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SYNTHESIS OF 3-(2-DEOXY-β-D-ERYTHRO-PENTOFURANOSYL)-2,3-DIHYDRO-1,3-6H-OXAZINE-2,6-DIONE ("3-OXA-2'-DEOXYURIDINE"), A NEW PYRIMIDINE NUCLEOSIDE ANALOG

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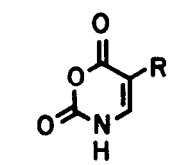
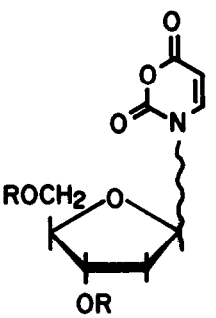
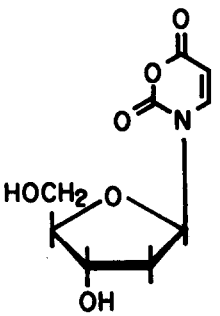
We have recently shown[1] that 2,3-dihydro-1,3-6H-oxazine-2,6-dione (I), the oxazine analog of uracil inhibits markedly the in vitro growth of some microbial and tumor cell lines. The thymine analog 5-methyl-2,3-dihydro-1,3-6H-oxazine-2,6-dione (II), on the other hand, exhibited little inhibitory activity in these systems. The inhibition exerted by I was reversed competitively by uracil and cytosine and by their nucleosides, characterizing this compound as a pyrimidine antagonist. This finding has now been confirmed by Skoda and his coworkers[2]. While I had been synthesized by Rinkes[3] in 1927 and by Washburne[4] in 1972, II had not previously been prepared.



I: R=H
II: R=CH_3



III: R=$CH_2C_6H_5$
VI: R=$COOCH_2C_6H_5$



VII

Our preparation of I proceeded by a new route involving ring closure of β-(N-ethoxycarbonylamino)acrylic acid[5] with phosphorus pentoxide in dimethylformamide, providing I in 55% yield. Simi-

larly, II [m.p. 134-135.5° from ethyl acetate, λ_{max} (ethanol) 271 nm (ε 6,500), nmr (DMSO-d_6) τ 2.43 (q, 1, J = 1.5 Hz, H-4), 8.17 (d, 3, J = 1.5 Hz, CH_3)] was obtained, in 56% yield, from β-(N-ethoxycarbonylamino)-α-methylacrylic acid[6] by treatment with polyphosphoric acid at 75-80°. We have also prepared II, at 70% yield, from citraconimide by treatment with sodium hypochlorite at 0-5°.

Although I inhibited the in vivo growth of leukemia L-1210 in mice, the therapeutic index obtained was relatively narrow. To possibly achieve greater selectivity, 3-(2-deoxy-β-D-erythro-pentofuranosyl)-2, 3-dihydro-1, 3-(6H)-oxazine-2, 6-dione (VII) has now been synthesized by the procedure reported herein.

The synthesis of VII presented a problem due to the instability of the 2, 3-dihydro-1, 3-6H-oxazine-2, 6-dione ring, which undergoes slow hydrolysis, even in ethanol at 20°. This fact precluded the use of conventional blocking groups, such as p-toluoyl or acetyl, for the protection of the sugar hydroxyls, because their removal requires conditions under which the oxazine ring of I is quickly hydrolyzed. Among alternative blocking groups, the benzyl group was considered, since it can be removed by hydrogenation under neutral conditions. This group has been used in the past for blocking 2-deoxy-D-erythro-pentofuranosyl chloride in a synthesis of thymidine[7]. The low yield of that synthesis was attributed to the marked tendency of the sugar chloride to eliminate hydrogen chloride and, thus, 3, 5-di-O-benzyl-2-deoxy-D-erythro-pentofuranosyl chloride was not considered a suitable intermediate for the synthesis of nucleosides[7]. Condensation of 3, 5-di-O-benzyl-2-deoxy-D-erythro-pentofuranosyl chloride with N-trimethylsilyl-2, 3-dihydro-1, 3-6H-oxazine-2, 6-dione[4] under various conditions produced no nucleoside material.

It has been shown[8] that in place of 1-halo sugars the more stable 1-acyloxy derivatives of protected sugars can be used for condensation with trimethylsilyl derivatives of pyrimidine bases in the presence of the Friedel-Crafts catalysts. Treatment of a mixture of 1-O-acetyl-3, 5-di-O-benzyl-2-deoxy-D-erythro-pentose[7] and the N-trimethylsilyl derivative of I in 1, 2-dichloroethane with $SnCl_4$, at -5° to 0°, gave an anomeric mixture of blocked nucleosides (III) in 25% yield, after purification by chromatography on silica gel [λ_{max} (ethanol) 268 nm, nmr (CCl_4) τ 2.81 (s, 10, benzyl), 2.32, 2.48 (2 d, 1, J_{4-5} = 8Hz, H_α - 4, H_β - 4), 4.06 (m, 1, H-1'), 4.73, 4.88 (2 d, 1, J_{4-5} = 8Hz, H_α - 5, H_β - 5)]. The ratio of the two anomers was approximately 1:2. Attempts to remove the protecting benzyl groups from III, by

hydrogenation in anhydrous dioxane using Pd-C catalyst failed, and the hydrogenolysis in ethanol was accompanied by some hydrolysis of the 1,3-oxazine ring. Hydrogenolysis in other solvents was not attempted, instead, the benzyloxylcarbonyl group was considered for the protection of the sugar hydroxyl groups. We presumed that, being an acyl type group, it would enhance the stability of the sugar chloride, and, at the same time, we expected that it would be more readily removed by hydrogenation than is the benzyl group. The starting material, methyl 3,5-di-O-benzyloxycarbonyl-2-deoxy-α, β-D-erythro-pentofuranosides (IV), was prepared in 67% yield from a mixture of methyl 2-deoxy-D-ribosides[9] by treatment at -20°, with benzyloxycarbonyl chloride in pyridine, and was purified by chromatography on silica gel. Treatment of IV with HCl in ethyl ether, at 0-5°, gave the syrupy 1-chloro derivative (V). Condensation of V with N-trimethylsilyl-2,3-dihydro-1,3-6H-oxazine-2,6-dione in benzene, and in the presence of a catalytic amount of $SnCl_4$, afforded a syrupy mixture of the α and β anomers of blocked nucleosides, VI, in 15% yield (λ_{max} (ethanol) 268 nm) which was purified by chromatography on silica gel. The protecting groups were readily removed from VI by catalytic hydrogenation in dioxane using Pd-C catalyst. The mixture of the free nucleoside VII and its α anomer VIII thus obtained, was purified by chromatography on silica gel (75% yield). The nmr spectrum of this mixture revealed the anomeric ratio of α/β to be approximately 1:1. The nucleoside VII was separated by fractional crystallization using an ethyl acetate-dioxane mixture as the solvent (35% yield). Assignment of the β configuration to the nucleoside VII was made on the basis of its nmr spectrum, wherein the anomeric proton appeared as the characteristic triplet. VII [m.p. 142-144° (dec.) λ_{max} (ethanol) 269 nm (ε 7,150), nmr (DMSO-d_6) τ 1.87 (d, 1, J_{4-5} = 8Hz, H-4), 3.92 (t, 1, $J_{1'-2'}$ = 6.5 Hz, H-1'), 4.32 (d, 1, J_{4-5} = 8Hz, H-5)]. The nmr of the syrupy nucleoside VIII revealed a pair of doublets for the α anomeric proton, and it showed the presence of a small amount of the β anomer VII. VIII [(λ_{max} (ethanol) 268 nm (ε 7,100), nmr (acetone-d_6) τ 1.98 (d, 1, J_{4-5} = 8Hz, H-4), 3.95 (d of d, 1, $J_{1'-2'-2''}$ = 2.0 and 7.0 Hz, H-1'), 4.32 (d, 1, J_{4-5} = 8Hz, H-5)]. Satisfactory analytical data were obtained for compounds II, IV and VII.

Although used extensively for the protection of the amino group, this use of the benzyloxycarbonyl group in the synthesis of a nucleoside has not, to our knowledge, been reported previously.

Preliminary evaluation showed that whereas I inhibited the growth of S. faecium by 50% at 6×10^{-5}M, the 2'-deoxy-β-D-erythro-pentofuranosyl derivative VII was inhibitory at 5×10^{-8}M, an approximately 1000 fold increase in potency. This inhibition was partially prevented by uridine, 2'-deoxy-

uridine, cytidine, 2'-deoxycytidine and by thymidine.

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