5′-O-ALKYL-5-FLUOROURIDINES: SYNTHESIS AND BIOLOGICAL ACTIVITY*

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Methyl 2,3-O-isopropylidene-D-ribofuranoside (*IV*) was alkylated with alkyl halides in the presence of sodium hydride and the products were transformed by acid hydrolysis and glycosylation into methyl 5-O-alkyl-D-ribofuranosides *VII*. Benzoylation of *VII* followed by acetolysis afforded 1-O-acetyl-2,3-di-O-benzoyl-5-O-alkyl-D-ribofuranoses *IX* which on reaction with 2,4-bis(trimethylsilyloxy)pyrimidine in the presence of tin tetrachloride in acetonitrile and subsequent hydrolysis gave 5′-O-alkyl-2′,3′-di-O-benzoyluridines *XIa*—*XIe*. Methanolysis of compounds *XI* furnished 5′-O-alkyluridines *III*. The 5-O-allyl derivative *XII* was hydroxylated in the presence of OsO_4 and transformed further to 5′-O-(*RS*)-(2,3-dihydroxypropyl)uridine (*IIIg*) and its tetrabenzoate *XVI*. Compounds *XI* and *XVI* on reaction with elemental fluorine in acetic acid afforded benzoyl derivatives of 5′-O-alkyl-5-fluorouridines *XVIIa*—*XVIIe* and *XIX* which were methanolyzed to give 5′-O-alkyl-5-fluorouridines *II*. This procedure afforded 5′-O-methyl (*IIa*), ethyl (*IIb*), n-butyl (*IIc*), n-hexyl (*IId*), n-octyl (*IIe*), and (*RS*)-(2,3-dihydroxypropyl) (*IIf*) derivatives of 5-fluorouridine. None of the compounds *II* exhibited antibacterial effect on *Escherichia coli* B or antiviral activity against HSV-1, HSV-2, vaccinia virus or vesicular stomatitis viruses. Compounds *IIc,d,e* suppressed the growth of L 1210 mice leukemic cells at concentrations of 10^{-5} to 10^{-6} mol l^{-1}; the 5′-O-n-butyl derivative *IIc* has the highest activity (ID_{50} 2·8 μmol l^{-1}) but does not prolong the life span of L 1210 leukemia bearing mice following repeated daily doses of 80 mg/kg.

5-Fluorouracil belongs to the oldest and best investigated cytostatics. Although this compound is undoubtedly successful in cancer chemotherapy, in the recent years much endeavour has been devoted to search for new derivatives of higher therapeutic potential, and, since 5-fluorouracil itself has only a very short half-life, for congeners of better pharmacokinetic parameters. Such a new drug is *e.g.* Ftorafur which is now widely used clinically (for a review see ref.[1]); another very active derivative, 2′-deoxy-5-fluorouridine (FUDR), is still not easily accessible on a large scale, in

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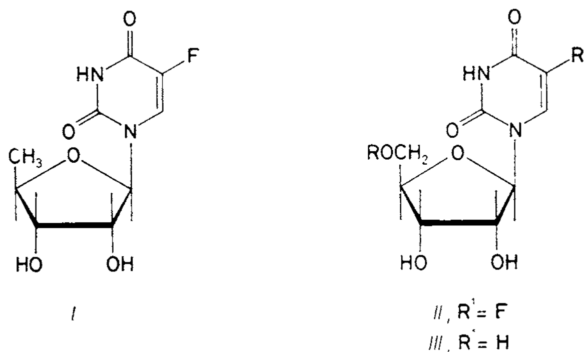
spite of some improvements of its synthesis[2]. Recently, another 5-fluorouracil derivative has been found that acts as its prodrug: 5′-deoxy-5-fluorouridine (*I*, Doxyfluridin). This compound was prepared from 5-fluorouridine[3-5] or by fluorination of 5′-deoxyuridine[6] and exhibits better antitumor properties, higher therapeutic index and lower bone marrow toxicity than all of the three above-mentioned compounds (for a review see refs[7,8]). Doxyfluridin is cleaved by uridine phosphorylase to give 5-fluorouracil[9] which is then almost quantitatively utilized for incorporation into nucleic acids[10,11]. In spite of these obvious advantages the results of the phase II clinical trials are so far not unambiguously successful[12].

A prodrug of 5-fluorouracil should achieve a sufficiently high concentration in the cellular pool and should persist for a sufficiently long time in the cell and finally be converted (*e.g.* with uridine phosphorylase) to 5-fluorouracil. Since both Ftorafur and compound *I* are cleaved to 5-fluorouracil, the sugar part of 5-fluorouracil nucleosides is probably not the decisive structural element for the function of eucaryotic uridine phosphorylases (*cf.* ref.[6]). It seems therefore unlikely that this phosphorolysis would be hindered by an O-substitution of the 5′-hydroxy group in 5-fluorouridine; on the contrary, a suitably chosen substituent might affect the cellular transport and, possibly, the pharmacokinetics of such compounds as well.

In this paper we describe 5′-O-alkyl derivatives of 5-fluorouridine *II* with etherically linked groups of varying hydrophobicity, and their cytostatic, antiviral and, antibacterial properties.

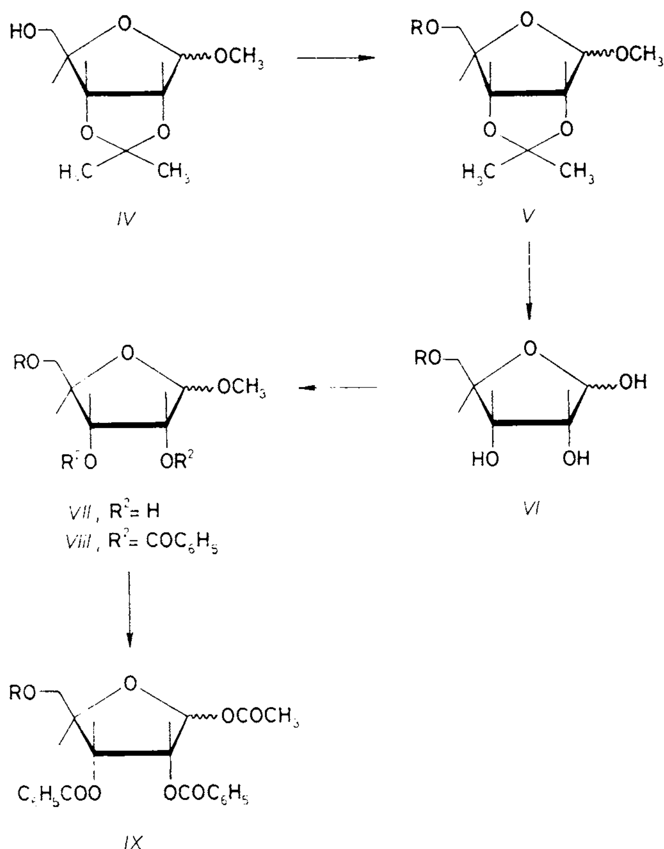


In formulae *II*–*XI*, *XVII*:

a, R = CH_3	*e*, R = C_8H_{17}
b, R = C_2H_5	*f*, R = $(CH_3)_2CHCH_2$
c, R = C_4H_9	*g*, R = $CH(OH)CH_2OH$
d, R = C_6H_{13}	*h*, R = H

Compounds of the type *II* cannot be prepared by direct alkylation of 5-fluorouridine since the pyrimidine ring is alkylated preferentially. Therefore, we have used

as a general method the Hilbert–Johnson nucleosidation of corresponding 5-O-alkyl-D-ribofuranoses with the bis(trimethylsilyl) derivative of uracil. Of the two alternatives, *i.e.* direct glycosylation of the 5-fluorouracil derivative or glycosylation of the uracil derivative followed by fluorination, we have chosen the latter one, because its intermediates, 5'-O-alkyluridines *III*, may also be of interest.

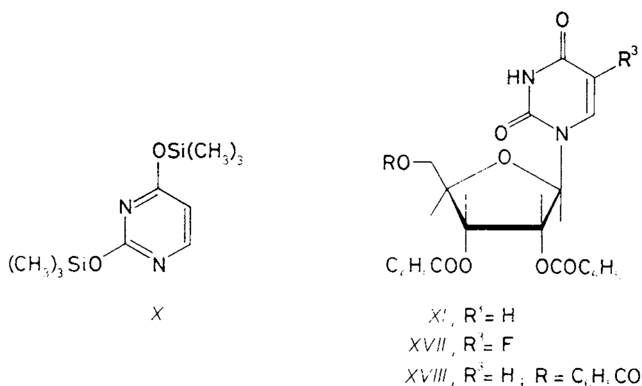


SCHEME 1

Preparation of the sugar synthons (Scheme 1) started from the well accessible[12] methyl 2,3-O-isopropylidene-D-ribofuranoside (*IV*): alkylation of the sodium alkoxide of this compound (generated *in situ* by reaction with sodium hydride) with the corresponding alkyl halides in dimethylformamide afforded methyl 5-O-alkyl-2,3-O-isopropylidene-D-ribofuranosides *V*, distillable *in vacuo*. These derivatives were acid-hydrolyzed to 5-O-alkyl-D-riboses *VI* and further transformed to methyl 5-O-alkyl-D-ribofuranosides *VII* which on benzoylation gave the 2,3-di-O-benzoyl

derivatives *VIII*. Acetolysis of *VIII* afforded finally 1-O-acetyl-5-O-alkyl-2,3-di-O--benzoyl-D-ribofuranoses *IX* as the desired synthons bearing a leaving group in position 1 and an acyl group in position O(2) which determines the β-configuration of the arising nucleoside (the anomeric composition of compounds *IX* has not been studied).

Compounds *IX* were condensed with 2,4-bis(trimethylsilyloxy)pyrimidine (*X*) in acetonitrile in the presence of tin tetrachloride[14] to give 2',3'-di-O-benzoyl derivatives *XI* which on the one hand were directly characterized by the ^{1}H NMR spectra (Table I), and on the other hand methanolyzed to 5'-O-alkyluridines *III*, characterized by ^{1}H NMR spectra (Table II) as well as UV spectra (Table III) and other techniques (Table IV). Using this approach, we prepared the 5'-O-ethyl, butyl, hexyl and octyl derivatives (*IIIb*–*IIIe*). (The 5'-O-methyl derivative *IIIa* and its dibenzoate *XIa* were prepared and characterized by us earlier[15].)



The hydrophilic 5'-O-(*RS*)-(2,3-dihydroxypropyl)uridine (*IIIg*) was synthesized starting from methyl 5-O-allyl-2,3-O-isopropylidene-D-ribofuranoside (*XII*) obtained by alkylation of compound *IV*. The derivative *XII* was converted by reaction with sodium chlorate in the presence of OsO_4 into the racemic 2,3-dihydroxypropyl ether *XIII* and its dibenzoate *XIV* which was further transformed into the sugar synthon *XV* and the nucleoside *XVI* (Scheme 2).

In the attempted preparation of 5'-O-(2-methylpropyl)uridine (*IIIf*) we found that compound *Vf* underwent dealkylation during acid hydrolysis and the reaction sequence gave predominantly 2',3',5'-tri-O-benzoyluridine (*XVIII*) which was identified by comparison with an authentic sample[16] using chromatography and ^{1}H NMR. Also uridine (*IIIh*), obtained by methanolysis of *XVIII*, was identical with an authentic sample according to chromatography. The desired derivative *IIIf* was obtained in low yield by methanolysis of the mother liquors from crystallization of *XVIII*.

TABLE I

^{1}H NMR spectra of perbenzoylated nucleosides (in deuteriochloroform)

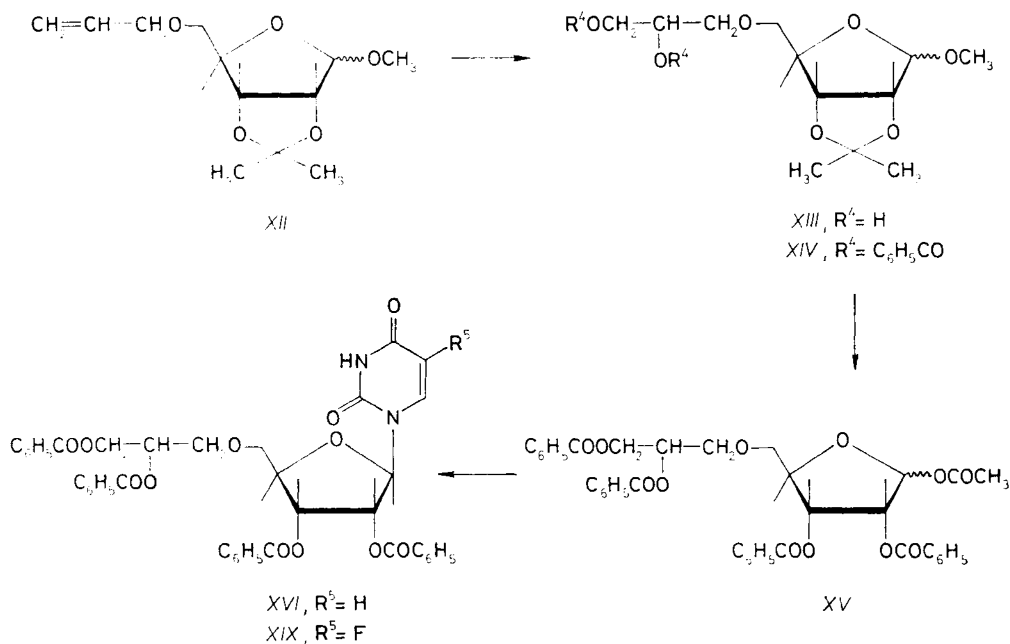
Compound	$H_{1'}$ ($J_{1',2'}$)	$H_{2'}$ ($J_{2',3'}$)	$H_{3'}$ ($J_{3',4'}$)	$H_{4'}$ ($J_{4',5'}$)	$H_{5'}$ ($J_{5',5''}$)	$H_{5''}$ ($J_{4',5''}$)	H_5 ($J_{1',F}$)	H_6 ($J_{6,F}$)	O—CH_2	C—CH_2	C—CH_3	NH ($J_{NH,5}$)
XIb	d 6·59 (6·8)	t 5·67 (5·5)	dd 5·78 (4·0)	q 4·50 (2·0)	2dd 3·81 (−11·0)	(2·0)	dd 5·80 —	[a] (8·0)[b]	q 3·68 (7·0)	—	t 1·32	9·20 (2·0)
XIc	d 6·58 (7·0)	dd 6·66 (5·5)	dd 5·79 (1·5)	brq 4·50 (3·0)	2dd 3·84 (−11·0)	(2·0)	d 5·80	[a] (8·0)[b]	t 3·62 (6·0)	1·30— —1·75	t 0·98 (J = 6·8)	8·60 (2·0)
XId	d 6·59 (6·8)	dd 5·65 (5·5)	dd 5·78 (1·6)	brq 4·50 (3·0)	2dd 3·81 (−11·0)	(2·0)	d 5·78	[a] (8·0)[b]	t 3·61 (6·5)	1·25— —1·85	t 0·91 (J = 6·5)	9·21 (2·0)
XIe	d 6·59 (6·8)	dd 5·65 (5·5)	dd 5·80 (1·5)	brq 4·49 (2·5)	2dd 3·81 (−11·0)	(2·5)	d 5·80	[a] (8·0)[b]	t 3·61 (6·5)	1·25— —1·85	t 0·88 (J = 6·5)	9·12 (2·0)
XVIIa	dd 6·57 (7·2)	dd 5·61 (5·5)	dd 5·79 (1·5)	brq 4·50 (2·3)	2dd 3·79 (−10·6)	(1·9)	— (1·9)	d 8·07 (6·4)	s 3·56[c]	—	—	9·06 (4·2)
XVIIb	dd 6·58 (7·0)	dd 5·66 (5·4)	dd 5·80 (1·5)	brq 4·51 (2·0)	dd 3·88 (−10·8)	dd 3·78 (1·7)	— (1·9)	d 8·27 (6·4)	q 3·71	—	t 1·37 (J = 7·0)	9·30
XVIIc	dd 6·58 (7·2)	dd 5·64 (5·4)	dd 5·79 (1·5)	brq 4·51 (2·0)	dd 3·88 (−10·8)	dd 3·76 (1·7)	— (1·9)	d 8·22 (6·3)	t 3·65 (6·5)	brp 1·72 broc 1·47	t 0·98 (J = 7·2)	9·02 (4·0)[d]
XVIId	dd 6·58	dd 5·64	dd 5·78	brq 4·51	dd 3·88	dd 3·76	—	d 8·22	t 3·64	brp 1·71	t 0·90	9·07
XVIId	(7·2)	(5·4)	(1·5)	(2·0)	(−10·8)	(1·6)	(1·9)	(6·5)	(6·2)	m 1·36		(4·0)[d]
XVIIe	dd 6·58 (7·2)	dd 5·64 (5·4)	dd 5·78 (1·5)	brq 4·51 (2·0)	dd 3·88 (−10·8)	dd 3·76 (1·7)	— (1·9)	d 8·22 (6·5)	t 3·64 (6·5)	m 1·71 m 1·29	t 0·87 (J = 6·5)	9·22 (4·0)[d]
XVIII	d 6·32 (5·4)	brt 5·75 (6·0)	dd 5·91 (4·0)	m 4·55—4·85		—	dd 5·62	7·60		—	—	8·71 (2·2)

[a] Obscured by aromatic protons 7·30—7·70 + 7·80—9·10; [b] $J_{5,6}$; [c] OCH_3; [d] $J_{NH,F}$.

TABLE II

^{1}H NMR spectra of nucleosides *III*

Compound	$H_{1'}$ ($J_{1',2'}$)	$H_{2'}$ + $H_{3'}$ + + $H_{4'}$ ($J_{4',5'}$)	$H_{5'}$ + $H_{5''}$ ($J_{5',5''}$) ($J_{4',5''}$)	O—CH_2	C—CH_2	C—CH_3	H_5 ($J_{5',6}$)	H_6	OH
IIIb	d 5·77 (4·7)	m 3·58 (2·0)	2d 3·58	q 3·49 (J = 7·0)	—	t 1·15	d 5·65 (8·0)	d 7·82	5·40 5·15
IIIc	d 5·77 (4·5)	m 3·90—4·05 (2·0)	2d 3·50 + 3·65 (−11·0)	t 3·44 (J = 6·0)	brp 1·50 brm 1·33 (J = 7·0)	t 0·89 (J = 7·2)	d 5·61 (8·0)	d 7·82	5·50 5·20
IIId	d 5·77 (3·5)	m 3·95	m 3·57	t 3·44	brp 1·53 m 1·28	brp 0·87	d 5·59 (8·0)	d 7·81	5·50
IIIe	d 5·77 (4·7)	m 3·95	m 3·57	t 3·44 (J = 6·3)	brp 1·52 m 1·25	t 0·86 (J = 7·0)	d 5·59 (8·0)	d 7·81	5·41 5·16
IIIf	d 5·77 (4·5)	m 3·92—4·05 (1·5)	2dd 3·23 (−12·5) (2·0)	dd 3·23	1·83	d 0·87	d 5·60	d 7·80	—
IIIg	d 5·79 (4·5)	m 3·96		m 3·25	3·75	—	d 5·60 (8·0)	d 7·97	5·37 5·14



SCHEME 2

The protected uracil nucleosides *XI* and *XVI* were fluorinated in acetic acid solution with elemental fluorine to the respective 5-fluorouridine derivatives *XVII* and *XIX*; the intermediate adduct of fluorine to the double bond in positions 5 and 6 of the uracil system underwent elimination to give the end product by treatment with triethylamine in ethanol. The obtained products were characterized by ^{1}H NMR

TABLE III

Ultraviolet spectra of 5′-O-alkyl-5-fluorouridines *II* and 5′-O-alkyluridines *III* (in methanol solution)

Compound	λ_{max}(nm)	ε_{max}	Compound	λ_{max}(nm)	ε_{max}
IIa	270	6 100	*IIIa*	262	10 500
IIb	270	6 300	*IIIb*	263	11 000
IIc	270	6 100	*IIIc*	262	10 800
IId	270	9 000	*IIId*	263	10 000
IIe	270	8 600	*IIIe*	263	10 200
IIg	270	9 200	*IIIf*	262	10 400
			IIIg	262	10 400

TABLE IV
Mass spectra (m/z)

Compound	M^+	$M - H_2O$	$M - 2H_2O$	$M - 3H_2O$	S^a	$S - H_2O$	B + CH_2CHOH^b	B + 30	BH_2	BH
IIa	276	258	—	—	147	129	—	—	131	130
IIb	290	272	254	—	161	143	—	161	131	130
IIc	318	300	—	—	189	171	—	—	131	—
IId	346	328	—	—	217	199	—	—	131	130
IIe	374	356	—	—	245	227	—	—	131	130
IIIb	272	254	236	218	161	143	155	141	113	112
IIIc	300	282	—	246	189	171	155	141	113	112
IIId	328[c]	310	—	274	217	199	155	141	113	112
IIIe	356[c]	338	—	302	245	227	155	141	113	112
IIIf	300	282	—	246	189	171	155	141	113	—
IIIg	318	300	—	—	207	189	155	141	113	112
IIIh	244[c]	226	208	—	133	—	155	141	113	112

[a] S sugar residue; [b] B base moiety; [c] accompanied by (M + 1).

spectra which confirmed their anomeric homogeneity (Table I). Upon methanolysis they afforded the 5'-O-alkyl-5-fluorouridines *II* characterized in Tables III–V.

During the isolation of compounds *II* we took particular care to remove 5-fluorouracil and 5-fluorouridine which could influence the results of biological tests. Since compounds *II* and *III* contained 5'-O-alkyl groups, we made use of chromatography on octadecyl-silica gel (this method proved to be applicable also to compound *IIg*). According to HPLC, the thus-purified products *II* contained neither the mentioned compounds nor other detectable impurities (Table VI).

Biological Activity of 5'-O-Alkyl-5-fluorouridines (II)

Antibacterial activity was tested on *Escherichia coli* B grown in a synthetic medium containing glucose, with a logarithmic series of concentrations of the test compounds. Under conditions of the experiments, none of the 5'-O-alkyl-5-fluorouridines *II* exhibited any significant antibacterial effect up to a concentration of 1 mg/ml. Interestingly enough, this observation contrasts with our previous finding that under the same conditions 5'-deoxy-5-fluorouridine (*I*) inhibits very effectively the growth of *E. coli* B[6]. Compound *I*, however, is to a considerable extent cleaved by the bacteria to release 5-fluorouracil which is an effective antibacterial agent. The negative results obtained in our tests with compounds *II* prove unequivocally that the test compounds are not contaminated with 5-fluorouracil ($ID_{50} = 4 . 10^{-9}$ mol l^{-1}, see ref.[6]) and that neither in the cells nor in the medium they give rise to detectable amounts of 5-fluorouracil (at concentration 1 mg/ml, ID_{50} of 5-fluorouracil would be achieved by 0·0001% cleavage of the nucleoside bond). Without use of labelled compounds *II* we cannot definitely decide whether the studied compounds penetrate into the cells or whether they are substrates of enzymes which split the nucleoside bond. The latter alternative, however, seems to be more probable. Also the 5'-O-alkyluridines *III*, prepared in this study, did not show any antibacterial effect.

Antiviral activity. Of the 5-fluorouracil nucleosides described so far only 5-fluoro-2'-deoxyuridine exhibits significant antiviral activity ($MIC_{50} = 4 . 10^{-4}$ mol l^{-1} *in vitro*) with a sufficiently high selectivity index against herpes simplex type 1 (*cf.* ref.[18]); the other compounds of this type have no significant antiviral effect (for a review see ref.[19]), except for thymidine kinase-deficient (TK^-) HSV mutants[20]. Antiviral evaluation of compounds *II* was performed by following the virus-induced cytopathogenicity in cell cultures infected with herpes simplex virus type 1 or 2 or vaccinia virus as representative of DNA viruses, and with vesicular stomatitis virus, representing RNA viruses. At the highest concentration used (400 μg/ml, *i.e.* $1 - 1{\cdot}5 . 10^{-3}$ mol l^{-1}), none of the compounds *II* showed significant activity (data not shown).

Cytostatic activity of 5'-O-alkyl-5-fluorouridines *II* was assayed *in vitro* in L 1210 mouse leukemic cells. The compounds were added 24 h after starting the

TABLE V

^{1}H NMR spectra of nucleosides *II* (in hexadeuteriodimethyl sulfoxide)

Compound	$H_{1'}$ ($J_{1',2'}$)	$H_{2'}$ ($J_{2',3'}$)	$H_{3'}+H_{4'}$ ($J_{4',5'}$)	$H_{5'}$ ($J_{5',5''}$)	$H_{5''}$ ($J_{4',5''}$)	OCH_2	C—CH_2	C—CH_3	H_5 ($J_{1',F}$)	H_6 ($J_{6,F}$)	OH
IIa	dd 5·73 (4·2)	brt 4·03 (4·5)	m 3·94 (2·0)	dd 3·62 (−11·0)	dd 3·51 (2·5)	s 3·34[a]		—	— (1·6)	8·02 (7·3)	5·37 5·86
IIb	dd 5·71 (3·6)	m 3·90—4·05 (2·5)		dd 3·67 (−11·0)	dd 3·53 (2·5)	q 3·53 + t 1·17 (J = 6·8)		—	— (1·9)	8·20 (7·2)	5·20 5·45
IIc	dd 5·71 (3·5)	m 4·05—3·90[b] (2·5)		dd 3·69 (−11·0)	dd 3·53 (2·5)	t 3·47 (J = 6·5)	brp 1·53 brsex 1·36	0·89 (J = 7·2)	— (1·8)	8·15 (7·5)	—
IId	dd 5·72 (3·5)	m 3·90—4·05 (2·5)		dd 3·68 (−11·0)	dd 3·52 (2·5)	t 3·46 J = 6·5)	m 1·54 m 1·27	t 0·85 (J = 6·5)	— (1·9)	8·14 (7·5)	5·17 5·46
IIe	dd 5·71 (3·5)	m 3·90—4·05 (2·5)		dd 3·68 (−11·0)	dd 3·52 (2·5)	t 3·46 (J = 6·5)	m 1·54 m 1·24	t 0·85 (J = 6·5)	— (2·0)	8·14 (7·3)	5·19 5·45
IIg	dd 5·79 (5·0)	m 3·30—4·00 (2·5)		—	—	—	—	—	— (1·8)	8·12 (7·0)	—

[a] OCH_3; [b] $H_{2'}$ br t 3·97 (after ^{2}H exchange).

TABLE VI
Chromatography data

Compound	R_F			k^a(S)	Compound	R_F(S1)	Compound	R_F	
	S2	S3	S5					S1	S4
IIa	0·34	0·40	0·52	2·30 (S7)	*Va*	0·20	*XIa*	0·37	0·18
IIb	0·38	0·50	0·56	2·50 (S8)	*Vb*	0·30	*XIb*	0·39	0·27
IIc	0·45	0·54	0·66	3·00 (S9)	*Vc*	0·38	*XIc*	0·52	0·30
IId	0·53	0·68	0·74	1·80 (S10)	*Vd*	0·40	*XId*	0·61	0·42
IIe	0·55	0·74	0·90	2·50 (S10)	*Ve*	0·47	*XIe*	0·78	0·44
IIg	0·12	—	0·42	1·49 (S6)	*Vf*	0·53	*XIf*	0·52	—
IIh	0·15	0·22	0·47	0·90 (S6)	*VIIIa*	0·18	*XIh*	0·31	—
IIIa	0·15	0·58	0·58	0·50 (S7)	*VIIIb*	0·21	*XVI*	0·43	0·30
IIIb	0·20	0·68	0·69	0·59 (S7)	*VIIIc*	0·25	*XVIIa*	—	0·27
IIIc	0·29	0·72	0·72	1·90 (S7)	*VIIId*	0·30	*XVIIb*	—	0·43
IIId	0·38	0·86	0·78	0·83 (S10)	*VIIIe*	0·40	*XVIIc*	—	0·49
IIIe	0·50	0·92	0·95	2·25 (S10)	*VIIIf*	0·25	*XVIId*	—	0·54
IIIf	0·29	0·72	0·72	—	*XII*	0·32	*XVIIe*	—	0·58
IIIg	0·02	0·15	0·48	0·97 (S6)	*XIII*	0·12	*XIX*	—	0·44
IIIh	0·07	0·25	0·50	0·70 (S6)	*XIV*	0·35	—	—	—

[a] HPLC in system S; $k = (t_R - t_0)$, t_R retention time, t_0 hold-up time.

culture *i.e.* during the logarithmic phase, at concentration of $2 . 10^{-7} - 10^{-5}$ mol l^{-1}. The IC_{50} values were evaluated after 72 h of exposure to the drug. 5-Fluorouracil and 5'-deoxy-5-fluorouridine (*I*) were used as standards. The inhibitory effects of compounds *II* are presented in Table VII. Significant cytostatic effects were observed with compounds *IIc*, *IId*, and *IIe*, *i.e.* derivatives with pronounced hydrophobic substituents in position 5'. Both the lower alkyl derivatives *IIa,b* and the hydrophilic *IIg*, were inactive. The 5'-O-n-butyl ether *IIc* was obviously the most effective member of the whole series; however, its IC_{50} value ($2{\cdot}8 . 10^{-6}$ mol l^{-1}) is still 14 times higher than that of the compound *I*. The concentration dependence for both compounds is shown in Fig. 1. Morphologic manifestation of the cytostatic effect of compounds *I* and *IIc* consisted of cellular desintegration and formation of polymorphous cell population with unusually high number of large cells.

In order to investigate possible effect of compound *IIc* on DNA or RNA synthesis we followed incorporation of ^{14}C-labelled uridine and 2'-deoxythymidine into these molecules in the presence of compound *I* or *IIc* at a concentration twice as high as their respective IC_{50} values. Under the conditions employed, neither of the two fluorouracil nucleosides had any effect on incorporation of the radiolabelled precursors (data not shown).

The effect of 5'-O-n-butyl-5-fluorouridine (*IIc*) was also evaluated *in vivo* in L 1210 leukemia bearing mice. The compound (80 mg/kg, 5 × daily administration) did not prolong the life span of the treated leukemic mice.

TABLE VII
Effect of 5'-O-alkyl-5-fluorouridines on the growth of L 1210 mouse leukemic cells *in vitro*

Compound	Concentration mol l^{-1}	% control	IC_{50} mol l^{-1}
I	10^{-5}	4	$2 . 10^{-7}$
5-Fluorouracil	10^{-5}	2	—
	10^{-6}	10	—
IIa	10^{-5}	33	—
IIb	10^{-5}	39	—
IIc	10^{-5}	6	$2{\cdot}8 . 10^{-6}$
	10^{-6}	77	—
IId	10^{-5}	12	$5{\cdot}2 . 10^{-6}$
	10^{-6}	83	—
IIe	10^{-5}	33	$6{\cdot}0 . 10^{-6}$
	10^{-6}	100	—
IIg	10^{-5}	90	—

Although the *in vivo* experimental results do not rank the above compounds *II* among promising cytostatics, it is not excluded that a continued investigation of 5-fluorouracil nucleosides bearing hydrophobic substituents at the sugar moiety could uncover compounds with more convenient pharmacological behavior to compare with these of 5-fluorouracil.

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 40°C/2 kPa and the compounds dried at 13 Pa over phosphorus pentoxide. Melting points were determined on a Kofler block and are uncorrected. Thin-layer chromatography (TLC) was carried out on Silufol UV_{254} plates (Kavalier, Votice, Czechoslovakia) in the following systems: S1 chloroform, S2 chloroform–methanol (9 : 1), S3 chloroform–methanol (75 : 25), S4 benzene–ethyl acetate (7 : 3). Preparative TLC was performed on loose layers of silica gel containing a fluorescent indicator (5 × 16 × 0·3 cm; Silpearl, Kavalier, Votice). Paper chromatography was done on a Whatman No 1 paper in the system S5 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). HPLC analyses were carried out on a 4 × 200 mm Silasorb C18 column in 0·05 mol l^{-1} triethylammonium acetate pH 7 containing the following concentrations (vol %) of methanol: S6 2·5%, S7 10%, S8 20%, S9 40%, S10 60%; flow rate 0·4 ml min^{-1}, detection at 254 nm. Column chromatography was performed on silica gel (Pitra, 30—40 μ) prepared in the Service Laboratories (Institute of Organic Chemistry and Biochemistry, Prague) or on octadecyl-silica gel (20 × 3 cm column) prepared by modification of Silpearl (30 μ). Ultraviolet absorption spectra were measured in aqueous solutions on a Specord UV-VIS spectrophotometer, ^1H NMR spectra on a Varian XL-200 (200 MHz) or a Tesla 100 MHz instrument in deuteriochloroform or hexadeuteriodimethyl sulfoxide with hexamethyldisilazane as internal standard; chemical shifts in ppm, coupling constants in Hz. Mass spectra were taken on an AEI 902 spectrometer (source temperature 120°C, 70 eV, direct inlet).

Methyl 5-O-Alkyl-2,3-O-isopropylidene-D-ribofuranosides (*V*)

A solution of methyl 2,3-O-isopropylidene-D-ribofuranoside[14] (*IV*; b.p. 86°C/9 Pa; 20·4 g, 0·1 mol) in dimethylformamide (50 ml) was added to an ice-cooled and stirred suspension of sodium hydride (2·5 g; 0·104 mol) in dimethylformamide (100 ml). After stirring at 0°C for

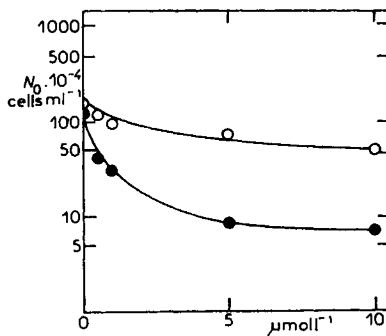


Fig. 1
Effect of different concentrations of compounds *I* (●) and *IIc* (○) on the growth of L 1210 mouse leukemic cells *in vitro*. Control — 1·7 . 10^6 cells *per* ml

30 min under exclusion of moisture, redistilled alkyl bromide (0·2 mol) was added. The mixture was stirred at 0°C for 2 h and at room temperature for 16–20 h and the solvent was evaporated at 60°C/2 kPa. The residue was mixed with ether (400 ml), the ethereal solution washed with water (3 × 100 ml), dried over magnesium sulfate and evaporated *in vacuo*. The product was distilled *in vacuo*. In this manner we prepared the following compounds *V* (yield, b.p.): *Va* (ref.[15]), *Vb* (81%, 76–80°C/9 Pa), *Vc* (48%, 86–94°C/7 Pa), *Vd* (60%, 110–115°C/7 Pa), *Ve* (69%, 134–135°C/7 Pa), *Vf* (64%, 84–86°C/7 Pa) and *XII* (86%, 90–93°C/20 Pa).

1-O-Acetyl-5-O-alkyl-2,3-di-O-benzoyl-D-ribofuranoses (*IX*)

To a solution of compound *V* (60 mmol) in a mixture of methanol (125 ml) and water (75 ml) was added conc. sulfuric acid (0·5 ml). After refluxing for 1 h, methanol was evaporated *in vacuo* and the residue extracted with ether (3 × 100 ml). The extract was washed with water, dilute (1 : 25) aqueous ammonia and water (2 × 50 ml), dried over magnesium sulfate and ether was evaporated *in vacuo* (finally at 60°C/13 Pa). This procedure was applied to the preparation of 5-O-alkyl-D-ribose *VId* (69%) and *VIe* (78%). In the other cases, the aqueous solution after evaporation of methanol was neutralized with saturated aqueous solution of barium hydroxide, the suspension was filtered through Celite and evaporated *in vacuo*. The residue was codistilled with dioxane (3 × 50 ml) and dried at 60°C/13 Pa over phosphorus pentoxide for 4 h. In this way were prepared compounds *VIb* (98%), *VIc* (82%) and *VIf* (73%) as oils.

Magnesium sulfate (7·5 g) and conc. sulfuric acid (0·75 ml) were added to a solution of compound *VI* (50 mmol) in methanol (150 ml), the mixture was stirred in a stoppered flask at 0°C for 3 h and then set aside at 0°C overnight. Calcium carbonate (15 g) was added and the mixture was stirred for 3 h, filtered through Celite, washed with methanol, and the neutral filtrate was made weakly alkaline with triethylamine. After evaporation *in vacuo*, the residue was codistilled with toluene (2 × 25 ml) and dried at 60°C/13 Pa. Samples of the thus-obtained methyl glycosides *VII* gave no Fehling reaction. Compound *VII* in pyridine (80 ml) was cooled in ice and benzoyl chloride (17 ml) was added with stirring. The mixture was stirred in ice for 2 h and allowed to stand in a refrigerator for 2 days. Water (10 ml) was added, followed by benzene (500 ml), and the organic layer was washed successively (100 ml portions) with water, saturated sodium hydrogen carbonate, water (2×), and dried over magnesium sulfate. After evaporation *in vacuo*, the residue was codistilled with toluene at 40°C/2 kPa (3 × 25 ml) until pyridine was removed. The remaining compound *VIII* (for chromatographic data see Table VI) was dissolved in acetic acid (200 ml) and acetic anhydride (50 ml). Concentrated sulfuric acid (15 ml) was added under ice-cooling and stirring and the mixture was stirred in an ice bath for 3 h, set aside in a refrigerator overnight and poured on ice (500 g). The product was taken up in chloroform (2 × 200 ml), washed with water (3 × 100 ml) and saturated sodium hydrogen carbonate solution was gradually added with stirring until the aqueous phase had a weakly alkaline reaction. The chloroform layer was dried over magnesium sulfate and the solvent was evaporated *in vacuo*, finally at 50°C/13 Pa. The residue in benzene (50 ml) was filtered through a column of alumina (200 ml) in the same solvent, the column was washed with benzene (300 ml) and the filtrate was taken down *in vacuo*. After drying at 13 Pa, the remaining compounds *IX* were used directly in the next reaction step. Thus were obtained: *IXb* (52%), *IXc* (76%), *IXd* (46%), and *IXe* (48%). Compound *Vf* gave a mixture of *IXf* and *IXh*.

1-O-Acetyl-5-O-(*RS*)-(2,3-dibenzoyloxypropyl)-2,3-di-O-benzoyl-D-ribofuranose (*XV*)

Compound *XII* (15·8 g; 65 mmol) and osmium tetroxide (40 mg) were added to a solution of sodium chlorate (13·8 g; 0·13 mol) in 50% aqueous ethanol (70 ml). After stirring under reflux

condenser at 60°C for 7 h, the mixture was concentrated until an oily product separated. It was extracted with chloroform (3 × 100 ml), the extract was washed with water, dried over magnesium sulfate, the solvent was evaporated *in vacuo* and the remaining almost homogeneous compound *XIII* (63 mmol) was dissolved in a solution of benzoyl cyanide (17 g; 130 mmol) in ether (150 ml). Triethylamine (1 ml) was added and after 1 h the solvent was evaporated *in vacuo*. Chromatography of the residue on silica gel (300 g) in chloroform afforded 20·8 g (63% based on *XII*) of compound *XIV* as a yellow oil. This product was subjected to acid hydrolysis under conditions described in the preparation of compounds *IX* (reflux for 3 h) and, after neutralization of the reaction mixture with triethylamine, methanol was evaporated *in vacuo*. The residue was extracted with chloroform (3 × 100 ml), washed with water (100 ml), dried over magnesium sulfate and taken down *in vacuo*. After stirring with methanol (150 ml), magnesium sulfate (8 g) and sulfuric acid (0·75 ml) at 0°C for 6 h, the mixture was set aside in a refrigerator overnight and neutralized with triethylamine. Methanol was evaporated *in vacuo*, the mixture was again extracted with chloroform (300 ml), washed with water (3 × 100 ml), dried and evaporated *in vacuo*. The residue was dissolved in pyridine (70 ml) and benzoyl chloride (15 ml) was added dropwise under stirring and ice-cooling. The mixture was stirred at 0°C for 6 h, allowed to stand overnight in a refrigerator, decomposed with water (10 ml), diluted with benzene (300 ml), washed with water, saturated sodium hydrogen carbonate solution, again water, and dried. After evaporation of the solvent *in vacuo*, the residue was subjected to reaction with acetic anhydride and acetic acid as described for compounds *IX*. Chromatography on alumina gave compound *XV* (58%) as a yellow foam.

5'-O-Alkyl-2',3'-di-O-benzoyluridines *XI*, *XVI*

Tin tetrachloride (5 ml) was added to a solution of compound *IX* or *XV* (50 mmol) and compound *X* (10·2 g; 40 mmol; b.p. 112—113°C/2 kPa) in acetonitrile (150 ml) and the mixture was allowed to stand at room temperature for 2 days. After evaporation of the solvent *in vacuo*, the residue was dissolved in chloroform (200 ml) and boiled with pyridine (10 ml) for 30 min. The mixture was filtered through Celite which was then washed with chloroform (200 ml), the filtrate was washed with water (3 × 100 ml), dried over magnesium sulfate and taken down *in vacuo*. Traces of pyridine were removed by codistillation with toluene (3 × 50 ml) *in vacuo* and the residue was chromatographed on a column of silica gel (300 g) in chloroform. The product fractions were combined and the product (*XI* or *XVI*) was crystallized from ether and light petroleum. According to this procedure were prepared: *XIb* (71%), *XIc* (48%), *XId* (79%), *XIe* (70%), and *XVI* (72%). Physical constants for these compounds are given in Table VIII, chromatographic data in Table VI. The reaction mixture from crude *IXf* afforded 60% of chromatographically pure 2',3',5'-tri-O-benzoyluridine (*XVIII*), identical with an authentic material[17] (compound *XIf* was concentrated in the mother liquor; *vide infra*).

5'-O-Alkyluridines *III*

A solution of compound *IX* or *XVI* (3 mmol) in 0·1 mol l^{-1} methanolic sodium methoxide (50 ml) was set aside at room temperature overnight, neutralized with Dowex 50 X 8 (H^+ form), filtered, and the Dowex was washed with methanol (100 ml). Methanol was evaporated *in vacuo* and the residue was codistilled with ethanol. 5'-O-n-Hexyluridine (*IIId*; 89%) or 5'-O-n-octyluridine (*IIIe*; 84%) was crystallized from chloroform–light petroleum. In other cases the residue after evaporation of methanol from the mixture was taken up in water (100 ml), washed with ether (2 × 50 ml), the ethereal layer was washed with water (2 × 20 ml) and the combined aqueous phases were taken down *in vacuo*. The residue was dissolved in water (20 ml) and applied on to a column of octadecyl-silica gel. The product was eluted with water under continuous

monitoring on a Uvicord instrument (LKB, Sweden). In this manner, the following pure (HPLC) products were obtained, after crystallization from ethanol–light petroleum: *IIIb* (81%) and *IIIg* (69%). Compound *IIIc* was eluted from the column with 30% aqueous ethanol; yield 60%. Physical constants and analyses of the products are given in Tables VI and VIII, other parameters in Tables I–V.

TABLE VIII

Analytical data of uridine derivatives

Compound (m.p., °C)	Formula (mol. weight)	Calculated/found		
		% C	% H	% N
IIIb (128)	$C_{11}H_{16}N_2O_6$ (272·2)	48·52 48·74	5·93 5·89	10·29 10·39
IIIc (85–88)	$C_{13}H_{20}N_2O_6$ (300·3)	51·99 51·63	6·68 6·64	9·33 9·15
IIId (102)	$C_{15}H_{24}N_2O_6$ (328·4)	54·86 54·71	7·37 7·18	8·53 8·32
IIIe (101–102)	$C_{17}H_{28}N_2O_6$ (356·4)	57·28 56·92	7·92 7·84	7·86 7·89
IIIf (103)	$C_{13}H_{20}N_2O_6$ (300·3)	51·99 52·21	6·68 6·83	9·33 9·27
IIIg (109)	$C_{12}H_{18}N_2O_8$ (318·3)	45·28 45·18	5·70 5·77	8·80 8·42
IIIh (164)	$C_9H_{12}N_2O_6$ (244·2)	44·26 43·91	4·95 4·93	11·47 11·43
XIb (96)	$C_{25}H_{24}N_2O_8$ (480·5)	62·49 63·01	5·03 5·13	5·83 5·52
XIc (147–150)	$C_{27}H_{28}N_2O_8$ (508·5)	63·77 63·50	5·55 5·44	5·51 4·98
XId (105–106)	$C_{29}H_{32}N_2O_8$ (536·6)	64·91 64·87	6·01 5·96	5·22 5·12
XIe (85)	$C_{31}H_{36}N_2O_8$ (564·6)	65·94 65·77	6·43 6·36	4·96 4·81
XVI (83)	$C_{40}H_{34}N_2O_{12}$ (734·7)	65·39 65·08	4·66 4·88	3·81 4·09
XVIII (145[a])	$C_{30}H_{24}N_2O_9$ (556·5)	64·74 64·41	4·35 4·54	5·03 4·70

[a] Ref.[17] gives m.p. 143°C.

5′-O-(2-Methylpropyl)uridine (*IIIf*)

The mother liquor from crystallization of *XVIII* (*vide supra*) was concentrated *in vacuo* and allowed to stand overnight with 0·1 mol l^{-1} methanolic sodium methoxide (100 ml). After neutralization with Dowex 50 X 8 (H^+ form), filtration and evaporation of the solvent, the residue was partitioned between ether and water (as described *e.g.* for *IIIb*) and chromatographed on a column of octadecyl-silica gel. Elution with water (4 ml min^{-1}) gave uridine (*IIIh*), further elution with 30% aqueous ethanol afforded pure *IIIf* which was crystallized from ether–light petroleum; yield 10% (based on crude *IXf*). For constants see Table VIII.

5′-O-Alkyl-2′,3′-di-O-benzoyl-5-fluorouridines *XVII* and *XIX*

A mixture of fluorine and nitrogen (1 : 10; v/v) was introduced into a solution of compound *XI* or *XVI* (10 mmol) in acetic acid (400 ml). When the reaction was complete (monitoring in system S4), the excess fluorine was removed by a stream of nitrogen, the mixture was taken down *in vacuo* and the residue was codistilled with acetic acid (2 × 100 ml) and ethanol (2 × 100 ml). The residue was dissolved in ethanol (100 ml) and the solution was neutralized with triethylamine. After evaporation of the solvent, the product was crystallized from ethanol. Mother liquors after crystallization of the main portion were purified by column chromatography on silica gel (200 g) in chloroform. This procedure furnished *XVIIa* (24%), *XVIIb* (58%), *XVIIc* (64·5%), *XVIId* (70%), *XVIIe* (51·5%), and *XIX* (46·5%). Physical constants and analytical data of the products are given in Tables VI and IX.

5′-O-Alkyl-5-fluorouridines *II*

A) A solution of compound *XVII* or *XIX* (5 mmol) in 0·1 mol l^{-1} methanolic sodium methoxide (100 ml) was allowed to stand at room temperature overnight, neutralized by addition of Dowex 50 X 8 (H^+ form), filtered and the Dowex was washed with methanol (100 ml). After evaporation, the residue was chromatographed on two loose layers of silica gel in methanol–chloroform (1 : 9). The product zones were eluted with methanol (500 ml) and evaporated *in vacuo*. Crystallization from ethyl acetate–ether (1 : 1) with addition of light petroleum to turbidity afforded the products *IId* and *IIe* in the respective yields 76·5% and 78%.

B) The crude *IIc*, obtained by neutralization with Dowex (see *A*) and evaporation, was dissolved in 10% aqueous methanol (30 ml) and applied onto a column of octadecyl-silica gel, pre-equilibrated with water. Successive elution with water (1 liter), 10% methanol (500 ml), 20% methanol (500 ml) and finally 50% methanol afforded *IIc* which, after evaporation, was crystallized from ethyl acetate–light petroleum; yield 62%.

C) Crude compound *IIa*, *IIb* or *IIg*, obtained by neutralization with Dowex (see *A*) and evaporation, was dissolved in water (200 ml) and washed with ether (3 × 50 ml). The ethereal extracts were washed with water (2 × 50 ml), the combined aqueous layers were made alkaline (pH 9) with ammonia and applied onto a column of Dowex 1 X 2 (acetate; 100 ml). After washing the column with water to loss of UV absorption and conductivity, the product was eluted with 0·05 mol l^{-1} acetic acid. The UV-absorbing eluate was taken down and the remaining acetic acid was removed by repeated distillation with water (2 × 50 ml) and ethanol (2 × 50 ml). The residue in water (10 ml) was applied on to a column of octadecyl-silica gel, pre-equilibrated with water. After removal of UV-absorbing impurities by elution with water, the product was eluted with 10% methanol (*IIa* or *IIb*) or 5% methanol (*IIg*). Fractions, homogeneous according to HPLC, were combined, taken down and the compounds were crystallized from ethanol–ether. The procedure furnished chromatographically pure compounds *IIa* (79%), *IIb* (84%), and *IIg*

(61%). Constants and analytical data of the products are given in Table IX, chromatographic data in Table VI, UV spectra in Table III and NMR and mass spectral parameters in Tables IV and V.

Biological Experiments

Antibacterial activity was tested using synthetic medium (10 ml) containing inorganic salts and D-glucose[21]. The sterile medium was added to the weighed compounds in sterile 25 ml Erlenmeyer flasks stoppered with cotton wool. After addition of 0·05 ml *Escherichia coli* B inoculum

TABLE IX

Analytical data of 5-fluorouridine derivatives

Compound (m.p., °C)	Formula (mol. weight)	Calculated/found			
		% C	% H	% F	% N
IIa (182)	$C_{10}H_{13}FN_2O_6$ (276·2)	43·48 43·46	4·74 4·66	6·88 6·79	10·14 9·92
IIb (158)	$C_{11}H_{15}FN_2O_6$ (290·3)	45·51 45·25	5·21 5·14	6·55 6·20	9·65 9·42
IIc (153)	$C_{13}H_{19}FN_2O_6$ (318·3)	49·06 48·88	6·02 5·94	5·97 5·84	8·80 8·51
IId (114)	$C_{15}H_{23}FN_2O_6$ (346·4)	52·01 52·34	6·69 6·57	5·49 5·71	8·09 7·84
IIe (118–119)	$C_{17}H_{27}FN_2O_6$ (374·4)	54·53 54·55	7·27 7·18	5·07 4·94	7·48 7·38
IIg (130–132)	$C_{12}H_{17}FN_2O_8$ (336·3)	42·86 42·40	5·10 5·35	5·65 5·22	8·33 7·78
XVIIa (158–159)	$C_{24}H_{21}FN_2O_8$ (484·4)	59·50 59·78	4·37 4·46	3·92 3·90	5·78 5·77
XVIIb (189)	$C_{25}H_{23}FN_2O_8$ (498·5)	60·24 59·84	4·65 4·30	3·81 3·62	5·62 5·54
XVIIc (214)	$C_{27}H_{27}FN_2O_8$ (526·6)	61·59 61·34	5·17 4·99	3·61 4·08	5·32 5·23
XVIId (165)	$C_{29}H_{31}FN_2O_8$ (554·6)	62·80 63·28	5·63 5·29	3·43 3·22	5·05 4·79
XVIIe (104–105)	$C_{31}H_{35}FN_2O_8$ (582·6)	63·90 63·67	6·05 5·77	3·26 3·06	4·81 5·00
XIX (65)	$C_{40}H_{33}FN_2O_{12}$ (752·7)	63·82 63·86	4·42 4·29	2·52 2·57	3·72 3·43

(prepared in the same synthetic medium), cultivation by stationary incubation was carried out at 37°C for 16 h. Growth of the bacteria was evaluated by absorbance measurements at 575 nm.

Antiviral assays. Primary rabbit kidney cells grown to confluency in Sterilin microtiter trays were inoculated with 100 $CCID_{50}$ (1 $CCID_{50}$ is the virus dose required to infect 50% of the cells) of either HSV-1 (KOS), HSV-2 (G), vaccinia virus or vesicular stomatitis virus. After 1 h virus adsorption, residual virus was removed and the cell cultures were incubated with Eagle's minimum essential medium supplemented with 3% calf serum, containing varying concentration of the test compounds (400, 200, 100 ... μg/ml). Virus induced cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures. The minimum cytotoxic concentration of the test compounds was evaluated in non-infected primary rabbit kidney cells under the same conditions as used for the antiviral assays and was defined as the minimum concentration required to cause a microscopically detectable alteration of normal cell morphology.

Cytostatic activity, animal experiments. Throughout the experiments, DBA/2 male mice (25 g) kept under standard conditions were used. L 1210 cells (10^5) were implanted intraperitoneally in a volume of 0·2 ml of saline. The drugs were dissolved fresh in saline and injected (0·2 ml) on day 2 following cell inoculation.

Cell growth experiments. The experiments were performed on L 1210 leukemic cells in RPMI 1640 Medium (Grand Island Biological Co., N.Y., U.S.A.) containing 15% horse serum (Bioveta, Ivanovice), penicillin (50 IU/ml), streptomycin (50 μg/ml) and 2-mercaptoethanol (10^{-6} mol . l^{-1}) using multiwell tissue culture plates (Nunc Product, Roskilde). For measurements of the effect of the drugs on cell growth, 2 ml of cell suspension (5 . 10^4 cells/ml) were placed in each well. After 24 h of exponential growth the cells were counted and 100 μl volume of inhibitor solution in RPMI medium without serum was added. Each sample was dispensed in triplicate and incubated in 10% CO_2 at 37°C in a humid atmosphere. After 72 h of exposure to drugs the cells were counted; for each data point, the counts agreed within ±12% limit.

Determination of DNA *and* RNA *synthesis in* L 1210 *cells.* L 1210 cells in culture were harvested during the exponential period of cell growth, washed in medium containing 15% horse serum and suspended in the same medium at 2 . 10^5 cells/ml. The cells (1·8 ml) were placed in plastic tubes containing 0·1 ml of inhibitor (*I*, 5 . 10^{-7} mol l^{-1}, *IIc*, 5 . 10^{-6} mol l^{-1}) and 0·1 ml of 2-^{14}C-2′-deoxythymidine (0·25 μCi, 5 nmol) (spec. activity 52 μCi/μmol) or U-^{14}C-uridine (0·25 μCi, 5 nmol) (spec. activity 320 μCi/μmol). The tubes were gassed with CO_2, stoppered and incubated in triplicate in a Dubnoff shaker bath at 37°C for 4·5 h. The incubation was terminated by adding 4 ml of ice cold 0·9% NaCl; after mixing on ice, the cell suspensions were filtered on Whatman GF/C glass fiber filters previously washed with saline. The filters were washed twice with 5 ml of cold 0·9% NaCl, twice with 5 ml of ice-cold trichloroacetic acid, once with 5 ml ethanol and dried. Thereafter, they were transferred into scintillation liquid (Omnifluor, New England Nuclear, Boston, U.S.A.) and counted. The counts in parallel experiments agreed within a ±15% limit.

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