Synthesis of Deoxyribosides of 5-Formyl-, 5-Hydroxymethyl- and 5-Benzyloxymethyluracil (1) (2).

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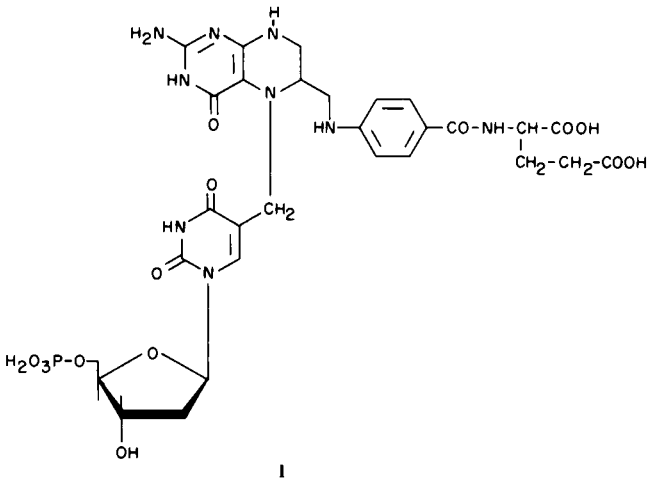
The enzyme thymidylate synthetase catalyzes conversion of 2′-deoxyuridine 5′-phosphate to the thymidine analog utilizing N_5,N_{10}-methylenetetrahydrofolic acid as the cofactor (3a-c). This reductive methylation has been postulated to occur through a bridged intermediate (I) (4) which then rearranges to thymidine 5′-phosphate and 7,8-dihydrofolic acid. A number of analogs of this bridged intermediate (I) involving 5-substituted uracils have been made (5a-g). The nature of the proposed intermediate and the known reactivity of tetrahydrofolic acid with carbonyl compounds (e.g., CH_2O) suggested the design of a potential inhibitor that would bind at the substrate site and react with the bound cofactor. 5-Formyl-2′-deoxyuridine (IXb) was synthesized in this study as an agent that could act in this manner giving enhanced inhibition of



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the enzyme. Condensation of the bis(trimethylsilyl) derivative of 5-formyluracil (II) (6,7,8) with 3,5-di-*O*-*p*-toluyl-2-deoxy-D-ribofuranosyl chloride (III) (9) in acetonitrile gave the corresponding α- and β-nucleosides (IVa and IVb) in 31% yield. A partial separation of the anomers was achieved on a silica gel column.

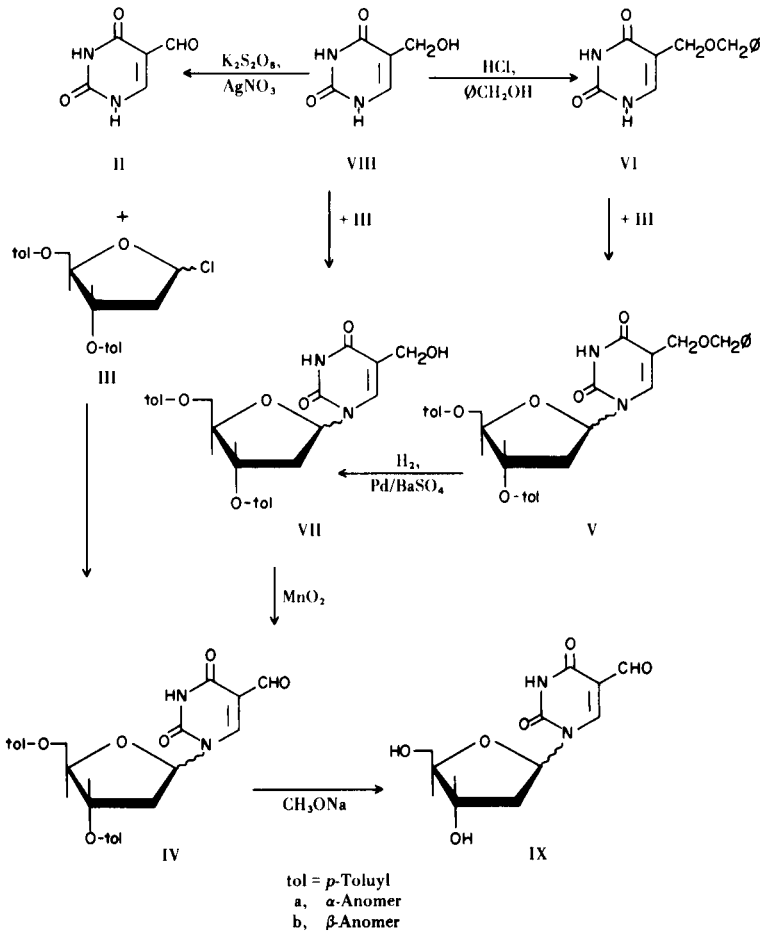
To secure large quantity of the formyl nucleoside IVb, it was necessary to investigate another route as the direct condensation gave a poor yield and also involved a lengthy column chromatographic procedure. The synthesis of 5-benzyloxymethyl-3′-5′-di-*O*-*p*-toluyl-2′-deoxyuridine (Vb) and its α-anomer (Va) has been reported by two groups (10,11,12).

The mercury procedure for the preparation of compounds to be tested in biological preparations, has been criticized (13a-d) as the trace amount of mercuric ions often complicates the results. In the present study, condensation of the silyl derivative of 5-benzyloxymethyluracil (VI) (12) with III in acetonitrile gave the α- and β-anomer (Va and Vb) in the ratio of 19:10 (total yield 71%). A larger proportion (10:13, total yield 66%) of the β-anomer was obtained when the reaction was carried out in benzene. This finding is consistent with reports (11,14) that the Hilbert-Johnson reaction of 2,4-dialkoxypyrimidines in nonpolar solvents usually leads to a higher proportion of the β-anomer. Compound Vb was conveniently separated by fractional crystallization. The benzyl group was removed in each case by hydrogenolysis in the presence of palladium on barium sulfate to give the known compounds VIIa and VIIb (12). These alcohols were oxidized with active manganese dioxide to the 5-formyl compounds IVa and IVb in good yield.

In an effort to bypass two steps, the protection with the benzyl group and its hydrogenolysis, condensation of the tris(trimethylsilyl) derivative of 5-hydroxymethyluracil (VIII) was attempted. The reaction mixture deposited a low yield of a crystalline product which was identified as the β-anomer, 5-hydroxymethyl-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (VIIb).

Transesterification of IVa by sodium methoxide gave the corresponding α-nucloeside (IXa), m.p. 92-95°. The anomeric assignment is based on nmr analysis in which the 1′ proton appeared as a multiplet of four peaks (13a,15) and the first order splitting pattern of the 2′ protons was consistent with that reported by Lemieux (15). A selective decoupling experiment (100 Mc) establishing a *cis* relationship between 1′H and 3′H in IXa confirmed the assignment.

Similarly, transesterification of the β-anomer (IVb) provided 5-formyl-2′-deoxyuridine (IXb), m.p. 175-175.5°. In the nmr spectrum the anomeric proton (1′H) appeared as a pseudo triplet (13a,15) and the spectrum of the remainder of the sugar protons resembled that of thymidine. The optical rotatory dispersion curve of IXb showed



a well defined positive Cotton effect which is consistent with the findings of Emerson *et al.* (16). Cline *et al.* (17) tentatively identified (paper chromatographic behavior and color reactions) the platinum dioxide oxidation product of 5-hydroxymethyl-2′-deoxyuridine (Xb) as 5-formyl-2′-deoxyuridine (IXb). In the present study compound IXb had the same comparative R_f value (uridine standard) as reported by these workers (17) in their paper chromatographic study.

Transesterification of VIIb yielded 5-hydroxymethyl-2′-deoxyuridine which was identical with an authentic sample.

EXPERIMENTAL

Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were recorded on Beckman IR-10 instrument in potassium bromide pellets. Nuclear magnetic resonance spectra were determined using Varian A-60 A instrument in 15-20% concentration with TMS or DSS as internal standards. Decoupling experiments were carried out on a Varian HR 100 instrument. Ultraviolet spectra were determined on Beckman DU spectrophotometer. Optical rotatory dispersion curves were obtained on Cary 60 instrument and rotation values were obtained on Perkin Elmer 141 polarimeter.

Silica gel for column chromatography refers to Brinkmann (0.05-0.20 mm) product activated at 110° and deactivated with 10% water. Pre-coated silica gel F-254 plates purchased from Brinkmann Instruments, Inc., were used for tlc. Paper chromatographs were run on standard size Whatman no. 1 papers using descending solvent technique. Microanalyses were run on an F and M 185 C, H, N, analyzer. All evaporations were carried out under reduced pressure.

Silylation of pyrimidines.

Silylation was carried out by the general procedure of Wittenburg (18). The pyrimidines were refluxed with excess hexamethyldisilazane with catalytic amount of ammonium sulfate under anhydrous conditions until (8-24 hours) a clear solution resulted. The excess silylating agent was evaporated under reduced pressure and the resulting oily silyl derivatives were used directly in the condensation reactions without further purification.

1-(3,5-Di-*O*-*p*-toluyl-2-deoxy-D-ribofuranosyl)-5-formyluracil (IVa and IVb).

The silyl derivative of 5-formyluracil (prepared from 1 g., 7.1 mmoles of II) (8) was dissolved in dry acetonitrile (25 ml.). After the addition of 3,5-di-*O*-*p*-toluyl-2-deoxy-D-ribofuranosyl chloride (III) (9) (2.2 g., 5.6 mmoles) and 4A molecular sieves (1 g.), the reaction mixture was stirred at 25° for two days under anhydrous conditions (7a-d). The molecular sieves were removed by filtration

and washed well with dry benzene. The filtrate and washings were combined and evaporated to a yellow oil which was decomposed with dry ethanol (10 ml.) to remove the silyl groups. The ethanol was removed by evaporation and the residue extracted with hot chloroform. The extract was filtered, evaporated, and chromatographed over a silica gel (300 g.) column (70 x 3.5 cm.). The column was eluted with chloroform and continuously monitored at 280 mμ. The fractions containing the α-anomer were pooled together and evaporated to yield 0.4 g. of IVa. Crystallizations from chloroform gave an analytical sample, m.p. 188-189°, nmr (deuteriochloroform) δ 10.07 (s, 1, CHO), 9.50 (broad s, NH), 8.57 (s, 1, olefinic H). The spectrum of the sugar part resembled that of α-thymidine 3′,5′-di-*O*-*p*-toluate (19).

Anal. Calcd. for $C_{26}H_{24}N_2O_8$: C, 63.41; H, 4.91; N, 5.69. Found: C, 63.40; H, 4.75; N, 5.62.

The fractions containing the anomeric mixture were pooled together and evaporated to yield 0.26 g. of the mixture containing IVa and IVb. The fractions containing the β-anomer were also pooled together and evaporated to yield 0.17 g. of IVb (total yield 0.83 g., 31%). Crystallizations from chloroform gave an analytical sample, m.p. 195-196°, nmr (deuteriochloroform) δ 9.95 (s, 1, CHO), 9.53 (broad s, 1, NH), 8.53 (s, 1, olefinic H). The spectrum of the sugar protons resembled that of thymidine 3′,5′-di-*O*-*p*-toluate (19).

Anal. Calcd. for $C_{26}H_{24}N_2O_8$: C, 63.41; H, 4.91; N, 5.69. Found: C, 63.42; H, 4.91; N, 5.77.

1-(3,5-Di-*O*-*p*-toluyl-2-deoxy-D-ribofuranosyl)-5-benzyloxymethyluracil (Va and Vb).

The silyl derivative of 5-benzyloxymethyluracil (prepared from 4 g., 17.3 mmoles of VI) (12) was dissolved in dry acetonitrile (100 ml.). After addition of 3,5-di-*O*-*p*-toluyl-2-deoxy-D-ribofuranosyl chloride (III) (9) (6 g., 15.4 mmoles) and 4A molecular sieves (4 g.), the reaction mixture was stirred at 25° for two days under anhydrous condition (7a-d). The reaction mixture was worked up as in the case of IV and the resulting oil was decomposed with absolute ethanol (40 ml.). The ethanol was removed by evaporation and the residual mass extracted with chloroform. The extract on evaporation gave the anomeric mixture of Va and Vb from which most of the β-anomer was separated by fractional crystallizations from methanol to yield 1.3 g. of Vb, m.p. 139-140° (lit. (12) m.p. 141-142°).

All the mother liquors from the fractional crystallizations were combined and chromatographed over a silica gel (300 g.) column (60 x 4 cm.) and eluted with chloroform. This provided 2.83 g. of the anomeric mixture containing Va and Vb in the ratio of 2:1 as indicated by the integration of the 5-C*H*$_2$OCH$_2$Ø proton peaks in the nmr (deuteriochloroform) spectrum. Further elution and subsequent evaporation of the eluant provided 2.26 g. of the α-anomer (Va) which was recrystallized from methanol, m.p. 99-102° (lit. (12) m.p. 99-101°) (total yield 6.33 g., 71%, ratio of α:β-anomer, 19:10).

A similar reaction in dry benzene as the solvent for 5 days at room temperature gave a higher proportion of the β-anomer, isolated by fractional crystallizations of the anomeric mixture, to yield 3.34 g. of Vb. The mother liquors were combined and chromatographed over a silica gel column (300 g.). Elution with chloroform:ethyl acetate (9:1) and the evaporation of the fractions containing the α-anomer provided 2.56 g. of Va (total yield 5.9 g., 66%, α:β-anomer, 10:13).

1-(3,5-Di-*O*-*p*-toluyl-2-deoxy-β-D-ribofuranosyl)-5-hydroxymethyluracil (VIIb).

Compound Vb (4.2 g., 8.5 mmoles) in 70 ml. of glacial acetic acid was added to the prereduced palladium-barium sulfate (5%, 1.75 g.) in the same solvent (70 ml.) and hydrogenated at atmospheric pressure till the uptake of hydrogen amounted to 185 ml. (175 ml. theoretical). The catalyst was removed by filtration and washed well with the solvent. The combined filtrate and washings were evaporated and the traces of acetic acid were removed by coevaporation with toluene. The product was crystallized from ethanol to yield 2.9 g. (82%) of VIIb.

Further crystallizations and drying in vacuum at 80° gave a sample melting at 169.5-170.5° (lit. (12) m.p. 167-169°).

Condensation of Tris(trimethylsilyl) Derivative of 5-Hydroxymethyluracil with III.

The tris(trimethylsilyl) derivative of 5-hydroxymethyluracil (prepared from 3 g., 21.1 mmoles of VIII) (17) was dissolved in dry acetonitrile (100 ml.). After addition of 3,5-di-*O*-*p*-toluyl-2-deoxy-D-ribofuranosyl chloride (III) (9) (9.3 g., 23.2 mmoles) and 4A molecular sieves (4 g.), the reaction mixture was stirred at 25° for two days under anhydrous conditions (7a-d). The reaction mixture was worked up as usual and the silyl groups were removed by the addition of 40 ml. of absolute ethanol. The tlc showed only a small amount of the desired deoxyribosides when developed with chloroform:ethyl acetate (1:1). However, a faster moving spot was the major product. Water (5 ml.) and ethanol (10 ml.) were added to the reaction mixture, which on standing at room temperature for several days, deposited a crystalline product. The product was purified by chromatography (silica gel) and crystallizations to yield 1.12 g. (10%) of VIIb identical in all respects with the product obtained from the hydrogenolysis of Vb.

1-(3,5-Di-*O*-*p*-toluyl-2-deoxy-α-D-ribofuranosyl)-5-hydroxymethyluracil (VIIa).

Compound Va (4.2 g., 8.5 mmoles) was hydrogenated as in the case of the corresponding β-anomer (Vb) and the product was crystallized from toluene to yield 2.8 g. (79%) of VIIa. Recrystallization from ethanol and drying at 70° gave a sample melting at 150-151° (lit. (12) m.p. 151-152°).

Manganese Dioxide Oxidation of VIIb.

Compound VIIb (2 g., 4 mmoles) was dissolved in 100 ml. of toluene and after addition of active manganese dioxide (20) (4 g.), the reaction mixture was refluxed with magnetic stirring. After four hours of refluxing, the reaction mixture was filtered through a Celite bed while hot and the manganese dioxide washed well with chloroform. The filtrate and washing were combined, evaporated and the product was crystallized from toluene to yield 1.3 g. (65%) of the product. Recrystallization from chloroform-cyclohexane gave a sample melting at 191-193° which was identical (m.m.p. and ir spectral comparison) with IVb prepared by the direct condensation of 5-formyluracil (II).

Manganese Dioxide Oxidation of VIIa.

Compound VIIa (2 g., 4 mmoles) was oxidized with active manganese dioxide (20) in 100 ml. of refluxing toluene as in the case of VIIb and the product was crystallized from chloroform to yield 1.1 g. (55%) of IVa, m.p. 184-186°. This sample was identical (m.m.p. and ir spectral comparison) with the compound prepared by the direct condensation of 5-formyluracil (II).

1-(2′-Deoxy-α-D-ribofuranosyl)-5-formyluracil (IXa).

Compound IVa (1.6 g., 3.2 mmoles) was suspended in 50 ml. of dry benzene and 50 ml. of dry methanol. A freshly prepared solution of sodium methoxide was added till the reaction mixture

was distinctly alkaline. The stoppered flask containing the reaction mixture was allowed to stand at room temperature for two days with occasional stirring. The precipitate was filtered and washed well with cyclohexane to yield 0.6 g. of the sodium salt of the nucleoside which was dissolved in required amount of water and passed through a column of Dowex 50 (H^+ form). The eluant and the washings from the column were coevaporated to yield 0.52 g. (63%) of the product. Repeated crystallizations from ethanol-ethyl acetate and drying in vacuum gave analytically pure light yellow hygroscopic crystals, m.p. 92-95°; $[\alpha]_D^{25°}$ +23.4° (c 1.40, water); nmr (deuterium oxide) δ 9.75 (s, 1, CHO), 8.70 (s, 1, olefinic H), 6.23 (q, $J_{H2'\alpha}$ = 7, $J_{H2'\beta}$ = 2.5, $W_{1/2}$ = 10.5 Hz, 1, anomeric H), the spectrum of the remainder of the sugar protons resembled that of α-thymidine (15); uv (1 *N* hydrochloric acid) λ max 282 (ε, 13,000), 234 (ε, 9,400), λ min 250 (ε, 2,900), (water), λ max 281 (ε, 12,100), 232 (ε, 9,300), λ min 251 (ε, 3,100), (1 *N* potassium hydroxide) λ max 283 (ε, 9,100), 237 (ε, 10,700), λ min 260 mμ (ε, 5,800).

Anal. Calcd. for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93. Found: C, 47.04; H, 4.55; N, 10.86.

5-Formyl-2′-deoxyuridine (IXb).

Compound IVb (0.73 g., 1.5 mmoles) was suspended in a mixture of dry benzene (15 ml.) and dry methanol (15 ml.) and transesterified by the addition of a freshly prepared solution of sodium methoxide until the solution was distinctly alkaline. The reaction mixture was allowed to stand overnight at room temperature in a stoppered flask with occasional stirring. The reaction mixture was diluted with 20 ml. of dry benzene and the precipitated sodium salt of the nucleoside was filtered and washed well with dry benzene. The precipitate was dissolved in water (10 ml.) and Dowex 50 (H^+ form) was added till the solution was neutral to slightly acidic. The neutralized solution upon concentration and cooling deposited 0.27 g. (71%) of the product as crystals. Repeated crystallizations from ethanol gave an analytically pure sample as tan yellow crystals, m.p. 175-175.5°; $[\alpha]_D^{25°}$ +33.0° (c 1.13, water), ORD (c, 0.00512, water, 22°) $[\Phi]_{315}$ +1,800°, $[\Phi]_{294}$ 0°, $[\Phi]_{286}$ −1,100°, $[\Phi]_{279}$ 0°, $[\Phi]_{242}$ +10,600°; nmr (deuterium oxide) δ 9.78 (s, 1, CHO), 8.85 (s, 1, olefinic H), 6.30 (t, J = 6.5, $W_{1/2}$ = 13 Hz, 1, anomeric H), the spectrum of the remainder of the sugar protons resembled that of thymidine (15); uv (1 *N* hydrochloric acid), λ max 281 (ε, 13,200), 232 (ε, 10,000), λ min 251 (ε, 3,100), (water), λ max 281 (ε, 13,400), 231 (ε, 10,400), λ min 250 (ε, 3,100), (1 *N* potassium hydroxide), λ max 282 (ε, 10,300), 238 (ε, 11,600), λ min 261 mμ (ε, 5,600). On chromatographic paper it had the same relative R_f value with uridine as reported by Cline *et al.* (17) in *t*-butyl alcohol, methyl ethyl ketone, formic acid and water (40:30:15:15) as the mobile phase.

5-Hydroxymethyl-2′-deoxyuridine.

Compound VIIb was transesterified as in the case of IVb and the product was crystallized from ethanol to yield 5-hydroxymethyl-2′-deoxyuridine, m.p. 174-178° which was identical (m.m.p. and ir spectral comparison) with an authentic sample melting at 176-178° (lit. (12) m.p. 180-182°).

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