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(Journal of the Korean Chemical Society)
Vol. 19, No. 6, 1975
Printed in Republic of Korea

5-할로겐 치환된 Uracil의 4-Thiosugar Nucleosides의 합성에 관한 연구(제2보). 5-Fluoro-4′-thiouridine 과 5-Chloro-4′-thiouridine의 합성

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(1975. 5. 5 접수)

Synthesis of 1-(4-Thio-α, β-*D*-ribofuranosyl)-5-halogenouracils(II). 5-Fluoro-4′-thiouridine and 5-Chloro-4′-thio-uridine

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(Received May 5, 1975)

요 약. Unusual pyrimidine nucleoside인 5-fluoro-4′-thiouridine과 5-Chloro-4′-thiouridine을 5-fluoro-와 5-chlorouracil의 2, 4-*bis* (trimethylsilyl) 유도체들을 만든후, 축합반응하여 합성하였다. Leukemia 1210 cell과 *Streptococcus faecium*에 대한 기초 생물화학적인 예비시험에 의하면 5-fluoro-4′-thiouridine은 5-fluorouridine보다 억제효과가 월등하여 항암제로서의 관망여하는 앞으로의 임상화학적인 결과로 구명될것 이라고 예상된다.

ABSTRACT. The synthheses of anomeric mixtures of 1-(4-thio-α, β-*D*-ribofuranosyl)-5-fluoro- and 5-chlorouracils from their corresponding *bis*(trimethylsilyl) derivatives of 5-halogeno-uracils and 2, 3, 5-tri-*O*-acetyl-4-thio-α, β-*D*-ribofuranosyl chloride are described.

Preliminary biochemical studies showed that in leukemia 1210 cells and *Streptococcus faecium*, the β-anomeric 5-chloro-4′-thionucleoside is not greatly different from the corresponding 4′-oxygen analog. However, the 5-fluoro-4′-thio-nucleoside showed a growth inhibitory effect more than that of the oxygen counterpart. The potential chemotherapeutic use of the analog is to warrant further study.

INTRODUCTION

As part of a program in synthesizing the nucleosides with biochemical potentials[1~5], we described the succesful syntheses (even the low yield (~7 %) obtained) of novel nucleoside analogs functionally substituted both on the heterocycle and on the carbohydrate. Such a structurally modified so-called, "thio-sugar" nucleosides represent a new class of compounds which may have unusual medicinal interests. Previous studies[2]

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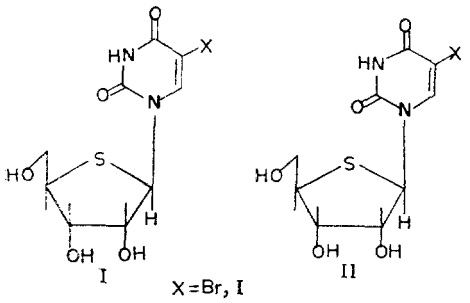
of tumor inhibitory evaluat-ion of 5-bromo-and 5-iodo-4′-thio-uridine nucleosides (I) *vs.* their corresponding 4′-oxygen counter-parts (II) indicated that they were qualitatively similar in their biological effects but the 4′-thio analogs had comparable high activities.

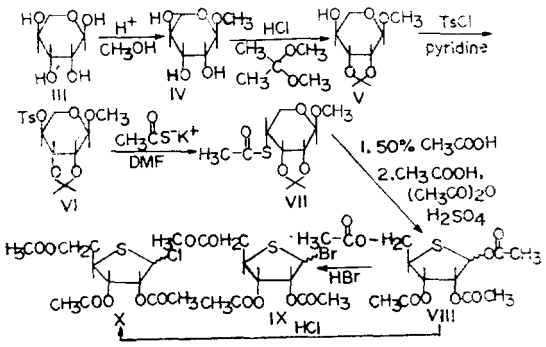


In a continuing study of 5-substituted 4′-thiopyrimidine nucleosides, two structural modifications of the 5-halogenated pyrimidine nucleoside molecules were made. In one, the ring oxygen of the carbohydrate moiety was replaced by sulfur; the other involved, in addition to this replacement, the substitution of the 5-hydrogen of the heterocycle with F and Cl groups; we undertook the synthesis of 1-(4-thio-α, β-*D*-ribofuranosyl)-5-fluorouracil (5-fluoro-4′-thiouridine, XIV_a and XV_a), and 1-(4-thio-α, β-*D*-ribofuranosyl)-5-chloro-uracil (5-chloro-4′-thiouridine, XIV_b and XV_b) in the hope of finding high antitumor activity with low toxicity and selectivity. The fluorinated pyrimidines (4′-*O*-analog) and their nucleosides haue demonstrated considerable clinical utility in the palliation of patients with advanced cancer[5].

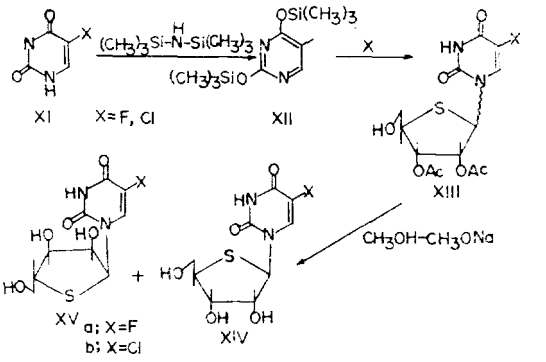
RESULTS and DISCUSSION

A standardized synthetic procedure was adopted here for the syntheses of the 5-halogeno-4′-thio-uridine nucleosides; Hilbert-Johnson[6] and the Mercury salt method[7]. The so-called, "thio sugar", 1, 2, 3, 5-tetra-*O*-acetyl-4-thio-α, β-*D*-ribofuranose (VIII) was synthesized from *L*-lyxose (III) by the following reaction sequences (see *Scheme* 1), and readily converted into the bromo-sugar, 2, 3, 5-tri-*O*-acetyl-4-thio-α, β-*D*-ribofuranosyl bromide (IX) by reaction with saturated HBr in glacial acetic acid. Initial attempts to prepare 5-halogeno-4′-thio-uridine using the generalized mercuric cyanaide-nitromethane method[9] failed, due, probabley, to the instability of the bromo sugars (1, 2, 3, 5-tetra-*O*-acetyl-4-thio-α, β-*D*-ribofuranose is relatively unstable compared with the corresponding 1, 2, 3, 5-tetra-*O*-acetyl-α, β-*D*-ribofuranose ; comparison of kinetic studies will be published in the future). Therefore this approach was abandoned in favor of a procedure using the *bis*(trimethylsilyl) derivatives of 5-halogeno-uracil. The 4′-thio uridine analogs of XIV a and b, and XV*a* and *b*, were prepared from the mercuric acetate

Scheme 1.



Scheme 2.



salt of 5-halogeno-2, 4-*bis*(trimethylsilyl) pyrimidine (XII)[10] and by application of the Hilbert-Johnson reaction[6] of 2, 3, 5-tri-*O*-acetyl-4-thio-α, β-*D*-ribofuranosyl chloride (X)[11]. A recent report from Wagner and his coworkers described the synthesis of the similar 4′-oxygen nucleosides from the Ag salt[12]. Similarly fluorinated 4′-oxygen pyrimidine nucleosides have been synthesized by Heidelberger and his students by the silyl method[13]. Condensation of 5-halogeno-2, 4-*bis*(trimethylsilyl) pyrimidine (XII) with 2, 3, 5-tri-*O*-acetyl-4-thio-α, β-*D*-ribofuranosyl chloride (X) gave low yields of the blocked α- and β-anomeric nucleosides (XIII*a* and *b*), with the β-anomer predominating in the approximate ratio of 6:1. The protected nucleosidic products, after chromatographic separations, were deacetylated to the free nucleosides by treatment with methanolic NaOMe, and chromatographically homogenous crystalline β-anomers, 5-halogeno-4′-thio-uridines (XIV*a* and *b*), were obtained 6 to 2 % yields, respectively. In all cases, glycosylation occurred to the site of glycosyl attachment at N-1 of the pyrimidine, as adduced from the close similarity of UV spectra of the products to that of uridine analogs (see Experimental). Firm evidence for the β-configuration was obtained from the NMR spectra of XIV*a* and *b* (in D_2O), in which the signal for the anomeric protons appeared at δ 6.13 and 6.01 for XIV*a* and XIV*b*, respectively as a singlet. This observation is in good agreement with the work of Walton, *et al*[14]. However, the coupling constant, J_{H-1-F} of 1∼2 Hz described by Cushley, *et al.*[15] in 5-fluorouridine was not observed in this 5-fluoro-4′-thiouridines. The CD (circular dichroism) spectrum of XIV*a* in H_2O provided further confirmation of the β-configuration in that it displayed a positive Cotton effect centered at 273 mμ, thus following the same pattern of the pyrimidine nucleosides proposed by Ulbricht, *et al*[16]. 1-(β-*D*-pentofuranosyl) uracils show positive Cotton effects in the region of the so-called B_{2u} spectral band (260∼280 mμ for most nucleosides) if the nucleoside possesses a preferred conformation owing to restricted rotation about the glycosyl bond. This rule appears to to be very valid for this nucleoside, 5-fluoro-4′-thio-uridine. Accordingly, its rule should be valid for all of the 5-halogeno-4′-thio uridines synthesized so far. Because of the usual lack of biological activity of α-pyrimidine nucleosides, the α-anomers obtained in these syntheses were not further characterized.

Preliminary tests of the 1-(4-thio-β-*D*-ribofuranosyl)-5-chlorouracil in leukemia 1210 cells and *Streptococcus faecium* is not greatly different from its corresponding 4′-oxygen nucleoside. However, 1-(4-thio-β-*D*-ribofuranosyl)-5-fluoro-uracil showed a sufficient growth inhibitory effect more than that of the oxygen counterpart. In conclusion, this 1-(4-thio-β-*D*-ribofuranosyl)-5-fluoro-uracil is to warrant further study for the potential chemotherapeutic use. Full details of antitumor screening of this compound will be published elsewhere[17], in cooperation with the Drug Research and Development Branch, National Cancer Institute, U. S. A.

EXPERIMENTAL

All melting points were taken on a Fisher-Johns apparatus and are corrected. Nmr spectra were recorded on a Varian Model A-60 spectrophotometer with TMS as an internal standard; *s* signifies singlet; *d*, doublet; *t*, triplet; *m*, multiplet. UV spectra were observed on a Beckman DB recording spectrophotometer. Cotton effect was observed on a Cary Model 60 spectropolarimeter operating in the circular dichroism mode. Elemental analyses were performed by Galbraith Laboratories, Tenn., U. S. A. Chromatographic determination of purity of nucleosides

was made at 25° on Whatman No. 1 filter paper, using 1-butanol-acetic acid-water (5:2:3, v/v) as developing solvent. The compounds or components were located by visual examination with an ultraviolet lamp. Thin layer chromatograms were made on silica gel G (Brinkman Instruments Inc., Great Neck, N. J.) coated plates and the spots were located by spraying with 5 % ethanolic sulfuric acid and charring.

An Improved Synthesis of Methyl 4-*S*-Acetyl-4-thio-2, 3-*O*-isopropylidene-β-*D*-ribopyranoside (VII). A mixture of freshly distilled *N*, *N*-dimethylformamide (80 m*l*), VI (7.12 g, 0.02 mole) and recrystallized potassium thioacetate (3.04 g, 0.027 mole) was heated at the oil bath temperature of 102~109° for 17~24 hours with stirring in a current of dry nitrogen. (The nitrogen used, was first passed through a solution of 8 g of potassium hydroxide, 0.8 g of sodium anthraquinone β-sulfonate and 6 g of sodium dithionite in 100 m*l* of water, then through sulfuric acid and finally through a 1×15 cm tube filled with potassium hydroxide). The dark brown reaction mixture was cooled to the room temperature, and evaporated *in vacuo* below the temperature of 40° uder reduced pressure. The residue was extracted from the dark brown insoluble material, several times with ether. The combined ether was washed and dried over anhydrous magnessium sulfate. Filtration and evaporation gave a dark brown oily residue which was chromatographed on silica gel column using *n*-hexane-ethyl acetate (20:1) to afford pure white crystalline products, 4.08 g (52 %); m. p 92~93° (lit[18]. 92~93); $[\alpha]^{25}D$-27° (*c* 0.75, ethanol).

Preparation of the *P*rotected *A*nomeric 1-(2, 3, 5-tri-*O*-acetyl-α, β-*D*-ribofuranosyl)-5-fluoro- and 5-chloro-uracils (XIII*a* and XIII*b*). A mixture of 0.01 mole of 2, 3, 5-tri-*O*-acetyl-4-thio-*D*-ribofuranosyl chloride (X)[11], 0.01mole of the 2, 4-*bis* (trimethylsilyl) derivative[10] of 5-fluoro-uracil (XII*a*) or 5-chlorouracil (XII*b*), and 0.01 mole of mercuric acetate in 100 m*l* of freshly distilled dry toluene was vigorously stirred at 90~100° for two days in a current of dry nitrogen. The cooled reaction mixture was concentrated *in vacuo* to a syrup, which was dissolved in 150 m*l* of ethyl acetate. This solution was successively washed with 20 % KI solution (2×80 m*l*), H_2O (2×100 m*l*), dried (Na_2SO_4) and was concentrated *in vacuo* to a syrup. This syrupy residue was purified by column (2×60 cm) chromatography on a silica gel in a benzene-acetone (8:1, v/v) mixture. The anomeric mixture of the protected nucleosides, XIII*a* and XIII*b* was obtained by evaporation, *in vacuo*, of the eluent as glassy residue, 6 % yield of XIII*b* and 2 % of XIII*a*: The ir ($CHCl_3$) spectrum showed absorption for free acetyl blocking group at 1735 cm^{-1}, and tlc showed only one spot in a benzne-acetone (20:1) (the α and β anomer did not show any significant R_f difference).

Preparation of the Anomeric 1-(4-thio-α, β-*D*-ribofuranosyl)-5-fluoro- and 5-Chloro-uracils (XIV*a* and *b*, and XV*a* and *b*). A syrupy mixture of α and β anomers of the acetylated nucleosides XIII*a* and *b* (0.001mole) was dissolved in 10 m*l* of absolute methanol, and 20 m*l* of NaOMe was added. The mixture was kept at 22° for 15 hours. Dowex-50 (H^+) ion exchange resin (1 m*l*) was added, the mixture was filtered, was filtered, and the resin was washed with 10 m*l* of methanol three times. The combined filtrate and washings were concentrated to a glassy residue, which was crystallized from ethanol to afford white crystals; for XIV*a*, 48% yield; m. p 200~201; λ_{max} 95 % EtOH nm ($E\times10^{-3}$) 271 (13.51), 211 (11.81); nmr (D_2O) δ6.13 (s, C_1—H), 4.18~4.72 (*m*, C_2—, C_3—, C_4—, C_5—, H), 8.24 (*d*, C_6—H), J_{H-1-F} 6 Hz; ORD

($c_2$0.00359, HO, 22°) $[\theta]_{270}+7700$.

Anal. Calc. for $C_9H_{11}FN_2SO_5$: C, 38.85; H, 4.00; F, 6.83; N, 10.07; S, 11.52. Found: C, 38.67; H, 4.24; F, 6.80; N, 10.35; S, 11.39.

For XIV*b*; 56 % yield; m.p 197~198°; $\lambda_{max}^{95\%\ EtOH}$ nm ($\epsilon\times10^{-3}$) 278 (10.29), 214 (11.76); nmr (D_2O) δ 6.01 (*s*, C_1–H), 4.18~4.72 (*m*, C_2—, C_3—, C_4—, C_5—H), 8.15 (*d*, C_6—H),

Anal. Calc. for $C_9H_{11}ClN_2SO_5$: C, 36.68; H, 3.76; Cl, 12.03; N, 9.50; S, 10.88. Found: C, 36.79; H, 3.59; Cl, 11.89; N, 9.38.

The mother liquors and washings were combined and concentrated *in vacuo* approximately to 2 m*l* which was applied to a column (2×60 cm) on a silica gel. The column was eluted with chloroform-methanol (6:1, v/v) mixture. A fraction containing the compound which was eluted from the column first, was collected and evaporated to obtain a glassy residue, and crystallized from ethanol; for XV*a*, 2 % yield; m.p 218~219°; $\lambda_{max}^{95\%\ EtOH}$ nm ($\epsilon\times10^{-3}$) 271 (13.51), 211 (11.26). For XV*b*, 5 % yield; m.p 221~223°; $\lambda_{max}^{95\%\ EtOH}$ nm ($\epsilon\times10^{-3}$) 277 (18.52), 214 (13.23).

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