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THE CHEMISTRY OF 2',3'-SECONUCLEOSIDES II. REACTIONS AND BIOLOGICAL PROPERTIES OF 2',3'-SECOPYRIMIDINE RIBONUCLEOSIDES

A. STANLEY JONES, MICHAEL J. McCLEAN, HIRONICHI TANAKA, RICHARD T. WALKER, JAN BALZARINI* and ERIK DE CLERCQ*

Chemistry Department, University of Birmingham, Birmingham B15 2TT

*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

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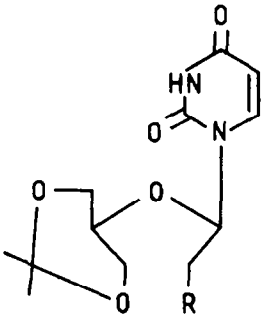
Abstract - Reaction of 2',3'-secouridine with acetone gave the 3',5'-O-isopropylidene derivative (1) which upon treatment with mesylchloride gave the 2'-O-mesyl compound (2). Replacement of the mesyl group of 2 with halide could be effected by reaction with a metal halide in DMF. The 3',5'-O-isopropylidene group was removed simultaneously to give a 2'-halogeno-2'-deoxy-2',3'-secouridine. 2',3'-Dichloro-2',3'-dideoxy-2',3'-secouridine upon treatment with base gave 6(R)-chloromethyl-2(R)-(uracil-1-yl)-1,4-dioxane in addition to O^2,2'-anhydro-3'-chloro-3'-deoxy-2',3'-secouridine, as previously reported. 2',3'-Dichloro-2',3'-dideoxy-5'-O-trityl-2',3'-secouridine was converted to 2',3'-dichloro-2',3'-dideoxy-2',3'-secocytidine (16) via a triazole derivative. Compound 16 was unstable and appeared to form O^2,2'-anhydro-3'-chloro-3'-deoxy-2',3'-secocytidine upon standing at room temperature. 5-Vinyl- and 5-(E)(2-bromovinyl)uridine dialdehydes have been made, as well as a number of other 5-substituted 2',3'-secouridine derivatives. None of the compounds obtained showed significant activity against a number of virus strains or tumor cell lines, except for 5-(E)(2-bromovinyl)uridine dialdehyde, which was inhibitory to the growth of human lymphoblast (Raji, Namalva) cells at a concentration of 28 μg/ml.

In the previous paper of this series the synthesis and properties of 2',3'-secouridine were described.[1] In particular it was found that the O^2 of the uracil ring could react with suitable substituents at C-2' to give O^2,2'-anhydro-2',3'-secouridine derivatives. Using these compounds it was possible to obtain 2',3'-secouridines selectively substituted at C-3'. By starting with a C-5'-substituted uridine it was possible to obtain 2',3'-secouridine selectively substituted at the 5'-position. The present paper describes a method of selectively substituting 2',3'-secouridine at C-2' and also the synthesis of 5-substituted 2',3'-secouridines and derivatives of 2',3'-secocytidine.

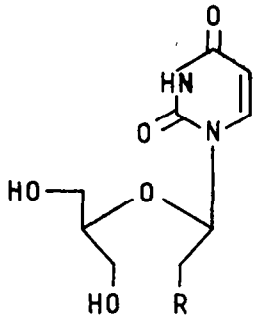
Treatment of 2',3'-secouridine with acetone under anhydrous acidic conditions gave 3',5'-O-isopropylidene-2',3'-secouridine (1) which upon treatment with mesyl chloride gave the 2'-O-mesyl derivative (2). The structures of these compounds were established by n.m.r. spectroscopy. In particular it was ascertained by proton decoupling that the mesyl group of (2) was on the 2'-position thus completely ruling out the unlikely possibility that the 2'-position was substituted by the isopropylidene group. Treatment of 2 with alkali metal halides in DMF replaced the mesyl group by halogen as expected, but surprisingly there occurred simultaneous removal of the isopropylidene group to give the 2'-halogeno compounds (3 and 4). This series of reactions provides a simple route to obtain selective substitution at the 2'-position of 2',3'-secouridine.

As the removal of the isopropylidene group from 2 under the conditions used was unexpected, experiments were carried out in order to provide an explanation. When 3',5'-O-isopropylidene-2',3'-secouridine (1) was treated with a metal halide in DMF there was no removal of the isopropylidene group. It appeared, therefore, that the presence of a good leaving group at C-2' is required.

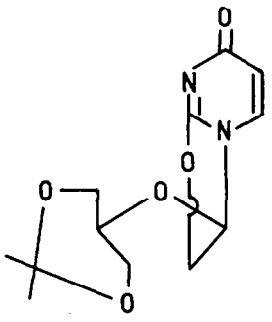
Furthermore no reaction occurred when 2 was heated in DMF in the absence of a metal halide. It was considered probable that the first stage in the reaction was the formation of O^2,2'-anhydro-3',5'-O-isopropylidene-2',3'-secouridine (5) and methanesulphonic acid.



1 R = OH
2 R = OMs



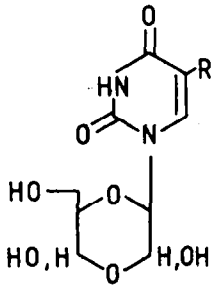
3 R = Cl
4 R = I



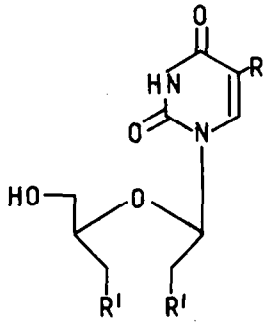
5

In order to substantiate this, 2 was treated with 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) to give 5 as a crystalline compound whose structure was established by n.m.r. and u.v. spectroscopy. Treatment of 5 with 1 molecular proportion of methanesulphonic acid and an excess of a metal halide in dry DMF did not result in rapid removal of the isopropylidene group unless 1 molecular proportion of water was added. As compound 2 was very hygroscopic, it appeared probably that water was present in the reaction so that the isopropylidene group was removed by acidic hydrolysis. However when 3',5'-O-isopropylidene-2',3'-secouridine was treated with 0.1 molecular proportions of methanesulphonic acid in DMF the isopropylidene group was only slowly removed; the rate of reaction was greatly increased by the addition of a metal halide. The mechanism of this effect was not investigated further.

In view of the known antiviral and antileukaemic activity of many nucleosides derived from 5-substituted uracil,[2] it was decided to prepare 5-substituted 2',3'-secouridine derivatives. The starting materials were the 5-substituted uridines which were converted into their 5'-O-trityl derivatives which were oxidised to the corresponding dialdehydes with periodate. In the case of 5-vinyl- and 5-(E)(2-bromovinyl) derivatives the dialdehydes (as acetals 6 and 7) were obtained by the direct periodate oxidation of the nucleosides.



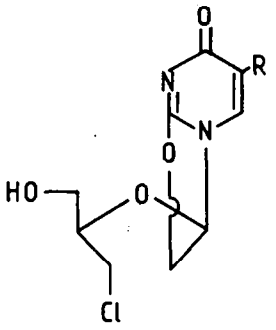
6 R = $-CH=CH_2$
7 R = (E)-CH=CHBr

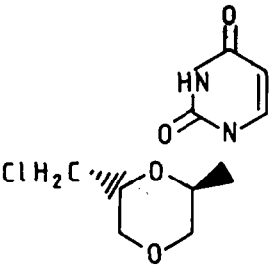


8 R = CH_3; R' = Cl
9 R = (E)-CH=CHBr; R' = OH
10 R = F; R' = OH
11 R = CF_3; R' = Cl

Reduction of 5'-O-trityl nucleosides gave the corresponding 5-substituted 5'-O-trityl-2',3'-secouridines which in some cases were converted into 2',3'-di-O-mesyl derivatives and 2',3'-dichloro-2',3'-dideoxy derivatives by the procedures previously described[1] to give the series of compounds 8-11.

Treatment of compound 8 with DBU gave the corresponding O^2,2'-anhydro compound 12.





12 R = CH_3
13 R = H

14

Whilst working on this reaction it was noticed that when making the corresponding uracil derivative (13) as previously reported[1] a minor component (16% yield) was also produced. This had a u.v. spectrum which was characteristic of a 1-substituted uracil and the n.m.r. spectrum showed the presence of an NH group and the absence of an OH group. A complex multiplet (7H) in the region δ 3.58-4.16 p.p.m. and a triplet (1H) at δ 5.92 are consistent with the compound being 6(R)-chloromethyl-2(R)(uracil-1-yl)-1,4-dioxane (14).

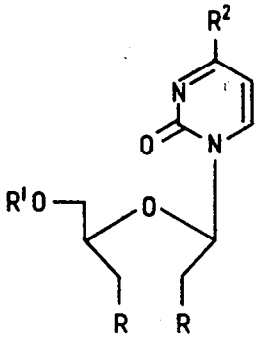
This assignment of structure was supported by the mass spectrum which showed a peak for $(M + 1)^+$ of 247 (^{35}Cl) and a strong peak of m/e 134 corresponding to $C_5H_7O_2Cl$. An increased yield of 14 was obtained when 13 was treated with potassium t-butoxide in tetrahydrofuran.

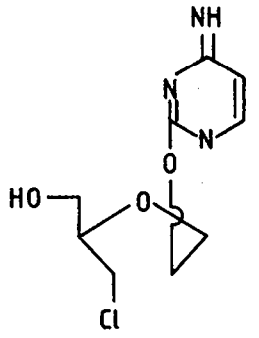
Derivatives of 2',3'-secocytidine can be obtained from cytidine by similar procedures to those described for 2',3'-secouridine,[1] although to effect selective substitution at the hydroxyl groups it is necessary to protect the amino group. An alternative, and in our case, more convenient procedure was to start with a 2',3'-secouridine derivative and convert it by established procedures into the 2',3'-secocytidine. Thus 5'-O-trityl-2',3'-dichloro-2',3'-dideoxy-2',3'-secouridine[1] was treated with 1,2,4-triazole and p-chlorophenylphosphorus dichloridate[3] to give 1(R)(1-chloro-4(R)-chloromethyl-5-trityloxy-3-azapentan-2-yl)-4-(1,2,4-triazol-1-yl)-1,2-dihydropyrimidine-2-one (15), which upon treatment with dilute ammonia and then with 80% acetic acid gave 2',3'-dichloro-2',3'-dideoxy-2',3'-secocytidine (16). This compound was unstable. After standing at room temperature for 24 h about 10% had decomposed to another compound which from its properties on tlc and its n.m.r. spectrum appeared to be the O^2,2'-anhydro compound (17). It should be noted that a similar transformation occurs with the corresponding 2',3'-secouridine derivative but more slowly. In the latter case the reaction is rapid when strong base is used.[1]

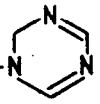
Compounds 3, 4, 6-12, 16 were tested for antiviral and antileukaemic activity. None showed significant antiviral activity; two of the compounds, 5-vinyluridine dialdehyde (6) and 4-(E)-(2-bromovinyl) uridine dialdehyde (7), showed low activity (ID_{50} 245 μg ml and 112 μg/ml respectively) against leukaemia L1210 cells. These were lower activities than has been found for uridine dialdehyde (ID_{50} 20 μg/ml)[1] so that in these cases the presence of a vinylic group at the 5 position did not enhance activity.

Compounds 3, 4, 6-12 and 17 were also evaluated for their cytostatic effects on murine leukemia (L1210) cells, and a thymidine (dThd) kinase-deficient (L1210/BdUrd) subline thereof, human lymphoblast (Namalva) cells, human lymphoblast (Raji/0) cells and a dThd kinase-deficient (Raji/TK$^-$) subline thereof. None of the compounds was endowed with an appreciable cytostatic activity (ID_{50} (inhibitory dose-50) > 100 μg/ml), except for 5-(E)(2-bromovinyl)uridine

dialdehyde (7) which was inhibitory to the growth of Namalva, Raji/0 and Raji/TK$^-$ cells at an ID_{50} of 28 μg/ml. There was no significant difference in the cytostatic effects of the compounds on the dThd kinase-deficient cell lines as compared to their effects on the corresponding parental cell lines. Thus, dThd kinase does not seem to play a significant role in the cell-growth inhibitory effects of the compounds. This is in marked contrast with the antitumor cell activity of the corresponding 2'-deoxy-ribonucleosides of compounds 6, 8, 10-12 which proved highly dependent on dThd kinase activity.[4]





15 R = Cl; R' = trityl; R^2 = 

16 R = Cl; R' = H; R^2 = $-NH_2$

17

Since the corresponding 2'-deoxyuridine analogues of compounds 10 and 11 are very potent inhibitors of thymidylate (dTMP) synthase,[5] additional experiments were carried out to determine the role of dTMP-synthase in the inhibitory effects of the 2',3'-secouridines on L1210 cell proliferation. To this end, we measured the cell growth-inhibitory effects of the compounds following addition of dUrd and dThd, and their inhibition of the incorporation of (1',2'-^{3}H)dUrd and (methyl-^{3}H)dThd into cell DNA. Indeed, (i) a more efficient reversal of cell growth inhibition upon addition of dThd than of dUrd, and (ii) a greater inhibitory effect on dUrd than on dThd incorporation into DNA, have previously been interpreted as valuable parameters for a selective inhibition of thymidylate synthase.[5] None of the compounds appeared to act as a thymidylate synthetase inhibitor.

Apparently, 5-fluoro-2',3'-secouridine (10) was not intracellularly converted to 5-fluoro-uracil, since the latter compound was about 1000-fold more inhibitory to the growth of L1210 cells and the incorporation of (1',2'-^{3}H)dUrd into L1210 cell DNA than compound 10 (data not shown).

EXPERIMENTAL

N.m.r. spectra were recorded on 100 MHz spectrometers (Perkin Elmer R14 and Varian XL100) with $(CD_3)_2SO$ as the solvent unless otherwise stated. U.v. spectra were measured in ethanol on a Perkin Elmer 552 spectrophotometer. Column chromatography was carried out on silica gel, Kieselgel 60 type 7734, 0.063-0.200 mm, 70-230 mesh ASTM (E. Merck A.G., Darmstadt, W. Germany). All experiments were carried out under scrupulously dry conditions unless otherwise stated and all evaporations of solvents were carried out under reduced pressure.

3',5'-O-Isopropylidene-2',3'-secouridine (1). To a solution of 2',3'-secouridine[1] (8 g) in methanol (30 ml) there was added acetone (2 l) and 60% perchloric acid (3 ml) and the solution kept at room temperature for 2 h. It was neutralised (K_2CO_3), filtered and the filtrate evaporated to give an oil which was fractionated by column chromatography. Elution of the column with chloroform-methanol (6:1) gave the product as a hygroscopic white solid (4.3 g, 45% yield). The unreacted starting material was recovered by elution with chloroform-methanol (3:1) and the above procedure repeated to give a further amount of product (1.5 g, overall yield, 61%) (Found: C, 50.1; H, 6.2; N, 9.9. $C_{12}H_{18}N_2O_6$ requires C, 50.3; H, 6.4; N, 9.8%); u.v. λ_{max} 260 nm (ε, 9,800); n.m.r. δ 1.3 (6H, s, $(CH_3)_2C$), 3.4-4.0 (7H, m, H-2', H-3', H-4', H-5'), 5.05 (1H, t, 2'-OH), 5.8 (2H, m, H-1' and H-5), 7.55 (1H, d, H-6), 11.0 p.p.m. (1H, s, -NH).

3',5'-O-Isopropylidene-2'-O-mesyl-2',3'-secouridine (2). To a solution of compound 1 (3.4 g) in pyridine (20 ml) at 0°C was added a solution of mesyl chloride (1.07 ml) in pyridine (20 ml) dropwise, with stirring over 30 min. The solution was stirred at 0°C for a further 90 min, and then ethanol (10 ml) was added. The solution was evaporated under high vacuum at low temperature to give an oil which was co-evaporated several times with toluene. The residual gum was purified by column chromatography by using chloroform–methanol (9:1) as the eluant. The product was finally obtained as a hygroscopic white solid (3.3 g, 77% yield) (Found: C, 43.1; H, 5.8; N, 7.8. $C_{13}H_{20}N_2O_8S$ requires C, 42.9; H, 5.5; N, 7.7%); u.v. λ_{max} 260 nm (ε, 9,400); n.m.r. δ ($CDCl_3$) 1.4 (6H, s, $(CH_3)_2C$), 3.1 (3H, s, CH_3SO_2), 3.5-4.1 (5H, m, H-3', H-4', H-5'), 4.35 (2H, d, H-2'), 5.75 (1H, d, H-5), 6.05 (1H, t, H-1'), 7.5 (1H, d, H-6), 9.75 p.p.m. (1H, s, NH).

2'-Chloro-2'-deoxy-2',3'-secouridine (3). Compound 2 (2.27 g) was added to dimethylformamide (20 ml) and the mixture heated at 100°C for 10 min. Then LiCl (1 g) was added and the mixture heated with stirring at 100°C for a further 2 h. The solvent was then removed by evaporation and co-evaporation with toluene and the residue purified by column chromatography using chloroform–methanol (4:1) as the eluant. Evaporation of the solvent from the appropriate fractions gave the product as a white hygroscopic solid (1.6 g, 97% yield) (Found: C, 39.9; H, 5.0; N, 9.9. $C_9H_{13}ClN_2O_6.0.5H_2O$ requires C, 39.5; H, 5.1; N, 10.2%); n.m.r. δ 3.46 (5H, m, H-3', H-4', H-5'), 3.98 (2H, d, H-2'), 4.96 (1H, t, 5'-OH), 5.19 (1H, t, 3'-OH), 5.66 (1H, d, H-5), 6.01 (1H, t, H-1'), 7.84 (1H, d, H-6), 11.36 p.p.m. (1H, br, NH).

2'-Deoxy-2'-iodo-2',3'-secouridine (4). To a solution of compound 2 (2.5 g) in dimethylformamide (10 ml) at 100°C there was added sodium iodide (2.6 g) and the mixture heated at 100°C with stirring for 30 min. The solvent was removed by evaporation to give an oil to which methanol (50 ml) was added. The resulting suspension was filtered and the filtrate evaporated to give an oil which was purified by column chromatography. Elution with chloroform–methanol (3:1) and evaporation of the appropriate fractions gave the product as a hygroscopic solid (1.76 g, 76% yield) (Found: C, 30.4; H, 3.9; N, 7.7. $C_9H_{13}IN_2O_5$ requires C, 30.3; H, 3.7; N, 7.9%); u.v. λ_{max} 260 nm (ε, 7,700); n.m.r. δ 3.1-3.5 (7H, m, H-2', H-3', H-4', H-5'), 3.7-4.1 (2H, bd, 3'-OH, 5'-OH), 5.6 (1H, d, H-5), 5.9 (1H, t, H-1'), 7.65 (1H, d, H-6), 11.05 p.p.m. (1H, s, NH).

O^2,2'-Anhydro-3',5'-O-isopropylidene-2',3'-secouridine (5). To a solution of compound 2 (2 g) in dichloromethane (50 ml) there was added DBU (0.9 g) and the solution kept at ~20°C for 30 min. The solvent was then removed by evaporation and the residual oil purified by column chromatography. Elution of the column with acetone–ethanol (4:1) and evaporation of the solvent gave the product (1.26 g, 86% yield), m.p. 149°C (Found: C, 53.4; H, 6.2; N, 10.3. $C_{12}H_{16}N_2O_5$ requires C, 53.7; H, 6.0; N, 10.4%), u.v. λ_{max} 247 nm (ε, 8,300); n.m.r. δ 1.3 (6H, s, $(CH_3)_2C$), 3.6-4.1 (5H, m, H-3', H-4', H-5'), 4.55 (1H, dd, H-2'), 4.8 (1H, dd, H-2'), 5.9 (1H, d, H-5), 6.05 (1H, dd, H-1'), 7.95 p.p.m. (1H, d, H-6).

5-Vinyluridine dialdehyde (6). To a solution of 5-vinyluridine (2.7 g)[6] in ethanol–water (2:1) (100 ml) there was added a solution of sodium periodate (2.25 g) in water (20 ml) and the solution left at ~20°C in the dark for 18 h. The solvent was then removed by evaporation and the resulting solid twice extracted with boiling ethanol (~50 ml) for 5 min. The insoluble material was rejected and the ethanol solution evaporated to dryness and the residue dissolved in ethanol. A small amount of insoluble material was filtered off and the filtrate evaporated to dryness to give a residue which was purified by column chromatography. Elution of the column with chloroform–methanol (4:1) and evaporation of the appropriate fractions gave the product as a monohydrate (2.68 g, 93% yield) (Found: C, 45.6; H, 5.0; N, 10.4. $C_{11}H_{12}N_2O_6.H_2O$ requires C, 45.2; H, 5.0; N, 9.8%); u.v. λ_{max} 290 nm (ε, 7,700).

(E)5-(2-Bromovinyl)uridine dialdehyde (7). This was obtained from 5-(E) (2-bromovinyl)uridine[7] in almost quantitative yield by the same procedure as described for compound 6. The compound was obtained as a solid which tenaciously retained ethanol (Found: C, 38.8; H, 4.1; N, 7.6. $C_{11}H_{11}BrN_2O_6.0.5EtOH$ requires C, 38.9; H, 3.8; N, 7.6%); u.v. λ_{max} 294 nm (ε, 10000).

5'-O-Trityl-5-methyl-2',3'-secouridine. A solution of 5-methyluridine (2.42 g) and tritylchloride (3.1 g) in pyridine (25 ml) was kept at ~20°C for 18 h and then heated at 100°C for 4 h. After cooling, the solution was poured onto ice/water (400 ml) with vigorous stirring. The resulting solid was separated from the aqueous phase and washed with water. The gummy solid was dissolved in ethanol (500 ml) and a solution of sodium periodate (2.35 g) in water (100 ml) added. The solution was kept at ~20°C in the dark for 18 h and then sodium borohydride (1.51 g) was added. After 16 h at ~20°C the solution was adjusted to pH 6 by the addition of hydrochloric acid. Evaporation of the solution to dryness gave a solid which was extracted twice with water and the residue was dissolved in acetone. The acetone solution was filtered and the filtrate evaporated to dryness to give a white solid which was purified by column chromatography. Elution with chloroform–methanol (9:1) and removal of the solvent by evaporation from the appropriate fractions gave the product (2.64 g, 51% yield (Found: C, 67.2; H, 5.8; N, 5.4. $C_{29}H_{28}N_2O_6.H_2O$ requires C, 67.2; H, 6.1; N, 5.4%); u.v. λ_{max} 263 nm (ε, 8000), n.m.r. δ 1.65 (3H, s, CH_3), 3.1 (2H, d, H-5'), 3.4-3.8 (5H, m, H-2', H-3', H-4'), 4.7 (1H, t, 3'-OH), 5.1 (1H, t, 2'-OH), 5.9 (1H, t, H-1'), 7.3 (15H, s, Ph_3C), 7.5 (1H, s, H-6), 11.05 p.p.m. (1H, s, -NH).

2',3'-Di-O-mesyl-5-methyl-5'-O-trityl-2',3'-secouridine. To a solution of 5'-O-trityl-5-methyl-2',3'-secouridine (5.2 g) in pyridine (40 ml) at 0°C there was added a solution of mesyl chloride (2.52 g) in pyridine (40 ml) dropwise, with stirring over 30 min, and the mixture stirred at 0°C for 2 h. The pyridine was removed by evaporation and co-evaporation with toluene and the residual gum triturated with water. The aqueous solution was removed and the gum dried by repeated co-evaporation with acetone. The resulting solid was purified by column chromatography using

toluene-acetone (7:3) as the eluant. Evaporation of the appropriate fractions gave the product (5.34 g, 85% yield) (Found: C, 56.2; H, 5.3; N, 4.6. $C_{31}H_{34}N_2O_{10}S_2$ requires C, 56.5; H, 5.2; N, 4.3%); u.v. λ_{max} 266 nm (ε, 7000); n.m.r. δ 1.65 (3H, s, $-CH_3$), 3.15 (5H, m, H-5' and CH_2SO_2-), 3.9 (1H, m, H-4'), 4.35 (2H, m, H-3'), 4.5 (2H, d, H-2'), 6.1 (1H, t, H-1'), 7.4 (15H, s, Ph_3C), 7.4 (1H, s, H-6), 7.6 (1H, s, NH), 11.4 p.p.m. (1H, s, NH).

2',3'-Dichloro-2',3'-dideoxy-5-methyl-5'-O-trityl-2',3'-secouridine. To a stirred solution of 2',3'-di-O-mesyl-5-methyl-5'-O-trityl-2',3'-secouridine (1.87 g) in dimethylformamide (30 ml) at 100°C there was added lithium chloride (0.62 g). The solution was stirred at 100°C for 90 min. and then the solvent was removed by evaporation to give an oil which was triturated with water. The resulting white solid was filtered off, washed with water and dried. Purification by column chromatography using toluene acetone (1:1) as the eluant gave the product as a white solid (1.33 g, 82% yield) (Found: C, 64.3; H, 5.4; N, 5.0. $C_{29}H_{28}Cl_2N_2O_4$ requires C, 64.6; H, 5.2; N, 5.2%); u.v. λ_{max} 265 nm (ε, 7700); n.m.r. δ 1.65 (3H, s, $-CH_3$), 3.1 (2H, d, H-5'), 3.8 (3H, m, H-3' and H-4'), 4.0 (2H, d, H-2'), 6.0 (1H, t, H-1'), 7.3 (15H, s, Ph_3C-), 7.6 (1H, s, H-6), 11.5 p.p.m. (1H, s, NH).

2',3'-Dichloro-2',3'-dideoxy-5-methyl-2',3'-secouridine (8). A suspension of 2',3'-dichloro-2',3'-dideoxy-5-methyl-5'-O-trityl-2',3'-secouridine (1.2 g) in acetic acid-water (4:1) (25 ml) was heated at 100°C for 30 min. The solvent was removed by evaporation to give a syrup which was co-evaporated with toluene to give a white solid. This was fractionated by column chromatography. The column was eluted first with toluene-acetone (3:2) and then with toluene-acetone (3:7) to give the product as a colourless glass (0.49 g, 74% yield) (Found: C, 40.4; H, 4.8; N, 9.1. $C_{10}H_{14}Cl_2N_2O_4$ requires C, 40.4; H, 4.7; N, 9.4%); u.v. λ_{max} 263 nm (ε, 7000); n.m.r. δ 1.8 (3H, s, $-CH_3$), 3.45 (2H, m, H-5'), 3.75 (3H, m, H-3', H-4'), 3.9 (2H, d, H-2'), 4.08 (1H, m, 5'-OH), 6.0 (1H, t, H-1'), 7.6 (1H, s, H-6), 11.3 p.p.m. (1H, s, NH).

5-(E)(2-Bromovinyl)-2',3'-secouridine (9). To a solution of compound 7 (3.7 g) in ethanol-water (1:2) (250 ml) there was added sodium borohydride (0.94 g) and the solution kept at ~20°C in the dark for 3 h. It was brought to pH 6 by the addition of ion exchange resin (Dowex 50 x 8 H^+ form). The resin was filtered off, washed well with methanol (3 x 100 ml) and the filtrate and washings evaporated to dryness and the residue co-evaporated several times with methanol to remove borate. The residual gum was purified by column chromatography using chloroform-methanol (4:1) as the eluant. The product (2.46 g, 70% yield) was obtained as a hygroscopic solid (Found: C, 37.6; H, 4.3; N, 7.7. $C_{11}H_{15}BrN_2O_6$ requires C, 37.6; H, 4.3; N, 7.9%); u.v. λ_{max} 294 nm (ε, 10,500); n.m.r. δ 3.2-3.7 (7H, m, H-2', H-3', H-4', H-5'), 4.4-4.7 (2H, m, 3'-OH, 5'-OH), 5.0 (1H, t, 2'-OH), 5.75 (1H, t, H-1'), 6.8 (1H, d, vinylic H, J = 13 Hz), 7.2 (1H, d, vinylic H), 7.8 (1H, s, H-6), 11.35 p.p.m. (1H, s, -NH).

5-Fluoro-2',3'-secouridine (10). 5-Fluoro-5'-O-trityl-2',3'-secouridine (1.64 g) (obtained from 5-fluorouridine by essentially the same procedure as described for the synthesis of 5'-O-trityl-2',3'-secouridine[1]) was dissolved in acetic acid-water (4:1) and the solution heated at 100°C for 3 h and then kept at ~20°C for 18 h. The mixture was filtered, the filtrate evaporated to dryness and the residue evaporated to dryness and the residue dissolved in acetone (100 ml), 60% perchloric acid (0.5 ml) added and the mixture kept at ~20°C for 1 h. It was then neutralised by the addition of potassium carbonate powder, inorganic material removed by filtration and the filtrate evaporated to dryness to give a residue which was purified by column chromatography. Elution of the column with chloroform-methanol (6:1) gave the product (0.30 g) (Found: C, 39.2; H, 4.8; N, 10.0. $C_9H_{13}N_2O_6F.0.5H_2O$ requires C, 39.6; H, 5.1; N, 10.2%); n.m.r. δ 3.2-3.8 (7H, m, H-2', H-3', H-4', H-5'), 5.80 (1H, t, H-1'), 7.95 p.p.m. (1H, d, H-6).

2',3'-Dichloro-2',3'-dideoxy-5-trifluoromethyl-2',3'-secouridine (11). This compound was obtained from 5-trifluoromethyluridine by a route and procedures which were similar to those described for the preparation of compound 8 from 5-methyluridine. The purification in the final stage was carried out by column chromatography using bezene-acetone (9:1) as the eluant followed by a second purification using chloroform-acetone (1:1) as the eluant to give the product (Found: C, 34.4; H, 3.4; N, 8.0. $C_{10}H_{11}Cl_2F_3N_2O_4$ requires C, 34.2; H, 3.2; N, 8.0%); u.v. λ_{max} 259 nm (ε, 9,100); n.m.r. δ 3.44 (2H, m, H-5'), 3.80 (3H, m, H-3', H-4'), 4.02 (2H, m, H-2'), 4.94 (1H, t, 5'-OH), 6.02 (1H, t, H-1'), 8.29 (1H, s, H-6), 10.28 p.p.m. (1H, bs, -NH).

O^2,2'-Anhydro-5-methyl-2',3'-secouridine (12). To a solution of compound 8 (250 mg) in dichloromethane (20 ml) there was added DBU (160 mg) and the solution kept at ~20°C for 1 h (t.l.c. in acetone-ethanol (4:1) showed the presence of one component which ran slower than the starting material). The solvent was removed by evaporation and the residue purified by column chromatography using acetone-ethanol (4:1) as the eluant. The product was obtained as a white crystalline solid (160 mg, 74% yield), m.p. 136-139°C (d) (Found: C, 45.9; H, 5.0; N, 10.5. $C_{10}H_{13}ClN_2O_4$ requires C, 46.1; H, 5.0; N, 10.8%); u.v. λ_{max} 251 nm; n.m.r. δ 1.8 (3H, s, $-CH_3$), 3.4-3.8 (4H, m, H-3', H-5'), 4.1 (1H, m, H-4'), 4.5 (1H, dd, H-2'), 4.7 (1H, dd, H-2'), 5.1 (1H, m, 5'-OH), 6.05 (1H, dd, H-1'), 7.8 p.p.m. (1H, s, H-6).

The action of bases on 2',3'-dichloro-2',3'-dideoxy-2',3'-secouridine

a) To a solution of 2',3'-dichloro-2',3'-dideoxy-2',3'-secouridine (500 mg) in dichloromethane (20 ml) there was added DBU (320 mg) and the solution kept at ~20°C for 3 days (t.l.c. showed that two products were present). The solvent was removed by evaporation and the residue fractionated by column chromatography. Elution with chloroform-ethanol (19:1) gave the minor product (70 mg, 16% yield) andsubsequent elution with chloroform-methanol (4:1) gave the major product (300 mg, 69% yield) which upon further purification and crystallisation from ethanol was shown to be O^2,2'-anhydro-3'-chloro-3'-deoxy-2',3'-secouridine, m.p. 125-126 C.[1] The minor product was shown to be 6(R)-chloromethyl-2(R)(uracil-1-yl)-1,4-dioxane (14) (Found: C, 43.9; H, 4.4; N, 11.3.

$C_9H_{11}ClN_2O_4$ requires C, 43.8; H, 4.5; N, 11.3%); u.v. λ_{max} 260 nm (ε, 9,700); n.m.r. δ ($CDCl_3$) 3.58-4.16 (7H, m, H-3, H-5, H-6 of dioxan ring and $ClCH_2$), 5.76 (1H, d, H-5 of uracil ring), 5.92 (1H, t, H-2 of dioxan ring), 7.94 (1H, d, H-6 of uracil ring), 9.70 p.p.m. (1H, bs, NH).
b) A solution of 2',3'-dichloro-2',3'-dideoxy-2',3'-secouridine (600 mg) in tetrahydrofuran (10 ml) was added to a solution of potassium t-butoxide (600 mg) in tetrahydrofuran (30 ml). The resulting pale-yellow suspension was stirred at ~20°C for 18 h. The mixture was then acidified with acetic acid and evaporated to dryness. The residue was dissolved in methanol-toluene (1:1), insoluble material filtered off and the filtrate evaporated to dryness in the presence of silica gel. The solid was applied to the top of a silica gel chromatography column and the column eluted with chloroform-ethanol (39:1). Evaporation of the solvent from the appropriate fractions gave compound 14 (240 mg, 46% yield) with identical physical properties to the product obtained by method (a).

1-(R)-(1-Chloro-4(R)-chloromethyl-5-trityloxy-3-oxapentan-2-yl)-4-(1,2,4-triazol-1-yl)-1,2-dihydropyrimidin-2-one (15). To a solution of 2',3'-dichloro-2',3'-dideoxy-5'-O-trityl-2',3'-secouridine (2.93 g) in pyridine (15 ml) there was added 1,2,4-triazole (2.05 g) and then p-chlorophenylphosphodichloridate (2.3 ml) was slowly added, the temperature being maintained at ~20°C. The mixture was kept at ~20°C for 18 h with stirring and then chloroform (50 ml) added. The chloroform solution was washed with water, separated and dried. The solvent was removed by evaporation and the residue co-evaporated with toluene to remove traces of pyridine. The residue was purified by column chromatography. Elution with benzene-acetone (9:1) and evaporation of the appropriate fractions gave the product (2.5 g, 79% yield) (Found: C, 62.8; H, 4.6; N, 11.9. $C_{30}H_{27}Cl_2N_5O_3$ requires C, 62.5; H, 4.7; N, 12.1%); n.m.r. δ ($CDCl_3$) 3.35 (2H, m, H-5'), 3.62 (3H, m, H-3', H-4'), 3.85 (2H, m, H-2'), 6.15 (1H, t, H-1'), 6.80 (1H, d, H-5), 7.26 (15H, m, trityl), 8.09 (2H, t, H-6 and triazole H), 9.21 p.p.m. (1H, s, triazole H).

2',3'-Dichloro-2',3'-dideoxy-2',3'-secocytidine (16). Compound 15 (2.5 g) was dissolved in dioxane (110 ml) and aqueous ammonia (sp. g 0.88, 60 ml) added, the solution kept at ~20°C for 80 min. and then evaporated to dryness. The residue was co-evaporated with methanol, dried and purified by column chromatography. Elution was carried out with chloroform-ethanol (19:1) and the fractions containing the required material further purified in a similar way on a second column in order to remove contaminating 1,2,4-triazole. The 5'-O-trityl derivative of 16 was obtained as a white solid (2.1 g, 90% yield) which was sufficiently pure for the next stage. This solid was dissolved in acetic acid-water (4:1) (50 ml) and the solution heated at 100°C for 90 min. and then kept at ~20°C for 18 h. The solution was then evaporated to dryness and the residue purified by column chromatography. The column was eluted with chloroform-methanol (3:1) and the appropriate fractions combined and evaporated to dryness to give the product (351 mg, 31% yield) (Found: C, 38.6; H, 4.9; N, 14.9. $C_9H_{13}Cl_2N_3O_3$ requires C, 38.3; H, 4.6; N, 14.9%); n.m.r. δ 3.35-3.90 (7H, m, H-2', H-3', H-4', H-5'), 5.84 (1H, d, H-5), 6.03 (1H, t, H-1'), 7.66 p.p.m. (1H, d, H-6).

It was apparent that compound 16 was unstable because upon standing in solution in dimethylsulphoxide at room temperature or upon storing the solid for a few days a compound which ran slower on t.l.c. than 16 was formed. This was identified as O2,2'-anhydro-3'-chloro-3'-deoxy-2',3'-secocytidine (17) from its n.m.r. spectrum (δ 3.35-3.90 (4H, m, H-3', H-5'), 4.30 (1H, m, H-4'), 4.68 (1H, dd, H-2'), 5.00 (1H, dd, H-2'), 6.37 (1H, dd, H-1'), 6.72 (1H, d, H-5), 8.55 p.p.m. (1H, d, H-6)) and similarities to the corresponding 2',3'-secouridine derivative.

BIOLOGICAL EVALUATION

Antiviral Activity. The antiviral activity assays were based upon the inhibition of virus-induced cytophathogenicity.[8] The assays were carried out with herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G), vaccinia virus and vesicular stomatitis virus in primary rabbit kidney cells; with vesicular stomatitis virus, Coxsackie virus type B4 and polio virus type 1 in HeLa cells; and with reovirus type 1, parainfluenza virus type 3, Sindbis virus, Coxsackie virus type B4 and measles virus in Vero cells. Cytotoxicity of the compounds for the host cells was monitored by microscopic examination of uninfected cell cultures exposed to varying concentrations of the test compounds and run in parallel with the virus-infected cell cultures.

Cytostatic and Antimetabolic Activity. Cytostatic activity assays were performed as previously described.[5] The L1210/0, L1210/BdUrd, Namalva, Raji/0 and Raji/TK$^-$ cell lines were characterised as indicated in ref. 4. Inhibition of the incorporation of (methyl-^{3}H)dThd and (1',2'-^{3}H)dUrd into cellular DNA, and the effect of addition of dUrd and dThd on the cytostatic activity of the compounds were assessed as described in ref. 5.

Radiochemicals. (Methyl-^{3}H)dThd (specific radioactivity 47 Ci/mmole) and (1',2'-^{3}H)dUrd (specific radioactivity 31 Ci/mmole) were from the Radiochemical Centre (Amersham, England).

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