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Synthesis and biological properties of 2'-deoxy-5-vinyluridine and 2'-deoxy-5-vinylcytidine

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ABSTRACT

Rapid and efficient syntheses for the preparation of 2'-deoxy-5-vinyluridine and 2'-deoxy-5-vinylcytidine are described starting from nucleoside precursors. Contrary to some previous reports, 2'-deoxy-5-vinyluridine has be found to be quite stable under normal laboratory conditions but when tested in animals shows neither toxicity nor anti-leukemic (L1210 cells) or anti-parasitic (Plasmodium berghei) activity. 2'-Deoxy-5-vinylcytidine appears to polymerise readily. It is much less toxic to cell cultures than 2'-deoxy-5-vinyluridine but is almost as active against herpes virus replication (ID_{50}:0.2μg/ml) for both type 1 and type 2 herpes virus (selectivity index:225).

INTRODUCTION

Several 5-substituted uracil or cytosine 2'-deoxynucleosides have been shown to possess significant and sometimes therapeutically-useful biological activity, since Prusoff and his colleagues originally reported the antiviral activity of 2'-deoxy-5-iodouridine. More specifically, the 2'-deoxy-5-halogenovinyluridines and -cytidines exceed the corresponding 2'-deoxy-5-halogenouridines and -cytidines in antiherpes activity by at least one order of magnitude, with a concomitant decrease in antimetabolic activity of around two orders of magnitude (1-4). From these investigations it has become evident that nucleoside analogues with selective antiviral activity can be developed, and this has given impetus to the discovery of methods of synthesis by transformation, usually via organometallic intermediates, at the nucleoside level. Thus, although E-5-(2-bromovinyl)-2'-deoxyuridine was originally prepared following a lengthy series of reactions, including base-sugar condensation and α-/β-anomer separation (1, 5), the methods used by Bergstrom and co-workers (6) have now been adapted such that the synthesis of 2'-deoxy-5-halogenovinyluridines from 2'-deoxyuridine can be achieved in three steps in high yield (7).

Here we report the use of related organometallic intermediates for the

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synthesis of 2'-deoxy-5-vinyluridine and 2'-deoxy-5-vinylcytidine. The biological properties of these compounds are also described.

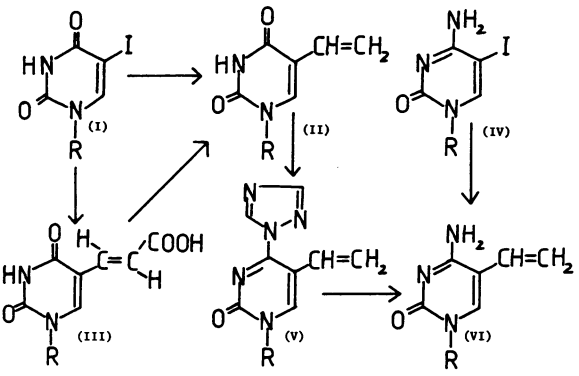
DISCUSSION

Since its synthesis was first reported in 1973 (8), several additional methods for the synthesis of 5-vinyluracil and its 2'-deoxynucleoside have appeared but none of the deoxynucleoside syntheses has proved to be entirely satisfactory (9). Condensation of a base moiety with a suitably-protected sugar derivative is fraught with difficulty and this product and those prepared at the nucleoside level frequently polymerise, although a recent preliminary account of a preparation *via* an organometallic intermediate from 2'-deoxy-5-iodouridine seems to be more promising (10).

Unfortunately, the obvious method using ethylene and a C-5 mercurated nucleoside with Li_2PdCl_4 gives rise to methoxylated products and only a very small yield, if any, of the required nucleoside (11). Direct vinylation using vinyl acetate has been used with 1,3-dimethyl-5-iodouracil or 2,4-dimethoxy-5-iodopyrimidine (12) but because of differences in starting material solubility, these sealed tube reaction conditions described by Arai and Daves (12) when applied to 2'-deoxy-5-iodouridine with their palladium catalyst (diacetato-bis[triphenyl phosphine]palladium [II]) were not feasible. Eventually, pre-activation of the catalyst followed by reaction in dimethyl-formamide at 70° for 5 1/2 hours resulted in the preparation of 2-deoxy-5-vinyluridine and 2'-deoxyuridine (formed by deiodination of this starting material). These could be separated easily to give an overall yield of 37% in one step (Scheme I).

It has also been possible to prepare 2'-deoxy-5-vinyluridine in one step by the decarboxylation of *E*-5-(2-carboxyvinyl)-2'-deoxyuridine, an intermediate in our preparation of *E*-5-(2-bromovinyl)-2'-deoxyuridine (7). When heated in DMF, in the presence of triethylamine at 100° for 35 hours, 2'-deoxy-5-vinyluridine can be isolated by chromatography in 24% yield (Scheme I). Neither of the products from these preparations, which have been obtained in gram quantities, has shown any tendency to polymerise, which confirms our suspicion that the product when made by previous routes was only unstable because of the presence of contaminating impurities which were difficult to separate because of the poor yields obtained.

The preparation of 2'-deoxy-5-vinylcytidine has only been recorded in the patent literature and a general claim of antiviral activity was made (13). However, no specific details were provided and the yields of the



SCHEME I SCHEME II R = 2'-Deoxyribosyl

product are likely to be low. We have now prepared this compound by two routes. One, starting from 5-iodo-2'-deoxycytidine, and using vinyl acetate and a palladium catalyst in dimethylformamide, is equivalent to one of the previously-described preparations of 2'-deoxy-5-vinyluridine (Scheme II). However, this method is not straightforward and the overall yield is less than 10%. Other identified products are 4-N-acetyl-2'-deoxycytidine and 2'-deoxycytidine, the latter being the major product because 2'-deoxy-5'-iodocytidine deiodinates at a much faster rate than does the corresponding deoxyuridine derivative. Decarboxylation of E-5-(2-carboxyvinyl)-2')-deoxycytidine (7) gave only unidentified fluorescent material.

An alternative way to prepare 2'-deoxy-5-vinylcytidine, now that the corresponding deoxyuridine derivative is readily available, is based on the conversion of the uridine into the corresponding cytidine derivative. A recently reported mild method for this transformation was attempted (14). 3'-5'-O-Acetylation of 2'-deoxy-5-vinyluridine was accomplished in good yield and reacted with 1,2,4-triazole and p-chlorophenylphosphorodichloridate in anhydrous pyridine. This intermediate triazole derivative could be decomposed with ammonia to give 2'-deoxy-3',5'-di-O-acetyl-5-vinylcytidine and further reaction with base gave the desired nucleoside (Scheme II). However, all preparations of 2'-deoxy-5-vinylcytidine have proved to be unstable. The product appears to polymerise very easily and also fluorescent material is rapidly formed upon storage. The fluorescent product is probably a pyrido[2,3-d]-pyrimidine caused by nucleophilic attack of the exocyclic amino group on the vinyl group.

Some of the biological properties of 2'-deoxy-5-vinyluridine have been described before. It is a potent anti-herpes virus agent (1,2,15), can be incorporated into the DNA in place of thymidine in E.coli (16) and a mycoplasma species (17), and the base, 5-vinyluracil, can be incorporated into phage T3 with a concomitant loss of phage viability (18). It also causes sister chromatid exchange at a concentration of 0.05 µg/ml in human fibroblasts (19). With the previously-reported preparations of 2'-deoxy-5-vinyluridine, it had been impossible to do any further biological work due to the inability to produce a sufficient quantity of the compound in a stable form. We have now made gram quantities available for testing 2'-deoxy-5-vinyluridine as an anti-cancer and an anti-malarial agent. Despite the toxicity of 2'-deoxy-5-vinyluridine for murine leukemia (L1210) cells in vitro [ID_{50}(inhibitory dose -50) for L1210 cell growth : 2.37 µg/ml (20)], no significant toxicity was seen in mice when the compound was administered at a dose of 250 mg/Kg or 320 mg/Kg. Neither did the compound show any significant protection against a lethal L1210 cell inoculation or Plasmodium berghei infection in mice. This indicates that 2'-deoxy-5-vinyluridine is probably inactivated before it can reach the target sites. It is also noteworthy that sister chromatid exchange is not observed with 2'-deoxy-5-vinyluridine in human lymphocytes up to a concentration that is by two orders of magnitude higher than that required for sister chromatid exchange in human fibroblasts (19).

It has been shown on several occasions (4,21) that 5-substituted 2'-deoxycytidines are more selective inhibitors of herpes virus replication than their 2'-deoxyuridine counterparts. This means that the 5-substituted 2'-deoxycytidines are significantly less cytotoxic, yet almost as active agains herpes virus replication, as the corresponding 2'-deoxyuridines. For example, the ID_{50} of 2'-deoxy-5-ethynyluridine and 2'-deoxy-5-ethynylcytidine for host cell metabolism ([2-^{14}C]2'-deoxyuridine incorporation into DNA) were 0.1 and 25 µg/ml, respectively (1,4). Their ID_{50} for herpes simplex virus type 1 replication were quite comparable, however (0.1 and 0.2 µg/ml, respectively). 2'-Deoxy-5-vinylcytidine continued this trend. Its ID_{50} for various strains of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) were similar, if not identical to those of 2'-deoxy-5-vinyluridine (Table 1). Yet, 2'-deoxy-5-vinylcytidine was much less inhibitory for the incorporation of [methyl-^{3}H]dThd and [1',2'-^{3}H]dUrd into host cell DNA (Table 2). As noted for other 5-substituted 2'-deoxycytidines (4), 2'-deoxy-5-vinylcytidine was also less inhibitory to vaccinia virus replication (Table 1). Thus, based

Table 1. Effects of 2'-deoxy-5-vinylcytidine and related compounds on virus-induced cytopathogenicity in primary rabbit kidney cell cultures.

Compound	ID_{50}[a] (μg/ml)				
	HSV-1 (KOS)	HSV-1 (McIntyre)	HSV-2 (Lyons)	HSV-2 (196)	Vaccinia Virus
2'-Deoxy-5-vinylcytidine	0.2	0.2	0.2	0.2	100
2'-Deoxy-5-vinyluridine	0.2	0.07	0.07	0.07	0.7
2'-Deoxy-3',5'-di-O-acetyl-5-vinylcytidine	1	1	1	2	100
E-5-(2-bromovinyl)-2'-deoxyuridine	0.008	0.01	1	4	7
E-5-(2-bromovinyl)-2'-deoxy-3',5'-di-O-acetyluridine	0.04	0.04	4	4	10

[a]Inhibitory dose-50 or dose required to reduce virus-induced cytopathogenicity by 50%. Viral cytopathogenicity was recorded as soon as it attained 100% in the untreated virus-infected cell cultures.

upon several parameters, i.e. ratio of ID_{50} for host cell metabolism to ID_{50} for HSV-1 or HSV-2 replication, or ratio of ID_{50} for vaccinia virus replication to ID_{50} for herpes virus replication, 2'-deoxy-5-vinylcytidine appeared to be a more selective inhibitor of herpes virus replication than the corresponding 2'-deoxyuridine derivative (Table 3). Furthermore, 2'-deoxy-5-vinylcytidine was not toxic for L1210 cells (ID_{50} for L1210 cell growth : $\geqslant$ 250 μg/ml) under conditions where 2'-deoxy-5-vinyluridine showed a marked cytotoxicity [ID_{50} for L1210 cell growth : 2.37 μg/ml (20)].

2'-Deoxy-5-vinylcytidine was equally effective against HSV-1 and HSV-2. This means that its spectrum of activity is quite unlike that of E-5-(2-bromovinyl)-2'-deoxyuridine and 2'-deoxy-5-halogenovinyluridines which show a preference for HSV-1 and is more like Acyclovir and 2'-deoxy-5-vinyluridine which hardly differentiate between HSV-1 and HSV-2 (2). Whether an inhibitory concentration of 0.2 μg/ml toward HSV-2 is sufficiently low for 2'-deoxy-5-vinylcytidine to have any promise as an effective therapeutic agent, remains to be investigated.

Table 2. Effects of 2'-deoxy-5-vinylcytidine and 2'-deoxy-5-vinyluridine on the incorporation of [methyl-^{3}H]dThd, [1',2'-^{3}H]dUrd and [5-^{3}H]dCyd into DNA of primary rabbit kidney cell cultures.

Compound	ID_{50}[a] (μg/ml)		
	[methyl-^{3}H]dThd	[1',2'-^{3}H]dUrd	[5-^{3}H]dCyd
2'-Deoxy-5-vinylcytidine	155	175	45
2'-Deoxy-5-vinyluridine	25	3	300

[a]Inhibitory dose-50 or dose required to inhibit the incorporation of [methyl-^{3}H]dThd, [1',2'-^{3}H]dUrd or [5-^{3}H]dCyd by 50%. The cells were allowed to proliferate for 16 h in the presence of the radiolabelled precursors. Input of the radiolabelled precursors per 10^5 cells : 10 pmoles (0.38 μCi) of [methyl-^{3}H]dThd, 6 pmoles (0.25 μCi) of [1',2'-^{3}H]dUrd and 11 pmoles (0.25 μCi) of [5-^{3}H]dCyd.

The 3',5'-di-O-acetyl derivative of 2'-deoxy-5-vinylcytidine retained much of the antiviral activity of the parent compound (Table 1). Similar results were obtained with the 3'-5'-di-O-acetyl derivative of E-5-(2-bromovinyl)-2'-deoxyuridine. These findings indicate that the O-acyl derivatives are readily hydrolyzed in cell culture to release the parent compound. Whether such O-acyl esters may have some therapeutic advantages

Table 3. Selectivity indexes of 2'-deoxy-5-vinylcytidine and 2'-deoxy-5-vinyluridine as anti-herpes agents in primary rabbit kidney cell cultures.

Selectivity Index	2'-deoxy-5-vinylcytidine	2'-deoxy-5-vinyluridine
(HSV-2/HSV-1)[a]	1	0.44
(Vaccinia Virus/HSV-1)[b]	500	4.4
(Host cell metabolism/HSV-1)[c]	225	19
(Host cell metabolism/HSV-2)[d]	225	43

[a]Ratio of average ID_{50} for three HSV-2 strains to average ID_{50} for three HSV-1 strains.
[b]Ratio of ID_{50} for vaccinia virus to average ID_{50} for three HSV-1 strains.
[c]Ratio of ID_{50} for either dThd, dUrd or dCyd incorporation, whichever was lowest, to average ID_{50} for three HSV-1 strains.
[d]Ratio of ID_{50} for either dThd, dUrd or dCyd incorporation, whichever was lowest, to average ID_{50} for three HSV-2 strains.

by virtue of a more adequate tissue distribution in vivo, is still subject to further study.

EXPERIMENTAL

Chromatography. TLC was carried out using silica gel, MN-Kieselgel G/UV$_{254}$ and column chromatography with Kieselgel 60, 70-230 mesh ASTM type 7734, both supplied by E. Merck AG, Darmstadt, W. Germany. Preparative HPLC separations were performed on a Dupont 830 system using Zorbax silica, Zorbax ODS and C_{18} silica columns (25 cm x 21.2 mm).

2'-Deoxy-5-vinyluridine (II) from 2'-Deoxy-5-iodouridine (I). Palladium (II) acetate (0.158 g, 0.7 mmol), triphenylphosphine (0.37 g, 1.4 mmol) and anhydrous triethylamine (10 ml, 73 mmol) were combined in anhydrous dimethylformamide (80 ml) and stirred at 70 °C until an intense red colour, caused by activation of the catalyst, developed. 2'-Deoxy-5-iodouridine (5 g, 14 mmol) and vinylacetate (49.45 g, 575 mmol) were then added and stirring maintained at 70 °C for 5.5 h [TLC of the reaction mixture in $CHCl_3$/MeOH/glacial acetic acid (8:1:1) showed the absence of starting material (R_f 0.20) and the formation of 2'-deoxy-5-vinyluridine (R_f 0.22)]. The resulting precipitate of palladium was removed by filtration, the filtrate evaporated to dryness and water (120 ml) added to the residue. Extraction of the aqueous mixture with methylene chloride (3 x 60 ml) and evaporation of the water layer gave a red residue which was fractionated by silica column chromatography in $CHCl_3$/MeOH (4:1). The coloured product isolated was washed with cold acetone (2 x 5 ml) to give a first crop of 2'-deoxy-5-vinyluridine (0.65 g). Combination of the acetone washings, evaporation to dryness and fractionation of the residue by preparative HPLC on C_{18} silica in H_2O/MeOH (3:1) afforded a second crop of product (0.68 g; total yield, 1.33 g, 37%) : UV (MeOH) λ_{max} 238 nm (ε 12200), 292 nm (ε 9188); λ_{min} 260 nm (ε 4015); NMR [$(CD_3)_2SO$] δ 11.53 (1H, bs, N-H), 8.15 (1H, s, H-6), 6.4 (1H, dd, vinylic H-1"), 6.13 (1H, t, H-1'), 5.86 (1H, dd, vinylic H-2"), 5.13 (3H, m, vinylic H-2", OH-3' and OH-5'), 4.24 (1H, m, H-3'), 3.77 (1H, m, H-4'), 3.57 (2H, m, H-5'), 2.14 (2H, t, H-2'). Anal ($C_{11}H_{14}N_2O_5$) C, H, N.

2'-Deoxy-5-vinyluridine (II) from E-5-(2-Carboxyvinyl)-2'-deoxyuridine (III). E-5-(2-carboxyvinyl)-2'-deoxyuridine (3 g, 10 mmol) was dissolved in triethylamine (11 ml, 78 mmol) and dimethylformamide (50 ml) and heated at 100 °C for 35 h. The reaction mixture was evaporated to dryness and the product, 2'-deoxy-5-vinyluridine, isolated as described above (0.6 g, 24%

yield). This product was identical in all respects with the product previously obtained.

2'-Deoxy-5-vinylcytidine (VI) from 2'Deoxy-5-iodocytidine (IV). Palladium (II) acetate catalyst (0.53 mmol) was prepared as described above and 2'-deoxy -5-iodocytidine (3.8 g, 10.6 mmol) and vinylacetate (36.3 g, 424 mmol) added and the mixture stirred at 90 °C for 2 h. TLC of the reaction mixture in $CHCl_3$/MeOH (4:1) showed the presence of one major product later identified as 4-N-acetyl-2'-deoxycytidine (R_f 0.35), starting material (R_f 0.2), 2'-deoxy-cytidine (R_f 0.05) and an unknown compound (R_f 0.14). A further 0.09 mmol of activated catalyst was added and the mixture heated at 95° for another two hours by which time the starting material had disappeared. The reaction mixture was evaporated to dryness, water (300 ml) was added and the aqueous mixture was extracted with methylene chloride (3 x 80 ml). Evaporation to dryness of the aqueous layer and chromatography on silica using $CHCl_3$/MeOH (9:1) gave crude 2'-deoxy-5-vinylcytidine (0.43 g). Contaminating triethylamine hydroiodide was removed using a reverse-phase silica column under normal pressure Merck 60, RP_{18}, 230-400 mesh, H_2O/MeOH (9:1) to give a purified sample of 2'-deoxy-5-vinylcytidine (0.13 g, 8% yield) : UV λ_{max} 229 (ε 9226), 300 nm (ε 6258), λ_{min} 267 nm (ε 1709) at pH2; NMR [$(CD_3)_2SO$] δ 8.16 (1H, s, H-6), 7.14 (2H, bs, NH_2), 6.56 (1H, dd, vinylic H-1"), 6.14 (1H, t, H-1'), 5.65-4.90 (4H, m, OH-3', OH-5' and vinylic H-2"), 4.25 (1H, bs, H-3'), 3.79 (1H, m, H-4'), 3.60 (2H, m, H-5'), 2.1 (2H, m, H-2'). Anal ($C_{11}H_{15}N_3O_4$. 0.25 CH_2Cl_2) C,H,N.

2'-Deoxy-5-vinylcytidine (VI) from 2'-Deoxy-5-vinyluridine (II) : 2-Deoxy-3',5'-di-O-acetyl-5-vinyluridine. A solution of 2'-deoxy-5-vinyluridine (0.8 g, 3.1 mmol) in anhydrous pyridine (10 ml) and acetic anhydride (1.1 ml, 12.6 mmol) was kept at 20°C for 10 h. The reaction mixture was evaporated to dryness and the residue co-evaporated with benzene and ethanol to remove traces of pyridine. The residue was washed with water (2 x 30 ml) and a solid residue could be removed by filtration to give 2'-deoxy-3',5'-di-O-acetyl-5-vinyluridine (0.81 g, 76% yield) : UV (EtOH) λ_{max} 237 nm (ε 12190), 292 nm (ε 9121), λ_{min} 260 nm (ε 4000); Mass Spectrum, CI m/e : 339 $(M+H)^+$; NMR [$CDCl_3$] δ 9.45 (1H, s, N-H), 7.59 (1H, s, H-6), 6.4 (1H, dd, vinylic H-1"), 6.26 (1H, t, H-1'), 5.9 (1H, dd, vinylic, H-2"), 5.22 (2H, m, H-3' and vinylic H-2"), 4.38 (3H, m, H-4' and H-5'), 2.12 (8H, m, H-2' and acetyl).

1-(β-D-2'-Deoxy-3',5'-di-O-acetylribofuranosyl)-4-(1,2,4-triazol-1-yl)-5-vinylpyrimidin-2(1H)-one (V). 2'-Deoxy-3',5'-di-O-acetyl-5-vinyluridine

(1 g, 2.9 mmol), 1,2,4-triazole (0.61 g, 8.9 mmol) and p-chlorophenyl-phosphorodichloridate (1.1 g, 4.4 mmol) were combined in anhydrous pyridine (20 ml) and stirred at 20°C for 84 h. The reaction mixture was evaporated to dryness, chloroform (40 ml) added to the residue and the organic solution washed with saturated aqueous sodium hydrogen carbonate solution (2 x 45 ml) and water (2 x 45 ml). The organic layer was dried, filtered, evaporated to dryness to give a residue which was fractionated by silica column chromatography in $CHCl_3$/EtOH (19:1). Isolation of the fluorescent material gave a contaminated product (0.66 g) which was purified by HPLC on ODS silica in H_2O/tetrahydrofuran (4:1) (Yield 0.17 g) : UV (EtOH) λ_{max} 241 nm (ε 18391), 341.5 nm (ε 5415), λ_{min} 293 nm (ε 847); NMR [$CDCl_3$] δ 9.35 (1H, s, H-5'''), 8.43 (1H, s, H-3'''), 8.22 (1H, s, H-6), 7.4 (1H, dd, vinylic H-1"), 6.34 (1H, t, H-1'), 5.35 (3H, m, H-3' and vinylic H-2"), 4.45 (3H, s, H-4' and H-5'), 2.1 (8H, m, H-2' and acetyl). Anal ($C_{17}H_{19}N_3O_6.H_2O$) C,H,N.

2'-Deoxy-3',5'-di-O-acetyl-5-vinylcytidine. The triazolyl compound (V) (0.16 g, 0.41 mmol) was kept in a solution of ammonia (0.880)/dioxan (1.3 v/v, 3 ml) at 20° for 50 min. Evaporation to dryness and silica column fractionation of the residue in $CHCl_3$/EtOH (4:1) afforded 2'-deoxy-3',5'-di-O-acetyl-5-vinylcytidine as a white solid (0.084 g, yield 61%) : UV (EtOH) λ_{max} 213 nm (ε 14985), 243 nm (ε 11033), 294 nm (ε 4225), λmin 229 nm(ε 8784), 273 nm (ε 2766) at pH 7. λ_{max} 236 nm (ε 9544), 305 nm (ε 5745), λ_{min} 268 nm (ε 1368) at pH 2; NMR [$(CD_3)_2SO$] δ 7.73 (1H, s, H-6), 7.27 (2H, bs, NH_2), 6.57 (1H, dd, vinylic H-1'), 6.18 (1H, t, H-1'), 5.75-4.9 (3H, m, H-3' and vinylic H-2"), 4.26 (3H, s, H-4' and H-5'), 2.36 (2H, m, H-2'), 2.07 (3H, s, acetyl), 2.03 (3H, s, acetyl). Anal ($C_{15}H_{19}N_3O_6$) C,H,N.

2'-Deoxy-5-vinylcytidine (VI). 2'-Deoxy-3',5'-di-O-acetyl-5-vinylcytidine (0.44 g, 0.13 mmol) was kept in a solution of dioxan/ammonia (0.880)/water (3:2:1, v/v, 3 ml) at 20° for 12 h. TLC in $CHCl_3$/EtOH (4:1) of the reaction mixture during this period showed a gradual disappearance of starting material (R_f 0.46), the formation of two intermediate monoacylated (?) components (R_f 0.32 and 0.23) which in turn disappeared to give the product, 2'-deoxy-5-vinylcytidine (R_f 0.09). Evaporation to dryness gave a contaminated product, which had a UV and NMR spectrum identical to that previously described. Attempts to remove contaminating salts failed when the material polymerised.

Antiviral and anitmetabolic assays. The method for measuring inhibition of virus-induced cytopathogenicity and incorporation of [methyl-^{3}H] dThd,

[1',2'-^{3}H]dUrd or [5-^{3}H]dCyd incorporation into DNA of primary rabbit kidney cells has been described previously (2,4).

Antileukemic activity in mice. BDF_1 ($C_{57}Bl$ x DBA/2) mice were inoculated intraperitoneally with 5 x 10^6 L1210 cells and a single dose of either 250 mg, 100 mg or 50 mg/Kg of 2'-deoxy-5-vinyluridine was injected intraperitoneally one day later. Control animals received an injection of phosphate buffered saline. Animals started to die after 18 days and all were dead after 40 days. No significant toxicity or prolongation of life was effected by the test compound.

Antimalaria activity in mice. The antimalaria experiments were carried out at the Walter Reed Army Medical Center under their standard conditions. ICR/HA Swiss mice received a standard inoculum of Plasmodium berghei KBG173 which produced a uniform disease fatal to 100% of the untreated animals within 6 to 8 days. A single dose of either 20 mg, 80 mg or 320 mg/Kg of 2'-deoxy-5-vinyluridine was administered subcutaneously 72 hours after infection. No significant toxicity or prolongation of life was effected by the test compound.

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