ε 3·97), $\lambda_{infl.}$ 220 nm (log ε 3·80), $\nu_{max.}$ 3300, 2899, 2841, 1634sh, 1605, 1515sh, and 1488 cm^{-1}.

5,6-*Dihydroisocytidine* (V).—2′,3′-*O*-Isopropylidene-5,6-dihydroisocytidine (VIII) (285 mg, 1 mmol) in anhydrous dioxan (20 ml) was treated with methanolic 3·5% hydrochloric acid (6·5 ml); the mixture was kept for 3·5 h at room temperature, and then diluted with methanol (14 ml). The solution was chromatographed through a DEAE cellulose column (4 g; Calbiochem anion exchange cellulose, Cellex-D) and the methanolic eluate was evaporated to dryness. The residue was rechromatographed in methanol–water on a silica gel column (7 g). The eluate was concentrated (yield 125 mg, 51%) and the product crystallized as *needles* on addition of ether, m.p. 223—225° (from methanol–ether), $[\alpha]_D^{26}$ −11·7° (*c* 0·5 in H_2O) (Found: C, 43·8; H, 6·35; N, 16·95. $C_9H_{15}N_3O_5$ requires C, 44·1; H, 6·2; N, 17·15%), $\lambda_{max.}$ 211 and 239 nm (log ε 4·04 and 4·07), $\lambda_{min.}$ 224 nm (log ε 3·94), $\lambda_{max.}$ (3% HCl–EtOH) 205 nm (log ε 3·94), $\lambda_{infl.}$ 221 nm (log ε 3·73), $\nu_{max.}$ 3311, 3165, 2882, 1678, 1603, 1548, 1493, and 1458 cm^{-1}.

2′,3′-O-*Isopropylidene*-2-N-*methylisocytidine* (XI).—2,5′-Anhydro-2′,3′-*O*-isopropylideneuridine [21] (1 g, 3·76 mmol) in anhydrous methanol (5 ml) was treated with methylamine (5 ml) in a sealed tube at room temperature for 3 days. The solution was evaporated to dryness and triturated with methylene chloride. The product (1·0 g, 90%) crystallized from methanol–methylene chloride as *needles*, m.p. 213—215°, $[\alpha]_D^{24}$ −60·6° (*c* 1 in H_2O) (Found: C, 52·25; H, 6·2; N, 14·05. $C_{13}H_{19}N_3O_5$ requires C, 52·5; H, 6·45; N, 14·15%), $\lambda_{max.}$ 213 nm (log ε 4·41), $\lambda_{infl.}$ 233, 264 nm (log ε 4·21 and 3·67), $\nu_{max.}$ 3413infl,br, 3268, 3067, 2817, 1645, 1621, 1548, 1536, and 1497 cm^{-1}.

2′,3′-O-*Isopropylidene*-2,2-*di*-N-*methyl isocytidine* (XII).—2,5′-Anhydro-2′,3′-*O*-isopropylideneuridine [20] (100 mg, 0·376 mmol) was treated with anhydrous dimethylamine (2 ml) in a sealed tube at room temperature for 2 days. The solution was evaporated to dryness and the residue was dissolved in methylene chloride and chromatographed on a silica gel plate, which was developed three times with acetone–methylene chloride (1 : 1). The fraction with R_F *ca.* 0·29 and the starting material (22 mg; R_F *ca.* 0·46) were eluted with methanol (yield 64 mg, 74%). Recrystallization from methylene chloride–hexane gave *needles*, m.p. 172—174°, $[\alpha]_D^{23}$ −14·2° (*c* 2·5 in H_2O) (Found: C, 53·9; H, 6·85; N, 13·4. $C_{14}H_{21}N_3O_5$ requires C, 54·0; H, 6·8; N, 13·5%), $\lambda_{max.}$ 232 nm (log ε 4·33), $\lambda_{infl.}$ 262 nm (log ε 3·86), $\nu_{max.}$ 3257, 3077, 2985, 2941, 1642, 1621, and 1520 cm^{-1}.

2′,3′-O-*Isopropylidene*-5,6-*dihydro*-2-N-*methylisocytidine* (IX).—2′,3′-*O*-Isopropylidene-2-*N*-methylisocytidine (XI) (297 mg, 1 mmol) in distilled water (15 ml) was shaken over 5% rhodium–carbon (240 mg) in hydrogen (50 lb in^{-2}) and the product was worked up as for compound (I). Recrystallization from methanol–methylene chloride yielded *needles* (260 mg, 87%), m.p. 106—108°, $[\alpha]_D^{24}$ −3·5° (*c* 1 in H_2O) (Found: C, 49·6; H, 7·15; N, 13·2. $C_{13}H_{21}N_3O_5,H_2O$ requires C, 49·2; H, 7·3; N, 13·25%), $\lambda_{max.}$ 240br nm (log ε 4·19), $\lambda_{max.}$ (3% HCl–EtOH) 206 nm (log ε 4·15), $\lambda_{infl.}$ 229 nm (log ε 3·44), $\nu_{max.}$ 3425infl,br, 3369, 3344, 3268br, 1645, 1580, and 1515 cm^{-1}.

2′,3′-O-*Isopropylidene*-5,6-*dihydro*-2,2-*di*-N-*methylisocytidine* (X).—2′,3′-*O*-Isopropylidene-2,2-di-*N*-methylisocytidine (XII) (103 mg, 0·3 mmol) in distilled water (6 ml) was hydrogenated over 5% rhodium–carbon (90 mg) at 50 lb in^{-2}; the product was worked up as for compound (I) and crystallized from acetone–hexane as *needles* (100 mg, 95%), m.p. 130—132°, $[\alpha]_D^{24}$ +75·1° (*c* 1 in MeOH) (Found: C, 53·65; H, 7·4; N, 13·35. $C_{14}H_{23}N_3O_5$ requires C, 53·65; H, 7·4; N, 13·4%), $\lambda_{max.}$ 248 nm (log ε 4·22), $\nu_{max.}$ 3367br, 3247, 2933br, 1639, and 1534br cm^{-1}.

2-N-*Methylisocytidine*.— 2′,3′-*O*-Isopropylidene-2-*N*-methylisocytidine (XI) (450 mg, 1·5 mmol) in 98% formic

[21] P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, 1934, **106**, 113.

acid (45 ml) was kept at room temperature for 6 h. After several evaporations of ethanolic solutions the residue was applied to a plate coated with silica gel which was developed twice with methylene chloride–methanol (5 : 1). The fraction with R_F *ca.* 0·5 was eluted with methanol; evaporation yielded a foamy *product* (328 mg, 85%), $[\alpha]_D^{24}$ −46·9° (*c* 1 in H_2O) (Found: C, 43·7; H, 6·35; N, 15·5. $C_{10}H_{15}N_3O_5,H_2O$ requires C, 43·65; H, 6·35; N, 15·5%), $\lambda_{max.}$ 213 nm (log ε 4·24), $\lambda_{infl.}$ 234 and 264 nm (log ε 4·00 and 3·49), $\nu_{max.}$ 3322vbr, 3012, 1650, 1637, 1613, and 1543 cm^{-1}.

5,6-*Dihydro*-2-N-*methylisocytidine* (VI).—2-*N*-Methylisocytidine (60 mg, 0·235 mmol) in distilled water (2 ml) was quantitatively hydrogenated over rhodium–carbon (56 mg) for 4 h at 50 lb in^{-2} as previously described. The *product* had m.p. 192—194° (from aqueous methanol–ether), $[\alpha]_D^{24}$ +9·3° (*c* 0·75 in MeOH) (Found: C, 46·6; H, 7·05; N, 16·45. $C_{10}H_{17}N_3O_5$ requires C, 46·4; H, 6·7; N, 16·2%), $\lambda_{max.}$ 240br nm (log ε 4·21), $\lambda_{max.}$ (3% HCl–EtOH) 209 nm (log ε 4·11), $\lambda_{infl.}$ 228 nm (log ε 3·86), $\nu_{max.}$ 3413, 3322, 3115br, 1629, 1563, and 1493 cm^{-1}.

5,6-*Dihydro*-2-*thiocytidine* (VII).—Anhydrous ammonia was bubbled for 1 h into a solution of 5,6-dihydro-2,4-dithiouridine [14] (209 mg, 0·75 mmol) in dioxan (50 ml). The solution was evaporated to dryness; the residue was dissolved in methanol (2 ml) to which a silica gel solution (4 g) was added, and the mixture was then transported in methylene chloride to a silica gel (12 g) column. Acetone (60 ml) eluted the impurities and acetone–ethanol (1 : 1) an amorphous fraction (133 mg, 68%) which on rechromatography gave *needles*, m.p. 177—179° (from methanol), $[\alpha]_D^{23}$ −15° (*c* 1 in H_2O) (Found: C, 41·2; H, 5·65; S, 12·2. $C_9H_{15}N_3O_4S$ requires C, 41·1; H, 5·8; S, 12·3%), $\lambda_{max.}$ 275 nm, $\lambda_{infl.}$ 292 nm (log ε 4·11 and 4·02), $\lambda_{min.}$ 226 nm (log ε 3·38), $\nu_{max.}$ 3367, 2915, 1639, 1536, (1307), and 1100 cm^{-1}.

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