NUCLEOSIDE CONJUGATES. 8. THE PREPARATION OF 5-FLUORO-2'-DEOXYURIDINE CONJUGATES OF CORTICOSTEROIDS

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ABSTRACT

Three 5'-(steroid-21-phosphoryl)-5-fluoro-2'-deoxyuridines (VI-VIII) have been prepared and characterized by uv, ir, ^{1}H-nmr, elemental analysis, chemical and enzymatic hydrolyses. These new compounds are 5-fluoro-2'-deoxyuridine conjugates of cortisol (VI), cortico-sterone (VII), and prednisolone (VIII). Besides the physical and analytical data, all of the conjugates were demonstrated to be enzymatically hydrolyzed to the corresponding steroid and 5-fluoro-2'-deoxyuridine 5'-monophosphate (III), and the latter was further shown to be hydrolyzed to 5-fluoro-2'-deoxyuridine (II) by phosphodiesterase I, 5'-nucleotidase, and acid phosphatase. However, they were shown to be resistant to hydrolysis by bacterial alkaline phosphatase.

INTRODUCTION

5-Fluorouracil (I) has been a primary drug for the treatment of common solid tumors, particularly breast, ovarian, and gastrointestinal carcinoma. Contrary to what was anticipated, its nucleoside, 5-fluoro-2'-deoxyuridine (II) never underwent wide clinical application, as it did not demonstrate toxicological or therapeutic superiority over 5-fluorouracil. However, in an attempt to improve the efficacy of I or II, a variety of analogs and prodrugs of I and II have been synthesized. Notable examples among them are the 5'-O-alkylphosphates (1), the cholesterol conjugate (2), and the 5'-phosphodiamidate (3) of II. Also included are Ftorafur [1-(2-tetrahydrofuranyl)-5-fluorouracil] (4),

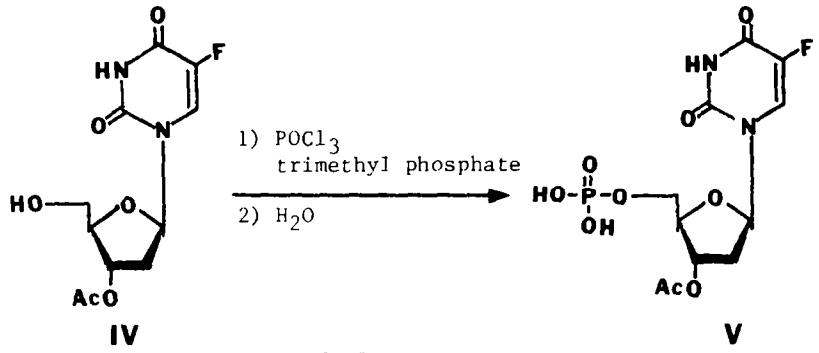
5'-deoxy-5- fluorouridine (5), the carbocyclic analog of II (6), 5'-substituted analogs of II (7), and analogs of 5'-deoxy-5-fluorouridine (8).

In our program to develop nucleoside conjugates as potential antitumor and antiviral agents, the synthesis and antitumor activity of 1-(β-D-arabinofuranosyl)cytosine and 9-(β-D-arabinofuranosyl)adenine conjugates of corticosteroids through a phosphodiester linkage have been reported recently (9-13). Most of the conjugates demonstrated significant antitumor activity against L1210 lymphoid leukemia in mice with reduced toxicity. This led us to synthesize conjugates of II with corticosteroids through a phosphodiester linkage. This paper describes synthesis and characterization of three conjugates of II with corticosteroids (Figure 1).

EXPERIMENTAL

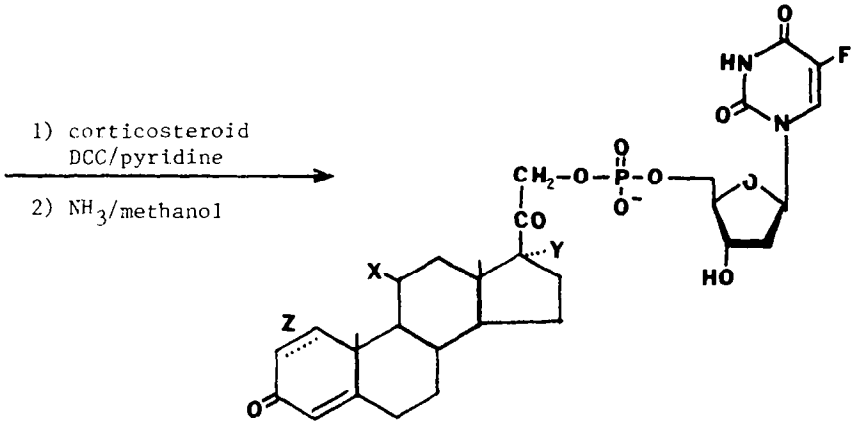
Melting points were determined in capillary tubes using a Mel-Temp apparatus and are uncorrected. The optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 297 IR Spectrophotometer. ^{1}H-nmr spectra were obtained with a Varian EM-390 spectrometer using tetramethylsilane as internal standard. UV absorption spectra were obtained on a Beckman Acta V spectrophotometer. AG1-X8 (formate, Bio-Rad), diethylaminoethylcellulose (DE-52, Whatman), and Amberlite CG-50 (100-200 mesh, Mallinckrodt) were used for column chromatography. Evaporation was performed in vacuo at 30°C. TLC was performed on glass plates coated with a 0.25-mm layer of silica gel PF-254 (Brinkman) and on polygram sil G UV 254 plates (Brinkman) using chloroform, methanol, or isopropyl alcohol-water-concentrated ammonium hydroxide (7:2:1 by vol) (solvent A). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

3'-O-Acetyl-5-fluoro-2'-deoxyuridine (IV) - A mixture of II (1 g, 4.1 mmol) and trityl chloride (1.36 g, 4.87 mmol) in pyridine (10 mL) was stirred at room temperature for 1 h and then at 50°C for 16 h. The reaction mixture was poured into ice-water (100 mL) with vigorous stirring. The precipitated gum was washed with water (2 x 20 mL) and dissolved in acetone (20 mL). The undissolved material was removed by filtration. The filtrate was evaporated to dryness below 30°C and dried in vacuo to give 5'-O-trityl-5-fluoro-2'-deoxyuridine (foamy) quantitatively; R_f=0.68 (solvent A). Without further purification, the residue



Ac = CH_3CO

DCC = N,N'-dicyclohexylcarbodiimide



VI, X=Y=OH, Z=single bond

VII, X=OH, Y=H, Z=single bond

VIII, X=Y=OH, Z=double bond

Figure 1. Synthetic scheme of the conjugates.

was stirred with pyridine (20 mL) and acetic anhydride (10 mL) at room temperature overnight. The excess acetic anhydride was destroyed with ice-water (20 mL) and the whole mixture was evaporated to a syrup. The resulting syrup was triturated with water to give a white precipitate, 5'-O-trityl-3'-O-acetyl-5-fluoro-2'-deoxyuridine; yield 2.13 g (97.9%), R_f=0.71 (solvent A), 0.15 (chloroform), 0.76 (methanol). Ir(KBr), 3040, 695 (aromatic CH), 1720, 1700(C=O), 1660 (benzene ring and amide), 1225(C-O) cm^{-1}. This was dissolved in 80% aqueous acetic acid (30 mL) and the mixture was refluxed for 30 min. The reaction mixture was cooled to room temperature to give a white precipitate, trityl alcohol. After removal of trityl alcohol by filtration, the filtrate was evaporated to a semisolid. The residue was dissolved in chloroform (5 mL) and chromatographed on a column of silica gel (2.5 x 15 cm). The column was eluted with chloroform (250 mL) to remove the residual trityl alcohol and then eluted with methanol (150 mL). The methanol effluent (150 mL) was evaporated to dryness. The residue was triturated with chloroform (5 mL) to give a white precipitate. The precipitate was collected by filtration and dried over P_2O_5 *in vacuo*; yield 1.0 g (84.7%), mp 188-190°C. Lit (14) mp, 207°C, R_f=0.61 (solvent A). ^{1}H-nmr (DMSO-d_6); δ 11.72(1H, bs, NH), 8.21(1H, d, J=7 Hz, H-6), 6.16(1H, t, J=7 Hz, H-1'), 5.23(2H, m, 3' and 5'OH), 4.02(1H, m, H-4'), 3.64(2H, d, J=3 Hz, H-5'+ 5''), 2.27(2H, m, H-2'+ 2''), 2.07(3H, s, CH_3) ppm.

3'-O-Acetyl-5-fluoro-2'-deoxyuridine-5'-monophosphate (V) - To an ice-cold solution of IV (1.50 g, 5.2 mmol) in freshly distilled trimethyl phosphate (15 mL) was added dropwise freshly distilled phosphorous oxychloride (2.39 g, 15.6 mmol), and the reaction mixture was stirred at 0-5°C overnight. The resulting clear solution was stirred 1 h more at room temperature. The mixture was extracted with n-hexane (3 x 25 mL) to remove the excess phosphorous oxychloride. The light brownish trimethyl phosphate solution was poured into ice-water (75 mL) and neutralized with concentrated NH_4OH to pH 7. The solution was evaporated to *ca.* 20 mL to result in some precipitation (NH_4Cl). After removing the precipitate by filtration, the filtrate was evaporated *in vacuo* to a small volume and the residue was dissolved in water (10 mL) and chromatographed on a column of AG1-X8 (formate, 2.5 x 25 cm) jacketed at 5°C. The column was eluted with water (300 mL) and then 0.3 M triethylammonium formate (400 mL). The triethylammonium formate effluent was passed through Dowex-50WX8 (H^+) column (2.5 x 30 cm). The column was washed with water until no UV-absorbing compound was detected, and the combined effluent was evaporated to a colorless syrup; yield 1.2 g (62.8%), R_f=0.15 (solvent A). The phosphate was used for the next step without further purification.

5'-(Cortisol-21-phosphoryl)-5-fluoro-2'-deoxyuridine (VI) - Compound V (1.66 g, 4.5 mmol) was stirred with cortisol (3.26 g, 9 mmol) and N,N'-dicyclohexylcarbodiimide (3.71 g, 18 mmol) in pyridine (100 mL) at room temperature for 48 h. Water (10 mL) was added and the mixture was stirred at room temperature for 16 h. The reaction mixture was evaporated to dryness and coevaporated with dry toluene (3 x 10 mL). The residue was extracted with 50% aqueous ethanol (150 mL) and

filtered. The filtrate was evaporated to dryness at 30°C, coevaporated with dry toluene (3 x 10 mL) and dried in vacuo. The residue was stirred with 2.0 N methanolic ammonia (100 mL) overnight at 5°C. The reaction mixture was evaporated to dryness at 30°C and dissolved in water (50 mL). After removing the insoluble white precipitate by filtration, the filtrate was chromatographed on a column of DE-52 (formate, 2.5 x 30 cm) jacketed at 5°C. The column was eluted with water (500 mL) and then with a linear gradient of 0-0.5 M triethylammonium formate (1 L each). The effluent (12 mL in each 20-mL tube) was collected. The appropriate fractions (43-52) were pooled and passed through Dowex-50WX8 (H^+) column (2.5 x 20 cm). The effluent was evaporated to dryness at 30°C. The residue was dissolved in the minimum amount of solvent A (5 mL) and chromatographed on a column of silica gel (2.5 x 30 cm). The column was eluted with solvent A and the effluent (12 mL in each 20-mL tube) was received. The appropriate fractions (6-9) were pooled, evaporated to dryness, and dried in vacuo, yielding pale yellowish powder, as an NH_4^+ salt, 1.48 g (47.9%), mp 106-108°C (effervescent) 200-220°C (slowly dec) R_f=0.46 (solvent A), $[\alpha]_D^{25}$= +86.21° (c, 0.58, methanol). Ir(KBr), 3400(OH), 2920(CH), 1720 (C=O), 1650, 1610(amide), 1230(P=O), 1075, 1050(P-O-C) cm^{-1}. ^1H nmr (DMSO-d_6), δ 8.08(1H, d, J=7 Hz, H-6), 6.11(1H, t, J=6 Hz, H-1'), 5.52 (1H, s, steroid 4), 3.09(2H, m, H-5'+ 5''), 3.78(1H, m, H-4'), 1.45 (3H, s, steroid 19), 0.80 (3H, s, steroid 18) ppm. Anal. Calc. for $C_{30}H_{43}FN_3O_{12}P \cdot 1.8H_2O$: C, 50.04; H, 6.52; N, 5.84; P, 4.30 Found: C, 50.23; H, 6.79; N, 5.46; P, 4.61.

The corticosterone (VII) and the prednisolone (11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione) (VIII) conjugates were synthesized in an analogous manner.

5'-(Corticosterone-21-phosphoryl)-5-fluoro-2'-deoxyuridine (VII) - Yield of 42.5%; mp 98-100°C (effervescent) 200-220°C (slowly dec); R_f= 0.62 (solvent A); $[\alpha]_D^{25}$= +83.6° (c, 0.54, methanol), Ir(KBr), 3400(OH), 2920(CH), 1720, 1710(C=O), 1660, 1610(amide), 1235(P=O), 1080, 1035(P-O-C) cm^{-1}. ^1H-nmr(DMSO-d_6 + D_2O), δ 7.74(1H, d, J=7 Hz, H-6), 6.17(1H, t, J=7 Hz, H-1'), 5.55(1H, s, steroid 4), 3.85(2H, d, J=5 Hz, H-5'+ 5''), 3.76(1H, m, H-4'), 1.40(3H, s, steroid 19), 0.80(3H, s, steroid 18) ppm. Anal. Calc. for $C_{30}H_{43}FN_3O_{11}P \cdot Na \cdot 2H_2O$:C, 48.98; H, 5.89; N, 3.81; P, 4.21. Found: C, 48.79; H, 5.69; N, 3.56; P, 4.55.

5'-(Prednisolone-21-phosphoryl)-5-fluoro-2'-deoxyuridine (VIII) - Yield of 37.2%; mp 95-98°C (effervescent) 200-220°C (slowly dec); R_f= 0.72 (solvent A); $[\alpha]_D^{25}$= +31.9° (c, 0.57 methanol). Ir(KBr), 3300, 3150(OH, NH), 3030(aromatic CH), 2940(CH), 1720, 1700(C=O), 1655, 1600 (amide), 1200(P=O), 1075, 1050(P-O-C) cm^{-1}. ^1H-nmr(DMSO-d_6 + D_2O), δ 8.05(1H, d, J=7 Hz, H-6), 7.35(1H, d, J=10 Hz, steroid 1), 6.10(2H, m, H-1' and steroid 2), 5.87(1H, s, steroid 4), 3.88(2H, m, H-5'+ 5''), 3.78(1H, m, H-4'), 1.39(3H, s, steroid 19), 0.80(3H, s, steroid 18) ppm. Anal. Calc. for $C_{30}H_{41}FN_3O_{12}P \cdot 4H_2O$: C, 47.55; H, 6.52; N, 5.55; P, 4.09. Found: C, 47.72; H, 6.77; N, 5.61; P, 3.91.

Hydrolysis of the Conjugates with Barium Hydroxide

The conjugate (0.01 mmol) and barium hydroxide (0.01 mmol) in 1 mL of water was heated at 90-95°C in a stoppered vial for 15 min. Aliquots (0.1 mL) were removed at the designated time, treated with Dowex-50WX8 (H^+) resin, and examined chromatographically in solvent A. Each band was extracted with 50% ethanol and quantitated by UV. Hydrolysis of the conjugate was completed in 5 min, and the products were the steroid 21-monophosphate and II. After standing at room temperature overnight, the steroid phosphate in the hydrolysate was further hydrolyzed to the corresponding steroid.

Enzymatic Hydrolysis

Enzymatic cleavage of the phosphodiester bond of the conjugates was studied by incubating the compounds (5 μmol) with phosphodiesterase I (EC 3.1.4.1) and 5'-nucleotidase (EC 3.1.3.5) from C. adamanteus, acid phosphatase (EC 3.1.3.2) from wheat germ, and bacterial alkaline phosphatase (EC 3.1.3.1) in appropriate buffer (final volume 1.0 mL) as described previously (10). Aliquots (0.1 mL) of the incubation mixtures at various lengths of time were streaked on TLC plates (0.05 x 10 x 20 cm) with authentic markers, and the plates were developed with solvent A. Each band was extracted with 50% ethanol and quantitated by UV.

RESULTS AND DISCUSSION

Chemistry

The conjugates (VI-VIII) were prepared by a method similar to that used for the preparation of the 1-(β-D-arabinofuranosyl)cytosine conjugates (9-11). Compound IV was prepared according to the method reported previously (14) with modified purification of the product using a silica gel column chromatography and chloroform and methanol as eluting solvents.

Phosphorylation of IV with the excessive amount (3 molar equivalents) of phosphorus oxychloride and trimethyl phosphate (15) afforded V in 63% yield. Condensation of V with 2 molar equivalents of the various steroids in the presence of N,N'-dicyclohexylcarbodiimide and pyridine at room temperature for 2 days and subsequent removal of the

acetyl group with 2 N methanolic ammonia gave the conjugates (VI-VIII) in 37-47% yield. The characteristic ir absorptions at 1720-1700 and 1660-1600 cm^{-1} supported the presence of the steroid (C=O) and the purine ring (C=C, C=N), and the strong absorptions at 1235-1220 and 1080-1035 cm^{-1} showed the presence of the phosphodiester bond (P=O, P-O-C). The presence of methyl groups of the steroids and the anomeric and C-6 protons of the nucleoside (II) was also supported by the ^1H-nmr spectra of the conjugates. Like the 1-(β-D-arabinofuranosyl)cytosine conjugates (9-11) and the 9-(β-D-arabinofuranosyl)adenine conjugates (13), confirmation of the molecular structures of the conjugates was further provided by chemical and enzymatic hydrolyses of the phosphodiester bonds. Hydrolysis of the conjugates (VI-VIII) by 0.05 M $Ba(OH)_2$ yielded the nucleoside (II) and the corresponding steroid 21-monophosphate, which was further hydrolyzed to the steroid. When enzymatic hydrolysis with phosphodiesterase I (EC 3.1.4.1) was used, the products were the nucleotide (III) and the steroid, and the former was further hydrolyzed to the nucleoside (II). Hydrolysis of the conjugates (VI-VIII) with 5'-nucleotidase (EC 3.1.3.5) and acid phosphatase (EC 3.1.3.2) for 24 h demonstrated that products were the steroid and the nucleoside (II). All conjugates were found to be resistant to enzymatic hydrolysis by bacterial phosphatase (EC 3.1.3.1). The results of antitumor evaluation will be published elsewhere.

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