NUCLEIC ACIDS COMPONENTS AND THEIR ANALOGUES. LI.*
SYNTHESIS OF 1-GLYCOSYL DERIVATIVES OF 5-AZAURACIL AND 5-AZACYTOSINE

A. Píškala and F. Šorm

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague

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For the preparation of 1-glycosyl derivatives of 5-azauracil and 5-azacytosine a method is described starting from peracetylglycosyl isocyanates. Addition of 2-methylisourea affords 1-peracetylglycosyl-4-methylisobiurets which are condensed with ethyl orthoformate to form 1-peracetylglycosyl-4-methoxy-2-oxo-1,2-dihydro-1,3,5-triazines. Deacetylation and demethylation of the latter compounds affords 1-glycosyl-5-azauracils whereas deacetylation followed by amination yields 1-glycosyl-5-azacytosines. By this procedure, glucopyranosyl, ribopyranosyl and ribofuranosyl derivatives of 5-azauracil as well as of 5-azacytosine have been prepared.

In the course of biochemical studies about cultivation of *Escherichia coli* in the presence of a subbacteriostatic concentration of 5-azauracil (2,4-dioxo-1,2,3,4-tetrahydro-1,3,5-triazine), ribosylbiuret[1] forming by decomposition of the unstable ribosyl-5-azauracil has been found in the medium. A detailed study of 5-azauracil anabolites has shown that cleavage of ribosyl-5-azauracil to ribosylbiuret proceeds in the culture of *E. coli via* N-formylribosylbiuret[2], *i.e.*, analogously to the cleavage of 5-azauracil to biuret[3]. Moreover, it has been demonstrated with the use of a cell-free extract of *E. coli* that 5-azauracil is a suitable substrate for microbial phosphorylases as well as pyrophosphorylases which makes possible the synthesis of ribosyl-5-azauracil and ribosyl-5-azauracil 5′-phosphate[4], resp. We have expected that ribosyl derivatives of 5-azauracil or 5-azacytosine (4-amino-2-oxo-1,2-dihydro-1,3,5-triazine) could exhibit marked biological effects similar to those of the corresponding 6-azaanalogues. In fact, preliminary experiments seem to support this assumption[5–7].

In this paper, we have studied the possibility of a chemical preparation of 1-glycosyl derivatives of 5-azauracil and 5-azacytosine which would represent the hitherto undescribed analogues of the appropriate pyrimidine nucleosides. Our attention has been focussed on the glucopyranosyl, ribopyranosyl and ribofuranosyl derivatives. The ribosyl derivatives were proposed for correlation with the above products of enzymic reactions.

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In a recent paper[8], we have described a general method for the preparation of 1-substituted 5-azauracils as well as 5-azacytosines which consists in the condensation of isobiurets with orthoesters followed by treatment with hydrogen chloride or ammonia. Since this method represents, according to our experience, the most suitable procedure for the preparation of this type of compounds, we have tried to synthesize also the required glycosyl derivatives in this manner. We have started from tetra-acetylglucopyranosyl isocyanate which was prepared by Fischer[9] in a 22% yield on treatment of 1-bromo-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (*I*) with silver cyanate. Later on, Johnson and Bergmann[10] described formation of a second, lower-melting modification. In several experiments, we isolated merely the higher-melting form. Furthermore, we were able to increase the yield (70%) by a suitable arrangement of the reaction conditions. Treatment with ammonia results in a product[9] to which the structure of 1-β-D-glucopyranosylurea was ascribed[11,12]. Consequently, the product of the treatment with silver cyanate may be considered as 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isocyanate (*II*).

Addition of 2-methylisourea afforded a high yield of a chromatographically homogeneous amorphous product analyzing in agreement with the expected 1-(2,3,4,6-

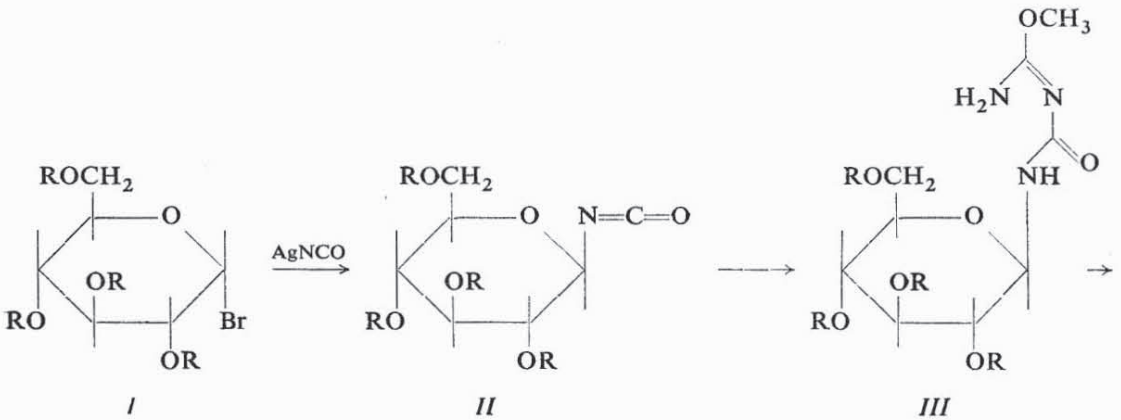
Table I

Ultraviolet Spectra

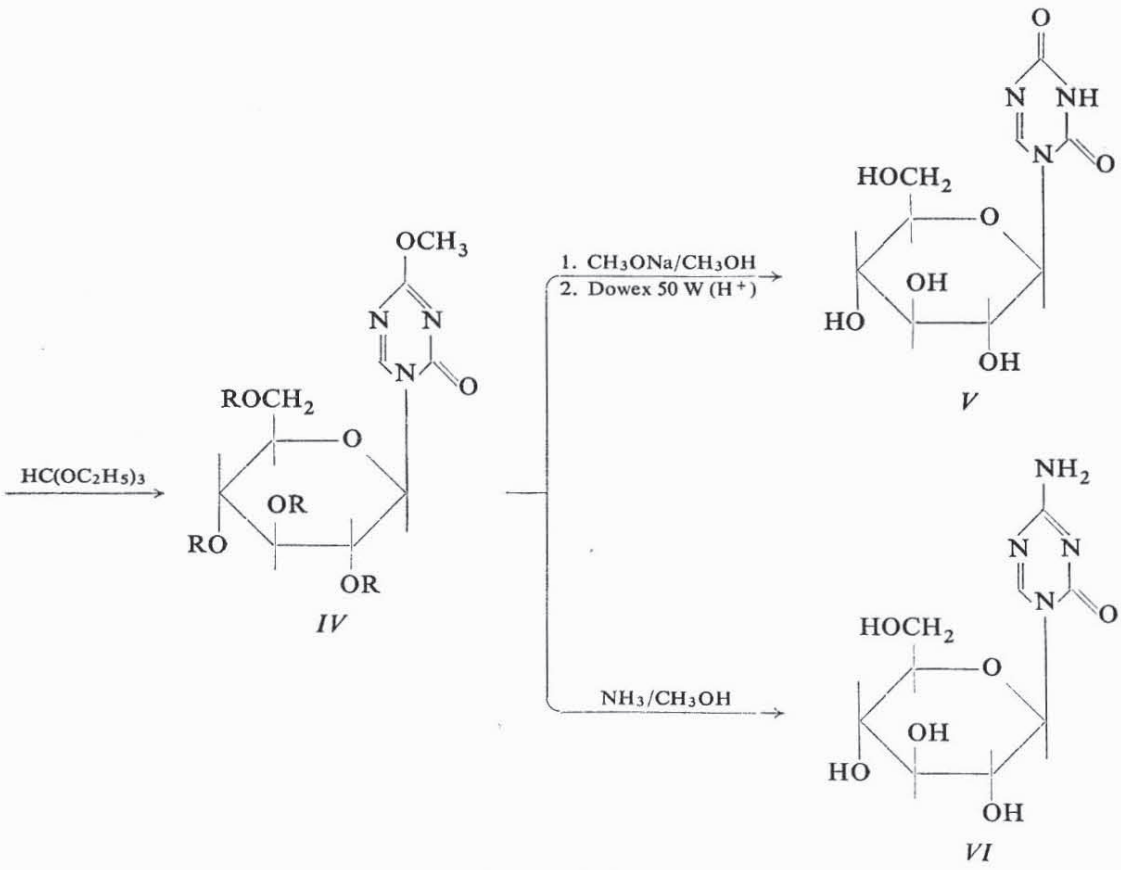
Compounds	Solvent	λ_{max} mμ	log ε
1,4-Dimethylisobiuret	96% ethanol	221	4·18
III	96% ethanol	221	4·26
X	96% ethanol	221	4·28
XVI	96% ethanol	221	4·27
1-Methyl-4-methoxy-2-oxo-1,2-dihydro-1,3,5-triazine	acetonitril	254	3·34
IV	acetonitril	254	3·40
XI	acetonitril	253	3·39
XVII	acetonitril	253	3·36
1-Methyl-5-azauracil	96% ethanol	245	3·20
V[a]	96% ethanol	240	3·25
XII[a]	96% ethanol	240	3·24
1-Methyl-5-azacytosine	water, pH 5·0	247	3·74
VI[a]	water, pH 5·0	243	3·81
XIII[a]	water, pH 5·0	243	3·80
XXII[a]	water, pH 5·0	246	3·78

[a] Spectra of the 5-azauracil and 5-azacytosine glycosyl derivatives were measured immediately after dissolution because of the time-dependence of their extinction coefficient[13,25].

tetra-O-acetyl-β-D-glucopyranosyl)-4-methylisobiuret (*III*). Its ultraviolet spectrum is practically identical with that of the analogous 1,4-dimethylisobiuret (see Table I). The above product was condensed at 135°C with ethyl orthoformate in the stream of dry nitrogen to yield crystalline 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-4-methoxy-2-oxo-1,2-dihydro-1,3,5-triazine (*IV*). Its structure was established on comparison of the ultraviolet spectrum with that of the analogous 1-methyl-4-methoxy-2-oxo-1,2-dihydro-1,3,5-triazine (Table I).



I *II* *III*

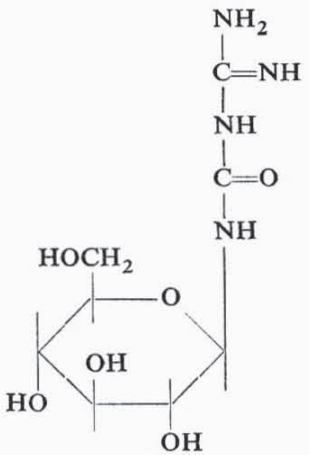


IV *V* *VI*

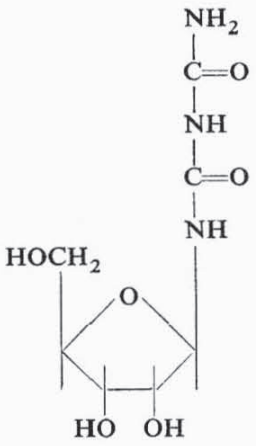
R = CH_3CO

Transformation of the glucoside *IV* into the required 1-β-D-glucopyranosyl-5-azauracil (*V*) was accomplished with methanolic hydrogen chloride or Dowex 50 W(H^+) ion exchange resin in methanol or sodium methoxide followed by Dowex 50 W (H^+) resin. When crystallised from aqueous ethanol, the substances formed a solvate with ethanol and water. Water may be removed by heating *in vacuo* at 130°C for 1 hour whereas removal of ethanol requires 10 hours at the same temperature. Its structure was verified on a comparison of the ultraviolet spectrum with that of the analogous 1-methyl-5-azauracil[13], and, furthermore, by an acidic hydrolysis yielding 5-azauracil and glucose. Moreover, the formation of a stable adduct with ethanol in the case of the glucoside *V* closely resembles the behaviour of 1-methyl-5-azauracil[14]. The structure of both these adducts will be discussed in detail in a subsequent paper of this Series[15].

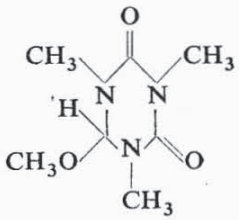
A short action of a methanolic solution of ammonia at room temperature on the glucoside *IV* afforded crystalline 1-β-D-glucopyranosyl-5-azacytosine (*VI*) in a good yield. Its structure was confirmed on comparison of the ultraviolet spectrum with that of the analogous 1-methyl-5-azacytosine (see Table I) and by acidic hydrolysis yielding 5-azacytosine and glucose. The prolonged action of the methanolic solution of ammonia led to a second product, namely, 1-β-D-glucopyranosyl-3-guanylurea (*VII*). This compound is obviously formed by cleavage of the triazine ring of the glucosyl derivative *VI*.



VII



XIX

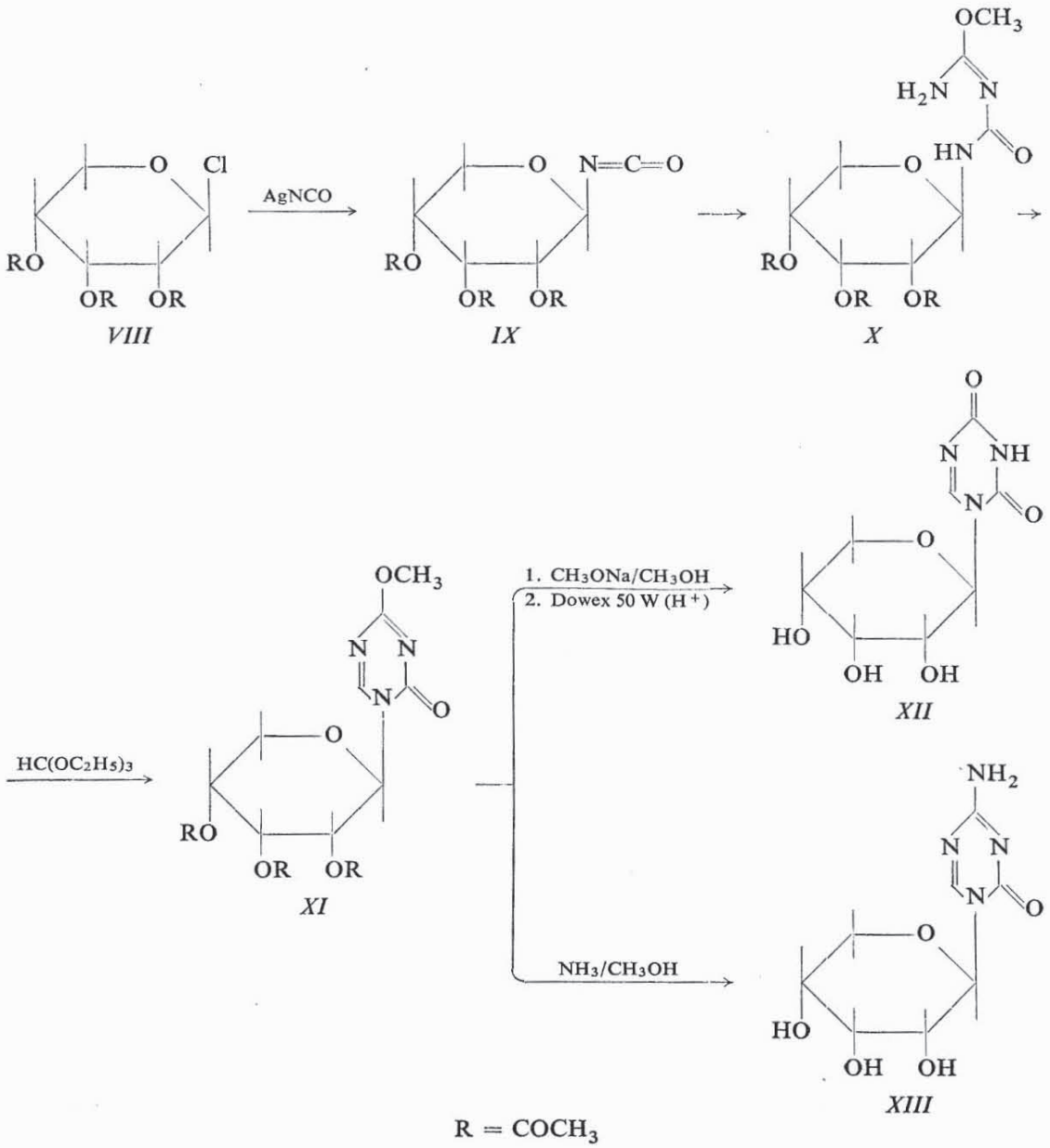


XXI

The above procedure was used also for the synthesis of 5-azauracil and 5-azacytosine ribopyranosyl derivatives. The starting 1-chloro-2,3,4-tri-O-acetyl-β-D-ribopyranose (*VIII*) was reacted with silver cyanate under similar conditions as in the preceding case. 2,3,4-Tri-O-acetyl-β-D-ribopyranosyl isocyanate (*IX*) was obtained as amorphous solid but its analysis as well as infrared spectrum, ν(N=C=O), 2252 cm^{-1}, closely resembling that of peracetylglucosyl isocyanate *II*, ν(N=C=O), 2253 cm^{-1}, both speak in favour of the supposed structure. Since in the reactions of halogenoses with silver cyanate the same steric course can be assumed as in the reactions with the

silver or mercury salts of pyrimidine and purine bases, we have used the rule of Baker[16] for allotment of the configuration at the glycosidic centre of the molecule. Treatment of the isocyanate *IX* with 2-methylisourea afforded a high yield of crystalline 1-(2,3,4-tri-O-acetyl-β-D-ribopyranosyl)-4-methylisobiuret (*X*) the structure of which was confirmed by the ultraviolet spectrum (see Table I). Condensation of this compound with ethyl orthoformate at 135°C resulted in crystalline 1-(2,3,4-tri-O-acetyl-β-D-ribopyranosyl)-4-methoxy-2-oxo-1,2-dihydro-1,3,5-triazine (*XI*) the ultraviolet spectrum of which corresponded to the proposed structure (Table I).

Successive treatment of the ribosyl derivative *XI* with sodium methoxide and Dowex 50 W (H^+) ion exchange resin yielded a product to which the structure of



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