〔Chem. Pharm. Bull.
15(10)1498~1502(1967)〕

UDC 547.963.3.07 : 547.854.2.07

191. Takao Sekiya and Tyunosin Ukita[*1]: Some Attempts on Substitution of 2′-Position of Uridine with Thiol Group.

(*Faculty of Pharmaceutical Sciences, the University of Tokyo*[*1])

The reactions of 2,2′-anhydrouridine (I) with hydrogen sulfide (Reaction A) and of I, 3′,5′-di-O-benzoyl-2,2′-anhydrouridine (Ⅲ) and 1-(3′,5′-di-O-benzoyl-2′-methanesulfonyl-β-D-arabinofuranosyl)uracil (V) with thiobenzoate or xanthogenate nucleophiles (Reactions B), were investigated with the purpose of introducing thiol substituents at the 2′-position. The Reaction A afforded 1-β-D-arabinofuranosyl-2-thiouracil (Ⅱ) as only sulfur containing product and the Reactions B gave no sulfur containing product but resulted in a partial liberation of uracil.

(Received November 22, 1966)

The analogues of natural nucleosides in which the hydroxyl groups of their sugar moieties are substituted with thiol groups should be interesting compounds not only as antimetabolite but also as useful tools in the research on biochemical significance of the hydroxyl groups in the natural nucleosides. Among several communications concerning with such modifications of nucleosides, especially in the pyrimidine nucleosides, there are reactions which involve an intermolecular attack of a nucleophilic reagent at an activated carbon atom in the sugar residue.

Leven and Tipson1) prepared 5′-deoxy-5′-thiobenzoyl-2′,3′-O-isopropylideneuridine by reaction of potassium thiobenzoate with 5′-O-tosyl-2′,3′-O-isopropylideneuridine. Chambers and Kurkov2) obtained 5′-deoxy-5′-thio-2′,3′-O-isopropylideneuridine besides 2-deoxy-2-thio-2′,3′-O-isopropylideneuridine by reaction of 2′,3′-O-isopropylidene-2,5′-anhydrouridine with hydrogen sulfide in the presence of triethyl amine. Miller and Fox3) reported a synthesis of 3′-deoxy-3′-thiobenzoylthymidine by thiobenzoate attack in the presence of benzoic acid at the 3′-position of 5′-O-trityl-2,3′-anhydrothymidine and the same authors4) also reported a similar nucleophilic attack by benzoate against 2′-position of 2,2′-anhydro-1-(3′,5′-di-O-benzoyl-β-D-arabinofuranosyl)uracil to convert the latter compound to a ribofuranosyluracil derivative. The present authors attempted these types of reaction with the purpose to obtain analogues of ribofuranosyluracil, the 2′-hydroxyl group of which is substituted by the thio-nucleophiles used.

The properties of the desired compound, which contains a more electron attracting substituent of larger atomic size instead of the hydroxyl group at 2′-position of natural nucleoside, if it could be obtained, should be valuable for understanding the correlations in structural and functional differences of RNA and DNA.

Analogously to the Chambers' method,2) the present authors treated 2,2′-anhydro-1-β-D-arabinofuranosyluracil (I)5) with hydrogen sulfide in the presence of triethylamine in dimethylformamide (DMF). On detection of the reaction products in paper chromatography, however, the only sulfur containing nucleoside found was 2-deoxy-2-thio-arabinofuranosyluracil (Ⅱ). The product (Ⅱ) was isolated as crystals in a yield of 70%. The structure of Ⅱ was confirmed by desulfurization of Ⅱ to 1-β-D-arabinofuranosyluracil with monochloro acetic acid according to the Brown's mdthod.6)

[*1] Hongo, Tokyo (関谷剛男, 浮田忠之進).

1) P.A. Levene, R.S. Tipson: J. Biol. Chem., **106**, 113 (1934).

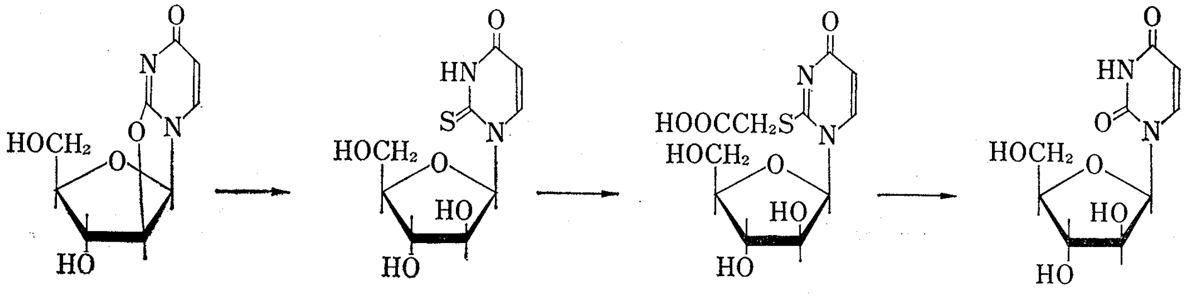
2) R.W. Chambers, V. Kurkov: J. Am. Chem. Soc., **85**, 2160 (1963).

3) N. Miller, J.J. Fox: J. Org. Chem., **29**, 1772 (1964).

4) J.J. Fox, N. Miller: *Ibid.*, **28**, 936 (1963).

5) J.E. Codington, I.L. Doerr, J.J. Fox: *Ibid.*, **29**, 558 (1964).

6) D.J. Brown: J. Soc. Chem. Ind., **69**, 353 (1950).



I II

Chart 1.

In the next experiment, the 2,2′-anhydrouridine was applied to the reaction similar to that reported by Miller and Fox.³⁾ The potassium thiobenzoate used in the reaction was freshly prepared and before the reaction, the stability of the reagent in hot DMF was checked. When the solution of the reagent in DMF was refluxed with bubbling of nitrogen, the typical absorption of the reagent at 245 mμ in acidic solution began to decrease with simultaneous increase of that of benzoate at 230 mμ in acidic solution. In Fig. 1, OD ratios of 245 mμ and 230 mμ of acidic solution of the salts, which were obtained after removal of the solvent from the mixture, were plotted against time, thus the Fig. shows that after 5 hours the majority of the reagent was converted to benzoate. In order to check the reactivity of the reagent, it was reacted with 5′-O-tosyl-2′,3′-O-isopropylideneuridine in DMF at 100° for 3 hours according to Leven and Tipson's report,¹⁾ and the production of 5′-deoxy-5′-thiobenzoyl-2′,3′-O-isopropylideneuridine was confirmed. The solution of the compound (I) in DMF was refluxed with freshly prepared potassium thiobenzoate in the presence of benzoic acid under bubbling of nitrogen. On separation by paper chromatography and spectrophotometric estimation, the products obtained from the mixture after 10 hours' reaction gave no sulfur containing product but it contained 31% of the starting compound (I), uracil (in a yield of 20%) and a monobenzoyl uridine which gave uridine on alkali hydrolysis and these products were already detected by paper chromatography in the reaction mixture after 1 hour's reflux.

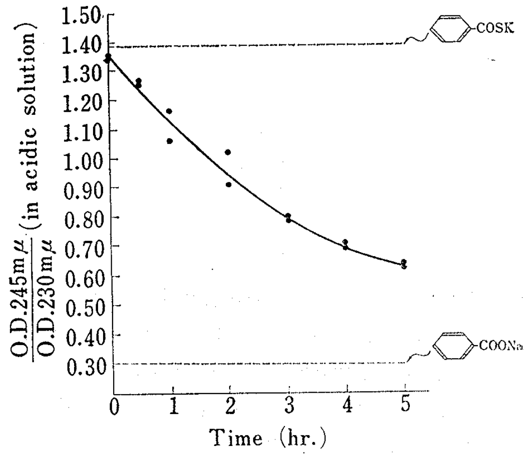


Fig. 1. The Decomposition of Potassium Thiobenzoate in Boiling Dimethylformamide. Experimental Detail in Text

Next, 3′,5′-di-O-benzoyl-2,2′-anhydrouridine (III) was submitted to the similar reaction and after 10 hours' reflux of the mixture, the product was hydrolysed with alkali to remove the benzoyl group. On detection and estimation of the product treated as described above, uridine, uracil and spongouridine were found in the respective yields of 25, 41.5 and 33.5%.

The production of considerable amounts of uracil in the both reactions suggested that the cleavage of the N-glycosyl bonds occurred as a result of attack by the nucleophile on 2′-carbon atom of the compounds (I) and (III) which involve structural hindrance by the 2,2′-anhydro bonds. In order to avoid the hindrance, the similar reaction was performed for 1-(3′,5′-di-O-benzoyl-2′-methanesulfonyl-β-D-arabinofuranosyl)uracil (V) which was prepared by mesylation by usual method of 1-(3′,5′-di-O-benzoyl-β-D-arabinofuranosyl)uracil (IV).⁷⁾ A solution of the mesylate (V) and potassium thiobenzoate in

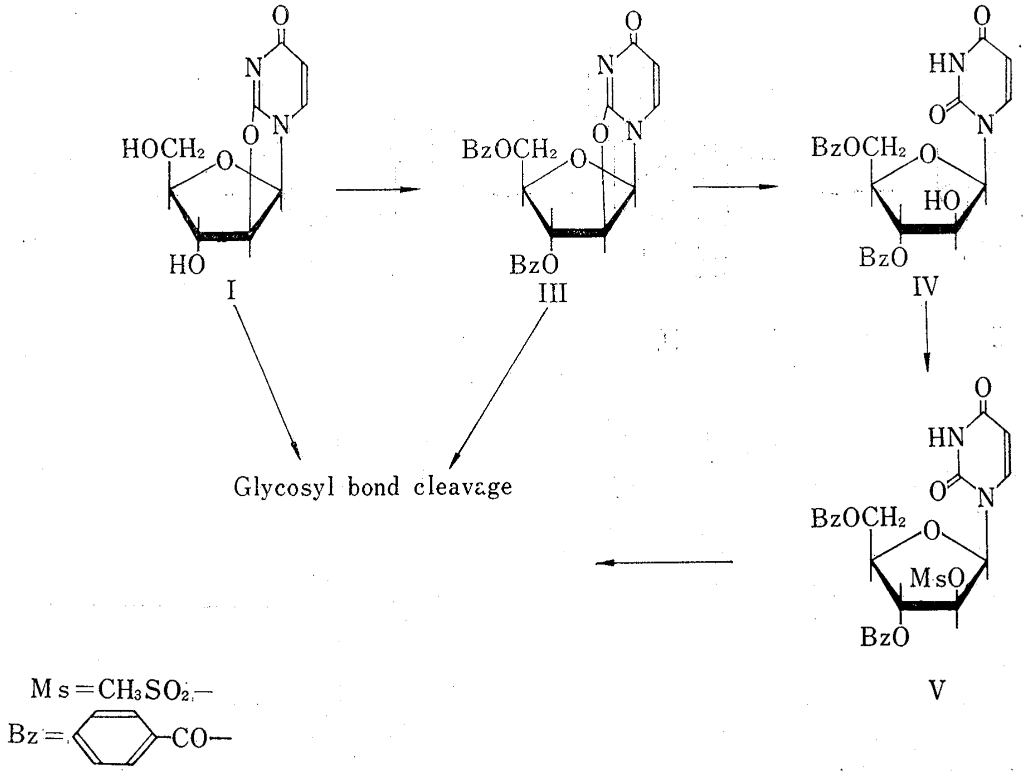


Chart 2.

DMF was refluxed under bubbling of nitrogen for 1.5 hours. The reaction mixture was separated into water soluble and insoluble fractions and the latter was submitted to alkali hydrolysis. On detection of the products by paper chromatography, uracil was found from the water soluble fraction and uridine and spongouridine from the alkaline hydrolysate.

The occurrence of uridine in the reactions described hitherto could be explained by an attack of benzoate nucleophile, which was produced from thiobenzoate, on 2′-carbon. In the reactions for III and V, however, similarly to the case reported by Codington, *et al.*,[5,7,8] a possibility of an intramolecular attack on the 2′-carbon by carbonyl group contained in benzoyloxy residue at 3′-position could be involved. The spongouridine may be the product of hydrolysis of unreacted anhydro nucleoside (III). It may also be produced from unreacted V *via* 2,2′-anhydrouridine.[*2]

The intermolecular nucleophilic reactions for III and V were performed using potassium xanthogenate instead of potassium thiobenzoate, but in these cases also no desired substitution of 2′-position of these compounds was observed.

Experimental

Paper Chromatography and Spectrophotometrical Determination——Unless otherwise described, paper chromatography was performed using Toyo Roshi No. 53 paper using two solvents: solvent 1,

---

[*2] From the reaction mixture of V and 0.75 mol. equivalent ethanolic NaOH, 2,2′-anhydrouridine and a compound which was assumed to be monobenzoyl 2,2′-anhydrouridine from UV spectrum were detected and when V was treated with 1.5 mol. equivalent ethanolic NaOH, the product contained 2,2′-anhydrouridine and spongouridine. 3.8 mol. equivalent ethanolic NaOH converted V to spongouridine as a sole product. Thus, V must be converted to spongouridine with alkali *via* 2,2′-anhydrouridine which should be formed from an 2′,3′-epoxy ribofuranosyl type precursor.

7) J.F. Codington, R. Fecher, J.J. Fox: J. Am. Chem. Soc., **82**, 2794 (1960).

8) *Idem*: J. Org. Chem., **27**, 163 (1962).

n-BuOH–H_2O (84:16) and solvent 2, iso-PrOH–conc. NH_4OH–H_2O (7:1:2) by ascending technique. The spectrophotometric determination of the spots was carried out with appropriate solvent using Hitachi Recording spectrophotometer Type EPS-2U.

2-Deoxy-2-thio-1-β-D-arabinofuranosyluracil (II)——To a solution of 200 mg. (0.88 mmol.) of 2,2′-anhydro-1-β-D-arabinofuranosyluracil[5] dissolved in 40 ml. of dry dimethylformamide (DMF), which was placed in a pressure tube, was added 2 ml. of triethylamine. The solution was cooled in an ice-bath and bubbled with dry hydrogen sulfide for 5 hr. The tube was sealed and the deep green reaction solution was set aside at room temperature for two days. The reaction mixture was evaporated to dryness and the brown residue was dissolved in a small amount of 50% aqueous EtOH and preparatively chromatographed on sheets of Toyo Roshi No. 514 papers in solvent 1. The ultraviolet light absorbing band which colored green with Grote's reagent[9] (Rf=0.40) was extracted with water, the extract once filtered and the filtrate was evaporated to leave white solid. On crystallization from EtOH, the solid gave 154 mg. of needles which melted at 193~195° (decomp.), yield 70%.

The product showed m.p. 199~202° (decomp.) after three recrystallizations from EtOH. $[\alpha]_D^{24}$ +107° (c=0.17, H_2O). UV $\lambda_{\max}^{\mathrm{pH\,1}}$ mμ (ε): 276 (14200), $\lambda_{\min}^{\mathrm{pH\,1}}$ 244 (3900), $\lambda_{\max}^{\mathrm{pH\,7}}$ 274 (14200), $\lambda_{\min}^{\mathrm{pH\,7}}$ 246 (5400), $\lambda_{\max}^{\mathrm{pH\,13.4}}$ 241 (21700), 267.5 (13950), $\lambda_{\min}^{\mathrm{pH\,13.4}}$ 261 (13850). *Anal.* Calcd. for $C_9H_{12}O_5N_2S$: C, 41.87; H, 4.65; N, 10.76; S, 12.32. Found: C, 42.11; H, 4.80; N, 10.75; S, 12.33.

To a solution of *ca.* 2 mg. of the product in a mixture of 0.3 ml. of EtOH and 0.2 ml. of H_2O was added 0.3 ml. of 10% aqueous monochloroacetic acid and the mixture refluxed for 40 min. After addition of 2 drops of conc. HCl, the reflux was continued for additional 2 hr. and the mixture neutralized with conc. NH_4OH. By this treatment the product (II) was converted to 1-β-D-arabinofuranosyluracil which was identified with authentic specimen, prepared according to the report described by Brown, *et al.*,[10] by paper chromatography run in solvents 1 and 2.

Preparation of Potassium Thiobenzoate and Its Stability in DMF——An excess of commercial thiobenzoic acid (2.0 g.: 14.5 mmol.) (Tokyo Kasei Kogyo Co., Ltd.) was dissolved in 8 ml. of aqueous KOH (containing 0.75 g.: 13.4 mmol. of KOH), the solution was shaken with ether to remove free thiobenzoic acid. The aqueous layer was then evaporated *in vacuo* under reduced pressure to dryness to give 2.0 g. of yellow powder. *Anal.* Calcd. for C_7H_5OSK: C, 47.70; H, 2.86. Found: C, 47.83; H, 3.03. This product colored green with Grote's reagent.

Twenty milligrams of the fresh potassium thiobenzoate thus obtained were dissolved in 1.5 ml. of DMF and the solution refluxed in a nigrogen atmosphere. Aliquotes of the reaction mixture were withdrawn in intervals and estimated spectrophotometrically to follow the absorbances at 245 mμ (absorption maximum of thiobenzoate) and at 230 mμ (that of benzoate) in acidic aqueous solution. The results is given in Fig. 1, thus it shows a time dependent decrease of the specific OD ratio at 245 mμ to 230 mμ of thiobenzoate and after 5 hr., the spectrum was entirely similar to that of benzoate instead of thiobenzoate.

The potassium thiobenzoate thus prepared was reacted with 5′-O-tosyl-2′,3′-O-isopropylideneuridine according to the report of Leven and Tipson[1] in DMF at 100° for 3 hr. and from the reaction mixture 5′-S-benzoyl-2′,3′-O-isopropylideneuridine was isolated as described by those authors.

1-(3′,5′-Di-O-benzoyl-2′-methanesulfonyl-β-D-arabinofuranosyl)uracil (V)——To a solution of 727 mg. (1.6 mmol.) of 1-(3′,5′-di-O-benzoyl-β-D-arabinofuranosyl)uracil (IV)[7] in 15 ml. of dry pyridine was added dropwise 0.35 ml. of methanesulfonyl chloride under ice cooling. The mixture was kept at 5° overnight and subsequently poured into 500 ml. of ice water. White precipitate that occurred was filtered and repeatedly washed with water. The precipitate was recrystallized from EtOH to afford 723 mg. of needles having m.p. 184~185°, from which sulfur was positively detected by fusion of the crystal with sodium and followed coloration with sodium nitroprusside reagent. The yield was 85%. The product (V) melted at 185.5~187° (decomp.) after two recrystallizations from EtOH. $[\alpha]_D^{23}$ +64° (c=0.46, $CHCl_3$). *Anal.* Calcd. for $C_{24}H_{22}O_{10}N_2S$: C, 54.34; H, 4.18; N, 5.28; S, 6.04. Found: C, 53.78; H, 4.19; N, 5.28; S, 6.26.

Reactions of 2,2′-Anhydro-1-β-D-arabinofuranosyluracil (I) and Its Di-O-benoate (III) with Potassium Thiobenzoate or Potassium Xanthogenate——To a solution of 23.6 mg. (0.1 mmol.) of the compound (I) in 5 ml. of DMF was added 12.2 mg. (0.1 mmol.) of benzoic acid and 35.2 mg. (0.2 mmol.) of potassium thiobenzoate, and the mixture was refluxed for 10 hr. in nitrogen atmosphere. The reaction mixture was then applied to paper chromatography in solvents 1 and 2. The chromatogram revealed three spots of the starting compound (I) (solvent 1: Rf=0.19 and solvent 2: Rf=0.66), uracil (solvent 1: Rf=0.39 and solvent 2: Rf=0.58) and a product presumably corresponding to a monobenzoyl derivative of uridine $\left(\frac{\mathrm{OD}_{230\,\mathrm{m}\mu}}{\mathrm{OD}_{260\,\mathrm{m}\mu}}=1.55,\ \text{solvent 1: Rf}=0.67\right)$ which gave uridine by alkaline hydrolysis. The spots of compound (I) and uracil were eluted with H_2O and that of monobenzoyl uridine with 50% EtOH. The extracts were estimated spectrophotometrically by usual method to give the respective yields of 31 and 20.5% for the compound (I)

---

[9] I.W. Grote: J. Biol. Chem., 93, 25 (1931).

[10] D.M. Brown, A. Todd, S. Varadarajan: J. Chem. Soc., **1956**, 2388.

and uracil. Thus, by this reaction *ca.* 20% of the starting material was decomposed to uracil and *ca.* 50% was converted to a monobenzoyluridine. These three products were already detected in similar chromatography even when the reaction time was limited to 1 hr.

2,2′-Anhydro-1-(3′,5′-di-O-benzoyl-β-D-arabinofuranosyl)uracil[4] (Ⅲ) (0.02 mmol.) was similarly reacted with potassium thiobenzoate (0.02 mmol.) in 1 ml. of DMF in the presence of benzoic acid (0.02 mmol.). To a 0.1 ml. aliquot of the mixture was added 0.5 ml. of EtOH and one drop of 5*N* NaOH solution and the mixture was refluxed for 30 min. to remove the benzoyl groups. Then the products was detected by paper chromatography run in solvents 1 and 2. Spots which were found on the chromatograms were eluted with water and estimated by spectrophotometry and the products found were uridine (yield: 25%) and spongouridine (41.5%) and uracil (33.5%).

The compound (Ⅲ) (0.02 mmol.) was similarly reacted with potassium xanthogenate (0.02 mmol.) in the presence of benzoic acid (0.02 mmol.) and the reaction mixture was treated as described above. Spongouridine (yield: 80%), uridine (17%) and small amount of uracil were found.

The Reaction of 1-(3′,5′-Di-O-benzoyl-2′-methanesulfonyl-β-D-arabinofuranosyl)uracil (Ⅴ) with Potassium Thiobenzoate or Potassium Xanthogenate——To a solution of 50 mg (0.1 mmol.) of the compound (Ⅴ) in 5 ml. of DMF was added 105 mg. (0.6 mmol.) of potassium thiobenzoate and the mixture was refluxed in nitrogen atmosphere for 1.5 hr. The mixture was evaporated *in vacuo* and the residue triturated with water. The aqueous extract was then detected in paper chromatography run in solvents 1 and 2, to find uracil as an only UV-absorbing spot. The water insoluble part of the residue was dissolved in EtOH and after hydrolysis with alkali of the products as was described in preceding paragraph, the hydrolysate was applied to the similar chromatography. Of the two UV-absorbing spots, the major one was that of spongouridine and the minor was uridine.

Similarly 103 mg. (0.2 mmol.) of the compound (Ⅴ) was reacted with 160 mg. (1.0 mmol.) of potassium xanthogenate in 5 ml. of DMF at 100~110° for 135 hr.. Then reaction mixture was treated as described above and subsequently applied to the paper chromatography in solvent 1. Spongouridine as major, uridine and uracil as minor UV-absorbing product were found.

The authors wish to thank Takeda Chemical Industries Co,. Ltd. for his gift of the starting material. They are also indebted to the members of the Central Analysis Room of this Faculty for their carrying out the elemental analyses.