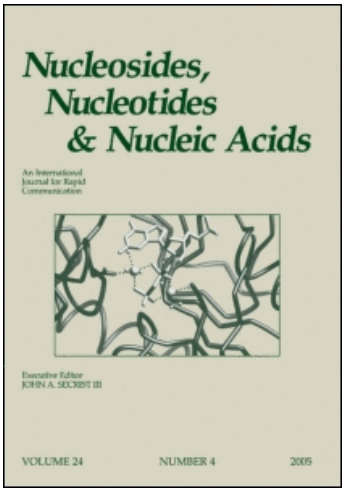

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The Carbocyclic Analogue of 5-(1-Propenyl)-2'-Deoxyuridine; Synthesis and Anti-Herpes Activity

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THE CARBOCYCLIC ANALOGUE OF 5-(1-PROPENYL)-2'-DEOXYURIDINE; SYNTHESIS AND ANTI-HERPES ACTIVITY.

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ABSTRACT - The title compound was synthesised and tested against HSV-1 in cell culture. Like related compounds, it was less active than the parent nucleoside.

Nucleosides have considerable potential as antiviral and anticancer agents and many chemical modifications have been tried in the search for useful biological activity [1]. A modification found to produce activity in the naturally occurring antibiotic aristeromycin [2] is replacement of the sugar ring oxygen atom by a methylene group. This substitution has been incorporated into a number of synthetic nucleosides giving the carbocyclic family of analogues [3]. These compounds often retain the biological properties of the parent nucleoside while showing greater resistance to the enzymes responsible for their catabolism and inactivation. For example, the antiviral agent 1-β-D-arabinofuranosyladenine is deactivated by adenosine deaminase whereas the carbocyclic analogue is resistant to deamination while retaining antiviral activity [4-6]. Furthermore, as carbocyclic

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derivatives lack a true glycosidic bond, they are also resistant to the phosphorylases and hydrolases responsible for the inactivation of many nucleosides by deglycosylation [4,7,8]. Deglycosylation is a major route for the metabolism and inactivation of the potent anti-herpetic (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU;3b)[9]. Recently, several reports have appeared on the carbocyclic analogue of this compound and other active 5-substituted deoxypyrimidine nucleosides [3,10-13].

We also have been studying the anti-herpes activity of this series of compounds and we wish to report a new member of the family, the carbocyclic analogue of 5-(1-propenyl)-2'-deoxyuridine (4a).

EXPERIMENTAL SECTION

Melting points were determined on an Electrothermal apparatus and are not corrected. All compounds were characterized by NMR on a Varian FT80A instrument and by chemical-ionization mass spectroscopy on a Finnigan 4000 instrument and have correct elemental analyses (to within ± 0.4%) or accurate mass determinations.

The (±)-carbocyclic analogues of 2'-deoxyuridine, 3'-deoxyuridine and 5-iodo-2'-deoxyuridine (1a,6,5a) were prepared by the methods of Shealy et al.[3,14,15]. Substitutions at the 5- position to give 2a, 3a,4a and 7 were performed by the same methods described for the parent nucleosides [16] based on reactions developed by Bergstrom and Ruth [17]. UV Spectra and coupling constants for protons in the 5- substituent were essentially the same as those for the parent nucleosides used to assign the E-stereochemistry [16]. The synthesis of the 5-(2-carbomethoxyvinyl) and 5-(2-bromovinyl) derivatives (2a and 3a) have been reported previously by other workers [11,13].

(±)-(E)-5-(1-Propenyl)-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1H,3H)-pyrimidinedione (4a). To a stirred solution of 1a (1.1g, 4.9mmol) in water (41mL) was added mercury(II) acetate (1.72g, 5.4mmol). After heating at 55° for 3h, a solution of sodium chloride (0.72g, 12.4mmol) in water (5mL) was added and the reaction stirred at room temperature for a further 16h. The precipitate was collected by filtration and washed with aqueous sodium chloride solution (0.1M), water, ethanol and finally ether. To a stirred suspension of the precipitate in methanol (20mL) was added a 0.1M methanolic solution

of Li_2PdCl_4 (5mL) and allyl chloride (1.5 mL, 18.4mmol). After 3h, hydrogen sulphide was passed through the solution for 1-2min followed by nitrogen (to remove excess H_2S). Precipitated metal sulphides were removed by centrifugation and washed twice with methanol. Solvent was evaporated from the combined supernatants and the residue dissolved in ethanol (25mL) and refluxed under nitrogen for 16h with $RhCl(PPh_3)_3$ (50mg, prepared by the method of Bennett and Longstaff [18]). The reaction was followed by UV spectroscopy (4a has λ_{max} 300nm compared to 277nm for the nonconjugated precursor) or by TLC on reverse phase silica in EtOH:0.1M NaCl (2:3) (where 4a moves slower than its precursor). If necessary, more catalyst was added and reflux continued for up to 36h. Solid material was removed by filtration through Whatman paper GF/A and the filtrate taken to dryness under vacuum. Desired product was extracted from the residual brown gum with ethyl acetate. Evaporation of the solvent gave a white solid which was taken up in water:methanol (2:3) and purified on a column of Sephadex LH20 by elution with water to give 4a (245mg, 19% overall yield). Recrystallization from ethyl acetate gave an analytically pure sample, mp 165-168°. Anal. ($C_{13}H_{18}N_2O_4$) C,H,N.

(±)-(E)-5-(1-Propenyl)-1-[(1α, 2β, 4α)-2-hydroxy-4-(hydroxymethyl) -cyclopentyl]-2,4(1H,3H)-pyrimidinedione (7). This compound was prepared from 6 by the same method used to prepare 4a. Crystallization from ethyl acetate gave an analytically pure sample, mp 198.5-199° (dec). Anal. ($C_{13}H_{18}N_2O_4$) C,H,N.

Antiviral assays. Antiviral activity was assessed by a microplaque reduction method using the S3 strain of HSV-1 [16]. Virus infectivity end-point titrations were performed by inoculation of serial 0.5 $\log_{10}$ dilutions of virus on monolayers of baby hamster kidney cells in flat-bottomed microtitre plates (Flow Laboratories, Irvine, Scotland). Test compounds were included at the required concentration in the overlay (Eagles minimal essential medium, Dulbecco's modification, containing 10% donor calf serum [Flow Laboratories] and 0.5% carboxymethylcellulose [Sigma Chemical Co.]). The minimum inhibitory concentration (MIC) of each compound was determined to be the least concentration that gave a reduction in virus

infectivity end-point of not less than 1 $\log_{10}$ compared with the non-drug-treated control.

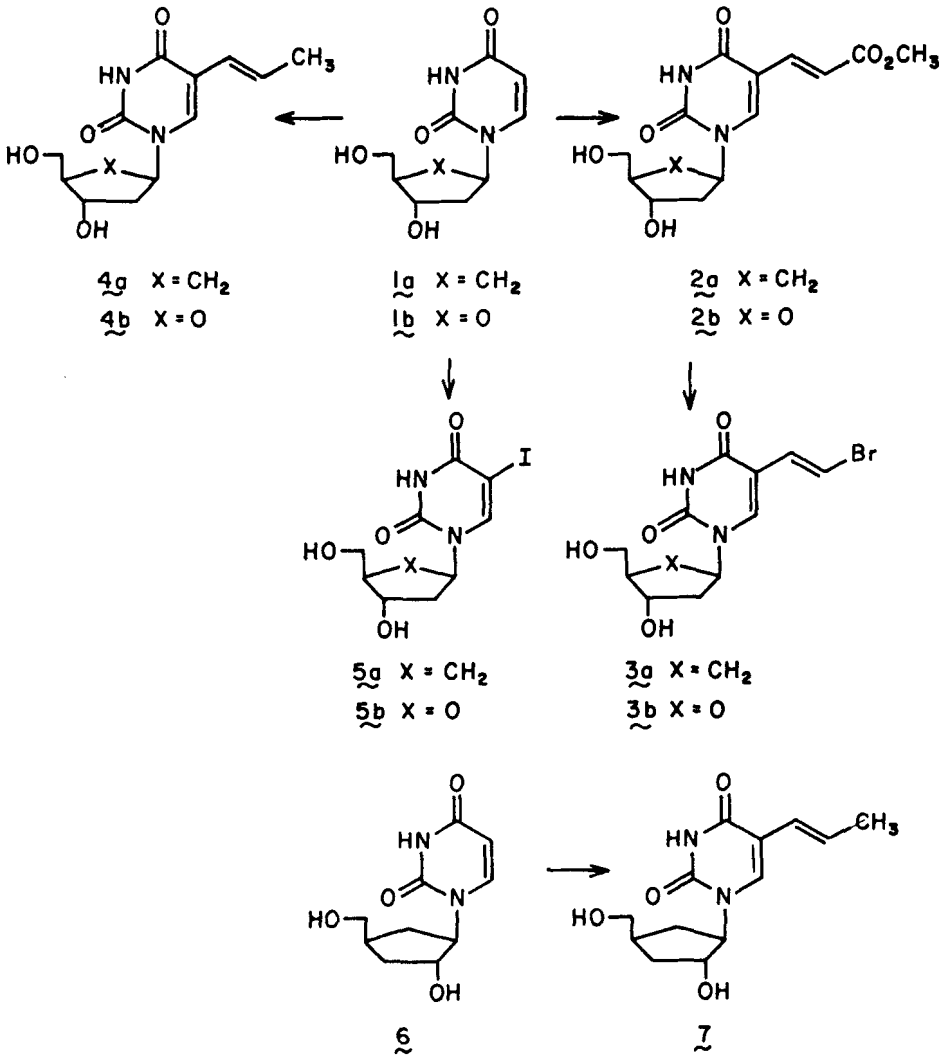
RESULTS AND DISCUSSION

5-Substituted 2'-deoxypyrimidine nucleosides have proven to be a class of compounds rich in anti-herpes activity [1,19,20]. The most potent of these against HSV-1 *in vitro* is BVDU (3b) [21]. A major route for the metabolism and inactivation of this compound *in vivo* seems to be deglycosylation [9] and so we synthesized its carbocyclic analogue (3a). In addition, we prepared carbocyclic analogues (4a,5a) of the known antiviral agents 5-propenyl- [22] and 5-iodo-2'- deoxyuridine [23]. These compounds were made by substitution of 1a. As the synthesis of 1a by the method of Shealy *et al*. [14,15] also yields the 3'-deoxy isomer 6, we prepared its propenyl derivative 7 for comparison.

The compounds were tested for antiviral activity against HSV-1 in a microplaque reduction assay and results are presented in the Table. Three carbocyclic compounds (3a-5a) showed antiviral activity but in all cases they were less active than their respective parent nucleosides and especially so in the case of 3a. Differences in antiviral activity amongst the compounds 3a - 5a were much less than for the corresponding parent nucleosides. In view of the moderate antiviral activity of the synthetic intermediate 2b determined previously [16], we also tested the carbocyclic analogue 2a but it was without activity at the highest concentration (100μg/ml). Of the isomers 4a and 7, differing only in the position of the secondary hydoxyl group, only the former was active. This is in accord with the studies of a related series where the 3'-deoxy analogues were found to be inactive as were the unsubstituted compounds 1a and 6 [3].

It would appear that, as in the 2'-deoxyuridine series, the 5-substituents in the carbocyclic analogues determine the degree of antiviral activity and that the different substituents produce qualitatively similar effects in the two series.

After we began this work, the synthesis and activity of 3a and 5a as well as the synthesis of 2a were reported by other workers [3,11-13]. Their studies of the anti-HSV-1 activity of 3a and 5a compared with



4a X = CH_2
4b X = O

1a X = CH_2
1b X = O

2a X = CH_2
2b X = O

5a X = CH_2
5b X = O

3a X = CH_2
3b X = O

6

7

TABLE

Comparison of the antiviral activity of carbocyclic 5-substituted deoxyuridines and their parent nucleosides against HSV-1 in cell cultures

Compound	5-Substituent	MIC[a] µg/ml	
		Carbocyclic	Deoxyribose
2a,b	(E)-CH=CHCO$_2$CH$_3$	>100	10
3a,b	(E)-CH=CHBr	5	0.005
4a,b	(E)-CH=CHCH$_3$	5	0.5
5a,b	I	1	0.5
1a	H	>100	
6	H	>100	
7	(E)-CH=CHCH$_3$	>100	

a Minimum inhibitory concentration = lowest concentration required to give a 1 $\log_{10}$ reduction in the infectivity end-point of HSV-1, strain S3, titrated on monolayers of BHK21 cells.

their parent nucleosides show qualitatively similar trends to ours but greater relative activity in the carbocyclic compounds.

In the compounds studied so far, the carbocyclic modification usually results in a reduction of activity of 5-substituted-2'-deoxyuridine against HSV-1 in cell culture and in one animal study [20]. Our findings with the 5-propenyl substituent further extend these observations.

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