192. Takao Sekiya and Tyunosin Ukitan: Synthesis of 2′-Deoxy-2′-thio-3′-deoxy-3′-aminouridine.†

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A complex neighboring approach provided a successful synthesis of 2′-deoxy-2′-thio-3′-deoxy-3′-aminouridine (X). 1-(3′-Deoxy-3′-amino-β-D-arabinofuranosyl)uracil (I) afforded, in three steps, the blocked dithiocarbamoyl mesylate (IV), which, on heating in pyridine, cyclized to the thiazoline (V), that was deblocked to VI and reduced to the thiazolidine (VII). Compound (VII) was successively treated with mercuric chloride and hydrogen sulfide to furnish the desired product (X).

(Received November 22, 1966)

Recently we reported that several trials to substitute the hydroxyl group of the 2′-position of uridine with thiol group by an intermolecular attack on the appropriately activated 2′-carbon atom with thiobenzoyl or xanthogenate nucleophiles resulted in a cleavage of N-glycosyl bond to liberate uracil. But in those reactions considerable amount of uridine was also obtained. As one of the possible explanations for the appearance of uridine, especially in the products obtained from 3′,5′-di-O-benzoyl-2′-anhydouridine or 1-(3′,5′-di-O-benzoyl-2′-O-methanesulfonyl-β-D-arabinofuranosyl)uracil, we have proposed an intramolecular nucleophilic attack at 2′-carbon by carbonyl group of benzoyloxy residue present at the neighboring 3′-position of the parent compounds.

In 1963, Goodman and Christensen reported the synthesis of methyl 3-deoxy-3-amino-2-deoxy-2-thio-α-D-ribofuranoside starting from methyl 3-deoxy-3-amino-α-D-arabinofuranoside by an intramolecular nucleophilic attack on 2-position by the thio-carbonyl residue previously substituted at neighboring 3-amino group of the mother compound.

The present authors attempted the application of this reaction to pyrimidine nucleoside and succeeded in synthesizing 2′-deoxy-2′-thio-3′-deoxy-3′-aminouridine. As the starting compound, 1-(3′-deoxy-3′-amino-β-D-arabinofuranosyl)uracil (I) was synthesized from uridine according to the report of Codering, et al., in a total yield of 40%. This compound was reacted with carbodisulfide in pyridine in the presence of triethylamine and successively methylated with methyl iodide. Upon separation of the product through cellulose column chromatography, the desired product (II), which has dithiocarbomethoxyamino group at the 3′-position of the starting compound (I), was obtained as needles in a yield of 73%. The elementary analysis of II coincided with those required for C11H15O5N3S3. The compound (II) colored green with Grote's reagent on paper chromatogram, gave UV-absorption spectrum similar to N4-substituted uracil and showed no positive charge in electrophoresis run in pH 4.0. In the next step, II was reacted with methyl chloroformate in pyridine to introduce methoxy carbonyl group at 5′-hydroxyl group of II. The product (III) was obtained as amorphous solid in 86.5% yield and the elementary analysis of the product was in accord with those calculated for

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† This paper was partially communicated in This Bulletin, 15, 542 (1967).
†† On paper chromatogram, this reagent which was reported by Grote (I. W. Grote : J. Biol. Chem., 93, 25 (1931)) for detection of organic sulfur compounds, colored green with compound containing C=S and C-SH, but it did not color when the thiol group was oxidized to C-S-S-C.
$\text{C}_{12}\text{H}_{17}\text{O}_{4}\text{N}_{3}\text{S}_{2}$ and $\text{III}$ revealed an IR-absorption at $1765\text{ cm}^{-1}$ which corresponded to ester $\text{C}=\text{O}$. The compound (III) was subsequently mesylated as usual with methanesulfonyl chloride and the $2'$-mesylester of III, (IV), was isolated as amorphous yellow powder in ca. 65% yield. This product gave a specific IR-absorption at $1180\text{ cm}^{-1}$ for sulfonate ester besides that for ester carbonyl. On refluxing a pyridine solution of the product (IV) in nitrogen atmosphere, the intramolecular nucleophilic attack of $2'$-carbon by the thiocarbonyl group substituted at $3'$-amino group caused a formation of a thiazoline ring. After evaporation of the solvent in vacuo, the product (V) was extracted with hot benzene to give a glass in ca. 53% yield. The product (V) showed a strong absorption
at 260 m\(\mu\) in acidic solution and its IR-spectrum revealed a new absorption at 1565 cm\(^{-1}\) caused by C=N group in thiazoline ring as well as the absorption for ester C=O but lacked that for sulfonate ester. The methoxycarbonyl group at 5'-position of \(\psi\) was removed by treatment of \(\psi\) with sodium methoxide and the product (\(\psi\)) was purified through cellulose column chromatography to give pure \(\psi\) as needles, the yield of \(\psi\) calculated from \(\Pi\) was 18\%. The product \(\psi\) also showed strong UV-absorption at 260 m\(\mu\) in acidic solution and IR-absorption for C=N group of thiazoline ring at 1558 cm\(^{-1}\) and lacked that for carbonyl group.

This compound (\(\psi\)) was also formed directly from \(\psi\) when \(\psi\) was treated with methanolic sodium methoxide. The compound (\(\psi\)) was reduced with aluminum-amalgam in tetrahydrofuran and the product (\(\psi\)) was obtained as amorphous glass in 68\% yield. On treatment of this compound (\(\psi\)) with aqueous mercuric chloride, the thiazolidine ring was decomposed to give mercuric chloride complex of 2'-deoxy-2'-thio-3'-deoxy-3'-aminouridine hydrochloride (\(\psi\)) as powder in a yield from \(\psi\) of 44\%. Removal of mercury from this complex was performed by bubbling hydrogen sulfide through a suspension of \(\psi\) in methanol and the product was obtained as amorphous white powder. Paper chromatography of the product run in solvent 1 (see Experimental) revealed a distinct spot (RF 0.07) of the main product and two additional faint spots at RF 0.01 (A) and 0.11 (B). The main spot, that of 2'-deoxy-2'-thio-3'-deoxy-3'-aminouridine hydrochloride (\(\psi\)), showed positive reactions to the sulfur test with Grote’s reagent and to periodate-benzidine. With ninhydrin it revealed similar yellow coloration to that for cytoine and cytoine hydrochloride. The compound (\(\psi\)) migrated to cathode on electrophoresis in pH 4.0, similarly to I, indicating its mono-positive net charge. The both faint spots also absorbed UV-light and the spot A was assumed to be that of disulfide derivative of \(\psi\), because when \(\psi\) was oxidized with iodine it was converted to a compound which gave RF 0.01 in the solvent 1 and, moreover, this compound as well as that of the spot A did not color with Grote’s reagent. As for the minor product which gave spot B, no further research was performed.

The compound (\(\psi\)) was benzoylated in pyridine as usual and the benzoate (X) was isolated as amorphous solid in 51\% yield calculated from \(\psi\) which run as single spot on thin-layer chromatography and gave analytic values required for 5'-O-benzoyl-2',3'-dideoxy-2'-benzoylthio-3'-benzoylaminouridine (X).

The study on the biological activities of the new analogues of uridine thus obtained is now under progress.

**Experimental**

**Paper Chromatography and Spectrophotometric Determination**—Paper chromatography was performed by ascending technique using Toyo Roshi No. 53 paper in three solvents, solvent 1, \(n\)-BuOH-H\(_2\)O (84:16), solvent 2, iso-PrOH-conc. NH\(_2\)OH-H\(_2\)O (7:1:2); and solvent 3, methyl ethyl ketone that was saturated with H\(_2\)O. The spectrophotometric determination of the separated spot was carried out for the extract of the spot with appropriate solvent in Hitachi Recording spectrophotometer Type EPS-2U and the molar absorptivity of the compounds was determined in Cary Recording Spectrophotometer 11.

**Paper Electrophoresis**—Paper electrophoresis was carried out at pH 4.0 in 0.01\(M\) acetate buffer on Toyo Roshi No. 53 at 22.5 V/cm. for 1 hr. \(M_{\text{uired}}\) represents the relative mobility towards cathode of the compound to that of uridine.

1-[3'-Deoxy-3'-(dithiocarbamothio)amino-\(\beta\)-D-arabinofuranosyl]uracil (\(\Pi\))—To a solution containing 3.0 g. (12.4 mmol.) of 1-[3'-deoxy-3'-amino-\(\beta\)-D-arabinofuranosyl]uracil (\(\Pi\)) and 1.74 ml. (12.5 mmol.) of triethylamine in 100 ml. of dry pyridine was added dropwise 0.75 ml. (12.5 mmol.) of carbon disulfide under stirring and ice cooling. After additional 1 hr. stirring under ice cooling, 0.78 ml. (12.5 mmol.) of methyl iodide was added dropwise to the cooled mixture under continuous stirring. The mixture was then set aside at 4-7\(^\circ\)C overnight and subsequently evaporated to dryness. The remaining pyridine in the residue was removed by repeated codistillation with EtOH below 40\(^\circ\). The residual yellowish brown gum was dissolved in a minimum volume of a mixture of \(n\)-BuOH and H\(_2\)O (84:16), the solution applied to a cellulose column (3.5x40 cm.) and elution was performed with the same mixed solvent. The fractions which,
on paper chromatography, gave single spot detectable by UV-absorption and by coloration with Grotes reagent at Rf 0.69, 0.72 and 0.77 (run in respective solvent 1, 2 and 3), were combined.

Solvant was evaporated from the solution in vacuo leave 4.02 g. (yield 81.5%) of white solid which was recrystallized from EtOH to give 3.13 g. of colorless needles having m.p. 172-174°C (decomp.) (yield 73%). After three recrystallizations from EtOH, the product (II) melted at 176.5-177°C (decomp.) and gave M mole, 8.88 (I) gave M mole, 2.82. \( \text{C}_{13}\text{H}_{26}\text{N}_2\text{O}_4 \) + 78°C (c = 0.44, MeOH). UV \( \lambda_{	ext{max}} \) (nm): 265 (22000), 275 (22000), 225 (4600), 290 (13700). Anal. Calcd. for C13H26N2O4S: C, 39.63; H, 4.55; N, 12.61; S, 19.24. Found: C, 39.55; H, 4.80; N, 12.35; S, 19.18.

1-[5'-O-Methoxy carbonyl-3'-deoxy-3'-(dithiocarboxymethoxy)amino-β-D-arabinofuranosyl]uracil (III)—To a solution of 2.37 g. (7.1 mmol.) of the compound (II) in 15 mL of pyridine was added dropwise a solution of 0.58 mL (7.5 mmol.) of methylichlorofomate dissolved in 7.5 mL of chloroform under ice cooling and stirring. After stirring for additional 30 min. at 0°C, the mixture was kept at room temperature overnight under continuous stirring. In order to complete the methoxy carbonylation, an additional 0.58 mL of methylichlorofomate dissolved in 7.5 mL of chloroform was added dropwise to the above mixture and stirring was continued overnight at room temperature. The solvent and the reagent were removed by distillation in vacuo and the residue was dissolved in CHCl3. The solution was then extracted twice with a saturated sodium bicarbonate solution and washed with water. On evaporation of the solvent from chloroform solution dried with Na2SO4, 2.4 g. of the product (II) was obtained as colorless glassy solid which gave a single spot (Rf 0.61) on paper chromatography run solvent in 1. yield was 86.5%; UV \( \lambda_{	ext{max}} \) (nm): 265, 275 (11000), 225 (22000), 290, IR \( \nu_{	ext{max}} \) cm\(^{-1}\): 1765 (ester C=O). Anal. Calcd. for C13H26N2O4S: C, 39.89; H, 4.38; N, 10.74. Found: C, 39.87; H, 4.65; N, 10.18.

1-[5'-O-Methoxy carbonyl-3'-deoxy-3'-(dithiocarboxymethoxy)amino-2'-O-methanesulfonyl-β-D-arabinofuranosyl]uracil (IV)—To a solution of 3.5 g. (9.0 mmol.) of the compound (II) in 20 mL of anhydrous pyridine was added dropwise 2.0 g. (1.75 mmol.) of methanesulfonyl chloride. The mixture was stirred for 1 hr. at 0°C and subsequently kept aside overnight at 4°C. The deep green reaction solution was poured into 1 L. of ice water with vigorous stirring and a pale yellow precipitate that occurred was filtered, thoroughly washed with water and dried to obtain 2.7 g. of the product (V) (yield 65%) as a pale yellow powder which gave a single UV-absorbing spot (Rf 0.85) on a paper chromatogram run in solvent 1. UV \( \lambda_{	ext{max}} \) (nm): 265, 275 (11000), 225, 290, IR \( \nu_{	ext{max}} \) cm\(^{-1}\): 1180 (sulfonate ester), 1760 (ester C=O).

1-[5'-O-Methoxy carbonyl-2', 3'-dideoxy-2'-thiomethyl-(3', 2': 4', 5')-2'-thiazoline)-β-D-arabinofuranosyl]uracil (V)—A 50 mL of anhydrous pyridine solution which contained 2.5 g. of the compound (V) was refluxed in nitrogen atmosphere for 2 hr. in an oil bath of 140°C. From the reaction mixture, solvent was evaporated in vacuo. The remaining pyridine in the residue was entirely removed repeated distillation with EtOH added. A brown glassy residue finally obtained was extracted ten times by boiling with each 50 mL of benzene. On removal in vacuo of the solvent from the combined benzene extract, 1.0 g. (yield ca. 53%) of a glassy solid was obtained which gave a single UV-absorbing spot (Rf 0.75) on a paper chromatogram run in solvent 1. UV \( \lambda_{	ext{max}} \) (nm): 259, 275, 285, 290, 260, IR \( \nu_{	ext{max}} \) cm\(^{-1}\): 1762 (ester C=O), 1695 (C=N).

1-[2', 3'-Dideoxy-2'-thiomethyl-(3', 2': 4', 5')-2'-thiazoline)-β-D-arabinofuranosyl]uracil (VI)—To a solution of 0.55 g. of the compound (V) in 20 mL of MeOH was added 1 mL of methanolic sodium methoxide which was prepared by dissolving 1 g. of sodium in 10 mL of MeOH. The mixture was stirred at room temperature overnight and subsequently neutralized with acetic acid and evaporated to dryness. The yellow glassy residue that obtained was dissolved in a minimum volume of a mixed solvent, n-ButOH-H2O (84:16), and the solution submitted to a cellulose column (3 x 42 cm). The column was eluted with the same solvent and fractions, which gave a spot of Rf 0.65 and 0.66 (detected by UV-absorption) on paper chromatography run in respective solvents 1 and 3, were combined. On evaporation of the solvent from the solution, a white solid was obtained which was recrystallized from EtOH to 0.26 g. of colorless needles (VI), m.p. 205-207°C (decomp.), yield was 56% calculated from V and 18% from II. After three recrystallizations from EtOH the product melted at 206.5-207°C (decomp.) and gave M mole, 0.82. \( \beta \) \( \beta \) -42°C (c = 0.42, MeOH). UV \( \lambda_{	ext{max}} \) (nm): 260 (22000), 260 (227, 5200), 275.5 (12500), 290 (3400), 290, IR \( \nu_{	ext{max}} \) cm\(^{-1}\): 1558 (C=N). Anal. Calcd. for C13H26N2O4S2: C, 41.89; H, 4.15; N, 13.32; S, 20.33. Found: C, 42.06; H, 4.10; N, 13.55; S, 20.40.

1-[2', 3'-Dideoxy-3', 2': 4', 5')-thiazolidine-β-D-arabinofuranosyl]uracil (VII)—The product (VI) (151 mg, 0.47 mmol.) was dissolved in 15 mL of tetrahydrofuran and the solution was added aluminum amalgam which was prepared from 600 mg of aluminum foil. After addition of 1.5 mL of water to the mixture, it was warmed on a water bath at 55-60°C for 1 hr. with occasional shaking. The mixture was then cooled and filtered on a celite filter. On evaporation of the filtrate, was obtained 88 mg. of a white glass which gave each single spot of Rf 0.26 and 0.27 (detected by UV-absorption) on paper chromatogram run in respective solvents 1 and 3, yield was 68%. UV \( \lambda_{	ext{max}} \) (nm): 261.5, 275, 233, 265.5, 267, 267, 290, 290, 340, 216. Mercuric Chloride Complex of 1-[2'-deoxy-2'-thio-3'-deoxy-3'-amino-β-D-arabinofuranosyl]uracil Hydrochloride (VIII)—To a solution of 88 mg. of the compound (VI) in 5 mL of water was added dropwise a saturated aqueous solution of mercuric chloride until no more white precipitate occurred. The mixture was warmed on a boiling water bath for several minutes to dissolve the precipitate. On setting aside the solution at cool place overnight, a pale yellow precipitate appeared, which was filtered, washed with water and dried. The
pale yellow powder (Ⅵ) thus obtained weighed 168 mg. (yield 44%), calculated from (Ⅴ), showed positive
coloration for sulfur test (coloration with sodium nitroprusside after fusion of the compound with sodium)
and melted at 195.5°(decomp.). Anal. Calcd. for C₉H₈O₉N₅S·HCl·HgCl·HgCl₂: C, 13.49; H, 1.63; N, 5.23;
S, 4.00. Found : C, 14.10; H, 1.76; N, 5.27; S, 4.25.
1-2′-Deoxy-2′-thio-3′-deoxy-3′-amino-β-D-ribofuranosyl)uracil Hydrochloride (Ⅸ)——The mercuric
complex (Ⅵ) (231 mg, 0.23 mmol.) was suspended in 10 ml of MeOH and the mixture was bubbled with hydrogen
sulfide for 2 hr. until all suspension turned into black precipitation. The mixture was filtered through a
celite filter, and the filtrate was evaporated in vacuo to dryness. The residue was triturated and washed
with ether. The ether insoluble residual white powder (Ⅹ), which was dried over P₂O₅ and weighed 83 mg.
(yield 98%), showed positive coloration to sulfur test, positive Beilstein test for chlorine and revealed the
similar mobility to cathode, Mₑ₅₅₅₅, 2.55, to that of 1-(3′-deoxy-3′-amino-β-D-arabinofuranosyl)uracil (Ⅰ).
Mₑ₅₅₅₅, 2.82, indicating mono positive net-charge of the compound at pH 4.0. On paper chromatography
run in solvent 1, this product gave one major (Rf 0.07) and two faint (Rf 0.11 and 0.01) UV-absorbing spots.
The major spot showed positive reaction with Grote’s reagent, periodate–benzidine test, and colored yellow
with ninhydrin similarly to cysteine and its hydrochloride. UV λₑ₈₅ₐ₅₁ mₚ : 232, λₑ₈₅ₐ₄₄₇ 232, λₑ₅₈₅₅₂₅, λₑ₅₈₅₅₄₂₆₃. When the major spot was extracted with water and oxidized with iodine, the oxidation product revealed
Rf 0.01, the same Rf value to one of the faint spots, and similarly to the latter it gave no coloration with
Grote’s reagents. Thus the minor product having Rf 0.01 must be a disulfide derivative of the major product.
As for the other minor product having Rf 0.11, no further research was performed.
1-(3′-O-Benzoyl-2′; 3′-dideoxy-2′-benzoylthio-3′-benzoylamino-β-D-ribofuranosyl) uracil (Ⅹ)——The mercuric
salt (Ⅵ) (290 mg, 0.29 mmol.) was treated with hydrogen sulfide as above and after removal of mercuric
sulfide by filtration, the solvent was removed from the filtrate in vacuo and the residue (Ⅹ) was dried over
P₂O₅. As soon as the residue was dried, it was dissolved in 5 ml of anhydrous pyridine and to the solution
was added dropwise 0.14 ml (1.5 mmol.) of benzoyl chloride under ice cooling. The mixture was shaken and
kept at 50° overnight, subsequently poured into an ice-cooled saturated aqueous sodium bicarbonate solution.
The aqueous mixture was extracted twice with CHCl₃, the chloroform layer washed three times with water
and dried over Na₂SO₄. Chloroform was removed and the residue was washed several times with ether and
dissolved in EtOH. On setting aside of the alcoholic solution at room temperature, precipitate appeared from
the white turbid solution, which solidified on gradual evaporation of EtOH at room temperature. The solid
was taken and dried over P₂O₅ to afford 85 mg. (yield 51% from Ⅵ) of the product (Ⅹ). This product, on
thin-layer chromatography run in EtOAc, gave a single spot. Rf 0.53, detected by UV-absorption and by
heating after 10% H₂SO₄-spray. [α]ₓ²₀ = −39°(c=0.59, CHCl₃). UV λₑ₅₅₅₅ mₚ (ε) : 232 (30900). λₑ₂₅₅₅ 260
(17290). Anal. Calcd. for C₉H₈O₉N₅S·1/₂H₂O: C, 62.06; H, 4.51; N, 7.34; S, 5.52. Found : C, 61.97; H,
4.59; N, 7.33; S, 5.08.

The authors wish to thank Dr. Setsuzo Tejima of the University of Hokkaido for his valuable discussion;
Takeda Chemical Industries Co., Ltd. and Dai-ichi Seiyaku Co., Ltd. for their supplies of the starting
materials; and members of the Central Analysis Room of this Faculty and those of Seikagaku-Kogyo Co.,
Ltd. for carrying out the elemental analyses.