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192. Takao Sekiya and Tyunosin Ukita*1 : Synthesis of 2′-Deoxy-2′-thio-3′-deoxy-3′-aminouridine.*2

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A complex neighboring approach provided a successful synthesis of 2′-deoxy-2′-thio-3′-deoxy-3′-aminouridine (Ⅸ). 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 (Ⅸ).

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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 thiobenzoate or xanthogenate nucleophiles resulted in a cleavage of N-glycosyl bond to liberate uracil.[1] 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-2,2′-anhydrouridine 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[2] 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 thiocarbonyl 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 Codington, *et al.*[3] in a total yield of 40%. This compound was reacted with carbondisulfide 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 $C_{11}H_{15}O_5N_3S_2$. The compound (Ⅱ) colored green with Grote's reagent*3 on paper chromatogram, gave UV-absorption spectrum similar to N_1-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 methyloxycarbonyl group at 5′-hydroxyl group of Ⅱ. The product (Ⅲ) was obtained as amorphus solid in 86.5% yield and the elementary analysis of the product was in accord with those calculated for

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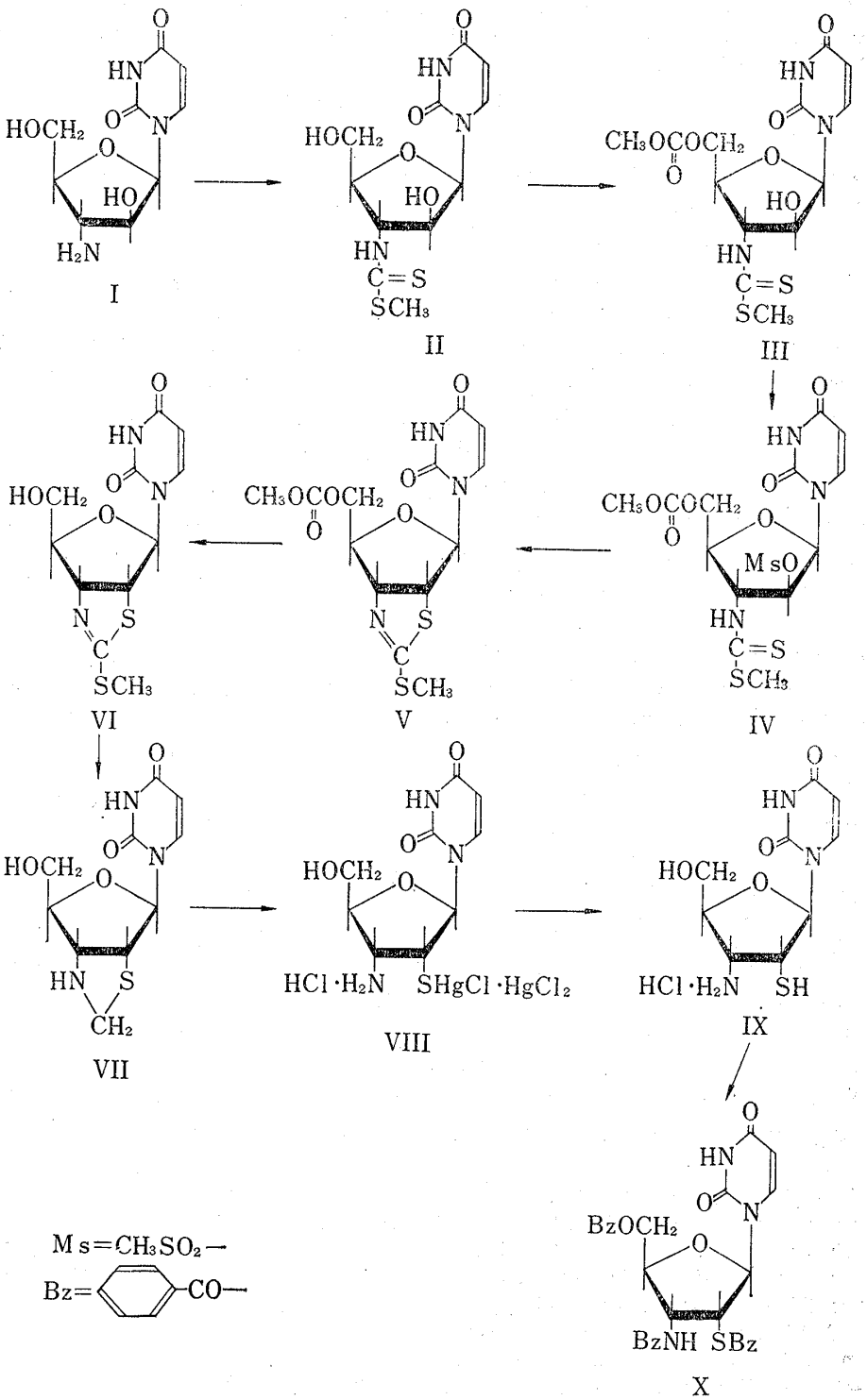
*2 This paper was partialy communicated in This Bulletin, **15**, 542 (1967).

*3 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.

1) T. Sekiya, T. Ukita : This Bulletin, **15**, 1498 (1967).
2) L. Goodman, J. E. Christensen : J. Org. Chem., **28**, 2610 (1963).
3) J. F. Codington, R. Fecher, J. J. Fox : J. Org. Chem., **27**, 163 (1962).

$C_{13}H_{17}O_7N_3S_2$ and III revealed an IR-absorption at 1765 cm^{-1} which corresponded to ester C=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 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 mμ 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 V was removed by treatment of V with sodium methoxide and the product (VI) was purified through collulose column chromatography to give pure VI as needles, the yield of VI calculated from II was 18%. The product VI also showed strong UV-absorption at 260 mμ 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 (VI) was also formed directly from IV when IV was treated with methanolic sodium methoxide. The compound (VI) was reduced with aluminum-amalgam in tetrahydrofuran and the product (VII) was obtained as amorphous glass in 68% yield. On treatment of this compound (VII) with aqueous mercuric chloride, the thiazolidine ring was decomposed to give mercuric chloride complex of 2′-deoxy-2′-thio-3′-deoxy-3′-aminouridine hydrochloride (VIII) as powder in a yield from VI of 44%. Removal of mercury from this complex was performed by bubbling hydrogen sulfide through a suspention of VIII 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 (IX), 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 cysteine and cysteine hydrochloride. The compound (IX) 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 IX, because when IX 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 (IX) was benzoylated in pyridine as usual and the benzoate (X) was isolated as amorphous solid in 51% yield calculated from VIII 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_2O (84:16), solvent 2, iso-PrOH–conc. NH_4OH–H_2O (7:1:2); and solvent 3, methylethylketone saturated with H_2O. 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. $M_{urid.}$ represents the relative mobility towards cathode of the compound to that of uridine.

1-[3′-Deoxy-3′-(dithiocarbomethoxy)amino-β-D-arabinofuranosyl]uracil (II)——To a solution containing 3.0 g. (12.4 mmol.) of 1-(3′-deoxy-3′-amino-β-D-arabinofuranosyl)uracil (I)[3] 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.'s 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° overnight and subsequently evaporated to dryness. The remaining pyridine in the residue was removed by repeated codistillation with EtOH below 40°. The residual yellowish brown gum was dissolved in a minimum volume of a mixture of *n*-BuOH and H_2O (84:16), the solution applied to a cellulose column (3.5×40 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 Grote's reagent at Rf 0.69, 0.72 and 0.77 (run in respective solvent 1, 2 and 3), were combined.

Solvent 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°(decomp.) (yield 73%). After three recrystallizations from EtOH, the product (II) melted at 176.5~177°(decomp.) and gave $M_{urid.}$ 0.88 ((I) gave $M_{urid.}$ 2.82). $[\alpha]_D^{22}$ +78°(c=0.44, MeOH). UV $\lambda_{max}^{pH\,1\text{ and }7}$ mμ (ε): 265 (22200), $\lambda_{min}^{pH\,1\text{ and }7}$ 232 (4600), $\lambda_{shoulder}^{pH\,13.4}$ 260 (13700). *Anal.* Calcd. for $C_{11}H_{15}O_5N_3S_2$: 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-Methoxycarbonyl-3'-deoxy-3'-(dithiocarbomethoxy)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 methylchloroformate dissolved in 7.5 ml. of chloroform under ice cooling and stirring. After stirring for additional 30 min. at 0°, the mixture was kept at room temperature overnight under continuous stirring. In order to complete the methoxycarbonylation, an additional 0.58 ml. of methylchloroformate 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 $CHCl_3$. The solution was then extracted twice with a saturated sodium bicarbonate solution and washed three times with water. On evaporation of the solvent from chloroform solution dried with Na_2SO_4, 2.40 g. of the product (III) was obtained as colorless glassy solid which gave a single spot (Rf 0.81) on paper chromatography run in solvent 1, yield was 86.5%. UV $\lambda_{max}^{pH\,1\text{ and }7}$ mμ: 265, $\lambda_{min}^{pH\,1\text{ and }7}$ 232, $\lambda_{shoulder}^{pH\,13.4}$ 260, IR ν_{max}^{KBr} cm^{-1}: 1765 (ester C=O). *Anal.* Calcd. for $C_{13}H_{17}O_7N_3S_2$: C, 39.89; H, 4.38; N, 10.74. Found: C, 39.87; H, 4.65; N, 10.18.

1-[5'-O-Methoxycarbonyl-3'-deoxy-3'-(dithiocarbomethoxy)amino-2'-O-methanesulfonyl-β-D-arabinofuranosyl]uracil (IV)——To a solution of 3.5 g. (9.0 mmol.) of the compound (III) 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° and subsequently kept aside overnight at 4~7°. 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 (IV) (yield 65%) as a pale yellow powder which gave a single UV-absorbing spot (Rf 0.83) on a paper chromatogram run in solvent 1. UV $\lambda_{max}^{pH\,1\text{ and }7}$ mμ: 265, $\lambda_{min}^{pH\,1\text{ and }7}$ 232, $\lambda_{shoulder}^{pH\,13.4}$ 260. IR ν_{max}^{KBr} cm^{-1}: 1180 (sulfonate ester), 1760 (ester C=O).

1-[5'-O-Methoxycarbonyl-2', 3'-dideoxy{2''-thiomethyl-(3',2': 4'',5'')-2''-thiazoline}-β-D-ribofuranosyl]uracil (V)——A 50 ml. of anhydrous pyridine solution which contained 2.5 g. of the compound (IV) was refluxed in nitrogen atmosphere for 2 hr. in an oil bath of 140°. 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, 1.05 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 $\lambda_{max}^{pH\,1}$ mμ: 259, $\lambda_{max}^{pH\,7}$ 258, $\lambda_{shoulder}^{pH\,13.4}$ 260. IR ν_{max}^{KBr} cm^{-1}: 1762 (ester C=O), 1565 (C=N).

1-[2',3'-Dideoxy-{2''-thiomethyl-(3',2':4'',5'')-2''-thiazoline}-β-D-ribofuranosyl]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*-BuOH-H_2O (84:16), and the solution submitted to a cellulose column (3×42 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° (decomp.), yield was 56% calculated from V and 18% from II. After three recrystallizations from EtOH the product melted at 206.5~207°(decomp.) and gave $M_{urid.}$ 0.82. $[\alpha]_D^{23}$ −42°(c=0.42, MeOH). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 260 (22600), $\lambda_{min}^{pH\,1}$ 227 (5200), $\lambda_{max}^{pH\,7}$ 257.5 (12500), $\lambda_{min}^{pH\,7}$ 230 (9400), $\lambda_{shoulder}^{pH\,13.4}$ 260 (9500). IR ν_{max}^{KBr} cm^{-1}: 1558 (C=N). *Anal.* Calcd. for $C_{11}H_{13}O_4N_3S_2$: C, 41.89; H, 4.15; N, 13.32; S, 20.33. Found: C, 42.06; H, 4.10; N, 13.53; S, 20.40.

1-[2',3'-Dideoxy-(3',2':4'',5'')-thiazolidine-β-D-ribofuranosyl]uracil (VII)——The product (VI) (151 mg., 0.47 mmol.) was dissolved in 15 ml. of tetrahydrofuran and to 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° 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 $\lambda_{max}^{pH\,1}$ mμ: 261.5, $\lambda_{min}^{pH\,1\text{ and }7}$ 233, $\lambda_{max}^{pH\,7}$ 263.5, $\lambda_{max}^{pH\,13.4}$ 262, $\lambda_{min}^{pH\,13.4}$ 246.

Mercuric Chloride Complex of 1-(2'-deoxy-2'-thio-3'-deoxy-3'-amino-β-D-ribofuranosyl)uracil Hydrochloride (VIII)——To a solution of 88 mg. of the compound (VII) 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 minuites 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 (Ⅷ) thus obtained weighed 168 mg. (yield 44%, calculated from (Ⅵ)), 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 $C_9H_{12}O_4N_3S \cdot HCl \cdot HgCl \cdot HgCl_2$: 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 (Ⅷ) (231 mg., 0.29 mmol.) was suspended in 10 ml. of MeOH and the mixture was bubbled with hydrogen sulfide for 2 hr. until all suspention 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 (Ⅸ), which was dried over P_2O_5 and weighed 83 mg. (yield 98%), showed positive coloration to sulfur test, positive Beilstein test for chlorine and revealed the similar mobility to cathode, $M_{urid.}$ 2.55, to that of 1-(3′-deoxy-3′-amino-β-D-arabinofuranosyl)uracil (I), $M_{urid.}$ 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 $\lambda_{max}^{pH\,1}$ mμ: 262, $\lambda_{min}^{pH\,1\text{ and }7}$ 232, $\lambda_{max}^{pH\,7}$ 262.5, $\lambda_{shoulder}^{pH\,13.4}$ 263. 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-(5′-O-Benzoyl-2′,3′-dideoxy-2′-benzoylthio-3′-benzoylamino-β-D-ribofuranosyl)uracil (X)——The mercuric salt (Ⅷ) (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 P_2O_5. 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 $CHCl_3$, the chloroform layer washed three times with water and dried over Na_2SO_4. 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 P_2O_5 to afford 85 mg. (yield 51% from Ⅷ) 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% H_2SO_4-spray. $[\alpha]_D^{24}$ −39°(c=0.59, $CHCl_3$). UV λ_{max}^{EtOH} mμ (ε): 232 (30300), $\lambda_{shoulder}^{EtOH}$ 260 (17200). *Anal.* Calcd. for $C_{30}H_{25}O_7N_3S \cdot \frac{1}{2}H_2O$: C, 62.06; H, 4.51; N, 7.24; S, 5.52. Found: C, 61.97; H, 4.50; N, 7.33; S, 5.08.

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