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STUDIES ON THE INHIBITION OF THE GROWTH OF KB CELLS BY 9- AND 7-(KETO-NUCLEOSIDES)

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Abstract—The effect of 7- and 9- [(6'-deoxy-L-*lyxo*-hexosyl-2' (and -4')-ulose] purines on the growth of KB cells *in vitro*, was investigated. These keto-nucleosides are the first keto-hexosyl-purines shown to inhibit cellular growth in contrast to their inactive parent nucleosides. A significative difference in activity between 7- and 9-keto-nucleosides has been shown.

IN RECENT papers, we have reported the novel synthesis of ketohexosyl and 6-deoxy ketohexosyl-purines[1–6] as key intermediates in the synthesis of nucleoside antibiotics. Additional interest in this new class of nucleosides has resulted from the discovery that 7 (2'-keto-β-L-"fucosyl") theophylline (II) exhibits growth inhibitory activity whereas the parent nucleoside (I) is inactive at the same dose levels.[7]

In this paper the action of the newly synthetized keto-deoxynucleoside derivatives on cellular growth *in vitro*, in comparison with the action of the parent deoxy-nucleosides is reported. The results obtained have been analysed with respect to structure–activity relationships.

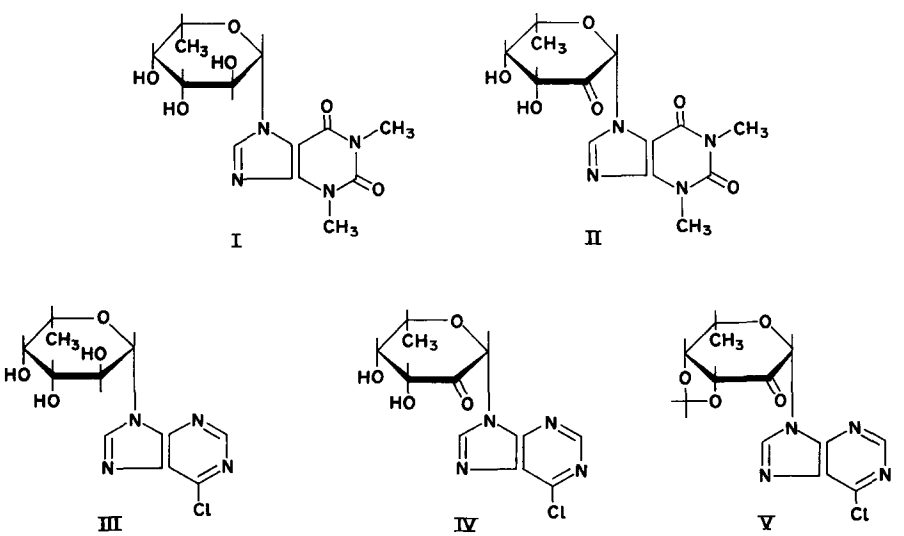

FIG. 1. 7- and 9-(2'-ketohexosyl) purines.

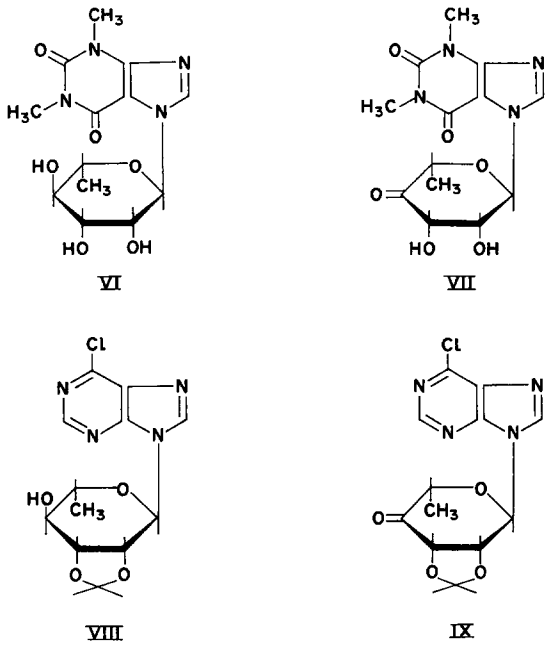

FIG. 2. 7- and 9-(4'-ketohexosyl) purines.

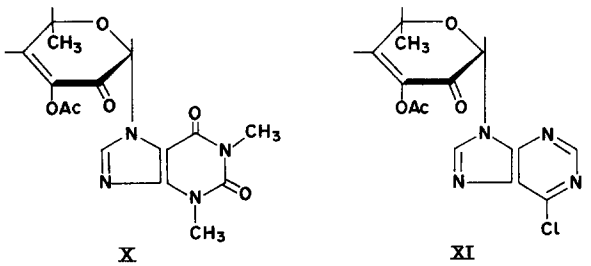

FIG. 3. 7- and 9- unsaturated keto-nucleosides.

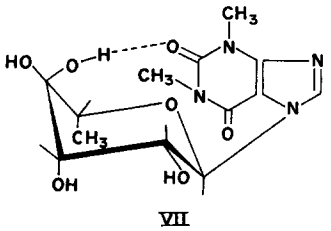

FIG. 4.

MATERIALS AND METHODS

Preparation of keto-nucleosides. 2'- and 4'-Keto-nucleosides (II) (IV) (V) (VII) (IX) were synthesized by direct oxidation of partially protected 7- and 9- (deoxy hexosyl)-purines.[1] The anomeric purity of the different models was shown by n.m.r. spectra which additionally confirmed the assigned conformation of the hexosyl-purines.

The unsaturated keto-nucleosides (X) (XI) were obtained by acetylation of the corresponding keto-nucleosides (II) and (IV) respectively, followed by β-elimination of an acetyl group.[6] The structure of these conjugated nucleosides was confirmed by n.m.r. spectra and their purity by spectroscopic and chromatographic methods.

Preparation of cultures. The biological activity of compounds I to XI was tested *in vitro* using KB tumor cells growing on glass coverslips in Leighton tubes that contained Eagles medium (Diploid basal medium, Gibco) to which 10% v/v calf serum (Sorga) had been added. Two millilitres of medium containing 10^5 KB cells was added to each tube. The next day the medium was changed and the compounds were added either dissolved directly in the medium or as a solution in dimethyl sulfoxide (DMSO). The final concentration of DMSO did not exceed 0·5 per cent. The test concentrations of the compounds were 0·7, 0·17 and 0·04 mg/ml.

Addition of reagent and method of counting. The activity was expressed as the lowest concentration giving at least 50 per cent inhibition following the method described below.

Each keto-nucleoside was compared with the corresponding non-oxidized nucleoside. Therefore 48 Leighton tubes containing KB cells, numbered from 1 to 48, were randomly distributed in 8 groups, each of six tubes.

The cell growth was measured using a method which employs a "proportional index". This "index" is determined from the number of dots out of the 31 dots that are randomly distributed over a circular area of the modified Chalkley microscope eyepiece[8,9] that overlap the cells when they are observed through this eyepiece.

On day zero, before the addition of any compound or solvent, the index was determined on 12 randomly selected tubes. On days 2 and 4 the measurement of the index was performed blind on the tubes, in numerical order. On each tube, four different microscopic fields were counted. In this way the arithmetical means for all treatment groups were obtained from 24 measurements, that for the control from 48.

RESULTS AND DISCUSSION

The 2'-keto-nucleosides (II) (IV) (V) (X) (XI) are all derivatives of L-fucose and have the β-configuration. The 4'-keto-nucleosides (VII) (IX) are derivatives of L-rhamnose and have the α-configuration. Attempts to obtain oth α- and β-anomers of the same keto-nucleoside were unsuccessful.

As can be seen in Tables 1, 2, 3 and 4 all deoxy-nucleosides possessing a keto-group in the sugar moiety inhibit cell growth whereas the parent nucleosides (I) (III) (VI) (VIII), before oxidation, are inactive under the same conditions. In addition no significant difference could be observed between the activity of α- and β-anomers. Before the addition of the compounds (day 0) the "proportional index" was practically the same for all the groups of tubes examined and the behavior of 2'-keto-nucleosides was very similar to that of 4'-keto-nucleosides.

TABLE 1. PROPORTIONAL INDEX OF GROWTH OF KB CELLS IN THE PRESENCE OF NUCLEOSIDES I, II, III, IV, V

Compound	Dose (mg/ml)	Days 0	2	4
	0·044		13·3	21
I	0·175	4·5 ± 0·7	13·1	22
	0·7		16 1	22·9
Control		3·3 ± 1·0	13·2 ± 3·1	19·1 ± 3·9
	0·044		17·7 ± 5·7	16·8 ± 7·8
II	0·175	3·7 ± 0·9	10·8 ± 3·6	18·5 ± 3·6
	0·7		2·6 ± 2·1	0·7 ± 0·1
Control		3·3 ± 1·0	13·2 ± 3·1	19·1 ± 3·5
	0·044		15·21 ± 2·62	25·42 ± 2·48
III	0·175	6·75 ± 0·75	13·63 ± 1·35	22·42 ± 2·82
	0·700		10·04 ± 1·17	9·63 ± 2·02
Control		6·73 ± 0·75	15·10 ± 1·51	25·29 ± 1·53
	0·044		13·96 ± 1·64	23·17 ± 1·85
IV	0·175	6·73 ± 0·75	12·38 ± 1·61	14·75 ± 1·46
	0·700		6·83 ± 1·80	4·67 ± 0·86
Control		6·73 ± 0·75	15·10 ± 1·51	25·29 ± 1·53
	0·044		9·79 ± 1·00	14·92 ± 1·51
V	0·175	5·95 ± 0·33	5·42 ± 0·98	4·21 ± 1·17
	0·700		1·54 ± 0·56	0·63 ± 0·28
Control	(DMSO)	5·95 ± 0·33	12·75 ± 0·92	21·79 ± 0·98

The results in Table 4 show that of the nucleosides derived from L-fucose, [7-nucleosides (I, II, X) and 9-nucleosides (III, IV and XI)], the 7- and 9-unsaturated keto-nucleosides X and XI had the highest inhibitory activity.

From the overall results (Table 4) it is clear that 9-keto-nucleosides (IV) (V) (IX) (XI) are more active than the 7-keto-nucleosides (II) (VII) (X); however this is the

TABLE 2. PROPORTIONAL INDEX OF GROWTH OF KB CELLS IN THE PRESENCE OF NUCLEOSIDES VI, VII, VIII, IX

Compound	Dose (mg/ml)	Days 0	2	4
	0·044		7·83	13·67
VI	0·175	4·40	10·83	20·00
	0·700		11·67	17·13
Control		4·40	10·18	14·00
	0·044		12·25 ± 1·02	20·75 ± 2·80
VII	0·175	5·95 ± 0·33	6·65 ± 0·94	3·17 ± 0·58
	0·700		2 08 ± 0·55	1·83 ± 0·64
Control	(DMSO)	5·95 ± 0·33	12·75 ± 0·92	21·79 ± 0·98
	0·039		9·71 ± 1·51	15·75 ± 1·40
VIII	0·155	4·83 ± 0·62	7·04 ± 1·63	9·67 ± 2·31
	0·588		4·92 ± 1·83	3·25 ± 1·28
Control		4·83 ± 0·62	13·40 ± 1·28	19·75 ± 1·34
	0·044		7·46 ± 1·44	11·13 ± 2·74
IX	0·175	4·83 ± 0·62	5 75 ± 0·73	4·30 ± 1·07
	0·700		3·58 ± 1 26	2·33 ± 1·26
Control		4·83 ± 0·62	13 40 ± 1·28	19·75 ± 1·34

TABLE 3 PROPORTIONAL INDEX OF GROWTH OF KB CELLS IN THE PRESENCE OF NUCLEOSIDES X and XI

Compound	Dose (mg/ml)	Days 0	2	4
	0·044		5·92 ± 1·54	11·08 ± 2·57
X	0·175	3·49 ± 0·54	3·71 ± 1·04	2·50 ± 1·00
	0·700		2·46 ± 1·31	1·67 ± 0·98
Control		3·49 ± 0·54	7·69 ± 1·34	14·02 ± 2·29
	0·044		16·00 ± 1·65	23·54 ± 2·30
XI	0·175	4·99 ± 1·00	12·13 ± 1·79	14·17 ± 2·17
	0·700		3·60 ± 1·74	1·17 ± 0·42
Control	DMSO	4·99 ± 1·00	17·83 ± 1·38	24·54 ± 1·81

first time that the growth inhibitory activity of 7-(hexosyl-purines) has been demonstrated. This difference cannot be attributed to the conformation of the hexosyl purines because either 7- or 9- (keto-hexosyl) purines possess both C 1 and 1 C conformations. It is of interest that in the case of (VII) the assigned conformation C 1 permits the establishment of hydrogen bonds between the *gem*-diol at 4'-position of the sugar (C = 0) hydrated, and the C = 0 group in 2-position of theophylline (Fig. 4). This is not possible with the 6-chloropurine derivative whatever its conformation.

Evidence for hydrogen bonding was obtained by the study of the dehydration of this molecule (VII) and from the n.m.r. spectrum. Thus attempts to obtain the pure keto-nucleoside by dehydration at 140° failed whereas this dehydration readily proceeded with other hydrated keto-nucleosides below 100°.[1] This exceptional stability can not be attributed to the formation of a cyclonucleoside (bonding between a keto group of the heterocyclic base and an activated sugar OH) since these cyclizations are obtained only under basic conditions. The *gem*-diol (VII) could also be dehydrated by azeotropic distillation in dimethyl formamide.

Furthermore in the n.m.r. spectrum measured in DMSO the OH signal appeared at a very low field confirming[11] that this proton was hydrogen bonded.

This important problem of hydration of keto-nucleosides is being investigated in our laboratory in connection with the mechanism of action of these nucleosides.

It is evident that in the keto-nucleosides examined, the carbonyl group plays an important rôle whatever their mechanism of action. Thus not only were compounds

TABLE 4. PHYSICAL DATA AND BIOLOGICAL ACTIVITY OF THE NUCLEOSIDES EXAMINED

Compound	Configuration (Conformation)		Temp. (°F)	$[\alpha]_D^{20}$	N.m.r. J 1'2'	Inhibitory effect against KB cells (mg/ml)
I	β-L-galacto	(1C)	272–273	−1 (H_2O)	9 Hz	inactive
II	β-L-lyxo	(1C)	265–270	−30 (H_2O)	—	0·7
III	β-L-galacto	(1C)	133	−35·5 (MeOH)	9 Hz	very weak at 0·7 mg/ml
IV	β-L-lyxo	(1C)	160–165	−19 (MeOH)	—	0·1
V	β-L-lyxo	(1C)	80–85	−47·5 (MeOH)	—	0·3
VI	α-L-manno	(C1)	153	−90 (H_2O)	—	inactive
VII	α-L-lyxo	(C1)	140–141	−55 (DMSO)	4 Hz	0·1
VIII	α-L-manno	(1C)	82		4 Hz	very weak at 0·17 mg/ml
IX	α-L-lyxo	(1C)	134–138	−45 (MeOH)	2 Hz	very active at 0·04 mg/ml
X.	β-L-glycero	(1C)	172–174	+80 (MeOH)	—	0·17
XI	β-L-glycero	(1C)	165–166	+75 (MeOH)	—	very active at 0·1 mg/ml

inactive before oxidation but those derived from reduction of the ketone (isomers or deoxynucleosides) did not inhibit cellular growth under the same conditions. The borohydride reduction of the various saturated and unsaturated keto-nucleosides has been examined in some detail[12, 13] since this provides additionally novel routes to rare sugar nucleosides and deoxy-nucleosides; the isolated and characterized new nucleosides[12, 13] 7(6'-deoxy-β-L-talo-pyranosyl)theophylline and 7(4', 6'-dideoxy-β-L-ribo-hexopyranosyl) theophylline were quite inactive against KB tumour cells at 0·7 mg/ml.

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