NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CLIX.* SYNTHESIS OF SOME 2-PYRIMIDONE NUCLEOSIDES**

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1-(β-D-Ribofuranosyl)-5-methyl-2-pyrimidone (*IVb*), 5-benzyloxy-2-pyrimidone (*IVc*) and 5-hydroxy-2-pyrimidone (*IVd*) were synthesized from the heterocyclic bases *I* and halogenose *II* by the mercury salt or Hilbert–Johnson procedure, followed by methanolysis of the intermediary tribenzoates *III*. 1-(β-D-Ribofuranosyl)-2-pyrimidinethione (*VIIIb*) was prepared by the reaction of 2-mercaptopyrimidine (*VIa*) sodium salt with halogenose *II* followed by S—N rearrangement, catalyzed by tin tetrachloride, and subsequent methanolysis. Desulfuration of 4-thiouracil or 4-thiothymine ribo- and 2-deoxyribonucleosides *XVIII*, *XIX* by deactivated Raney nickel produced the corresponding 2-pyrimidone and 5-methyl-2-pyrimidone ribonucleosides *IVa,b* and 2-deoxyribonucleosides *XXa,b*. Phosphorylation of compounds *IVb*, *XXa,b* with phosphoryl chloride in triethyl phosphate afforded the corresponding 5'-nucleotides *XXIII*.

Recently, 1-(β-D-ribofuranosyl)-2-pyrimidone (*IVa*) was found to inhibit selectively DNA synthesis in bacteria[1,2]. In order to gain additional information concerning its mechanism of action it was thought desirable to prepare some analogues of this compound and investigate their behaviour in bacterial cells *in vivo*. Preliminary investigations on this topic resulted in an assumption that the actual biochemically active species responsible for the inhibition of DNA synthesis *de novo* might be the corresponding 2'-deoxyribonucleoside or its 5'-phosphate formed from compound *I* *in vivo*. Therefore, special attention was turned to the synthesis of this particular type of nucleoside or -tide analogues.

Compound *IIIa* can be easily synthesized by the condensation of 2-pyrimidone (*Ia*) with 2,3,5-tri-O-benzoyl ribofuranosyl chloride (*II*) using the mercury salt method[3]. Similarly, the 5-methyl-2-pyrimidone derivative *IIIb* was synthesized from the mercury salt of the corresponding base *I* and the free nucleoside *IVb* was then obtained by alkali-catalyzed deblocking of the protecting groups. The 5-benzyloxy-2-pyrimidone derivative *IVc* was obtained from the mercury salt of the heterocyclic

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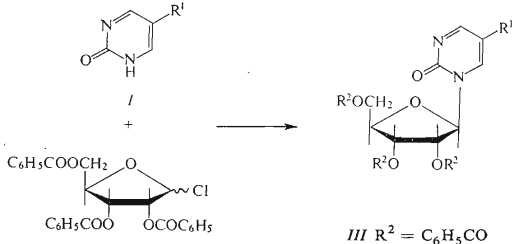
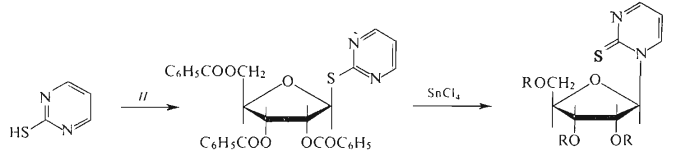
base[4] *Ic*, but attempted hydrogenolysis of this compound to 1-(β-D-ribofuranosyl)-5-hydroxy-2-pyrimidone (*IVd*) failed owing to an extreme sensitivity of the heterocyclic ring towards hydrogenation. Also, direct condensation of halogenose *II* with the mercury salt of 2,5-dihydroxypyrimidine (*Id*) was not successful. A silyl modification of the Hilbert–Johnson reaction[5,6] afforded much better yields. Thus, compound *Id* was transformed by the reaction with trimethylsilyl chloride and hexamethyldisilazane into a 2,5-di(trimethylsilyl) derivative which was used *in situ* for the condensation reaction. Aqueous hydrolysis resulted in the protected derivative *IIId* and subsequent alkaline deblocking afforded the end-product *IVd* (Scheme 1).



In formulae *I*, *III*, *IV*: *a*, $R^1 = H$; *c*, $R^1 = C_6H_5CH_2O$; *b*, $R^1 = CH_3$; *d*, $R^1 = OH$.

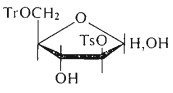
SCHEME 1

The particularly interesting 5-hydroxy derivative *IVd* – an isomer of uridine – was synthesized by a different pathway, namely, the condensation of tritylated 2-O-*p*-toluenesulfonyl-D-arabinose *V* with the sodium salt of 2,5-dihydroxypyrimidine[7,8] (*Id*). The condensation product after acidic hydrolysis of the trityl group yielded compound *IVd* (Scheme 2).

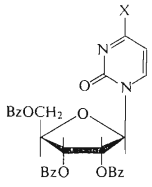


SCHEME 2

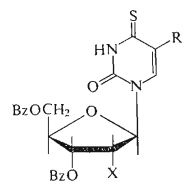
The last analogue of compound *IVa* synthesized in this connection was 1-(β-D-ribofuranosyl)-2-pyrimidinethione (*VIIIb*). Reaction of halogenose *II* with the mercury salt of 2-mercaptopyrimidine (*VI*) produced a mixture of S- and N-glycosides *VII*, *VIIIa*. However, by using the sodium salt of base *VI* the S-glycoside *VII* was obtained as the main product, in analogy with the corresponding glucopyranoside derivative[9]. In contradiction to the referred paper no S → N migration was achieved by treatment with mercuric bromide; nonetheless, a quantitative conversion to the N-riboside derivative *VIIIa* took place on treatment with tin tetrachloride (as a more powerful Lewis' acid). Alkaline methanolysis afforded the 2-thio nucleoside *VIIIb* (Scheme 3).



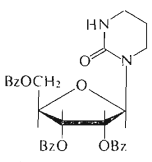
V
Tr = triphenylmethyl
Ts = *p*-toluenesulfonyl



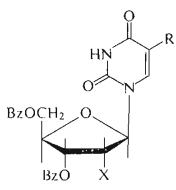
IX, X = Cl
XIV, X = SCH_3
Bz = C_6H_5CO



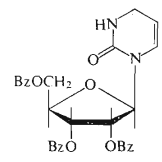
Xa, R = H, X = OBz
b, R = H, X = H
c, R = CH_3, X = OBz
d, R = CH_3, X = H
Bz = C_6H_5CO



XI, Bz = C_6H_5CO



XII, X = OBz
XIII, X = H
a, R = H, *b*, R = CH_3
Bz = C_6H_5CO



XV, Bz = C_6H_5CO

The nucleoside condensation reaction was also investigated as a means of synthesizing 1-(2-deoxy-β-D-ribofuranosyl)-2-pyrimidone. Condensation of the corresponding mercury salt with 3,5-di-O-*p*-tolyl-2-deoxyribofuranosyl chloride under the usual conditions[10] yielded a trace amount of a product with the appropriate properties.

As this compound must be expected to consist of two anomers, this method was abandoned as impractical for preparative purposes.

It was claimed that 2-pyrimidone nucleosides were formed during reduction of uracil or thymine nucleosides by amalgamated sodium[1,11-13]. Our attempts to reproduce these experiments failed repeatedly; none of the products obtained possessed properties identical with those of our authentic specimens in the ribo or 2-deoxyribo series. There is no apparent reason why, as the authors claimed[1], reduction of the $C_{(4)}$-carbonyl group should predominate especially as polarographic reduction of the starting compounds (uracil and thymine nucleosides) proceeds even at strongly negative potentials[14]. Also, the products are very susceptible to reductive processes.

Nevertheless, pyrimidine nucleosides were considered the most plausible starting material for the preparation of 2-pyrimidone ribo- and 2-deoxyribonucleosides. Probably the greatest advantage would be the anomeric homogeneity of the products (due to the β-configuration of starting materials). The first attempt consisted of the transformation of protected uracil nucleosides into the 4-chloro-2-pyrimidone derivatives (achieved easily by thionyl chloride treatment[15]) followed by reductive dehalogenation. With 2′,3′,5′-tri-O-benzoyluridine as starting material, the 4-chloro derivative *IX* was obtained but treatment of this compound with excess tri-n-butyltin hydride with aza-bisisobutyronitrile catalysis[16,17] did not result in the formation of the required product. The same failure was met in an attempt to remove the chlorine atom from *IX* by the action of activated zinc powder in acetic acid. The only product of the reaction was starting uridine derivative formed most probably by the nucleophilic reaction of compound *IX* with acetate anions followed by hydrolysis. These findings were rather unexpected as the chlorine atom has a very high reactivity in various ionic reactions and although the organometallic reagent is believed to act predominantly *via* a radical mechanism, nevertheless, conversions like carboxylic acid chloride – aldehyde are also known[18].

Another possibility for the transformation of uracil nucleosides into 2-pyrimidone derivatives is by desulfuration of 4-thiouracil derivatives. This procedure has often been investigated with Raney nickel as desulfuration catalyst. Thus, N^1-alkyl-4-thiouracil derivatives were reported to afford a complex mixture of products including 2-pyrimidone, its 3,4- or 4,5-dihydro- and 3,4,5,6-tetrahydro derivatives[19-22], depending upon reaction conditions and, more importantly, upon the quality of the Raney nickel preparation. The particular problem in the nucleoside series was also investigated by Fox and coworkers[21] who treated 4-thiouridine tribenzoate *Xa* with Raney nickel and assumed that the 3,4,5,6-tetrahydro derivative *XI* was the sole product. Their conclusion was supported by an experiment with N^1-methyl-2-pyrimidone (*XVIIa*) as a model of the intermediate expected. This derivative underwent a perhydrogenation by Raney nickel treatment to form the methyl analogue of compound *XI*. The negative conclusions resulting in this latter paper are somewhat surprising in view of the fact that the experimental data demonstrated that the 2-pyrimidone nucleoside was initially formed in the reaction and its isolation should therefore be merely a matter of experimental modification. Therefore, we felt entitled to reexamine the desulfuration of 4-thiouracil nucleosides by various Raney nickel catalysts.

Generally, the starting uracil or thymine ribo- or 2′-deoxyribonucleosides were transformed into their 2′,3′,5′- or 3′,5′-benzoates *XII*, *XIII* by the action of 10%

excess benzoyl cyanide in the presence of triethylamine[23,24]. These compounds were transformed to the 4-thio derivatives *X* by the action of phosphorus pentasulfide in dioxane[25] and purified by preparative thin-layer chromatography. On treatment of the compound *Xa* with excess Raney nickel W 6 in boiling ethanol for a prolonged time, the perhydro derivative *XI* was readily obtained as a single product and its structure confirmed by analysis and NMR spectrum. The same compound was also obtained from the 4-methylthio derivative *XIV* under the same conditions. On the other hand, analysis of the reaction mixture by thin-layer chromatography indicated that product *IIIa* was formed during the course of the reaction, as well as another compound which gives a very sensitive red color reaction with *p*-dimethylaminobenzaldehyde in acid solution. This latter intermediate is probably the dihydro derivative of compound *III*, presumably the 3,4-isomer *XV*. During the reaction, both compound *IIIa* and *XV* disappear and derivative *XI* appears.

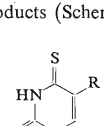
Similarly, N^1-methyl-4-thiouracil (*XVIa*) and N^1-methyl-4-thiothymine (*XVIb*) (both prepared from the corresponding uracil or thymine derivative by treatment with phosphorus pentasulfide in dioxane) afford the 2-pyrimidone derivatives *XVII* as minor components together with compounds giving the color reaction with *p*-dimethylaminobenzaldehyde. The same pattern was obtained on Raney nickel treatment of compound *XVIIa* which was prepared from 2-pyrimidone (*Ia*) by treatment with methyl iodide (no methoxy derivative was formed under the reaction conditions).

These observations led to a systematic comparative investigation of the effect of catalyst deactivation upon the composition of reaction mixture. In addition to Raney nickel catalysts, nickel boride was examined as a potential desulfurating agent reported as unable to hydrogenate activated olefin linkages[26]. However, the latter was not practically useful when compared with Raney nickel W 6 deactivated by refluxing in acetone for two hours. By using a large excess of the catalyst and a short reaction time, both ribonucleoside derivative *Xa* and the model compound *XVIa* were transformed into the compounds *IIIa*, *XVIIa* resp., as the major products of the reaction. Nevertheless, the other contaminants mentioned above were always present in the reaction mixture. The identity of compound *IIIa* so obtained was also confirmed by methanolysis to the nucleoside *IVa* identical with authentic material[3].

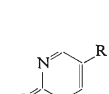
A substantial improvement of the procedure was achieved by the use of free 4-thiouracil or -thymine nucleosides *XVIII*, *XIX*, as starting materials for the desulfuration reaction. The starting materials were prepared by the methanolysis of compounds *X* and purified by silica loose layer chromatography.* The compounds *XVIII*, *XIX* were obtained as amorphous solids with spectral properties indentical

* Under the same conditions the 4-methylthio derivative *XIV* afforded 4-methoxy-2-pyrimidone riboside *XXI* as the methanolysis product (similar substitution reaction was observed with a 4-methylthio derivative in the 6-azauridine series as well[27]).

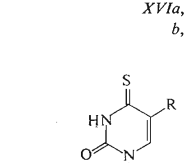
to those described in the literature[28-30]. Their NMR spectra were also consistent with the structures proposed. After deactivation by boiling in acetone for 2 hours, excess of either W 6 or W 7 Raney nickel[31] in aqueous ethanol for a short reflux time transformed the above compounds *XVIII*, *XIX* into the 2-pyrimidone derivatives *IV*, *XX* as the sole products (Scheme 4). The reaction yields are somewhat lower



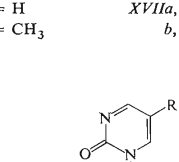
XVIa, R = H
b, R = CH_3



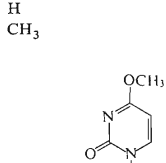
XVIIa, R = H
b, R = CH_3



XVIII, X = OH
XIX, X = H
a, R = H, *b*, R = CH_3



IV, X = OH
XX, X = H
a, R = H, *b*, R = CH_3



XXI

due to adsorption on the catalyst, however, there is no substantial contamination with the dihydro- or tetrahydro derivatives. The reaction was applicable both for uracil and thymine derivatives in either the ribo- or 2-deoxyribo series, with essentially the same results. Thymine derivatives are substantially less prone to over-reduction. A minor contamination encountered in some cases was identified as the corresponding uracil or thymine derivative (apparently resulting from the hydrolysis of the starting material *XVIII* or *XIX*) and was easy to remove. The use of free 4-thiouracil nucleosides for the above transformation is convenient not only for the simplified reaction pattern it gives, but also because no subsequent alkaline treatment of the intermediates is required to remove the protecting groups (the products are alkali-labile[14]).

The spectral properties of the compounds *IV* and *XX* are in agreement with the expected values. The chromatographic behaviour of the ribosides *IV* is identical with that of the authentic materials. The structure of the 2-deoxyribosides *XX* also follows from their splitting to the corresponding base which was found to take place in the presence of trace amounts of acid (or silica).

TABLE I

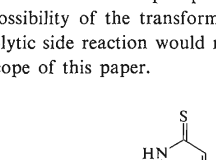
Chromatography and Electrophoresis

Compound	R_F[a]			E_2[b]
	S 1	S 2	S 5	
Uridine	0·50	0·42	0·22	1·00
2′-Deoxyuridine	0·60	0·50	0·29	0
5-Methyluridine	0·61	0·49	0·28	1·00
5-Methyl-2′-deoxyuridine	0·72	0·57	0·43	0
Ia	0·59	0·53	0·28	0·31
Ib	0·58	0·64	0·33	−0·15
Ic	0·69	—	—	—
Id	0·37	—	—	—
IVa	0·66	0·46	0·18 (0·70)[b]	1·00
IVb	0·67	0·56	0·26	0·95
IVc	0·80[c]	—	—	0·90
IVd	0·38	—	—	1·22
VIIIb	0·57	0·63	—	0·98
XVIa	0·60	0·74	0·90	—
XVIb	0·72	0·80	0·94	—
XVIIa	0·68	0·64	0·35	—
XVIIb	0·78	0·71	0·50	—
XVIIIa	0·53	0·56	0·45	1·31
XVIIIb	0·59	0·65	0·49	0·85
XIXa	0·56	0·67	0·56	0·70
XIXb	0·68	0·74	0·62	—
XXa	0·73	0·57	0·24	−0·10
XXb	0·66	0·64	0·33	−0·15
XXI	0·85	0·54	0·50	
Tetrahydro-*IVa*[d]	0·65	0·50	0·42[b]	0·87
Tetrahydro-*IVb*[d]	0·69	0·58	0·35[b]	0·87
				E_1[e]
Uridine 2′(3′)-phosphate	0·11	0·23	—	1·00
XXII	0·13	0·25	—	1·12
XXIIIa	0·10	0·20	—	1·03
XXIIIb	0·23	0·30	—	1·03
XXIIIc	0·14	0·27	—	1·00
XXIIId	0·25	0·35	—	1·00

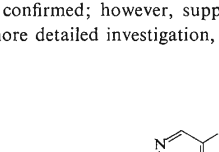
[a] R_F in S 3: *IIIa* 0·20, *IIIb* 0·30, *IIIc* 0·32, *VII* 0·35, *VIIIa* 0·38, *IX* 0·44, *XIIa,b* 0·30, *XIIIa,b* 0·30; [b] referred to uridine; [c] S 4, R_F 0·50; [d] detected by periodate-benzidine reaction; [e] referred to uridine 2′(3′)-phosphate.

An interesting feature was observed in the ultraviolet absorption spectra of the 5-methyl-2-pyrimidone derivatives *IVb*, *XXb*. In contrast with the non-methylated derivatives *IVa*, *XXa*, there is a significant bathochromic shift of the absorption maxima in the change of pH from neutral to acidic (or methanol). This suggests a contribution of the 5-methyl group to the stabilisation of the protonated (N_3) form of the 2-pyrimidone base. No such significant effect has been encountered with thymine derivatives.

2-Pyrimidone ribofuranoside 5'-diphosphate is a poor substrate for polynucleotide phosphorylase[32]. Therefore, it was of interest to investigate the possibility of transforming 4-thiouridine containing polymers into the species containing compound *IVa* by the above mentioned reaction. This investigation line was supported by the fact that 4-thiouridylic acid polymers are easy to prepare[33] and preliminary experiments with codons containing compound *IVa* has demonstrated them as non-functional[1]. As a model compound for the investigation of the transformation mentioned on the nucleotide level 4-thiouridine 5'-phosphate (*XXII*) was chosen. This compound has already been described in the literature[33,34]; in the present work, the necessity of protecting the *cis*-diol system in compound *XVIIIa* was avoided by application of the recently improved procedure involving the free nucleoside and phosphoryl chloride in triethyl phosphate[35]. In this way, the required compound *XXII* was prepared in a high yield and isomerically uniform. On treatment of this compound with the deactivated Raney nickel catalyst (see above) in aqueous ethanol some of the product *XXIIIa* was also formed. The main reaction components were, however, identified as uridine 5'-phosphate and, probably, its 3',5'-cyclic phosphate. Thus, the possibility of the transformation was confirmed; however, suppression of the hydrolytic side reaction would require a more detailed investigation, which exceeds the scope of this paper.



XXII



XXIIIa, R = H, X = OH
b, R = H, X = H
c, R = CH_3, X = OH
d, R = CH_3, X = H

None of the 2-pyrimidone nucleosides *IV* (with the exception of *IVa* (ref.[1]), *XX*, or the thio nucleosides *VIIIb*, *XVIII*, *XIX*, exhibited any significant bacteriostatic activity towards *Escherichia coli B* grown on a synthetic media with glucose, up to

a concentration of 1 000 μg/ml.* Still, there is no information as to the ability of the compounds tested to penetrate the bacterial cells. As was shown earlier[2], compound *IVa* enters into the bacterial cells in a very small overall amount but still exhibits a pronounced biochemical activity. We would like to emphasize the theoretical interest in investigating the properties of the 5-hydroxy derivative *IVd* which was unknown up to now. Its preparation, due to simplification of the synthesis of the corresponding base[4] has been substantially improved and this uridine analogue now becomes accessible. Unlike uridine, this compound cannot form hydrogen bonds involving either the N^3 or C^4 positions but still possesses the hydrophilic character of the former. Therefore, its derivatives might be interesting as a probe for the investigation of some enzymes in nucleic acid metabolism.

The ineffectiveness of the nucleosides mentioned towards bacterial growth is consistent with the hypothesis concerning the mode of action of compound *IVa* (see above). Thymidylate synthetase, an enzyme catalyzing the transformation of dUMP into dTMP on behalf of N^4, N^{10}-methylenetetrahydrofolate[36] can use only the 5'-monophosphate as a type of 2'-deoxyuridine substrate. Therefore, as the last part of this study the 2-pyrimidone nucleosides were converted into the corresponding 5'-phosphates *XXIII*. This was achieved by phosphorylation of the free nucleosides *IV*, *XX* by phosphoryl chloride in triethyl phosphate followed by neutral hydrolysis. The compounds *XXIII* thus obtained were isolated as ammonium salts homogeneous by paper chromatography and electrophoresis. Their structure was confirmed by spectral properties and enzymatic (snake venom 5'-nucleotidase and alkaline bacterial phosphatase) degradations to the corresponding nucleoside.

On examining the effect of compounds *XX* upon thymidylate synthetase activity *in vitro*, compound *XXIIIb* was found to be a very potent inhibitor of the enzyme mentioned. These results and their implications will be described elsewhere[37].

EXPERIMENTAL

Melting points were taken on the Kofler block and are uncorrected. If not stated otherwise, solutions were evaporated at 35°C/15 Torr. The compounds were dried at 0·1 Torr over P_2O_5. Paper chromatography was performed on Whatman No 1 paper (preparative runs on Whatman No 3 MM paper) in systems, S 1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), S 2,1-butanol–acetic acid–water (5 : 2 : 3). Paper electrophoresis was performed on Whatman No 3MM paper in an apparatus of Markham and Smith[38] at 40 V/cm (1 h) in buffer solutions *E* 1, 0·1M triethylammonium hydrogen carbonate pH 7·5, *E* 2, 0·1M triethylammonium borate pH 7·5. The R_F values and electrophoretic mobilities are summarized in Table I. Thin layer chromatography was performed on Silufol U_{235} silica plates with fluorescent indicator (Kavalier, Czechoslovakia) in systems S 3, chloroform–ethanol (97 : 3), S 4, chloroform–ethanol (90 : 10) or S 5, chloroform–ethanol (75 : 25). Preparative chromatography was made on loose layers of silica

* These experiments were performed by Dr I. Votruba, Department of Molecular Biology of this Institute.

(30—50 mesh) with fluorescent indicator (produced by the Service Laboratories of the Institute), compounds were eluted with ethanol. Enzymatic degradation was performed with 10—15 A_{max} (2—3 μmol) of compound *XXI* in 50 μl 0·05M Tris-HCl buffer pH 8·5 containing 10 μg alkaline phosphatase *E. coli* (Worthington) or snake venom (*Crotalus adamanteus*) 5'-nucleotidase (Worthington). Incubation, 4 h 37°C. Ultraviolet absorption spectra were measured on a Beckman DU apparatus in aqueous (or 0·01M-HCl) solutions. NMR Spectra were taken on Varian 100 apparatus in deuterio chloroform or hexadeuteriodimethyl sulfoxide with hexamethyldisilazane as internal standard. CD-Spectra were taken on a Jouan Dichrograph apparatus in water.

1-(β-D-Ribofuranosyl)-5-methyl-2-pyrimidone (*IVb*)

To a solution of 5-methyl-2-pyrimidone[4] (*Ib*) (4·40 g, 40 mmol) and sodium hydroxide (1·6 g) in water (60 ml) there was added, dropwise with stirring a solution of mercuric chloride (10·8 g) in ethanol (240 ml). After brief heating to the boiling point, the mixture was allowed to come to room temperature, filtered by suction, washed with water, ethanol and ether and finally dried *in vacuo*. Yield, 11·6 g (86·5%) mercuric chloride salt of *Ib*. A suspension of this salt (11·0 g, 32 mmol) in toluene (250 ml) was evaporated twice, resuspended in toluene (250 ml) and stirred; a solution of *II* (30 mmol, *cf.*[38]) in toluene (100 ml) was then added dropwise. Thereafter, mercuric bromide (10 g) was added and the whole refluxed and stirred for 90 min. After cooling, the solution was decanted, the precipitate washed by toluene (50 ml) and the combined solutions taken down. The residue in chloroform (250 ml) was successively washed with 40% potassium iodide, 10% sodium thiosulfate and twice water (100 ml each), dried over magnesium sulfate, filtered and evaporated. The residue was applied to a column (400 g) of silica (40—60 mesh, prepared according to Pitra) in chloroform and eluted by the same solvent. The fractions containing product *IIIb* (S 3, R_F 0·36; *IIIa* R_F 0·27) were pooled, evaporated and the residue crystallized from ethanol (brought to turbidity with light petroleum) at 4°C. Yield, 10·5 g *IIIb*, m.p. 203°C. For $C_{31}H_{26}N_2O_8$ (554·5) calculated: 67·14% C, 4·72% H, 5·05% N; found: 66·97% C, 4·74% H, 5·10% N.

A solution of *IIIb* (5·0 g, 9·2 mmol) in 100 ml methanol 30% saturated with ammonia was left to stand at 4°C for 2 days. The reaction mixture was evaporated *in vacuo*, the residue dissolved in water (100 ml), extracted twice with ether (25 ml), and the aqueous phase evaporated. The residue was applied to two plates of silica and chromatographed in System S 5. The product *IVb* was eluted with methanol (500 ml), the eluate evaporated and the residue precipitated from ethanol (4 ml) by ether (100 ml). The product was collected by centrifugation, washed with ether and dried. Yield, 1·8 g (7·5 mmol, 81·5%) *IVb*, chromatographically (S 1, S 2, S 5) and electrophoretically homogeneous; m.p. 128—130°C. For $C_{10}H_{14}N_2O_5$ (242·2) calculated: 49·58% C, 5·82% H, 11·56% N; found: 49·97% C, 5·80% H, 11·39% N. UV-Spectrum (pH 2): λ_{max} 324 nm (ε_{max} 5200), λ_{min} 260 nm.

1-(β-D-Ribofuranosyl)-5-benzyloxy-2-pyrimidone (*IVc*)

The preparation of the mercuric chloride salt of compound[4] *Ic* and its condensation with halogenose *II* was performed on the same scale and analogously as given for compound *IVb*. The crude reaction mixture after the removal of mercuric salts was taken to dryness and dissolved in 0·1M sodium methoxide solution in methanol (100 ml). After standing for 2 days at room temperature the mixture was neutralized by dry Dowex 50 X 8 (acid form), filtered, evaporated, redissolved in water (100 ml) and extracted by ether (two 25 ml portions). The aqueous phase was evaporated and the residue purified by chromatography on two silica plates in system S 1. After elution with methanol (200 ml), the compound was evaporated to get a foamy material (4·0 g, 40%) homo-

geneous on paper chromatography (S 1, S 2, S 4) and electrophoresis (*E* 1, *E* 2). For $C_{16}H_{18}N_2O_6$ (334·3) calculated: 57·48% C, 5·42% H, 8·38% N; found: 57·84% C, 6·78% H, 8·47% N. UV-Spectrum (pH 2 and 6): λ_{max} 335 nm (ε_{max} 5100), λ_{min} 290 nm. A solution of *IVc* (0·67 g, 2 mmol) in ethanol (100 ml) with 5% palladium-on-charcoal catalyst (0·5 g) was hydrogenated under atmospheric pressure. The uptake of hydrogen did not cease after the theoretical volume of 44·3 ml but amounted to about 95 ml. Analysis of the mixture by chromatography in system S 1 revealed unreacted material *IVc* and a series of unidentified fluorescing compounds, with only a trace of compound corresponding to *IVd*.

1-(β-D-Ribofuranosyl)-5-hydroxy-2-pyrimidone (*IVd*)

A) A suspension of *Id* (0·7 g, 5·4 mmol; *cf.*[4]) in a mixture of toluene (5 ml), hexamethyldisilazane (6 ml) and trimethylchlorosilane (0·5 ml) was refluxed for 4 h, evaporated and codistilled with toluene (10 ml). To this residue dissolved in acetonitrile (15 ml) was added, with stirring a solution of halogenose *II* (6 mmol, *cf.*[39]) in acetonitrile (20 ml) and the mixture refluxed with exclusion of atmospheric moisture for 5 hours. The mixture was evaporated, the residue dissolved in chloroform (100 ml) and washed with water and saturated sodium hydrogen carbonate solution (twice 25 ml each). After drying with magnesium sulphate, and evaporating, the residue was left to stand in 0·1M sodium methoxide in methanol (100 ml) for two days at room temperature. The mixture was then neutralized by dry Dowex 50 X 8 (acid form), filtered, washed with methanol (50 ml) and the filtrate taken down. The residue was dissolved in water (100 ml), extracted with ether (2 × 25 ml), the aqueous phase concentrated *in vacuo* to a small volume and applied on a column (80 × 4 cm) of DEAE-cellulose (Cellex D, std. capacity in HCO_3' form). Elution was performed with a linear gradient of triethylammonium hydrogen carbonate buffer (pH 7·5) using 2 l of water in the mixing chamber and 2 l 0·1M buffer solution in the reservoir (elution rate 3 ml/min, fractions taken at 10 min intervals). The course of elution was followed by continuous measurement of UV-absorption on an Uvicord apparatus. The fractions containing *IVd* (0·01 to 0·03M buffer) were pooled, evaporated and the residue chromatographed on one plate of silica in system S 1. The band of product was eluted by methanol (250 ml), taken to dryness and the residue precipitated from ethanol (2 ml) by ether (100 ml). The product was isolated by centrifugation, washed with ether and dried. Yield, 208 mg (0·84 mmol, 15·5%) *IVd*, chromatographically (S 1, S 2) and electrophoretically (*E* 1, *E* 2) homogeneous. For $C_9H_{12}N_2O_6$ (244·2) calculated: 44·26% C, 4·95% H, 11·47% N; found: 44·82% C, 5·12% H, 11·72% N.

B) Compound *Id* (224 mg, 2 mmol; *cf.*[4]) in 0·1M sodium methoxide solution in methanol was evaporated and the residue codistilled with toluene (2 × 20 ml). To this residue, a solution of *V* (5 mmol, *cf.*[7,8]) in dimethylformamide (5 m,) was added and the mixture stirred overnight with exclusion of moisture. Water (200 ml) was then added, the precipitate filtered by suction, washed with water and dissolved in chloroform (100 ml). After drying with magnesium sulfate, the solution was evaporated and the residue refluxed with 80% acetic acid (50 ml) for 40 min. After evaporating the mixture, the residue in water (100 ml) was filtered, the filtrate extracted with ether (2 × 25 ml), the aqueous phase evaporated and the residue purified on one plate of silica in the system S 1. The product was processed as given under method *A*. Yield, 120 mg *IVd* (24·6%), identical with the above product in all systems mentioned. UV-Spectrum (pH 2 and 6): λ_{max} 272 nm (ε_{272} 4500), 326 nm (ε_{32} 5200), λ_{min} 235, 295 nm.

2-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)thiopyrimidine (*VII*) and 1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-2-pyrimidinethione (*VIIIa*)

A) Compound *VI* (3·5 g, 31 mmol; *cf.*[40]) was dissolved in NaOH (1·25 g, 31 mmol) in water (50 ml) and a solution of mercuric chloride (8·5 g) in ethanol (200 ml) was added with stirring.

After heating to the boiling point then cooling, the mixture was filtered, the precipitate washed with water, ethanol, ether and dried. Yield, 10·4 g (95%) of mercuric chloride salt of *VI*. This salt, (10·4 g, 30 mmol) in toluene (100 ml) was treated under stirring with halogenose *II* (25 mmol, *cf.*[39]) in toluene (100 ml) and mercuric bromide (10 g). The mixture was refluxed and stirred for 2 h, filtered while hot through Celite and the filtrate processed as given above for compound *IVb*. The mixture was applied on a column of silica (250 g) and the column washed with chloroform (2 l) and 2% ethanol in chloroform (1 l). The latter eluate was evaporated and the residue chromatographed on two plates of silica (see above) in system S 3. The bands of compound *VIIIa* (R_F 0·10, yellow band) and *VII* (R_F 0·14) were eluted with methanol (200 ml) and the eluates evaporated and dried. Yield, 1·8 g (13%) of the N-glycoside *VIIIa* and 1·8 g (13%) of the S-glycoside *VII*. For $C_{30}H_{27}N_2O_7S$ (559·6) calculated: 64·38% C, 4·86% H, 5·00% N, 5·73% S; found in *VII*: 64·81% C, 4·69% H, 5·11% N, 5·71% S; found in *VIIIa*: 64·71% C, 4·56% H, 5·26% N, 5·95% S.

B) To a solution of *VI* (8·4 g, 75 mmol) in 1M sodium methoxide in methanol (80 ml) ether was added till turbidity and the mixture left to crystallize for 1 h; ether (200 ml) was added, the product filtered, washed with ether and dried. Yield, 10·2 g (100%) of the sodium salt of compound *VI*. This salt (7·0 g, 52 mmol) was added to a solution of halogenose *II* (50 mmol, *cf.*[39]) in acetonitrile (100 ml), the mixture stirred overnight at room temperature and evaporated. The residue was dissolved in chloroform (200 ml), the solution washed with saturated $NaHCO_3$ (three 50 ml portions), water (50 ml) and dried over magnesium sulfate. The mixture was processed as given above (final purification on 6 silica plates in system S 3. Yield, 3·5 g (6·3 mmol, 12·6%) of the N-glycoside *VIIIa* and 18·0 g (32·4 mmol, 64·8%) of the S-glycoside *VII*, both identical with the respective compounds described above.

Isomerisation: A solution of *VII* (1·0 g) and mercuric bromide (125 mg) in toluene (25 ml) was refluxed 8 h, the mixture evaporated, the residue taken in chloroform (25 ml), washed with 10% sodium thiosulfate solution (twice 10 ml), water (10 ml) and evaporated. The chromatography in system S 3 revealed unchanged *VII* as the only compound present.

To a solution of *VII* (10 g) in acetonitrile (200 ml), 3·2 ml (7·1 g) $SnCl_4$ was added dropwise under stirring. The mixture was left to stand overnight at room temperature, evaporated, the residue taken in chloroform (200 ml) and washed successively with 10% sodium thiosulfate (twice 50 ml) and water (50 ml). After being dried with magnesium sulfate and evaporated, the residue (8·5 g, 85%) on chromatography in system S 3 revealed compound *VIIIa* contaminated by traces of the starting material.

1-(β-D-Ribofuranosyl)-2-pyrimidinethione (*VIIIb*)

To a boiling solution of *VIIIa* (3·5 g, 6·25 mmol) in methanol (250 ml), 6 ml 1M sodium methoxide in methanol were added and the whole refluxed for an additional 10 min. After cooling the mixture was neutralized by dry Dowex 50 X 8 (acid form), filtered and the filtrate evaporated. The residue in 50 ml water was extracted with ether (two 20 ml portions), the aqueous phase evaporated, dried by codistillation with ethanol and precipitated from methanol (2 ml) by ether (100 ml). The product was collected by centrifugation, washed by ether and dried. Yield, 1·0 g (4·1 mmol, 65·5%) compound *VIIIb*, homogeneous on paper chromatography (S 1, S 2) and electrophoresis (E 1, E 2). For $C_9H_{12}N_2O_4S$ (244·3) calculated: 44·24% C, 4·95% H, 11·47% N, 13·12% S; found: 43·91% C, 5·01% H, 11·20% N, 13·79% S. UV-Spectrum (pH 6): λ_{max} 283 nm (ε_{283} 13000), 345 nm (ε_{345} 1300), λ_{min} 240 nm (ε_{240} 1000), 310 nm (ε_{310} 600).

Attempted Transformation of the 4-Chloro Derivative *IX* Into Compound *IIIa*

A) The compound *IX* (0·50 g, *cf.*[15]) and tri-n-butyltin hydride (0·6 g, *cf.*[16]) in benzene (5 ml) was refluxed in the presence of aza-bisisobutyronitrile (10 mg) for 2 h. Chromatography in S 3 revealed the starting material contaminated by some slower moving decomposition products. No substantial amount of *IIIa* could be detected.

B) The chloro derivative (1·0 g) in acetic acid (5 ml) was treated with zinc powder (98 mg) 6 h under stirring at room temperature. The mixture was then diluted with water (100 ml), filtered, washed with water, dissolved in chloroform (100 ml), filtered through Celite, dried with magnesium sulfate and evaporated. The residue was homogeneous on chromatography in system S 3 and identical with authentic 2′,3′,5′-tri-O-benzoyluridine (*XIIa*); crystallisation from ethanol afforded 0·5 g of the compound, m.p. 141—143°C (authentic 2′,3′,5′-tri-O-benzoyluridine, m.p. 142—143°C for $C_{30}H_{24}N_2O_9$ (556·5) calculated: 64·74% C, 4·34% H, 5·03% N; found: 65·20% C, 3·87% H, 4·68% N. NMR Spectrum of this material is identical with that of 2′,3′,5′-tri-O-benzoyluridine.

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-4-methylthio-2-pyrimidone (*XIV*)

2′,3′,5′-Tri-O-benzoyluridine (*XIIa*) (2·8 g, 5 mmol) was dissolved in dioxan (100 ml), to this solution was added phosphorus pentasulfide (2·6 g) and the mixture refluxed for one hour with exclusion of moisture. The mixture was then filtered while hot and the filtrate evaporated. The residue was dissolved in chloroform (250 ml), extracted successively with water (50 ml), saturated aqueous sodium hydrogen carbonate (3 × 50 ml) and water (50 ml) then dried (magnesium sulfate), filtered and evaporated. The syrupy residue was dissolved in dioxan (75 ml) and water (25 ml) then methyl iodide (1 ml, 2·26 g, 15 mmol) was added. To this solution sodium methoxide (4·5 mmol in 15 ml methanol) was added dropwise with stirring at room temperature. After 5 min a white solid began to appear and the mixture was stirred for 30 mins at room temperature. Water (250 ml) was added, the solid filtered and washed with water, ethanol and ether. Crystallization from ethanol (50 ml) and dioxan (22 ml) afforded needles, m.p. 212—214°C, 2·3 g (78·5%). For $C_{31}H_{26}N_2O_8S$ (586·5) calculated: 63·48% C, 4·47% H, 4·78% N, 5·46% S; found: 63·85% C, 4·41% H, 4·65% N, 5·87% S. When an attempt was made to remove the benzoyl groups employing the standard procedure (0·1M-NaOH in anhydrous methanol) the 4-methoxy derivative *XXI* was obtained, m.p. 135—138°C, $\lambda_{max}(H_2O)$ 272 nm; (lit.[41] gives m.p. 141—142°C, $\lambda_{max}(H_2O)$ 274 nm.

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-3,4,5,6-tetrahydro-2-pyrimidone (*XI*)

Compound *XIV* (800 mg) was dissolved in dioxan (50 ml) and ethanol (50 ml), Raney nickel W 6 (6 g wet paste) was added, the mixture refluxed for 45 min and filtered. The filtrate was evaporated and the residue purified on loose silica layer (3% ethanol in chloroform) to give a foam (620 mg). For $C_{30}H_{28}N_2O_8$ (544·5) calculated: 66·17% C, 5·18% H, 5·14% N; found: 66·17% C, 5·17% H, 4·74% N. NMR Spectrum (deuteriochloroform): 1·86 p.p.m. (m, 5-CH_2), 3·10—3·50 (m, 4-CH_2 + + 6-CH_2), 4·53 (m, $H_{4'}$), 4·66 (m, 2 $H_{5'}$), 5·38 (m, NH), 5·74 (m, $H_{3'}$ + $H_{2'}$), 5·99 (m, $H_{1'}$). This compound is identical with the material prepared by desulfuration of 4-thiouridine tribenzoate (*Xa*) under the same conditions.

4-Thio Nucleosides

The 3′,5′-di-O-benzoyl (*XIII*) or 2′,3′,5′-tri-O-benzoyl nucleosides (*XII*) (10 mmol) were dissolved in dioxan (200 ml), phosphorus pentasulfide (2·5 g, 9·5 mmol) was added and the mixture refluxed for 1 h. The reaction mixture was filtered while hot and the filtrate evaporated. The residue was

dissolved in chloroform (250 ml) and washed successively with water (50 ml), saturated $NaHCO_3$ (3 × 50 ml), water (50 ml) then dried with magnesium sulfate and evaporated. The residue in methanol (190 ml) was treated with 1M sodium methoxide solution (10 ml) and the resulting solution was allowed to stand overnight at room temperature. This solution was then neutralized with Dowex 50 × 8 (H^+ form) and filtered; the filtrate was evaporated, the residue dissolved in water (100 ml) extracted with ether (2 × 50 ml) and the aqueous phase evaporated. Purification on loose silica gel plates yielded a foam representing about 90% of the theoretical yield.

4-*Thiouridine* (XVIIIa): UV Spectrum (H_2O) λ_{max} 330, 243 nm, λ_{min} 275 nm. NMR Spectrum (hexadeuteriodimethyl sulfoxide): 3·50—4·40 p.p.m. (complex multiplet, 2 $H_{5'}$, $H_{2'}$, $H_{3'}$, $H_{4'}$), 5·96 (multiplet, 1 $H_{1'}$), 6·31 (d, 1 H_5), 7·38 (d, 1 H_6). 4-Thiothymidine (*XVIIIb*): UV Spectrum (H_2O) λ_{max} 335, 232, 265 sh, λ_{min} 280 nm. NMR Spectrum: 1·95 (s, 3 H, 5-CH_3), 2·20 (m, 1 $H_{2'}$), 3·77 (m, 2 $H_{5'}$), 3·97 (m, 1 $H_{4'}$), 4·13 (m, 1 $H_{3'}$), 5·77 (m, 1 $H_{1'}$), 7·81 (brs, 1 H_6). 4-Thio-2′-deoxyuridine (*XIXa*): UV Spectrum (H_2O): λ_{max} 330, 243 nm, λ_{min} 275 nm; NMR Spectrum: 2·22 (m, 2 $H_{2'}$), 3·71 (m, 2 $H_{5'}$), 3·96 (m, 1 $H_{4'}$), 4·37 (m, 1 $H_{3'}$), 6·15 (t, 1 $H_{1'}$, $J_{1',2'} = 5·8$), 6·30 (d, 1 H_5), 7·81 (d, 1 H_6, $J_{5,6} = 8·0$). 4-Thio-2′-deoxythymidine (*XIXb*): UV spectrum (H_2O) λ_{max} 335, 232, 265 sh, λ_{min} 280 nm. NMR Spectrum: 1·97 (s, 3 H, 5-CH_3), 2·15 (m, 2 $H_{2'}$), 3·70 (m, 2 $H_{5'}$), 390 (m, 1 $H_{4'}$), 4·35 (m, 1 $H_{3'}$), 6·15 (t, 1 $H_{1'}$, $J_{1',2'} = 6·0$), 7·78 (brs, 1 H_6).

2-Pyrimidone Nucleosides *IV*, *XX*

The preferred catalyst was Raney nickel W 6 deactivated by refluxing in acetone for 2 h, then stored under acetone. Essentially similar in reactivity was catalyst prepared according to Burgstaller[31] and then deactivated by refluxing for 2 h with acetone. Although yields varied somewhat depending on the batch of catalyst or the type of substrate a reproducible reaction pattern was apparent, as described. The 4-thio nucleoside *XVIII*, *XIX* (1 mmol) and Raney nickel catalyst (approx. 5 g of wet paste) in ethanol (150 ml) was refluxed for 15 min, filtered and washed with hot methanol. The combined filtrates were evaporated and the residue fractionated *via* loose layer silica gel chromatography in system S 5. The major components of the mixture were 2-pyrimidone nucleosides *IV*, *XX* (30—40%), unreacted material (10—20%) and sometimes uridine or thymidine (0—5%). Total recovery of material averaged 50—60% and the unreacted starting material could be recycled. 1-(β-D-Ribofuranosyl)-2-pyrimidone (*IVa*): UV Spectrum (methanol) λ_{max} 309, λ_{min} 247 nm, identical with the authentic specimen[3]. CD-Spectrum: 304 nm (+13400), 251·5(s) (+1000), 239 (0), 218 (−9200), 212·5 (−2150), 210 (−6900). 1-(2-*Deoxy*-β-D-*ribofuranosyl*)-2-*pyrimidone* (XXa): UV-Spectrum (methanol) λ_{max} 309 nm, λ_{min} 247 nm, ε_{max} 4900. Amorphous material. CD-Spectrum: 303 nm (+10950), 259 (+750), 242 (+1700), 233(0) 223 (−5900), 216 (0), 209 (+3650), 203 (0). 1-(β-D-*Ribofuranosyl*)-5-*methyl*-2-*pyrimidone* (IVb), m.p. 166—170°C (ethanol–ether). For $C_{10}H_{14}N_2O_5$ (242·2) calculated: 49·58% C, 5·82% H, 11·56% N; found: 48·27% C, 6·00% H, 10·98% N. UV-Spectrum (H_2O) λ_{max} 315 nm, λ_{min} 245 nm, ε_{max} 5400. pH 2: λ_{max} 322 nm, λ_{min} 255 nm, ε_{max} 6300. CD-Spectrum: 315 nm (+11600), 266·5 (+750), 248 (1200), 239·5 (0), 227·5 (−6650), 217 (−250), 210! (−6500). 1-(2-*Deoxy*-β-D-*ribofuranosyl*)-5-*methyl*-2-*pyrimidone* (XXb): m.p. 148—151°C (ethanol–ether). For $C_{10}H_{14}N_2O_4$ (226·2) calculated: 53·09% C, 6·24% H, 12·38% N; found: 52·53% C, 6·18% H, 12·02% N. UV-Spectrum (H_2O, pH 7): λ_{max} 315 nm, λ_{min} 245 nm, ε_{max} 5300; (H_2O, pH 2): λ_{max} 322 nm, λ_{min} 255 nm, ε_{max} 5440. CD-Spectrum: 315 nm (+8400), 266·5 (+150), 245 (+1850), 235 (0), 227 (−4000), 221 (0), 216 (+4250), 209 (0).

2-Pyrimidone 5′-Nucleotides *XXIII*

2-Pyrimidone nucleoside *IV*, *XX* (0·2 mmol) was suspended in triethyl phosphate (0·5 ml) and stirred at 0°C. To the suspension was added phosphorus oxychloride (0·04 ml, 0·067 g, 0·44 mmol)

and the flask stoppered. After 10—15 min the solution became homogeneous and was allowed to stand at 0°C until no starting material remained as judged by thin layer chromatography (system S 5) (usually 2 h). Excess triethylammonium hydrogen carbonate buffer (0·5M, 3—4 ml) was added and the resulting solution brought to pH 8·5 with triethylamine (2—3 drops). This solution was allowed to stand at room temperature for 4 h, then was applied to Whatman No 3 MM paper (2 sheets) and developed in system S 1. The appropriate band was eluted with very diluted ammonia, evaporated and the yield (usually 70—80%) was estimated spectrophotometrically. Samples were freezedried, sealed and stored in the refrigerator. UV Spectra of the compounds obtained were essentially identical with those of starting nucleosides *IV*, *XX*.

1-Methyl-2-pyrimidone (*XVIIa*)

To a solution of 2-pyrimidone (*Ia*); 1·1 g, 11·5 mmol) in methanolic sodium methoxide (0·5M, 24 ml) methyl iodide (0·8 ml) was added. The mixture was stirred overnight at room temperature, neutralized with acetic acid, evaporated and applied in water (20 ml) to a column of Dowex 50 X 8 (100 ml, H^+ form). Elution was performed with water until both the UV absorption and conductivity of the eluate decreased then continued with 2·5% ammonia. The latter eluate was evaporated and the residue was crystallized from hot ethanol (5 ml) and cyclohexane (added to turbidity). Yield, 0·80 g (63%), m.p. 128—129°C (lit.[21] gives m.p. 127—128°C). For $C_5H_6N_2O$ (110·1) calculated: 54·54% C, 5·49% H, 25·45% N; found: 54·67% C, 5·44% H, 25·33% N. UV-Spectrum (water): λ_{max} 303 nm (ε_{max} 5300).

1,5-Dimethyl-2-pyrimidone (*XVIIb*)

XXIIb was prepared from 5-methyl-2-pyrimidone (*Ib*) by a procedure identical to that described above for compound *XVIIa*. Yield, 57%, m.p. 134—135°C (ethyl acetate) (Lit.[22] gives m.p. 132°C). For $C_6H_8N_2O$ (124·1) calculated: 58·06% C, 6·49% H, 22·58% N; found: 57·83% C, 6·19% H, 22·58% N. UV Spectrum (water): λ_{max} 314 nm (ε_{max} 4700).

1-Methyl-4-thiouracil (*XVIa*) and 1-Methyl-4-thiothymine (*XVIb*)

Compounds *XVI* were prepared from 1-methyluracil or 1-methylthymine by treatment with phosphorus pentasulfide in dioxan using the procedure described above for the preparation of 4-thio nucleosides (*XVIa*): yield 91%, m.p. 193—195°C. UV Spectrum (water): λ_{max} 244, 334 nm, λ_{min} 278 nm (lit.[21] gives m.p. 191—193°C, λ_{max} 244, 333 nm, λ_{min} 277 nm). *XVIb*: yield 90%, m.p. 230—232°C (lit.[21] gives mp. 211—212°C). For $C_6H_8N_2OS$ (156·1) calculated: 46·15% C, 5·16% H 17·94% N; 20·50% S; found: 45·70%, 5·12% H, 17·90% N, 20·95% S. UV-Spectrum (water): λ_{max} 238, 338 nm, λ_{min} 284 nm.

Treatment of Compounds *XVIa*, *XVIb*, *XVIIa* with Raney Nickel

A) A solution of *XVIIa* (200 mg) in ethanol (25 ml) was refluxed with Raney nickel catalyst[31] (2·0 g wet paste, not previously deactivated) for 30 min. On TLC, a faster moving, UV absorbing spot appeared which, when sprayed with a solution of *p*-dimethylaminobenzaldehyde, gave a pink colour. After 60 min reflux all starting material had disappeared and after 2—3 hours the amount of intermediate was greatly diminished. The reaction mixture was filtered and the residue from the filtrate chromatographed on a loose layer of silica gel (system S 5). There was isolated after sublimation a solid (m.p. 40—50°C) which had UV maximum at 250 nm (water) and a poorly resolved NMR spectrum indicating the possibility of a mixture of the corresponding 3,4 and 5,6-dihydro compounds.

B) A solution of *XVIa* (50 mg) in ethanol (15 ml) was treated with deactivated Raney nickel catalyst (1·0 g wet paste). After refluxing for 15 min the mixture was filtered and the filtrate evaporated to yield a residue of 22 mg. The major components of this residue, as identified by chromatographic comparison (S 1, S 2, S 5) with authentic compounds and UV spectra (in water) were unchanged *XVIa* and *XVIIa*. A faint indication of the "pink" product (see above) could be detected by treatment with *p*-dimethylaminobenzaldehyde.

C) A solution of *XVIb* (50 mg) in ethanol (15 ml) was refluxed in the presence of deactivated Raney nickel (1·0 g, wet paste) for 30 min. After filtration and evaporation the residue was chromatographed on a plate of loose silica gel (system S 4). The major (80% of the mixture) component was isolated to yield 20 mg. After sublimation and crystallisation from ethyl acetate, mp. (131—134°C), and mixed mp. (131—133°C), chromatographic comparison (S 1, S 2, S 5) and UV-spectrum (water) identified the product as *XVIIb*.

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