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Antiviral activity of some natural and synthetic sugar analogues

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A number of natural and synthetic sugar analogues have been tested for their antiviral activity, using an influenza virus strain as a model Hemagglutinating titres (HA) and cytopathic effect (CPE) were surveyed to estimate the virus production It was found that introduction of the benzyl group into these sugars generally causes them to become antivirally active Substitution with methyl, acetyl, uridyl and thiocyanyl groups or derivatization with azido, isopropylidene and benzylidene groups were without effect All sugars containing the 2-deoxy-2-acetamido group were inactive

Sugar analogue, Synthetic sugar, Antiviral activity, Hemagglutinating titre, Influenza virus

1 INTRODUCTION

Some naturally occurring sugars and their structurally related compounds are known to inhibit virus multiplication 2-Deoxy-D-glucose and D-glucosamine cause impairment of glycosylation of viral glycoproteins at high concentrations [1–4] 1-Deoxynojirimycine, 1-deoxy-mannojirimycine and castanospermine disturb glycosylation by inhibiting the trimming enzymes needed to complete the synthesis of sugar chains [5–9] Recently many amino sugar derivatives have been shown to inhibit HIV [10] In this work, a number of sugar analogues differently modified at various positions were screened for their antiviral activity. An influenza virus, the fowl plague virus, was used for this study The results obtained provided an insight into the structural features which may or may not be responsible for the antiviral activity of these sugar analogues

2 MATERIALS AND METHODS

2 1 *Cell culture and virus multiplication*

MDCK cells (Madin–Darby canine kidney cells) were grown to confluency in plastic Petri dishes, 3 5 cm in diameter, using Dulbecco's complete medium containing 10% new-born calf serum An influenza virus (the fowl plague virus, strain Rostock) was used to infect these cells at a multiplicity of 50 plaque forming units After 1 h of adsorption, the excess virus was removed by washing and the plates were maintained in the same Dulbecco's medium (without serum) containing 10 mM of the various sugar analogues used These plates and the control plate without sugar analogues were further incubated for 7 h at 37°C

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2 2 *Virus quantification*

The medium was tested for virus-specific hemagglutinating activity at the end of an 8-h replication cycle Cell-associated hemagglutinating activity was also tested after 3 cycles of freezing and thawing treatment Normally, the control plates containing no inhibitors had a hemagglutinating activity of about 32 or 64

2 3 *Cytopathic effect (CPE) and cytotoxic effect (CTE)*

CPE was marked in the table (Table I, CPE) as positive (+), when cells rounded up and detached from the plate at 8 h after infection as a result of virus infection CTE was defined in this work as the lowest concentration of sugars that caused about 5% of the total cells to round up and detach from the plates within 8 h Both CPE and CTE were observed under a light microscope (Zeiss inverse microscope) to estimate the extent of cell damage

2 4 *SDS-gel electrophoresis*

To determine the effect of sugars on glycosylation and protein synthesis, 50 μCi of L-[1-^{3}H]fucose and 2 μCi of ^{14}C-labelled protein hydrolysate were added to the medium 2 h after infection After a pulse period of 4 h, the cells were harvested with a rubber policeman and analysed by SDS-gel electrophoresis in a 10% round gel (0 5×10 cm) The gels were cut into 1-mm slices and counted in a scintillation counter

2 5 *Sugars*

All natural sugars and synthetic analogues were obtained from Bayer AG Purity of the compounds and correctness of their structures were checked

3 RESULTS AND DISCUSSION

All sugars tested were used at a concentration of 10 mM. This concentration of 2-deoxy-D-glucose and D-glucosamine is known to be just sufficient to completely inhibit many enveloped viruses without being toxic to cells [1–4]. Deoxynojirimycine, an inhibitor of glucosidases, can act at a much lower concentration [5–9]. However, for the purpose of comparison, derivatives of deoxynojirimycine were also employed at 10 mM in the

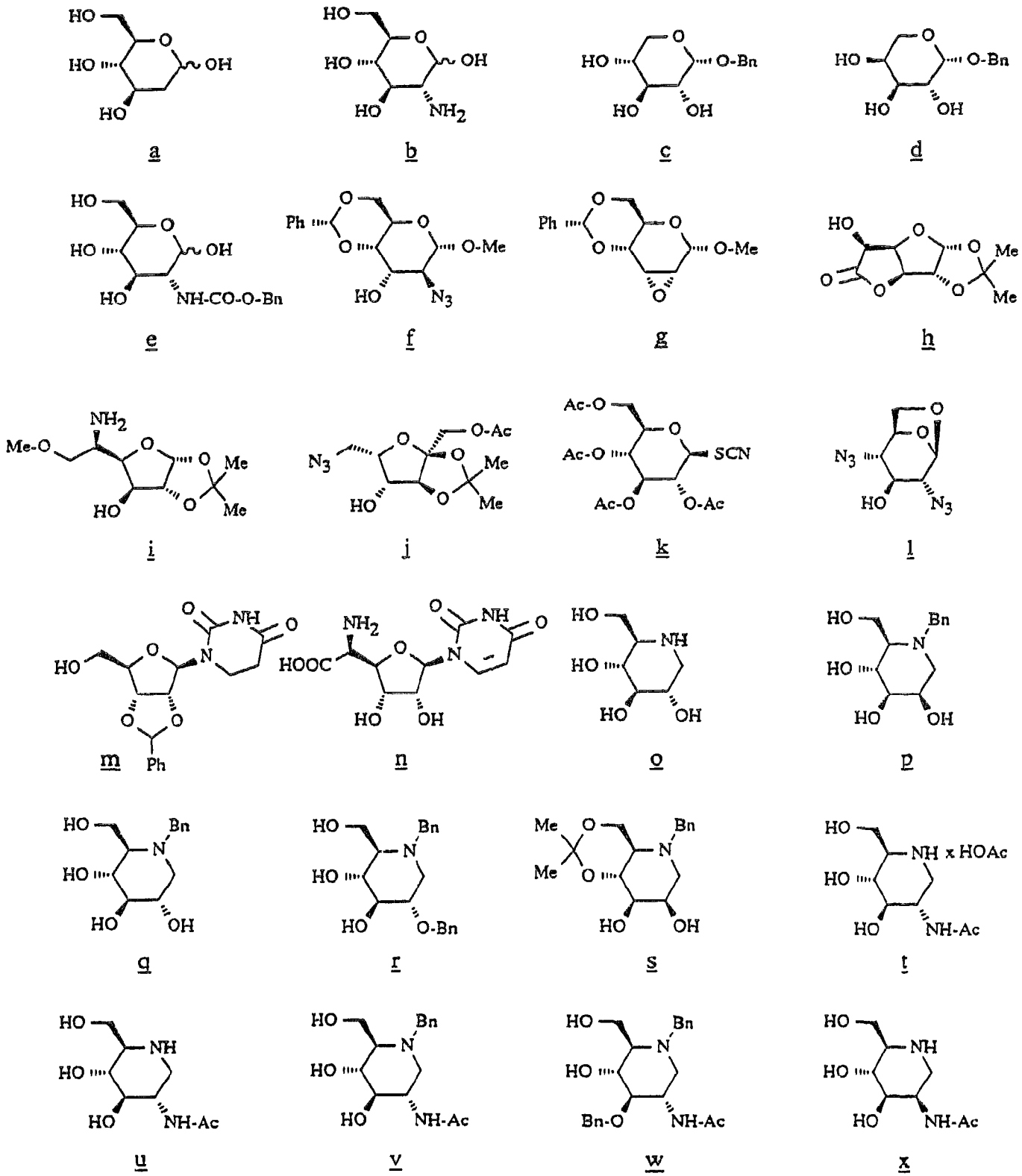

Fig 1. a*, 2-Deoxy-D-glucopyranose, b*, 2-Amino-2-deoxy-D-glucopyranose, c*, Benzyl α-D-xylopyranoside, d*, Benzyl β-L-arabinopyranoside, e*, 2-(Benzyloxycarbonylamino)-2-deoxy-D-glucopyranose, f, Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside; g, Methyl 2,3-anhydro-4,6-*O*-benzylidene α-D-allopyranoside, h, 1,2-*O*-Isopropylidene-α-D-glucofuranurono 6,3-lactone, i, 5-Amino-5-deoxy-1,2-*O*-isopropylidene-6-*O*-methyl-α-D-glucofuranose, k, 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosil-thiocyanate, l, 1,6-Anhydro-2,4-diazido-2,4-dideoxy-β-D-glucopyranose, m, 2′,3′-*O*-Benzylidene-uridine, n, 5′-Amino-5′-deoxy-β-D-glucofuranuronosyl-uridine, o*, 1,5-Dideoxy-1,5-imino-D-glucitol (1-Deoxynojirimycine), p*, *N*-Benzyl-1,5-dideoxy-1,5-imino-D-mannitol, q*, *N*-Benzyl-1,5-dideoxy-1,5-imino-D-glucitol, r*, *N*,2-*O*-Dibenzyl-1,5-dideoxy-1,5-imino-D-glucitol, s*, *N*-Benzyl-1,5-dideoxy-1,5-imino 4,6-*O*-isopropylidene-D-mannitol; t, 2-Acetamino-1,5-imino-1,2,5-trideoxy-D-glucitol hydroacetate, u, 2-Acetamino-1,5-imino-1,2,5-trideoxy-D-glucitol, v, 2-Acetamino-*N*-benzyl-1,5-imino-1,2,5-trideoxy-D-glucitol, w, 2-Acetamino-*N*,3-*O*-dibenzyl-1,2,5-trideoxy-D-glucitol, x, 2-Acetamino-1,2,5-trideoxy-D-mannitol

present study A preliminary test showed that MDCK cells incubated with 10 mM of each of the individual sugars used were able to grow normally in Dulbecco's medium, indicating a lack of toxicity of these sugars to MDCK cells under the experimental conditions At this concentration, none of the sugars tested had any effect on HA by themselves Antiviral effect of the sugars was not tested at concentrations higher than 10 mM, because this is an unseemly high dose in the case of a therapeutic use.

At the end of an 8-h replication cycle, hemagglutinating activity released into the medium and that associated with the cells were compared Only those compounds that completely inhibited hemagglutinating activity are marked with an asterisk in the legend of Fig 1 The unmarked compounds were not inhibitory. In keeping with results of others, we also found that 2-deoxy-D-glucose, D-glucosamine and 2-deoxynojirimycine (Fig. 1a, b and o) were capable of completely inhibiting virus multiplication. Of the other compounds tested, those containing a benzyl group (Fig. 1c, d and e) were antivirally active, with the exception of those also containing an acetamido group (Fig 1v and w) In contrast, the phenyl group in the benzylidene structure (Fig 1f and m) did not confer antiviral activity. Other modifications of the sugars, such as methylation (Fig 1m and n) or introduction of isopropylidene (Fig 1h, i and j) and azido (Fig 1f, j and l) groups also did not elicit antiviral activity. Sugars containing an acetamido group (Fig. 1t, u, v, w and x), including those also substituted with the benzyl group (Fig 1n and w) were found to be ineffective as antiviral agents This is reminiscent of the previous findings, that glucosamine was antivirally active whereas *N*-acetylglucosamine was not [1] As it can be seen from Table I, the antiviral effect of the sugars was similarly reflected in the amounts of HA released into the medium and that associated with the cell, showing that the sugars were not affecting the process of virus release into the medium The cytopathic effect (CPE), that occurred as a result of a virus infection, corresponded well with the above data in all cases (Table I) To find out how toxic the sugars were by themselves, concentrations of up to 50 mM were tested for CTE. The cytotoxic concentrations of the sugars were found to be at least 2.5 to 10 times higher than the antivirally active doses (Table I, CTE).

Table I

Antiviral activity of natural and synthetic sugar analogues

	Sugar analogues	HA titre[1]		CPE[2]	CTE (mM)[3]
		Medium	Cell		
	Control (without sugar)	5	4	+	
a)	2-Deoxy-D-glucopyranose	0	0	–	>100
b)	2-Amino-2-deoxy-D-glucopyranose	0	0	–	50
c)	Benzyl α-D-xylopyranoside	0	0	–	50
d)	Benzyl β-L-arabinopyranoside	0	0	–	50
e)	2-(Benzyloxycarbonylamino)-2-deoxy-D-glucopyranose	0	0	–	50
f)	Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside	5	4	+	>100
g)	Methyl 2,3-anhydro-4,6-*O*-benzylidene α-D-allopyranoside	5	4	+	>100
h)	1,2-*O*-Isopropylidene-α-D-glucofuranurono-6,3-lactone	5	5	+	>100
i)	5-Amino-5-deoxy-1,2-*O*-isopropylidene-6-*O*-methyl-α-D-glucofuranose	5	4	+	>100
j)	1-*O*-Acetyl-6-azido-6-deoxy-2,3-*O*-isopropylidene-α-L-sorbofuranose	4	5	+	>100
k)	2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylthiocyanate	5	4	+	>100
l)	1,6-Anhydro-2,4-diazido-2,4-dideoxy-β-D-glucopyranose	4	5	+	>100
m)	2',3'-*O*-Benzylidene-uridine	5	4	+	>100
n)	5'-Amino-5'-deoxy-β-D-glucofuranuronosyl-uridine	5	5	+	>100
o)	1,5-Dideoxy-1,5-imino-D-glucitol (1-Deoxynojirimycine)	0	0	–	>100
p)	*N*-Benzyl-1,5-dideoxy-1,5-imino-D-mannitol	1	1	–	25
q)	*N*-Benzyl-1,5-dideoxy-1,5-imino-D-glucitol	2	1	+	25
r)	*N*,2-*O*-Dibenzyl-1,5-dideoxy-1,5-imino-D-glucitol	0	0	–	25
s)	*N*-Benzyl-1,5-dideoxy-1,5-imino-4,6-*O*-isopropylidene-D-mannitol	1	1	–	50
t)	2-Acetamino-1,5-imino-1,2,5-trideoxy-D-glucitol hydroacetate	5	4	+	>100
u	2-Acetamino-1,5-imino-1,2,5-trideoxy-D-glucitol	5	4	+	>100
v)	2-Acetamino-*N*-benzyl-1,5-imino-1,2,5-trideoxy-D-glucitol	5	5	+	>100
w)	2-Acetamino-*N*,3-*O*-dibenzyl-1,2,5-trideoxy-D-glucitol	5	5	+	>100
x)	2-Acetamino-1,2,5-trideoxy-D-mannitol	5	4	+	>100

[1]HA, titre found in the culture medium at the end of an 8 h reproduction cycle HA titre is the number of twofold dilutions that still cause hemagglutination HA of 0 designates absence of hemagglutination (total inhibition of virus production),
[2]CPE, cytopathic effect caused by virus infection at 8 h after infection (see section 2),
[3]CTE, concentrations (mM) in twofold dilutions that caused cytotoxic effect within 8 h (see section 2)

Tracing experiments using [^{3}H]fucose and [^{14}C]amino acids to label the virus (not shown) indicated that the synthesis of viral proteins and viral glycoproteins is suppressed by sugars that contain a benzyl group Sugar analogues have recently become of interest as antiviral drugs because of the availability of a large number of derivatives and the relatively small cytotoxicity of the sugars. In many cases, the antiviral activity was shown to be related to the synthesis and trimming of carbohydrate chains [1–9,11]. In other instances, impairment of protein biosynthesis and destabilization of proteins in general have been observed [11,12] These investigations serve to indicate some structural features that may be of use for designing antivirally active sugar analogues

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