PREPARATION, ANTIBACTERIAL EFFECTS AND ENZYMATIC DEGRADATION OF 5-FLUOROURACIL NUCLEOSIDES

Beatrice SCHWARZ[a], Dieter CECH[a], Antonín HOLÝ[b] and Jan ŠKODA[b]

[a] *Sektion Chemie, Humboldt Universität, Berlin, GDR and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

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Reaction of perbenzoylated aldopentafuranosyl derivatives of uracil with fluorine in acetic acid afforded perbenzoylated 5-fluorouracil nucleosides. Their methanolysis gave the following free nucleosides of 5-fluorouracil: β-D-ribofuranoside (*Id*), 2-deoxy-β-D-ribofuranoside (*IId*), their enantiomers *VIII* and *IX*, α-D-ribofuranoside (*XIII*), 2-deoxy-α-D-ribofuranoside (*XV*), β-D-arabinofuranoside (*IV*) and its L-enantiomer *X*, β-D-xylofuranoside (*V*) and its α-D-anomer *XI*, α-L-lyxofuranoside (*VI*) and 2-deoxy-α-L-lyxofuranoside (*VII*) and the enantiomers of the latter two compounds, *XII* and *XIV*, respectively. Analogously were obtained 5-deoxy-β-D-ribofuranoside (*III*), β-D-ribopyranoside (*XVI*) and 1-(*S*)-(2,3-dihydroxypropyl)-5-fluorouracil (*XVIIc*). 1-Allyl-5-fluorouracil (*XVIII*) was prepared by reaction of allyl bromide with 2,4-bis-(trimethylsilyloxy)-5-fluoropyrimidine.

The cell-free extract from *Escherichia coli* cleaves all the 5-fluorouracil nucleosides in which the nucleoside carbon atom has the *R*-configuration and the 3'-hydroxyl of the sugar moiety is in *trans*-relation to the base. Compounds which have not these structural features are resistant. Besides nucleosides which on enzymatic cleavage afford 5-fluorouracil, also the non-cleavable 1-(2-deoxy-β-L-ribofuranosyl)-5-fluorouracil (*IX*), 1-(2-deoxy-α-D-ribofuranosyl)-5-fluorouracil (*XV*) and 1-(2-deoxy-α-D-lyxofuranosyl)-5-fluorouracil (*XIV*) exhibit an antibacterial effect towards *E. coli* (ID_{50} 1·0—2·5 . 10^{-5}M). This effect can be reversed by 2'-deoxyuridine but not by thymidine.

5-Fluorouracil and its derivatives have become useful tools of the molecular-biological research and attracted attention of many experimental as well as clinical oncologists and virologists[1,2]. On the other hand, substantially less is known about the interactions of 5-fluoropyrimidines with microbial cells, although some of the compounds mentioned (first of all 5-fluorocytosine of low toxicity[3]) exhibit pronounced antimicrobial activity.

Having in hand a relatively large series of nucleosides, derived from 5-fluorouracil, we decided to compare their antibacterial activity towards *Escherichia coli*. We chose a 5-fluorouracil-sensitive prototrophic strain, growing on media, containing only inorganic salts and glucose without any components which could antagonize the effects of the studied analogues.

Since most of microbial, and first of all bacterial, cells have a very potent enzymatic equipment, particularly concerning nucleosidases and phosphorylases, it

could be expected that many 5-fluorouracil nucleosides would be degraded by microorganisms to give 5-fluorouracil – an extraordinarily potent growth-inhibitor of *Escherichia coli*. Since this antibaterial activity is as high as that of strongly active antibiotics[4] we can assume that in such case the biological activity is caused or influenced by the arising 5-fluorouracil. Therefore, we first studied cleavage of the 5-fluorouracil nucleosides with a non-fractionated cell-free extract from *Escherichia coli*. The aim of this study was also to find the minimum structural conditions required for the enzymatic hydrolysis or phosphorolysis of the nucleoside bond in the anomalous nucleoside by the complex of bacterial enzymes.

Preparation of 5-Fluorouracil Nucleosides

Nucleoside derivatives of 5-fluorouracil can in principle be prepared by nucleosidation reactions of 5-fluorouracil[5], its 2,4-di-O-methyl[6] or 2,4-bis(trimethylsilyl) derivatives[6,7], by conversion of 5-fluorouridine[7–12] or by fluorination of uracil nucleosides[13,14]. Recently, we elaborated an anvantageous synthesis of fluorouracil nucleosides[15] consisting in the direct fluorination of perbenzoylated uracil nucleosides with fluorine dissolved in an appropriate solvent (chloroform, acetic acid). This method was applied to preparation of 2'-deoxy-5-fluorouridine (*IId*) and other related compounds[16,17]. We elaborated also synthetic procedures leading to some hitherto unknown or not easily accessible uracil nucleosides with modified sugar moiety. Since the mentioned fluorination reaction is sufficiently mild and accompanied neither by nucleoside bond fission nor change in the sugar moiety, it was applied also to compounds *IIIb–XVIIb*. The starting compounds were obtained either directly in the corresponding syntheses or by benzoylation of the free nucleosides with benzoyl cyanide[18].

5'-Deoxyuridine (*IIIa*) was prepared from 5'-iodo-5'-deoxy-2',3'-O-isopropylideneuridine[19] by reductive dehalogenation with tri-n-butyltin hydride and acid hydrolysis. Reaction of *IIIa* with benzoyl cyanide afforded the 2',3'-dibenzoate *IIIb*. 1-(β-D-Xylofuranosyl)uracil (*Va*) was obtained by nucleosidation of 2,4-dimethoxypyrimidine with 2,3,5-tri-O-acetyl-D-xylofuranosyl chloride, subsequent demethylation of the product with hydrogen chloride and methanolysis.

The perbenzoylated nucleosides *Ib–XVIIb* were then halogenated with a small excess of fluorine in acetic acid and after evaporation of the solvent *in vacuo* the reaction mixture was treated with an alcoholic solution of triethylamine in order to convert the intermediate 5,6-difluoro-5,6-dihydrouracil derivative[16] into the desired 5-fluorouracil derivative. Crystallization or purification on silica gel afforded the pure compounds *Ic–XVIIc*. Their ^{1}H-NMR spectra exhibit, besides signals of sugar and aromatic protons, only the signal of the proton H_6 ($J_{F,H} = 6{\cdot}0$ Hz; the coupling constant of the anomeric proton, $J_{H_{1'},F}$, is about 1–1·5 Hz); the H_5 signal is absent in spectra of these compounds[20].

Free nucleosides *Id–XVIId* were prepared from the above-mentioned perbenzoyl derivatives by methanolysis. Since – as already mentioned – our studies required removal of even trace amounts of 5-fluorouracil (and also fluoride ions), all the prepared chromatographically pure nucleosides were subjected to additional chromatography on a column of strongly basic ion exchange resin (Dowex 1X2) which removed both these contaminants. All the nucleosides *Id–XVIId* were then isolated by crystallization, their purity being higher than 99·95% (estimated on the basis of UV-absorption).

The UV spectra of these compounds exhibit a maximum at 269–270 nm throughout the whole pH region and a 22–24% hyperchromy in alkaline media. Molar extinction coefficients in an acid or neutral medium are $8{\cdot}92 \pm 0{\cdot}5 \cdot 10^3$ for all the studied compounds, in accord with the values published[5] for 5-fluorouridine (*Id*). The slightly acidic character of all the investigated nucleosides is manifested

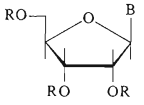
TABLE I
Properties of 5-Fluorouracil Nucleosides

Compound	M.p., °C[a]	R_F[b]	ID_{50} (M)	Cleavage[c]	Configuration			
					1′	2′	3′	4′
Id	184	0·19	$2{\cdot}5 \cdot 10^{-7}$	+	*R*	*R*	*R*	*R*
IId	150	0·26	$3{\cdot}0 \cdot 10^{-7}$	+	*R*	—	*S*	*R*
IIId	187	0·67	$6{\cdot}5 \cdot 10^{-7}$	+	*R*	*R*	*R*	*R*
IVd	182	0·30	$6{\cdot}0 \cdot 10^{-6}$	+	*R*	*S*	*R*	*R*
Vd	203	0·30	$3{\cdot}5 \cdot 10^{-4}$	—	*R*	*R*	*S*	*R*
VId	181	0·24	$9{\cdot}0 \cdot 10^{-7}$	+	*R*	*R*	*R*	*S*
VIId	152	0·35	$4{\cdot}0 \cdot 10^{-5}$	+	*R*	—	*S*	*S*
VIIId	184	0·19	$3{\cdot}5 \cdot 10^{-4}$	—	*S*	*S*	*S*	*S*
IXd	143—147	0·42	$2{\cdot}5 \cdot 10^{-5}$	—	*S*	—	*R*	*S*
Xd	183	0·30	$9{\cdot}0 \cdot 10^{-4}$	—	*S*	*R*	*S*	*S*
XId	—	0·43	$5{\cdot}0 \cdot 10^{-4}$	—	*S*	*R*	*S*	*R*
XIId	182	0·24	$9{\cdot}0 \cdot 10^{-5}$	—	*S*	*S*	*S*	*R*
XIIId	153	0·38	$1{\cdot}0 \cdot 10^{-4}$	—	*S*	*R*	*R*	*R*
XIVd	160—163	0·30	$9{\cdot}0 \cdot 10^{-6}$	—	*S*	—	*R*	*R*
XVd	150	0·23	$1{\cdot}5 \cdot 10^{-5}$	—	*S*	—	*S*	*R*
XVId	204	0·28	$5{\cdot}0 \cdot 10^{-5}$	—	*R*	*R*	*R*	*R*
XVIId	144	0·28	$7{\cdot}0 \cdot 10^{-4}$	—	—	*S*	—	—
XVIII	124	—	$1{\cdot}0 \cdot 10^{-3}$	—	—	—	—	—
XIX	—	—	$4{\cdot}0 \cdot 10^{-9}$	[d]	—	—	—	—
XX	—	—	$1{\cdot}0 \cdot 10^{-7}$	[d]	—	—	—	—

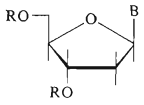
[a] Ethanol–ether; [b] in S3; [c] by cell free extract *E. coli*. [d] not cleavable.

by their electrophoretic mobility in a weakly alkaline buffer (pH 7·5), constant for the whole series and identical with the mobility of compound *Id*. Paper electrophoresis represents also the best method for separation of compounds *Id*–*XVIId* from the more acidic 5-fluorouracil.

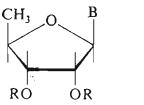
In addition to the mentioned nucleoside derivatives of 5-fluorouracil we prepared also 1-(*S*)-(2,3-dihydroxypropyl)-5-fluorouracil (*XVIIc*) by fluorination of the previously described[21] dibenzoate *XVIIa*, followed by methanolysis. Its UV spectra and electrophoretical mobility correspond to those of 5-fluorouracil nucleosides.



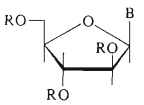
I
(β-D-*ribo*-)



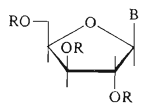
II
(2-deoxy-β-D-*ribo*-)



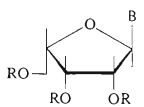
III
(5-deoxy-β-D-*ribo*-)



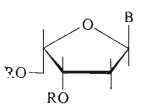
IV
(β-D-*arabino*-)



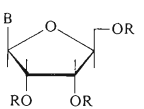
V
(β-D-*xylo*-)



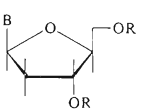
VI
(α-L-*lyxo*-)



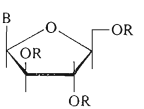
VII
(2-deoxy-α-L-*lyxo*-)



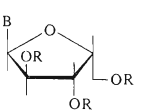
VIII
(β-L-*ribo*-)



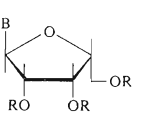
IX
(2-deoxy-β-L-*ribo*-)



X
(β-L-*arabino*-)

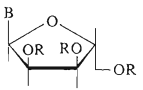


XI
(α-D-*xylo*-)



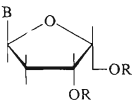
XII
(α-D-*lyxo*-)

Another compound prepared within the framework of this study is 1-allyl-5-fluorouracil (*XVIII*), obtained by reaction of 2,4-bis(trimethylsilyloxy)-5-fluoropyrimidine with allyl bromide in acetonitrile. The structure of this compound was proved by its ^{1}H-NMR spectrum; its UV maximum is bathochromically shifted (2–3 nm) as compared with 5-fluorouracil nucleosides.



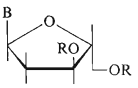
XIII

(α-D-ribo-)



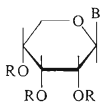
XIV

(2-deoxy-α-D-lyxo-)



XV

(2-deoxy-α-D-ribo-)



XVI

(β-D-ribo-)

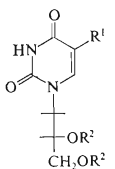
In compounds *I*—*XVI*, *XXI*:

a) R = H, B = uracil-1-yl

b) R = COC_6H_5, B = uracil-1-yl

c) R = COC_6H_5, B = 5-fluorouracil-1-yl

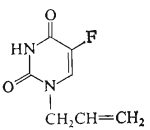
d) R = H, B = 5-fluorouracil-1-yl



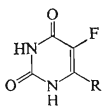
XVIIa; R^1 = H, R^2 = COC_6H_5

XVIIb; R^1 = F, R^2 = COC_6H_5

XVIIc; R^1 = F, R^2 = H

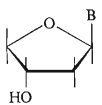


XVIII



XIX, R = H

XX, R = COOH



XXI

Biological Activity of 5-Fluorouracil Nucleosides

We studied the *in vitro* cleavage of compound *Id–XVIId*, *XVIII* by cell-free extract from *E. coli* under conditions causing quantitative degration of 5-fluorouridine (*Id*) and 5-fluoro-2'-deoxyuridine (*IId*) to 5-fluorouracil. In order to take into account the possible participation of enzymes, bonded to the cell debris, the whole study was carried out both with the centrifuged and the total cell homogenate. The tests were performed in the presence, as well as in the absence, of magnesium ions and also in the presence of ATP. The results, summarized in Table I, are as follows: the nucleoside bond is cleaved exclusively in compounds containing a structural grouping of the type *XXI*, *i.e.* in aldofuranosides in which the hydroxyl in the position 3' has a configuration, corresponding to "D-*ribo*", and is *trans*-oriented to the 5--fluorouracil moiety (which has the 1*R*-configuration). The presence and configuration of hydroxyl in the position 2' is not decisive and affects only the reaction rate. Also neither the presence nor absolute configuration of hydroxymethyl group in the position 4' is important. Compounds which do not fulfil the mentioned conditions, *i.e.* derivatives with 1*S*-configuration of the nucleoside bond and 3*R*-configuration of the hydroxyl-bearing carbon in the aldopentafuranose moiety (or 3*S*-configuration in the case of 2-deoxyaldopentafuranoses, see *XXI*)* are under the employed conditions completely resistant toward the studied complex of enzymes.

The specificity of the bacterial enzyme complex toward the structure *XXI* is in accord with the fact that compounds *Id*, *IId* and *IVd* are cleaved with cell-free extract from Ehrlich ascites tumor[22]. No other transformation was observed *in vitro* for the whole studied group of compounds, even in the presence of ATP.

The fission of the nucleoside bond can be a or hydrolytic phosphorolytic process; however, it was not the aim of the present study to identify the enzymes or to study in detail the character of their effect.

The antibacterial effect was investigated with *Escherichia coli* on a synthetic medium with glucose in the static arrangement, using a logarithmic order of concentrations of the studied compounds. 5-Fluorouracil (*XIX*) and 5-fluoroorotic acid (*XX*) were used as standards. From the observed groups of values the ID_{50} values were calculated (Table I); the growth-inhibitory effect of 5-fluorouracil in our experiments was considerably higher than was found[3] for the strain *E. coli* K-12. According to the obtained results, all the studied compounds can be divided into three groups: *a*) a group of compounds with ID_{50} in the region $10^{-6}-10^{-8}$M, *i.e.* with activity comparable with both the standards; it involves the compounds *Id–IVd*, *VId* and *VIId*. Since all these compounds are *in vitro* cleaved to 5-fluorouracil, we can assume that their activity is caused mainly by this compound. *b*) A se-

* It is worth mentioning that the symbols, designating the absolute configuration, are different for the same spatial arrangement at 3' carbon atom because of different priorities of the groupings at $C_{(2)}$ and $C_{(4)}$, postulated by the *RS* nomenclature.

cond group of compounds which can be regarded upon as inactive *in vivo* (ID_{50} = $= 5 . 10^{-4}$ to 10^{-3}M); here belong all the aldofuranosides, resistant in *in vitro* systems. *c*) Finally, the third and the most interesting is the group of compounds *IX*, *XIV* and *XV* with $ID_{50} = 10^{-5}$M. The common feature of these compounds is their resistance *in vitro*; their biological activity cannot therefore be caused by 5-fluorouracil, liberated by cleavage. All these compounds are 2-deoxyaldofuranosides with the 1*S*-configuration of the nucleoside bond.

Naturally, it is necessary to expect that the activity of these compounds is influenced (besides their structure) also by their permeation into the bacterial cells. It cannot therefore be excluded that in eukaryotic systems, in which the membrane transport differs from the permeation through the bacterial cell wall, also other compounds of the group *b*) can be effective. Interestingly enough, the compound *IXd* shows an antibacterial effect: it has been found earlier[23,24] that neither β-L-*ribo*- nor 2-deoxy-β-L-ribonucleosides (nor α-L-lyxofuranosides) of pyrimidine and purine bases permeate into bacterial cells of *E. coli*. No information on the permeation of our compounds is available so far. The acidic character of 5-fluorouracil nucleosides could of course influence their permeation into cells; also other type of interaction with the membrane cannot be excluded.

Finally, we investigated the antagonism between two of the new inhibitory 5-fluorouracil nucleosides and some of natural pyrimidine 2'-deoxyribonucleosides with the aim to obtain a preliminary information on molecular mechanism of their growth-inhibitory effects. The results are summarized in Fig. 1. The inhibitory

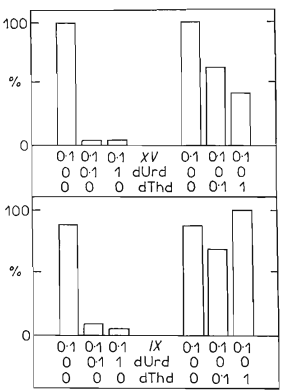


FIG. 1

Antagonism between Antibacterial Activity of Compounds *IX* and *XV* and Natural 2'-Deoxyribonucleosides

The numbers designate mg of the compounds in 1 ml of the medium. % Indicate the inhibition of grown.

action of compound *XVd* can be completely suppressed by 2'-deoxyuridine already in the ratio 1 : 1. Thymidine has only a partial antagonistic effect; obviously it is cleaved very rapidly by the *E. coli* cells to give thymine which is not re-utilized. Also the inhibitory effect of compound *IXd* was practically completely suppressed by 2'-deoxyuridine: with this analogue, thymidine exhibited again low antagonistic activity and in higher concentrations even stimulated the inhibitory activity of *IXd*. This stimulation can be explained by enzymatic transdeoxyribosylation leading to 5-fluoro-2'-deoxyuridine which is then rapidly cleaved to the highly active 5-fluorouracil. A more detailed evaluation of our findings would require to know how permeation of the studied analogues and of the natural antagonist into the bacterial cells influence each other. Nevertheless, we can localize the inhibition effect of the compound *IXd* and *XVd* on the bacterial growth to the biosynthesis of thymine nucleotides.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. The solutions were taken down at 40°C/2 kPa and the compounds were dried over phosphorus pentoxide at 3 Pa. Paper chromatography (descending arrangement) was carried out on paper Whatman No 1 in the systems S1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), S2, 1-butanol–acetic acid–water (5 : 2 : 3), thin-layer chromatography on silica gel plates (Silufol UV_{254}, Kavalier, Czechoslovakia) in the systems S3, chloroform–ethanol (4 : 1), S4, ethyl acetate–benzene (7 : 3), S5, ethyl acetate–2-propanol–water (12 : 1 : 6), S6, chloroform–ethanol (9 : 1). Preparative chromatography on silica gel was performed on loose layers (40 × 16 × 0·3 cm) of silica gel, containing a fluorescence indicator (Service Laboratory of the Institute). UV absorption spectra were measured in aqueous solutions on a Specord UV-VIS spectrophotometer (Carl Zeiss, Jena, GDR), ^{1}H-NMR spectra on a Varian 100 instrument in deuteriochloroform (hexamethyldisiloxane as internal standard, chemical shifts in ppm, coupling constants in Hz).

The employed 2'-deoxy-5-fluorouridine (*IId*) was a Fluka product, ATP was purchased from Calbiochem (San Diego, USA). The following nucleosides were prepared according to the already described procedures: *IVa* (ref.[25]), *VIa* (ref.[26]), *Xa* (ref.[27]). *XIa* (ref.[28]), *XIIa* (ref.[25]) and *XIIIa* (ref.[29]).

5'-Deoxyuridine (*IIIa*)

A mixture of 5'-iodo-5'-deoxy-2',3'-O-isopropylideneuridine[19] (10·1 g; 24·6 mmol), tri-n-butyltin hydride[30] (20 g), benzene (100 ml) and aza-bis-isobutyronitrile (50 mg) was refluxed for 1 h, filtered through celite and the filtrate was taken down *in vacuo*. The residue was mixed with light petroleum (200 ml), filtered, washed with light petroleum and dried *in vacuo*. The obtained product was stirred with a mixture of methanol (70 ml), water (50 ml) and Dowex 50X8 (30 ml, H^+-form) for 5 h at room temperature, the mixture was filtered, the solids on the filter washed with water and the filtrate taken down. Crystallization from ethanol afforded 4·45 g (79·3%) of *IIIa*, m.p. 182—184°C (reported[31] m.p. 182—183°C), R_F 0·50 (S1). For $C_9H_{12}N_2O_5$ (228·2) calculated: 47·36% C, 5·30% H, 12·27% N; found: 48·08% C, 5·50% H, 12·12% N. ^{1}H-NMR spectrum: 1·30 (d, 3 H, $J = 6·0$) 5'-CH_3; 3·10 (t, 1 H, $J_{4',5'} = 6·0$) $H_{4'}$; 3·70 (t, 1 H, $J_{2',3'} = 5·4$) $H_{3'}$; 4·08 (d, 1 H, $J_{1',2'} = 5·0$) $H_{2'}$; 5·62 (dd, 1 H, $J_{5,6} = 8·0$) H_5; 7·52 (d, 1 H) H_6; 11·23 (bs, 1 H) NH.

1-(β-D-Xylofuranosyl)uracil (*Vb*)

A solution of 1,2,3,5-tetra-O-acetyl-D-xylofuranose (49·3 g; 0·155 mol) in 1,2-dichloroethane (200 ml) was saturated with hydrogen chloride at 0°C, set aside overnight at room temperature and taken down *in vacuo*. The residue was codistilled with toluene (3 × 50 ml), dissolved in acetonitrile (200 ml) and refluxed with 2,4-dimethoxypyrimidine[6] (28 g; 0·2 mol) for 12 h. After evaporation, the residue was dissolved in chloroform (500 ml), the solution saturated at 0°C with hydrogen chloride, allowed to stand for 2 days at room temperature and taken down. The residue was taken up in chloroform (200 ml), washed with saturated sodium hydrogen carbonate solution (50 ml portions, to neutral reaction) and with water, dried over sodium sulfate and taken down *in vacuo*. The mixture was chromatographed on a silica gel column (200 g, 20—60 mesh; according to Pitra) in chloroform, the product-containing fractions (R_F 0·28 in S6) were combined, taken down *in vacuo* and allowed to stand with 0·1M methanolic sodium methoxide (200 ml) overnight. After neutralization with dry Dowex 50X8 (H^+-form) the mixture was filtered, the solids on the filter washed with methanol and the filtrate taken down *in vacuo*. Crystallization from ethanol (70 ml) afforded 14·5 g (38·5%) of *Va*, identical (S1, S2) with the authentic compound[32]; m.p. 162°C (reported[23] m.p. 161—162°C).

TABLE II

Properties of Perbenzoylated Uracil Nucleosides

Compound Yield, %	M.p., °C[a] $[\alpha]_D^{20}$ [b]	Formula (mol.weight)	Calculated/Found		
			% C	% H	% N
IIIb	196	$C_{23}H_{30}N_2O_7$	(63·29	4·62	6·42)
93	—89·5°	(436·4)	63·96	4·63	7·03
IVb	196—198	$C_{30}H_{24}N_2O_9$	(64·74	4·34	5·03)
95	+36·4°	(556·5)	64·49	4·40	5·38
Vb	122[c]	*cf. IVb*		*cf. IVb*	
92	+58·1°		65·26	4·58	5·20
VIb	115—119	*cf. IVb*		*cf. IVb*	
87	—124·0°		64·56	4·33	5·50
Xb	199—200	*cf. IVb*		*cf. IVb*	
89	—40·4°		65·49	4·40	5·38
XIb	99—103	*cf. IVb*		*cf. IVb*	
70	—13·2°		64·50	4·70	5·13
XIIb	121	*cf. IVb*		*cf. IVb*	
66	+122·4°		64·51	4·32	5·13
XIIIb		*cf. IVb*		*cf. IVb*	
83			64·89	4·60	5·22

[a] Crystallized from ethanol–petroleum ether; [b] $c = 0·5$, dimethylformamide; [c] literature[33] gives m.p. 112—118°C.

Preparation of Perbenzoylated Uracil Nucleosides

A solution of benzoyl cyanide (20 mmol, or 15 mmol in the case of deoxynucleosides) in acetonitrile (40 ml) was added to a suspension of the nucleoside *Ia*—*XVI* (5 mmol) in acetonitrile (20 ml), and after addition of triethylamine (1 ml) the mixture was stirred at room temperature for 2 h, diluted with ether (150 ml) and filtered. The product was washed with ether and crystallized from ethanol with addition of light petroleum. Yields, physical constants and analyses of thus-prepared compounds are given in Table II, their ^{1}H-NMR spectra in Table III. In addition, using the already described procedures, following compounds were prepared: *Ib* (ref.[18]), *VIIb* (ref.[28]), *VIIIb* (ref.[26]), *IXb*(ref.[27]), *XIVb* (ref.[26]), *XVb* (ref.[29]), *XVIb* (ref.[34]) and *XVIIb* (ref.[21])

TABLE III

^{1}H-NMR-Spectra ($CDCl_3$)[a]

Compound	$H_{1'}$ ($J_{1',2'}$)	$H_{2'}$ ($J_{2',3'}$)	$H_{3'}$ ($J_{3',4'}$)	$H_{4'}$ ($J_{4',5'}$)	$H_{5'}$	H_5 (J_5, NH)	H_6 ($J_{5,6}$)
IIIb	d 6·07 (4·5)	dd 5·75 (6·0)	5·48 (6·0)	m 4·42 (6·0)	d 1·55[b]	d 5·72	[c] (8·0)
IIIc	d 6·10 (5·0)	dd 5·76 (6·0)	t 5·46 (6·0)	m 4·43 (6·0)	d 1·56[b]	—	d 7·70
IVb	d 6·50 (4·0)	dd 5·86 (2·0)	dd 5·71 (4·0)	bq 4·57 (5·0)	d 4·85	d 5·53 (1·0)	d 7·66 (8·0)
IVc	dd 6·47 (4·5)	dd 5·85 (2·0)	dd 5·70 (4·0)	m 4·59	m 4·87	—	[c]
Vb	d 6·25 (2·4)	t 5·67 (1·5)	dd 5·86 (3·5)	4·70—4·95		d 5·72 (1·0)	d 7·75 (8·0)
Vc	t 6·23 (1·5)	t 5·61 (1·5)	m 5·83	4·55—5·0		—	[c]
Xb	d 6·48 (4·2)	dd 5·86 (2·0)	dd 5·72 (4·0)	m 4·57 (5·0)	d 4·85	d 5·51 (1·0)	d 7·65 (8·0)
XIc	d 6·58 (4·0)	dd 5·90 (2·0)	dd 6·01 (4·5)	m 5·10 (5·5)	m 5·65	—	[c]
XVc	d 6·32 (6·5)	d 2·98 d 2·51 (6·5)[d]	d 5·64 (6·0)	t 4·99 (4·0)	m 4·54	—	d 7·78 (6·5)

[a] Aromatic protons 7·10—8·10; [b] methyl group; [c] superimposed by aromatic protons; [d] J_{gem} = = 15·5.

Preparation of Perbenzoylated 5-Fluorouracil Nucleosides *Ic—XVIIc*

A solution of fluorine (5·2 mmol) in acetic acid (about 50 ml) was added with stirring at room temperature to a solution of the perbenzoate *Ib—XVIIb* (5 mmol) in acetic acid (55 ml). After stirring for 1 h, the mixture was taken down *in vacuo*, codistilled several times with acetic acid and then with ethanol and the residues was dissolved in a 5% ethanolic triethylamine solution (20 ml). After standing for 1 h and evaporation, the residue was crystallized from ethanol. Yields of chromatographically pure compounds *Ic—XVIIc* were 60—90%. Compounds *XIc*, *XIVc* and *XVc* after the treatment with triethylamine were isolated by chromatography on a loose layer of silica gel (*vide supra*) in the system S4 and elution of the product band with methanol (500 ml). Properties and analyses of the prepared compounds are given in Table IV, their ^{1}H-NMR spectra in Table III.

TABLE IV
Properties of Perbenzoylated 5-Fluorouracil Nucleosides

Compound	M.p., °C[a]	$[\alpha]_D^{20}$ [b]	R_F [c]	Formula (mol.weight)	Calculated/Found			
					% C	% H	% F	% N
IIIc	94—96	— 76·6°	0·52	$C_{23}H_{19}FN_2O_7$ (454·5)	(60·96 61·12	4·20 4·24	4·18 4·40	6·17) 6·30
IVc	217	+ 53·8°	0·54	$C_{30}H_{23}FN_2O_9$ (574·5)	(62·95 62·13	4·04 4·13	3·32 3·44	4·89) 4·53
Vc	181	+ 41·2°	0·58	*cf. IVc*	62·74	4·12	3·20	4·74
VIc	[d]	—115·6°	0·46	*cf. IVc*	63·21	4·15	3·14	4·51
VIIc	[d]	— 20·0°	0·32	*cf. IIIc*	59·29	4·32	5·04	6·20
VIIIc	204	+ 76·8°	0·59	*cf. IVc*	61·46	4·73	3·38	4·45
Xc	213—216	— 52·8°	0·53	*cf. IVc*	61·52	4·57	3·61	5·45
XIc	[d]	— 28·4°	0·56	*cf. IVc*	60·98	3·83	3·42	4·91
XIIc	187	+109·5°	0·46	*cf. IVc*	61·35	4·07	3·12	4·53
XIIIc	[d]	— 87·4°	0·58	*cf. IVc*	61·31	3·88	3·20	4·71
XIVc	87—90	+ 28·9°	0·32	*cf. IIIc*	58·66	4·44	4·00	5·55
XVc	171—174	— 58·7°	0·25	*cf. IIIc*	59·28	3·68	4·72	5·87
XVI	128	— 84·4°	0·59	*cf. IVc*	63·04	4·43	3·28	4·58
XVIIc	162	—117·2°	0·21	$C_{21}H_{17}FN_2O_6$ (412·4)	(61·25 60·02	4·12 4·29	4·61 3·98	6·80) 6·40

[a] Crystallization from ethanol; [b] $c = 0·5$, dimethylformamide; [c] in S4; [d] foam.

Preparation of 5-Fluorouracil Nucleosides *Id—XVIId*

1M Sodium methoxide was added to a solution of compound *Ic—XVIIc* in methanol (10 ml per 1 mmol) till the mixture became strongly alkaline (moist pH-paper). After standing overnight at room temperature, the mixture was neutralized with dry Dowex 50X8 (H^+-form), filtered, the solids on the filter were washed with methanol and the filtrate was taken down *in vacuo*. The residue was dissolved in water (50 ml) the solution washed with ether (3 × 25 ml), the aqueous layer taken down *in vacuo* and the residue dissolved in water (3—5 ml) and applied on a column of Dowex 1X2 (40 × 3 cm, 100—200 mesh; acetate form). The column was washed (2 ml min^{-1}) with water (100 ml) and then with 0·02—0·3M acetic acid (linear gradient, à 0·5 l). The product-containing fractions were taken down *in vacuo*, the residue codistilled with ethanol (3 × 25 ml) and crystallized from ethanol (with addition of ether until the solution became turbid). The products were obtained in 60—90% yield and were homogeneous and completely free of 5-fluorouracil (S1, S2, S5). Their purity, determined spectrophotometrically after chromatography of 2 mg of compound in the system S2 or after electrophoresis in 0·1M triethylammonium hydrogen carbonate, pH 7·5, was higher than 99·95%. Properties of the compounds are given in Table I.

1-Allyl-5-fluorouracil (*XVIII*)

A mixture of 5-fluorouracil[15] (*XIX*) (3·0 g; 23 mmol), hexamethyldisilazane (20 ml) and ammonium sulfate (0·2 g) was refluxed for 4 h and distilled *in vacuo*. The obtained 2,4-bis(trimethylsilyloxy)-5-fluoropyrimidine (b.p. 132°C/1·3 kPa) was immediately diluted with acetonitrile (15 ml) and treated with allyl bromide (15 ml) under stirring. The mixture was stirred at room temperature for 2 h, then refluxed for other 2 h and then set aside at room temperature overnight. After evaporation, the residue was codistilled with ethanol and chromatographed on two layers (*vide supra*) of silica gel in the system S6. The product bands were eluted with methanol (500 ml), the solvent was evaporated and the residue crystallized from ethanol–light petroleum, yielding 1·90 g (48·5%) of *XVIII*, m.p. 123—124°C, R_F 0·35 (S3), 0·57 (S6). For $C_7H_7FN_2O_2$ (170·1) calculated: 49·41% C, 4·14% H, 11·16% F, 16·46% N; found: 49·26% C, 3·97% H, 11·33% F, 16·47% N. ^{1}H-NMR spectrum: 4·35 (bd, 2 H, $J_{CH_2,CH}$ = 5·3) N—CH_2; 5·20 (dq, 1 H, J = 7·5) = CH_2; 5·30 (m, 2 H) —CH= + =CH_2; 7·26 (d, 1 H, $J_{6,F}$ = 4·5) H_6; 10·13 (bs, 1 H) NH. UV spectrum (pH 1—7) λ_{max} 273 nm, (pH 12) λ_{max} 271 nm.

Cleavage of Nucleosides *Id—XVIId* and *XVIII* with Cell-Free Extract from *E. coli*

A) Incubation mixture: a solution of 1·9 μmol of the compound in 50 μl of 0·2M TRIS-buffer, pH 7·4 (containing no or 10^{-4}M magnesium chloride) and 25 μl (or 50 μl) of the cell-free extract from *E. coli*, prepared according to ref.[35] (protein content, determined according to Lowry[36], 41 mg per ml) was made up with water to 250 μl. Incubation at 37°C, aliquotes chromatographed after 20 min, 30 min, 60 min, and 15 h in the system S5. With compounds resistant after 15 h, the experiment was repeated using 50 μl of the cell-free extract. Under these conditions, compounds *Id* and *IId* were cleaved quantitatively after 20 min, compounds *IId*, *IVd*, *VId* and *VIId* reacted at various rates in the course of 20—60 min whereas the remaining compounds were not cleaved with 50 ml of the extract even after 15 h.

B) The incubation mixture was of the same composition as in the experiment *A*) but in addition it contained 5 μmol of ATP (as sodium salt). The results of cleavage were the same as under *A*).

Tests for Antibacterial Activity

The growth-inhibitory activity was tested using synthetic medium (10 ml), containing inorganic salts and glucose[37]. The sterile medium was added to the weighed compounds in sterile 25 ml Erlenmayer flasks stoppered with cotton wool. After addition of 0·05 ml of *Escherichia coli* inoculum (prepared in a synthetic medium of the above-mentioned composition), cultivation by stationary incubation was carried out at 37°C for 16 h. Growth of the bacteria was evaluated by absorbance measurement at 575 nm. In the determination of antagonism between the compounds tested and natural deoxyribonucleosides both components were added into the medium simultaneously prior to the inoculation.

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