PREPARATION OF OROTIDINE, 2'-DEOXYOROTIDINE, AND RELATED COMPOUNDS*

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Received June 20th, 1974

By reaction of 2-amino-β-D-(or β-L-)arabinofuro[1',2' : 4,5]oxazoline (*II*) with dimethyl acetylenedicarboxylate, 6-methoxycarbonyl-O-$^{2,2'}$-anhydrouridine (*III*) and its L-enantiomer, resp., were prepared. On treatment with benzoyl cyanide, compound *III* was converted to the 3',5'-dibenzoyl derivative *IV* which was refluxed with stannic chloride in methanol to afford a mixture of 2',5'-di-O-benzoyl-6-methoxycarbonyluridine (*VI*) and its 3',5'-isomer *VII*. Methanolysis of this mixture furnished orotidine methyl ester (*VIII*) while the alkaline hydrolysis led to orotidine (*I*). In addition to dibenzoates *VI* and *VII*, there was also obtained 1-(2-O-methyl-3,5-di-O-benzoyl-β-D-ribofuranosyl)-4-methoxy-6-methoxycarbonylpyrimidin-2-one (*IX*), the methanolysis of which yielded 1-(2-O-methyl-β-D-ribofuranosyl)-4-methoxy-6-methoxycarbonylpyrimidin-2-one (*X*). The L-orotidine (L-*I*) was prepared analogously. By the action of hydrogen chloride in dimethylformamide, *IV* was converted into a mixture of 1- and 3-isomeric 2-chloro-2-deoxy-β-D-ribofuranosyl derivatives *XV* and *XVI* of methyl orotate. On treatment with triethylamine, *IV* was recovered from *XV* while an isomeric anhydronucleoside *XVII* was obtained from the 3-isomer *XVI*. Bromination of 3',5'-di-O-benzoyl-2'-deoxyuridine (*XIXa*) with N-bromosuccinimide yielded the 5-bromo derivative *XIXb* which was converted into the 6-cyano derivative *XX* by the action of sodium cyanide in pyridine. Alkaline hydrolysis of *XX* afforded 2'-deoxyorotidine (*XVIII*).

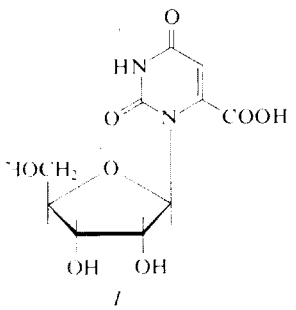
Orotidine (6-carboxyuridine, *I*) is in the form of its 5'-phosphate (orotidylic acid) very important as intermediate in the synthesis of nucleic acids *in vivo*. Decarboxylation of the orotidylate in the presence of orotidylate decarboxylase affords uridine 5'-phosphate. This route represents the sole synthesis of uridine and its derivatives *de novo* and constitutes consequently one of the most important processes in the synthesis of nucleic acids.

The accessibility of orotidine and its derivatives is limited since the main source of these substances are fermentation methods using some mutants of *Neurospora crassa*[1] or methods based on selective blocking of orotidylate decarboxylase *in vivo* by some substances, for example 6-azauridine, leading to cumulation of orotidine or its 5'-phosphate. Both these methods require a laborious isolation technique. The direct ribosylation of orotic acid or its derivatives (esters) is not suitable for the preparation of orotidine because of the almost predominant formation of the 3-ribosyl derivative[2] while the protected orotidine derivative is formed in a trace amount only. The recently published synthesis of orotidine by reaction of 5-bromouridine with cyanides proceeds in fair yields *via* 6-cyanouridine as intermediate[3].

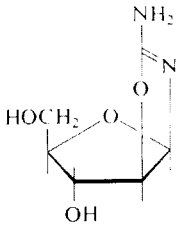
* Part CLXIII in the series Nucleic Acid Components and their Analogues; Part CLXXII: This Journal *40*, 187 (1975).

Recently, an original synthesis of pyrimidine nucleosides has been developed consisting in reaction of sugar 2′-aminooxazolines with derivatives of acetylenemonocarboxylic acid[4,5]. In this Laboratory, the oxazoline method has been extended for various sugar derivatives but particularly for the preparation of 6-substituted nucleoside derivatives by the use of substituted acetylenemonocarboxylic acids and their esters[6,7] or preferably, esters of β-substituted β-haloacrylic acids[8]. It was obvious that the sugar 2′-aminooxazolines also could react with esters of acetylenedicarboxylic acid and thus afford derivatives of 6-alkoxycarbonyluracil or its nucleosides, as reported in the present paper. Analogous problems have also been examined by other authors[5,9]. Reaction of 2-amino-β-D-arabinofuro[1′,2′ : 4,5]oxazoline (*II*) (*cf.*[5]) with dimethyl acetylenedicarboxylate afforded 6-alkoxycarbonyl-$O^{2,2'}$-anhydrouridine (*III*).

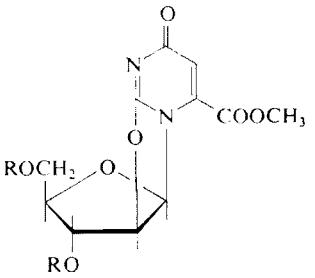
As with other $O^{2,2'}$-anhydrouridine derivatives, the hydrolysis of compound *III* proceeds *via* an exclusive attack of the anhydro bond at position 2 of the uracil nucleus with the formation of the derivative of the *arabino* configuration[9]. In spite of the successful synthesis of orotic acid nucleoside derivatives specifically ribosylated at position N^1, the direct conversion of these derivatives to orotidine (*I*) is not possible. Recently however, a method has been developed for the reversed opening of the anhydronucleoside bond in $O^{2,2'}$-anhydrouridine and its derivatives[10,11]; this method is based on participation of the benzoyl group at position vicinal to the anhydro bond ($C_{(3')}$) in the methanolysis catalysed by Lewis acids. Since this reaction is stereospecific and proceeds in high yields, an advantageous preparation of uridine and its 6-alkyl derivatives from arabinose was made possible. The character of the Lewis acid is of great importance in this reaction. Thus with unsubstituted uracil derivatives,



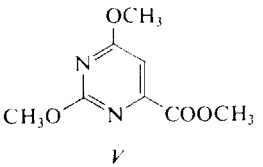
I



II



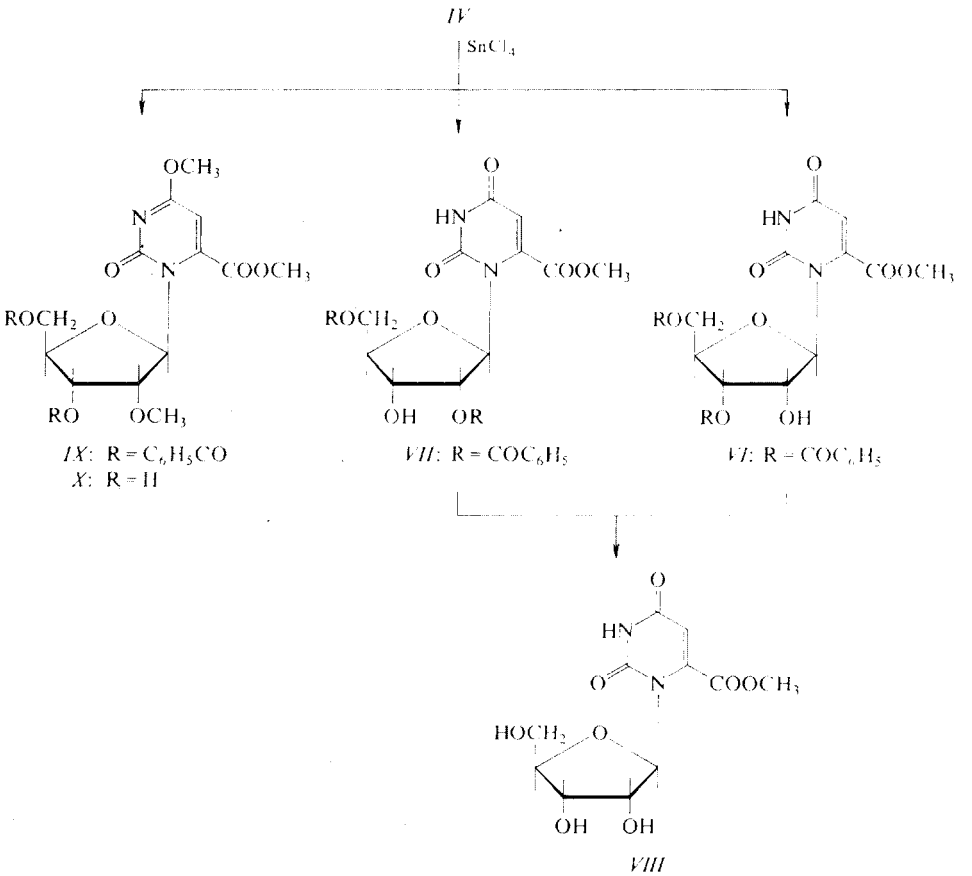
III, R = H
IV, R = COC_6H_5



V

the reaction proceeds in the presence of boron trifluoride etherate[10] while stannic chloride must be used as a stronger acid in the case of $O^{2,2'}$-anhydrouridine 6-alkyl derivatives[11]. It was therefore of interest to attempt this method of the reversed anhydro bond opening also in the case of the orotidine $O^{2,2'}$-anhydro derivatives of the type *III*.

The reaction course was effected separately with both enantiomers *II* derived from D-arabinose[5] on the one hand and L-arabinose[12] on the other (in schemes and formulae, the D-enantiomers are shown). Reaction of compound *II* with dimethyl acetylenedicarboxylate afforded both the enantiomeric derivatives of 6-methoxycarbonyl-$O^{2,2'}$-anhydrouridine (*III*); these substances were converted (by reaction with benzoyl cyanide in acetonitrile[13]) into the 3',5'-di-O-benzoyl derivatives *IV* which served as starting material in the subsequent steps. Reaction of *IV* with boron trifluoride in methanol[10] did not afford the required product; under the reported[10] conditions,



SCHEME 1

compound *IV* was recovered from the reaction mixture while longer reaction time led to a destruction of the molecule due to the cleavage of the nucleoside bond. Thus, 2,4-dimethoxy-6-methoxycarbonylpyrimidine (*V*) was isolated as the single product. The relative lability of the nucleoside bond in orotidine (when compared with that in uridine) is generally known and probably is the cause of the formation of compound *V* by an attack of the methoxide ion at position N^1 followed by cleavage of the labile $C_{(2)}$-$O^{2'}$ bond and methylation of the intermediate.

A similar cleavage of the nucleoside bond leading finally to compound *V* has also been observed in the reaction of compound *IV* with stannic chloride in methanol[11] when longer periods of time were used. The application of this stronger Lewis acid however, brought about the required course of the reaction under milder conditions. In this case, the isomeric 6-methoxycarbonyluridine 2',5'- and 3',5'-dibenzoyl derivatives *VI* and *VII* were isolated. Their structure was established by analysis, NMR spectra, and alkaline hydrolysis leading to orotidine (*I*). Compound *I* is a strong acid (p*K* 2·7); the isolation of this substance in the form of the free acid is not suitable. In this work, the lithium salt (prepared on ion exchange resins) has been advantageously used in the isolation process. Methanolysis of the mixture of compounds *VI* and *VII* afforded 6-methoxycarbonyluridine (*VIII*) as expected. One of the main problems in the chemistry of orotidine relates to its acidic carboxylic function which also affects the chemical reactions occurring on the sugar residue[14]; it is therefore desirable to protect this function by esterification (*cf.* the methyl ester *VIII*). However, the usual esterification agents either fail in this case or afford products of a simultaneous alkylation at position N^3 (such as diazomethane or dimethyl sulfate). The single known method allowing conversion of orotidine (*I*) to the ester *VIII* in a low yield consisted in the reaction of the silver salt of compound *I* with methyl iodide[15]. The above mentioned preparation of the ester *VIII* from the anhydronucleoside *IV* is more advantageous (Scheme 1).

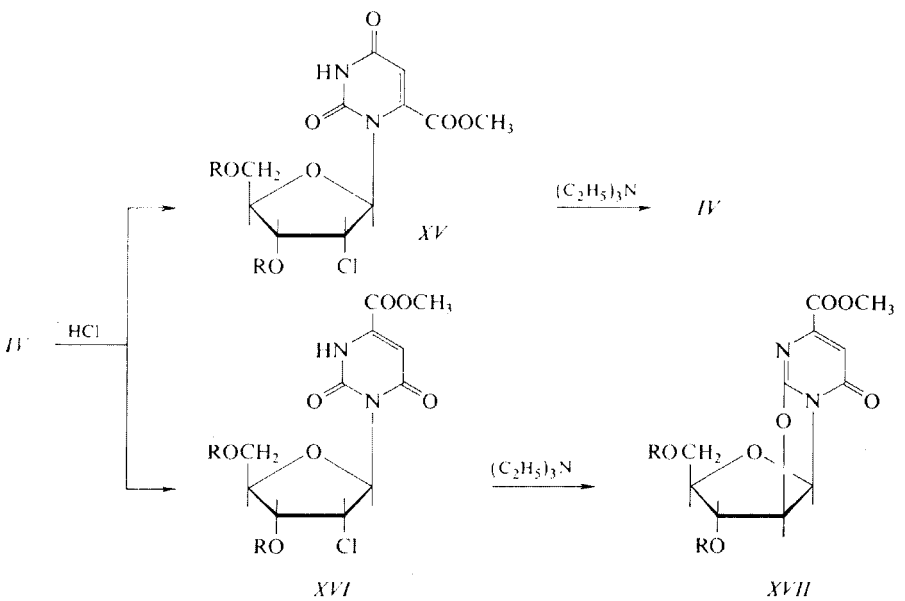
The formation of the dibenzoyl derivatives *VI* and *VII* from compound *IV* on treatment with stannic chloride in methanol is not a uniform reaction but compounds *VI* and *VII* are easy to isolate by chromatography on silica gel. This purification in the stage of benzoylated derivatives is necessary since after removal of the protecting benzoyl groups orotidine (*I*) may be separated only with difficulty from the accompanying contaminants. Notwithstanding, the above mentioned formation of orotidine (*I*) is advantageous from the preparative point of view since arabinose and simple derivatives are used as the starting material[16]. Application of the same procedure to the L-enantiomers led to the first preparation of the hitherto unknown L-orotidine (L-*I*).

The dibenzoates *VI* and *VII* are products of the expected attack of the 3'-O-benzoyl function of compound *IV* at position $O^{2'}$ accompanied by the methoxide ion attack on the intermediary acylonium ion. In addition to the above dibenzoates, another reaction product was also isolated by chromatography on silica gel. Cautious metha-

nolysis of this by-product afforded a trimethyl derivative of orotidine. Analysis of the main peak in the mass spectrum of the methanolysis product indicated the brutto formula $C_7H_9N_2O_4$ corresponding to the presence of two methyl groups on the skeleton of orotic acid; one of these methyl groups must be bound in the form of an ester to the carboxylic function at position 6, as suggested by the reaction scheme. The third methyl group must be consequently attached to the sugar moiety. In accordance with this idea, the NMR spectrum indicates the presence of one methyl group on the sugar residue (3·43 p.p.m.), the other on the heterocyclic nucleus (3·98 p.p.m.), and the third ester-bound methyl group (4·05 p.p.m.). The NMR spectrum also confirmed the presence of two exchangeable protons on the sugar residue and the absence of a NH function on the heterocyclic nucleus. The NMR spectrum of the starting dibenzoyl derivative confirmed the presence of three methyl groups (also occurring in the spectrum of the debenzoylation product) as well as the presence of two benzoyl groups in the sugar moiety. On the basis of the NMR spectrum, the sugar residue O-methyl group was located into position 2′ of the dibenzoyl derivative. All these indications suggest the structure *IX* for the by-product of the reaction between compound *IV* and stannic chloride and the structure *X* for the corresponding methanolysis product. The final proof was based on benzoylation of compound *X* with benzoyl cyanide in acetonitrile with the uniform formation of compound *IX*, identical with the original by-product. The removal of benzoyl groups from the sugar moiety of compound *IX* by methanolysis is thus not accompanied by any side reactions. The O-methylation of the sugar residue at position 2′ is of special interest since neither $O^{2,2'}$-anhydrouridine 3′,5′-di-O-benzoyl derivative *XIa* nor 6-methyl-$O^{2,2'}$-anhydrouridine 3′,5′-di-O-benzoyl derivative *XIb* (*cf.*[11]) undergo an analogous reaction in spite of prolonged reaction times. In both cases only uridine (*XIIa*) and 6-methyluridine (*XIIb*), resp., were obtained as single products after removal of the benzoyl groups. The reaction is thus exclusively limited to the derivative *IV* and might be ascribed to activation of the $C_{(2')}$ carbon atom by the presence of the alkoxycarbonyl group on the heterocyclic nucleus. In the first stage of the reaction, a stannic chloride complex is obviously formed at position N^3 of the heterocyclic nucleus and the $O^{2,2'}$-anhydro bond of compound *IV* is activated; the subsequent methoxide ion attack at position 2′ is a stereospecific S_N2 reaction and leads to the formation of a 2′-O-methyl derivative with the *ribo* configuration. The final methylation at position O^4 is obviously mediated by the complex of stannic chloride, methanol, and the intermediate under participation of the position N^3 and its course appears to be intramolecular. Since the yield is rather high and the nucleoside bond of orotidine derivatives may be readily cleaved in acid media, the above reaction might be of value in preparations of 2-O-methylribose and derivatives.

The anhydro bond opening of 3′,5′-di-O-benzoyl-$O^{2,2'}$-anhydrouridine and derivatives (*IV*, *XI*) by the action of methanol in the presence of a Lewis acid (leading in the case of compound *IV* to orotidine and compounds *IX* or *X*) does not represent

the single possibility how to utilize compounds of the type *XI*. As shown in numerous papers[7,11,12,17–20], these compounds are also useful in the preparation of 2′-deoxy-ribo derivatives. The reaction consists in treatment of compounds *XI* with hydrogen chloride in dimethylformamide to afford the corresponding 2′-chloro-2′deoxy derivative *XIII* with the *ribo* configuration of the C-Cl bond; the derivative *XIII* may be then reductively dehalogenated by the action of tri-n-butyltin hydride with the formation of the 3′,5′-di-O-benzoyl-2′-deoxyribofuranosyl derivative *XIV*. With the use of benzoylated derivatives *XI*, the first reaction step and the isolation of intermediates is particularly easy. Furthermore, benzoylation of the sugar moiety results in stabilisation of the nucleoside bond against the action of acidic agents. After treatment of compound *IV* with anhydrous hydrogen chloride in dimethylformamide at 100°C, there were isolated two isomeric 2′-chloro-2′-deoxy derivatives with very similar NMR spectra differing only in the sugar moiety by the chemical shift value for $H_{1'}$. The $H_{1'}$ doublet shape and the coupling constant ($J_{1',2'} = 4{\cdot}0$) are identical in both cases and indicate thus the presence of a 2-chloro-2-deoxyribofuranosyl residue to which a little different heterocyclic residue is attached; the whole molecule possesses in both cases the β configuration. The proton signals of the heterocyclic base confirm in both cases the presence of a methoxycarbonyl group, NH function and a H_5 proton, the chemical shift values of the two latter being only little different in both



In formulae *XV*–*XVII*, R = C_6H_5CO group

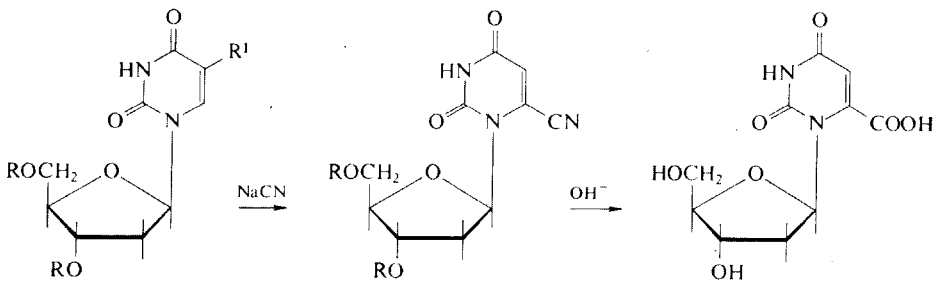
SCHEME 2

substances. On the basis of these findings, the two isomers may be ascribed structures *XV* and *XVI*, namely, the structures of methyl orotate glycosylated at positions N^1 and N^3, resp. (Scheme 2).

The assignment of both structures and their final proof was performed by reaction of compounds *XV* and *XVI* with triethylamine in acetonitrile leading in both cases to the recovery of $O^{2,2'}$-anhydronucleosides, *i.e.*, to the quantitative formation of the starting *IV* from compound *XV* and of the isomeric anhydronucleoside *XVII* from compound *XVI* under the same conditions. The NMR spectra of compounds *IV* and *XVII* differ only in the chemical shift value of the $H_{1'}$ proton; a downfield shift may be thus observed with compound *XVII*, similarly to compounds *XV* and *XVI*. Compounds *IV* and *XVII* are pure anomers with the β-*arabino* configuration.

Migration* of the heterocyclic portion of the modified nucleoside *IV* on treatment with hydrogen chloride might be explained by an attack of the halide ion at position 1′ combined with liberation of the heterocyclic base which, however, remains attached through the $C_{(2)}—O^{2'}$ bond and undergoes an intramolecular reaction with the intermediarily formed halogenose, this time in the sterically more accessible N^3 position. The anomeric purity of the product would be in accordance with this reaction sequence. The intermediary anhydronucleoside *XVII* (a small amount of which was detected in the reaction mixture by chromatography) is then rapidly opened by the action of hydrogen chloride (a S_N2 reaction) with the formation of compound *XVI*.

From the standpoint of the 2′-deoxyorotidine (*XVIII*) preparation, only the N^1--derivative *XV* is of interest. The attempted reaction of compound *XV* with tri-n--butyltin hydride in the presence of 2,2′-azobis(2-methylpropionitrile[12]) failed in spite of several experiments. The single product which was isolated after a longer reaction period of time was the anhydronucleoside *IV*, the formation of which may be ascribed to the weakly basic character of the reagent. It is the first case in the series



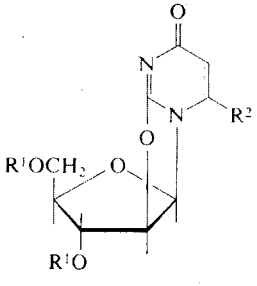
XIXa; $R^1 = H$ *XX* *XVIII*
XIXb; $R^1 = Br$
In formulae *XIX*, *XX*, $R = C_6H_5CO$

SCHEME 3

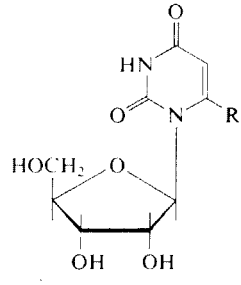
* For an analogous migration see ref.[9] (hydrolysis of compound *II*).

of the type *XIII* derivatives that reduction of the C—Cl bond failed under the above mentioned conditions. This failure is not due to whatever substituents of compound *XV* at position 6 since 6-alkyl derivatives of type *XIII* readily afford the 2'-deoxyribosides *XIV* (ref.[7,11]); this effect might be rather ascribed to the character (electronegativity) of the alkoxycarbonyl residue which deactivates the C—Cl bond in radical reactions.

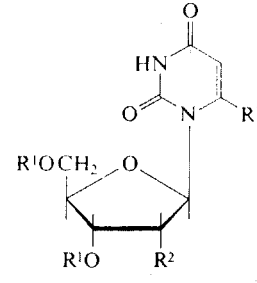
For purposes of comparison, the authentic 2'-deoxyorotidine (6-carboxy-2'-deoxyuridine, *XVIII*) has been now prepared by a method which makes use of a principle reported in the literature[3]. Thus, bromination of 3',5'-di-O-benzoyl-2'-deoxyuridine (*XIXa*) with N-bromosuccinimide afforded the 5-bromo derivative *XIXb* which was converted into the 6-cyano derivative *XX* by the action of sodium cyanide in pyridine. Alkaline hydrolysis of compound *XX* afforded 2'-deoxyorotidine (*XVIII*) which was isolated as the lithium salt (Scheme 3). The UV spectrum of compound *XVIII* (λ_{max} 267 nm at pH 7 and 12; hyperchromy at pH 12) is almost identical with that of orotidine (*I*) and confirms the presence of a NH function on the heterocyclic nucleus as well as of an orotic acid residue glycosylated at position N^1. This synthesis of 2'-deoxyorotidine (*XVIII*) is advantageous from the preparative standpoint since the starting compound *XIXa* is readily accessible from compound *XI* (from D-arabinose)[12].



XIa; $R^1 = COC_6H_5$, $R^2 = H$
XIb; $R^1 = COC_6H_5$, $R^2 = CH_3$



XIIa; R = H
XIIb; $R = CH_3$



$R^1 = COC_6H_5$, R^3 = H, alkyl
XIII; R^2 = Cl
XIV; R^2 = H

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block) and are uncorrected. Unless stated otherwise, solutions were taken down on a rotatory evaporator at 35°C/15 Torr and analytical samples were dried over phosphorus pentoxide at 0·1 Torr. Paper chromatography was performed by the descending technique on paper Whatman No 1 (preparative runs on paper Whatman No 3 MM) in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2); S_2, 1-butanol–acetic acid–water (5 : 2 : 3); and S_3, 1-butanol–ethanol–water (4 : 1 : 2). Thin-layer chromatography on silica gel was performed on ready-for-use Silufol UV_{235} silica

gel plates (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems S_4, chloroform; S_5, chloroform–ethanol (98 : 2); S_6, chloroform–ethanol (95 : 5); S_7, chloroform–ethanol (9 : 1); S_8, benzene–ethyl acetate (75 : 25); S_9, benzene–ethyl acetate (3 : 2); and S_{10}, ethyl acetate alone. Paper electrophoresis was performed by the reported technique[21] on paper Whatman No 3 MM at 20 V/cm/1 h in buffer solutions E_1, 0·1M triethylammonium hydrogen carbonate (pH 7·5) and E_2, 0·1M triethylammonium borate (pH 7·5). Preparative column chromatography was performed on silica gel according to Pitra (100 g; 30–50 mesh) in the appropriate solvent, 30 ml fractions being taken (Table I). Chromatography on loose layers (40 × 16 × 0·4 cm) was performed on a fluorescent indicator-containing silica gel (produced by Service Laboratories of this Institute). The UV absorption spectra were taken in aqueous solutions on a recording Zeiss Specord spectrophotometer. The NMR spectra were measured on a Varian 100 apparatus in deuteriochloroform or hexadeuteriodimethyl sulfoxide with the use of hexamethyldisiloxane as internal standard (the chemical shift values are expressed in p.p.m., the coupling constant data are given in Hz).

6-Methoxycarbonyl-$O^{2,2'}$-anhydro-L-uridine (L-*III*)

A mixture of 2-amino-L-arabinofuro[1′,2′ : 4,5]oxazoline[12] (L-*II*; 17·4 g; 0·1 mol), dimethyl acetylenedicarboxylate (25 ml), and ethanol (250 ml) was refluxed for 2 h, cooled down with ice, the solid collected with suction, washed with acetone, and recrystallised from an 1 : 1 mixture of ethanol and acetone. Yield, 21·2 g (74·7%) of compound L-*III*, m.p. 241–242°C. For $C_{11}H_{12}N_2O_7$ (284·2) calculated: 46·48% C, 4·25% H, 9·86% N; found: 46·26% C, 4·27% H, 10·00% N.

3′,5′-Di-O-benzoyl-6-methoxycarbonyl-$O^{2,2'}$-anhydrouridine (*IV*)

To a mixture of compound[5] *III* (21·4 g; 75 mmol), benzoyl cyanide (21 g; 160 mmol), and acetonitrile (150 ml) there was added dropwise with stirring triethylamine (15 ml) until the exothermic reaction set in. The mixture was stirred for 1 h, diluted with ether (1 000 ml), the solid collected with suction, washed with ethanol (200 ml), and recrystallised from ethanol. Yield, 27·3 g (74%) of compound *IV*, m.p. 212–213°C, $[\alpha]^{25}$ −125·6° (*c* 0·5, dimethylformamide). For $C_{25}H_{20}N_2O_9$ (492·4) calculated: 60·97% C, 4·09% H, 5·69% N; found: 59·96% C, 4·14% H, 5·83% N. NMR spectrum ($CDCl_3$): 3·91 (s, 3 H) $COOCH_3$; 4·43 (m, 2 H) $H_{5'}$; 4·72 (m, 1 H) $H_{4'}$; 5·54 (d, 1 H, $J_{1',2'} = 5·7$, $0 < J_{2',3'} < 1·0$) $H_{2'}$; 5·76 (d, 1 H, $J_{3',4'} = 2·0$) $H_{3'}$; 6·67 (s, 1 H) H_5; 7·13 (d, 1 H, $J_{1',2'} = 5·7$) $H_{1'}$; 7·30–7·70 + 7·80–8·10 (aromatic protons).

The L-*isomer* (L-*IV*) was prepared analogously to *IV* in 75·5% yield; m.p. 213°C; $[\alpha]_D^{25}$ +123·5° (*c* 0·5, dimethylformamide). Found: 60·58% C, 4·05% H, 5·94% N. NMR spectrum ($CDCl_3$): identical with that of compound *IV*.

2′,5′- and 3′,5′-Di-O-benzoyl-6-methoxycarbonyluridine (*VI* and *VII*)

To a mixture of compound *IV* (24·6 g; 50 mmol) and methanol (800 ml) there was added dropwise with stirring stannic chloride (18 ml) over 10 min. The mixture was refluxed for 3 h under exclusion of atmospheric moisture (calcium chloride tube), evaporated, the residue diluted with chloroform (300 ml), the solution washed with water (100 ml), two 100 ml portions of 10% aqueous sodium thiosulfate, and water (100 ml) again, dried over anhydrous magnesium sulfate, filtered, and the filtrate evaporated. The residue was applied in benzene to a column of silica gel (50 g; packed in benzene) and the elution was performed with 500 ml of benzene (*cf.* compound *IX*). The subsequent elution with a mixture of benzene and ethyl acetate (9 : 1) afforded a mixture of compounds *VI* and *VII*. After drying under diminished pressure, the yield was 7·2 g (28%) of a foam which was chromatographed on four loose layers of silica gel in the solvent system S_9.

Bands of the product were eluted with methanol, the eluates evaporated, an the residue dried under diminished pressure to afford 3·4 g of compound *VI*, homogeneous on chromatography. For $C_{25}H_{22}N_2O_{10}$ (510·5) calculated: 58·81% C, 4·34% H, 5·48% N; found: 59·29% C, 4·46% H, 5·71% N. There was also obtained 3·6 g of compound *VII* as a foam; found: 58·78% C, 4·49% H, 5·62% N. The final elution of the silica gel column was performed with methanol (500 ml). The eluate was evaporated and the residue crystallised from ethanol (150 ml) to afford 1·4 g (5·7%) of the starting compound *IV*, m.p. 214°C, undepressed on admixture with an authentic specimen; its identity was confirmed by chromatography in S_5 and S_{10}.

The L-*isomers* L-*VI* and L-*VII* were obtained analogusly as a mixture (7·8 g; 30·6%) and were chromatographically identical (S_9) with D-enantiomers.

Prolonged reaction time. A mixture of compound *IV* (4·9 g; 10 mmol) and methanol (200 ml) was treated dropwise with stannic chloride (4 ml) with stirring, the whole refluxed (calcium chloride tube) for 7 h, evaporated, the residue diluted with chloroform (100 ml), the solution washed with two 50 ml portions of 10% aqueous sodium thiosulfate and water (50 ml), dried over anhydrous magnesium sulfate, evaporated, and the residue processed as above by chromatography on

TABLE I

Paper Chromatography (R_F) and Electrophoresis

Compound	S_1	S_2	S_3	E_1[a]	E_2[b]
Uridine	0·41	0·50	0·35	—	1·00
I	0·24	0·23	0·13	0·35	1·80
III	0·46	0·51	0·50	0·44	1·00
VIII	0·28	0·28	0·25	0·47	0·90
X	0·86	0·93	—	0·43	0·92
XVIII	0·42	0·25	0·22	0·55	1·20

Compound	S_4	S_5	S_8	S_9	S_{10}
IV	—	0·17	—	0·05	0·25
V	—	—	0·40	—	—
VI	—	—	0·10	0·20	0·85
VII	—	—	0·10	0·33	0·85
IX	0·77	—	0·57	0·73	—
XV	—	0·45	0·55	—	—
XVI	—	0·80	0·70	—	—
XVII	—	0·42	—	—	—
XIXa	0·24	—	0·14	—	—
XIXb	0·24	—	0·37	—	—
XX	0·20	—	0·37	—	—

[a] Referred to 3′-uridylic acid; [b] referred to uridine.

a silica gel column in benzene. The benzene eluate was evaporated and the residue rechromatographed on two layers of silica gel in the solvent system S_8 (elution with methanol) to afford 0·82 g (15%) of compound *IX*, homogeneous on chromatography, and 0·69 g (35%) of compound *V*, m.p. 104–106°C, undepressed on admixture with an authentic specimen. For $C_8H_{10}N_2O_4$ (198·2) calculated: 48·47% C, 5·08% H, 14·13% N; found: 48·22% C, 5·12% H, 14·54% N. Elution of the column with a mixture of ethyl acetate and benzene (1 : 1) and rechromatography on two silica gel layers in S_9 (elution with ethyl acetate) afforded 13·5% of a mixture of compounds *VI* and *VII* and 4% of the starting material *IV*.

1-(3,5-Di-O-benzoyl-2-O-methyl-β-D-ribofuranosyl)-4-methoxy-
-6-methoxycarbonylpyrimidin-2-one (*IX*)

A. The benzene eluate from the above preparation of compounds *V* and *VI* was evaporated and the residue (12 g) chromatographed on four silica gel layers in the solvent system S_8. The separated bands were eluted with methanol and the eluates evaporated. Yield, 9·0 g (33·5%) of compound *IX*, homogeneous on chromatography. For $C_{27}H_{26}N_2O_{10}$ (538·5) calculated: 60·21% C, 4·86% H, 5·20% N, 17·27% OCH_3; found: 59·93% C, 5·05% H, 5·25% N, 17·08% OCH_3. NMR spectrum ($CDCl_3$): 3·43 (s, 3 H) + 3·72 (s, 3 H) + 3·86 (s, 3 H) 2'-OCH_3 + + 4-OCH_3 + 6-$COOCH_3$; 4·50 (m, 1 H, $J_{4',3'} = 5·0$, $J_{4',5'} = 4·0$, $J_{4',5''} = 5·5$) $H_{4'}$; 4·52 (m, 2 H) 2 $H_{5'}$; 5·22 (s, 1 H, $J_{1',2'} = 1·0$) $H_{1'}$; 5·50 (d, 1 H, $J_{2',1'} = 1·0$, $J_{2',3'} = 1·5$) $H_{2'}$; 5·55 (br d, 1 H, $J_{3',2'} = 1·5$, $J_{3',4'} = 5·0$) $H_{3'}$; 7·01 (s, 1 H) H_5; 7·25–7·60 (m, 6 H) + 7·95 to 8·0 (m, 4 H) aromatic protons.

B. A mixture of compound *X* (662 mg; 2 mmol), benzoyl cyanide (0·9 g; 6·9 mmol), and acetonitrile (15 ml) was treated with triethylamine (0·5 ml), the whole stirred at room temperature for 1 h, evaporated, and the residue chromatographed on one layer of loose silica gel in the solvent system S_4. Band of the product *IX* was eluted with methanol and the eluate evaporated to afford 800 mg (74%) of the chromatographically homogeneous (solvent systems S_4 and S_8) compound *IX*, identical with specimen obtained by procedure *A*.

6-Methoxycarbonyluridine (*VIII*)

A mixture (4·8 g; 9·4 mmol) of compounds *VI* and *VII* was kept at room temperature in 0·1M methanolic sodium methoxide (100 ml) overnight, neutralised with dry Dowex 50 X 8 (H^+) ion exchange resin, the resin filtered off, and washed with methanol (50 ml). The filtrate and washings were evaporated under diminished pressure, the residue diluted with water (100 ml), extracted with three 25 ml portions of ether, the aqueous phase evaporated, and the residue codistilled with ethanol to afford 2·5 g of an amorphous foam which was chromatographed on two silica gel layers in the solvent system S_7. Bands of the product were eluted with methanol, the eluate evaporated under diminished pressure, and the residue purified by precipitation from a methanolic solution (10 ml) with ether (100 ml). The precipitate was collected with suction, washed with ether, and dried to afford 2·1 g (74%) of compound *VIII*, homogeneous on chromatography (S_1–S_3) and electrophoresis (E_1, E_2). For $C_{11}H_{14}N_2O_8$ (302·2) calculated: 43·70% C, 4·66% H, 9·27% N, 10·26% OCH_3; found: 44·28% C, 4·35% H, 9·22% N, 11·70% OCH_3. NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·58 multiplet centre (2 q, 2 H, $J_{4',5'} = 3·0$, $J_{4',5''} = 6·5$, $J_{gem} = 11·5$) 2 $H_{5'}$; 3·78 (m, 1 H) $H_{4'}$; 3·84 (s, 3 H) $COOCH_3$; 4·06 (t, 1 H, $J_{3',4'} = 6·0$) $H_{3'}$; 4·50 (q, 1 H, $J_{2',3'} = 6·5$) $H_{2'}$; 5·44 (d, 1 H, $J_{1',2'} = 4·5$) $H_{1'}$; 5·86 (s, 1 H) H_5; 3·20 (br s, 3 H) 3 OH. UV spectrum, water: λ_{max} 275 nm; pH 12: λ_{max} 273 nm (hyperchromy, 10%).

Orotidine (lithium salt) (*I*)

A mixture (7·2 g; 14 mmol) of compounds *VI* and *VII* was kept at room temperature in 0·2M methanolic sodium methoxide (100 ml) for two days and then processed analogously to the preparation of compound *VIII*. The residue of the crude compound *VIII* was dissolved in water (50 ml), the solution was treated with 10% aqueous lithium hydroxide (20 ml), the whole kept at room temperature overnight, neutralised with Dowex 50 X 8 (H^+) ion exchange resin, the resin filtered off, and washed with water (50 ml). The filtrate and washings were concentrated to the volume of about 50 ml and the concentrate applied to a column (100 ml) of Dowex 50 X 8 ion exchange resin in the Li^+ cycle. The UV-absorbing fraction was eluted with water through the Uvicord apparatus and the eluate concentrated under diminished pressure to the volume of about 10 ml. Ethanol (50 ml), acetone (100 ml), and ether (200 ml) were added to the concentrate, the precipitate was collected with suction, washed with a mixture (200 ml) of acetone and ether (1 : 1) and dried *in vacuo* to afford 2·8 g (75%) of the lithium salt *I*, homogeneous on chromatography (S_1-S_3) and electrophoresis (E_1 and E_2) and identical with an authentic specimen (Calbiochem, Los Angeles, USA). For $C_{10}H_{11}LiN_2O_8$ (294·2) calculated: 9·52% N; found: 9·84% N. UV spectrum, pH 7: λ_{max} 270 nm, ε_{max} 9700; pH 12: λ_{max} 271 nm, ε_{max} 7000.

L-*Orotidine* (lithium salt) (L-*I*) was prepared from a mixture (10 mmol) of compounds L-*V* and L-*VI* in 71% yield as a chromatographically (S_1-S_3) and electrophoretically (E_1 and E_2) homogeneous substance; content, 95% (as determined spectrophotometrically).

1-(2-O-Methyl-β-D-ribofuranosyl)-4-methoxy-6-methoxycarbonyl-pyrimidin-2-one (*X*)

A solution of compound *IX* (2·7 g; 5 mmol) in 0·1M methanolic sodium methoxide (100 ml) was kept at room temperature overnight, neutralised with dry Dowex 50 X 8 (H^+) ion exchange resin, the resin filtered off, and washed with methanol (50 ml). The filtrate and washings were evaporated and the residue was kept with a little water in a refrigerator overnight to deposit a solid which was collected with suction, washed with a mixture (200 ml) of ethanol and ether (1 : 2), and crystallised from water. Yield, 1·0 g (60%) of compound *X*, m.p. 158–159°C. For $C_{13}H_{18}N_2O_8$ (330·3) calculated: 47·26% C, 5·49% H, 8·48% N, 28·15% OCH_3; found: 47·28% C, 5·49% H, 8·40% N, 28·21% OCH_3. Mass spectrum: M + 1 (331, $C_{13}H_{18}N_2O_8$), M − 1 (329), M − 18 (299, $C_{12}H_{15}N_2O_7$), m/e = 269 ($C_{11}H_{13}N_2O_6$), m/e = 185 (BH_2, $C_7H_9N_2O_4$). NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·34 (s, 3 H) 2′-OCH_3; 3·94 (s, 3 H) $COOCH_3$; 3·65 (2 q, 2 H, $J_{5',4'}$ = 2·5, $J_{5'',4'}$ = 4·5, J_{gem} = 12·0) $2H_{5'}$; 3·90 (m, 2 H) $H_{3'}$ + $H_{4'}$; 4·72 (br s, 1 H) 5′-OH; 4·92 (s, 1 H, $J_{1',2'} < 1·0$) $H_{1'}$; 4·98 (dd, 1 H, $J_{2',1'} < 1·0$, $J_{2',3'}$ = 3·0) H_2; 5·38 (br d, 1 H) 3′-OH; 6·99 (s, 1 H) H_5. UV spectrum, pH 7: λ_{max} 284 nm, ε_{max} 5500, λ_{min} 241 nm; pH 12: λ_{max} 270 nm, ε_{max} 5400, λ_{min} 238 nm.

Reaction of Compound *IV* with Boron Trifluoride Etherate

A mixture of compound *IV* (4·9 g; 10 mmol), methanol (100 ml), and boron trifluoride etherate was refluxed for 6 h, evaporated, the residue dissolved in chloroform (100 ml), the solution washed (50 ml portions) with water, three times with saturated aqueous sodium hydrogen carbonate and water again, dried over anhydrous magnesium sulfate, evaporated, and the residue chromatographed on two silica gel layers in the solvent system S_4. The starting compound *IV* band at the start line was eluted with chloroform and ethyl acetate (200 ml each), the eluates evaporated, and the residue crystallised from ethanol to afford 3·4 g (70·0%) of the starting material *IV*, m.p. 213°C, undepressed on admixture with an authentic specimen. Band of the reaction product *V* was eluted with methanol, the eluate evaporated, and the residue crystallised from

ethanol to afford 0·50 g (25%) of compound *V*, m.p. 105—106°C. For $C_8H_{10}N_2O_4$ (198·2) calculated: 48·47% C, 5·08% H, 14·13% N, 46·92% OCH_3; found: 48·99% C, 5·18% H, 14·24% N, 47·36% OCH_3. NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·98 + 3·95 + 3·99 (3 × s, 3 × 3 H) 3 × OCH_3; 6·98 (s, 1 H) H_5.

Reaction of Compound *IV* with Hydrogen Chloride in Dimethylformamide

A stirred solution of compound *IV* (27 g; 55 mmol) in dimethylformamide (150 ml) was heated to 100°C under exclusion of atmospheric moisture, treated with 6M hydrogen chloride in dimethylformamide (100 ml), the whole heated with stirring for 1 h at 100°C, and poured into 2 liters of water. The solid was collected with suction, washed with water until the washings were neutral, and dissolved in chloroform (200 ml). The chloroform solution was dried over anhydrous magnesium sulfate, filtered, the filtrate evaporated, and the residue chromatographed on a column of silica gel (150 g). Elution with chloroform afforded 7·6 g (26·2%) of compound *XV* as a foam, homogeneous on chromatography in solvent systems S_4, S_5, and S_8. For $C_{25}H_{21}ClN_2O_9$ (528·9) calculated: 56·77% C, 4·00% H, 6·70% Cl, 5·29% N; found: 56·35% C, 3·98% H, 6·24% Cl, 5·28% N. NMR spectrum (deuteriochloroform): 3·97 (s, 3 H) $COOCH_3$; 4·64 (m, 3 H) $H_{4'}$ + + 2 $H_{5'}$; 5·42 (q, 1 H, $J_{2',1'} = 4·0$, $J_{2',3'} = 6·8$) $H_{2'}$; 5·88 (br t, 1 H, $J_{3',2'} = 6·8$, $J_{3',4'} = 6·5$) $H_{3'}$; 6·18 (br s, 1 H) H_5; 6·20 (d, 1 H, $J_{1',2'} = 4·0$) $H_{1'}$; 9·54 (br s, 1 H) NH; 7·25—7·65 + + 7·95—8·15 (10 H) aromatic protons. The other product was compound *XVI*, a homogeneous foam in the solvent systems S_4, S_5, and S_8; yield, 4·4 g (15%). For $C_{25}H_{21}ClN_2O_9$ (528·9) calculated: 56·77% C, 4·00% H, 6·70% Cl, 5·29% N; found: 56·92% C, 4·15% H, 6·22% Cl, 5·70% N. NMR spectrum (deuteriochloroform): 3·90 (s, 3 H) $COOCH_3$; 4·68 (m, 3 H) $H_{4'}$ + + 2 $H_{5'}$; 5·43 (q, 1 H, $J_{2',1'} = 4·0$, $J_{2',3'} = 6·7$) $H_{2'}$; 6·0 (br t, 1 H, $J_{3',2'} = 6·5$) $H_{3'}$; 6·38 (d, 1 H, $J_{5,NH} = 2·0$) H_5; 6·78 (d, 1 H, $J_{1',2'} = 4·0$) $H_{1'}$; 9·28 (br s, 1 H) NH; 7·25—7·65 + + 7·95—8·15 (10 H) aromatic protons.

Reaction of 3′,5′-Di-O-benzoyl-$O^{2,2'}$-anhydrouridine (*XIa*) and 3′,5′-Di-O-benzoyl-6-methyl-$O^{2,2'}$-anhydrouridine (*XIb*) with Stannic Chloride

To a suspension of 5 mmol of compound *XIa* (*cf.*[14]) or *XIb* (*cf.*[13]) in methanol (100 ml) there was added dropwise with stirring stannic chloride (2 ml), the whole mixture refluxed for 7 h under exclusion of atmospheric moisture (calcium chloride tube), and evaporated. The residue was dissolved in chloroform (100 ml), the solution washed with two 50 ml portions of 10% aqueous sodium thiosulfate and water (50 ml), dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was heated in methanolic sodium methoxide (0·1M, 100 ml) for 3 h at 50°C, the mixture neutralised with dry Dowex 50 X 8(H^+) ion exchange resin, filtered, and the resin washed with methanol (50 ml). The filtrate and washings were evaporated, the residue was diluted with water (50 ml) and washed with two 20 ml portions of ether. The aqueous phase was evaporated and the residue was analysed by chromatography in the solvent systems S_1 and S_2 as well as electrophoresis in buffer solutions E_1 and E_2 to show the ribonucleoside *XIIa* and *XIIb*, resp., as the single product; no by-products were detected in concentration higher than 5%.

$O^{2,2'}$-Anhydro-3-(3,5-di-O-benzoyl-β-D-arabinofuranosyl)-6-methoxycarbonyluracil (*XVII*)

A solution containing compound *XVI* (2 g; 3·8 mmol), triethylamine (5 ml), and acetonitrile (20 ml) was refluxed for 4 h (quantitative reaction as shown by chromatography in the solvent system S_5), evaporated, the residue codistilled with ethanol and then crystallised from ethanol (100 ml)

to afford 1·2 g (64%) of compound *XVII*, m.p. 167—168°C. For $C_{25}H_{20}N_2O_9$ (492·4) calculated: 60·97% C, 4·09% H, 5·69% N; found: 60·80% C, 4·11% H, 5·75% N. NMR spectrum (deuteriochloroform): 3·81 (s, 3 H) $COOCH_3$; 4·32 (q, 1 H, $J_{5',4'} = 5{\cdot}0$, $J_{5',5''} = 12{\cdot}0$) H_5; 4·50 (q, 1 H, $J_{5'',4'} = 5{\cdot}6$, $J_{5',5''} = 12{\cdot}0$) $H_{5'}$; 4·70 (br d, 1 H, $J_{4',3'} = 2{\cdot}5$) $H_{4'}$; 5·52 (d, 1 H, $J_{2',3'} < 1{\cdot}0$, $J_{2',1'} = 6{\cdot}0$) $H_{2'}$; 5·69 (br d, 1 H, $J_{3',4'} = 2{\cdot}5$) $H_{3'}$; 6·62 (d, 1 H, $J_{1',2'} = 6{\cdot}0$) $H_{1'}$; 6·77 (s, 1 H) H_5; 7·25—7·65 + 7·80—8·05 (10 H) aromatic protons.

The analogous reaction of compound *XV* with triethylamine in acetonitrile afforded compound *IV* (m.p. 214—215°C, undepressed on admixture with an authetic specimen; yield, 78%) the identity of which was confirmed by chromatography in the solvent systems S_5 and S_{10} and by NMR spectrum (deuteriochloroform).

Reaction of Compound *XV* with Tri-n-butyltin Hydride

A mixture of compound *XV* (1·05 g; 2 mmol), tri-n-butyltin hydride (2·5 g), 2,2'-azobis (2-methylpropionitrile) (0·05 g), and benzene (20 ml) was refluxed for 5 h and evaporated. The residue was triturated with light petroleum (100 ml), the solid collected with suction, washed with light petroleum, and chromatographed on one layer of silica gel in the solvent system S_5. Elution of UV-absorbing bands with ethanol and evaporation of eluates afforded 0·7 g (66·5%) of the starting material *XV* and 0·25 g (25%) of compound *IV*, m.p. 214°C (ethanol), undepressed on admixture with an authentic specimen and identical on chromatography in the solvent systems S_5 and S_{10}.

3',5'-Di-O-benzoyl-5-bromo-2'-deoxyuridine (*XIXb*)

A mixture of 3',5'-di-O-benzoyl-2'-deoxyuridine[13] (*XIXa*; 2·2 g; 5 mmol), N-bromosuccinimide (2 g), and ethanol-free chloroform (60 ml) was refluxed until the starting material disappeared (as shown by chromatography in the solvent system S_5), evaporated, the residue refluxed briefly with ethanol (100 ml), and the whole poured into water (1 000 ml). The resulting solution was kept at room temperature overnight, the solid collected with suction, washed with water and ethanol, and crystallised from ethanol, light petroleum being added until the solution was turbid. Yield, 2·0 g (77·5%) of the chromatographically pure compound *XIXb*, m.p. 190—190·5°C, $[\alpha]_D^{25}$ +35·4° (*c* 0·5, dimethylformamide). For $C_{23}H_{19}BrN_2O_7$ (515·4) calculated: 53·59% C, 3·71% H, 15·51% Br, 5·43% N; found: 53·82% C, 3·79% H, 16·05% Br, 5·34% N.

3',5'-Di-O-benzoyl-6-cyano-2'-deoxyuridine (*XX*)

A mixture of compound *XIXb* (1·9 g; 3·7 mmol), sodium cyanide (2 g), and pyridine (20 ml) was refluxed for 2 h, diluted with water (50 ml), neutralised with dilute (1 : 1) hydrochloric acid, and concentrated under diminished pressure to the volume of about 20 ml. Chloroform (100 ml) was added to the concentrate and the aqueous phase extracted with additional two 50 ml portions of chloroform. The combined organic phase was washed with two 50 ml portions of water, dried over anhydrous magnesium sulfate, evaporated, and the residue crystallised from ethyl acetate with the addition of light petroleum to the turbidity. Yield, 1·2 g (70%) of compound *XX*, m.p. 153—155°C. For $C_{24}H_{19}N_3O_7$ (461·4) calculated: 62·47% C, 4·15% H, 9·10% N; found: 62·42% C, 4·32% H, 9·03% N.

2'-Deoxyorotidine (lithium salt) (*XVIII*)

A solution of compound *XX* (1·0 g; 2·17 mmol) in 0·1M methanolic sodium methoxide was kept at room temperature overnight, neutralised with dry Dowex 50 X 8 (H^+) ion exchange resin,

the mixture filtered, and the resin washed with methanol (50 ml). The filtrate and washings were evaporated, the residue dissolved in water (50 ml), and the solution washed with two 25 ml portions of ether. The aqueous phase was evaporated, the residue refluxed in 1M-NaOH (50 ml) for 2 h, the mixture neutralised with Dowex 50 X 8 (H^+) ion exchange resin, and filtered. The filtrate was concentrated under diminished pressure and the concentrate applied to a column (100 ml) of Dowex 50 X 8 (Li^+ cycle) ion exchange resin. The UV-absorbing product was eluted with water (3 ml per min) with the use of the Uvicord apparatus. The eluate was evaporated, the residue dried by coevaporation with three 25 ml portions of ethanol and then by precipitation from methanol (10 ml) with ether (100 ml), The precipitate was collected with suction, washed with ether, and dried under diminished pressure. Yield, 0·4 g (66·5%) of the lithium salt, homogeneous on chromatography (S_1-S_3) and electrophoresis (E_1 and E_2). For $C_{10}H_{11}LiN_2O_7$ (278·2) calculated: 10·07% N; found: 10·54% N. Ultraviolet spectrum, pH 7: λ_{max} 267 nm, ε_{max} 9 500, λ_{min} 234 nm; pH 12: λ_{max} 268 nm, ε_{max} 6 900.

The author wishes to thank Dr M. Masojídková for measurement and interpretation of NMR spectra and Dr. A. Trka for measurement and interpretation of mass spectra. Thanks are due to Professor Dr J. Škoda of this Institute for a gift of a sample of the authentic orotidine. The excellent technical assistance of Mrs B. Kopecká is gratefully acknowledged.

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Translated by J. Pliml.