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On the Benzylation of Nucleosides. I. Reaction of Uridine with Benzyl Bromide in the Presence of Sodium Hydride

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Uridine was benzylated with benzyl bromide in the presence of sodium hydride in dimethyl sulfoxide or dimethylformamide to give two products. The one was a dibenzyl uridine (I), yield 33%, and the other N_3–benzyluridine (II), yield 30.5%. The product (I) was converted to the product (II) by catalytic hydrogenation and was identified with N_3, 2′–O–dibenzyluridine which was synthesized by detritylation of N_3,2′–O–dibenzyl–3′,5′–di–O–trityluridine (IV) derived by the similar benzylation of the known 3′,5′–di–O–trityluridine (III).

The proper protection of 2′–hydroxyl group of ribonucleoside or ribonucleotide is known to be important problem in the synthesis of oligonucleotides. The protecting functions must fulfil the requirement that they have to remain in place until the final stage and that it must be removed under conditions which do not promote hydrolytic cleavage or migration of the internucleotidic linkages. Griffin, *et al.*[1] have recommended the use of benzyl ether group as one of the protecting functions, because this group may be cleaved by catalytic hydrogenolysis under very mild conditions, which avoid the difficulties outlined above. It is desirable to have a procedure for specific benzylation of 2′–position of a ribonucleoside, by a simple reaction and in a better yield than that hitherto reported.

It has been communicated by Hakomori[2] that the free hydroxyl groups in a complex carbohydrate such as cerebroside could be methylated in high efficiency with methyl iodide catalyzed by methylsulfinyl carbanion in the presence of sodium hydride in dimethyl sulfoxide. The method has originally been established by Corey and Chaykovsky[3] and involved a stimulation of the alkoxide formation of hydroxyl groups by strongly basic carbanion and a subsequent methylation with methyl iodide.

$$\mathrm{ROH + CH_3SOCH_2^-Na^+ \longrightarrow RONa + CH_3SOCH_3}$$

$$\mathrm{RONa + CH_3I \longrightarrow ROCH_3 + NaI}$$

We applied this reaction for the benzylation of uridine using benzyl bromide in place of methyl iodide and found that beside the benzylation of N_3–position, a selective benzylation on the sugar moiety occurred at the 2′–hydroxyl group.

From the ether soluble part of a reaction mixture of uridine and benzyl bromide under the existence of methylsulfinyl carbanion which was prepared from dimethyl sulfoxide (DMSO) and sodium hydride, a product (I) was obtained as colorless needles, mp 189.5—190.5° in a yield of 33%. On the other hand, from the water soluble part, another product (II) was isolated in a yield of 30.5% as colorless crystals which melted at 175.5—176.5°.

The elemental analysis of the product (II) coincided with that for a monobenzyluridine and its ultraviolet absorption spectra indicated that the benzyl group must be attached at N_3–position of the uracil moiety. The product (I) gave the analytical data corresponding to those of a dibenzyl uridine. From the characteristic ultraviolet absorptions of this com-

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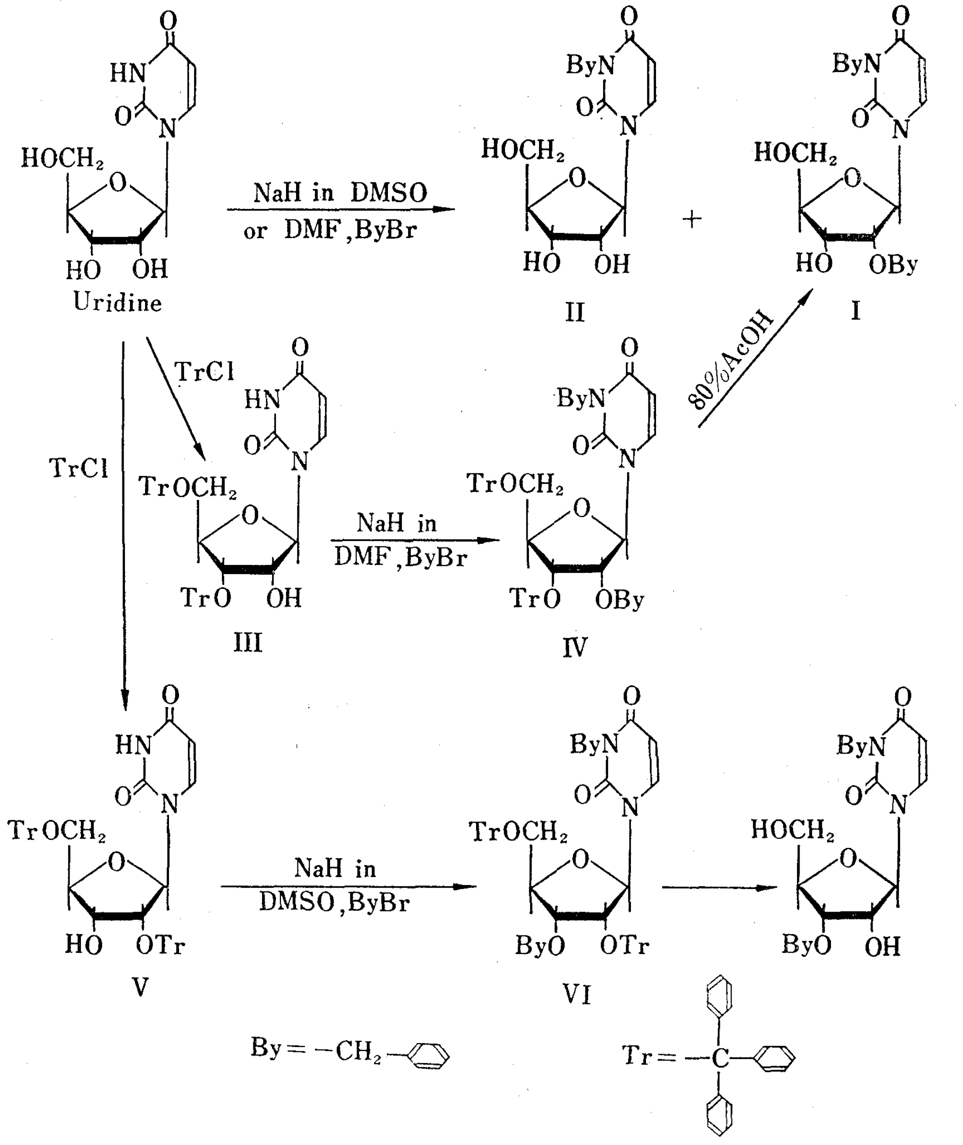
1) B.E. Griffin, C.B. Reese, G.F. Stephenson, and D.R. Trentham, *Tetrahedron Letters*, **1966**, 4349.
2) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
3) E.J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **84**, 866 (1962).



pound (I) as those for a N_1,N_3-dialkyluracil and the observation that the compound (I) did not consume periodate, a structure of N_3, 2′(or 3′)-di-O-benzyluridine was proposed to this dibenzyluridine (I). On catalytic hydrogenation with palladium-charcoal, the compound (I) lost one of the two benzyl groups and was converted to a monobenzyl uridine identical with the product (II). The remaining one benzyl group in the compound (II) could not be removed by prolonged hydrogenolysis using palladium-charcoal or -barium sulfate as catalyst.

The benzylation reaction of uridine was proceeded quite similarly even when the solvent, DMSO, in above described reaction was replaced by dimethylformamide (DMF) to give the compound (I) in a yield of 21%. Thus, it was found that for the benzylation of uridine to yield the products (I and II), the presence of the methylsulfinyl carbanion in the reaction system is not indispensable.

In order to have a more confirmatory evidence for the structure of the product (I), 3′,5′-di-O-trityluridine (III), which was synthesized by the method of Žemlička,[4] was reacted with benzyl bromide in DMF. The product (IV) was obtained as pale yellow needles, which melted at 110—117°, and gave elemental analysis corresponding to that of a dibenzyl ditrityl uridine. The ultraviolet absorption spectra of this product indicated the characteristics of N_1,N_3-

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[4] J. Zemlička, *Collection Czech. Chem. Commun.*, 29, 1734 (1964).

dialkyluracils. On detritylation of the compound (IV) by warming it with 80% acetic acid, IV gave a product which melted at 189.5—190.5° and showed no depression on mixed fusion with the compound (I) derived from uridine by direct benzylation. The both infrared and ultraviolet absorption spectra and the behaviours in paper- and thin-layer-chromatographies of the detritylation product were in good accordance with those of the compound (I). Thus, the structure of I was unambiguously determined to be N_3,2′-O-dibenzyluridine. In the benzylation of III to form the compound (IV), DMF was used as the solvent instead of DMSO, because the reaction in DMSO gave heavily colored side-products which made the isolation of the desired product difficult.

On the other hand, in the benzylation of 3′-position of 2′,5′-di-O-trityluridine (V), which was synthesized according to Fox, *et al.*,[5] the reaction did not proceed smoothly when DMF was used as solvent, but it gave the desired compound (VI) when DMSO was used for the reaction. The product (VI) was obtained as colorless needles which melted at 198—204°. The elemental analysis, ultraviolet absorption spectra of this product indicated that VI is a dibenzyl ditrityl uridine, one of the benzyl groups of which attached at the N_3-position of the pyrimidine moiety.

The removal of trityl groups from VI was performed by treating it with 80% acetic acid at 96° for 25 min or with $CHCl_3$ saturated with hydrogen chloride at 0° for 2 hours. The main product of these reactions was separated by thin-layer chromatography. This product did not consume periodate and gave characteristic ultraviolet absorption spectra for a N_1, N_3-disubstituted uridine and showed different *Rf* value (Rft_2 0.45) from that of the product I (Rft_2 0.35).

The results of the investigations described above revealed that the reaction between uridine and benzyl bromide in the presence of sodium hydride and DMSO or DMF was found to give two benzylated products: the one N_3-benzyluridine and the other N_3,2′-O-dibenzyluridine in which 2′-hydroxyl group of the ribofuranosyl residue was benzylated. The reaction could be recommendable as it can benzylate the 2′ hydroxyl function of the ribofuranosyl moiety of uridine by a simple procedure. The availability of this reaction in the synthesis of oligonucleotide, however, is restricted unless the simultaneous benzylation of N_3-position could be avoided. The improvement of the method in such direction has been achieved by the use of 4-methylthiouridine as starting material and the results of the research will be reported in the subsequent paper.

Experimental

Methods——Paper chromatography was performed by ascending technique on Toyo Roshi No. 53 paper using solvent of BuOH-H_2O (84:16) and the movement of the spots was represented by *Rf*. Thin-layer chromatography was perfomred by ascending technique on Silica Rider (Daiichi Pure Chemicals Co., Ltd.) thin-layer ($0.25 \times 5 \times 20$ cm^3) in $CHCl_3$-C_2H_5OH (99:1) and AcOEt. The movements of the spot in these solvents were given by Rft_1 and Rft_2, respectively.

N_3, 2′-O-Dibenzyluridine (I)—A) To 48 mg (*ca.* 1 mmole) of commercial NaH (50% purity), which was washed three times with each 3 ml of petroleum ether (dried over Na), was added 6 ml of DMSO (dimethyl sulfoxide). The solution was warmed at 50—60° for 50 min. After finishing the evolution of hydrogen gas and completing the dissolution of NaH, to the solution was added 122 mg (0.5 mmole) of uridine dissolved in 2 ml of DMSO, and the mixture was stirred for 45 min at room temperature. Under stirring and careful cooling with ice-water not to solidify the solution, 0.15 ml (1.3 mmoles) of benzyl bromide was added to the solution and the stirring was continued for additional 30 min at room temperature.

After the reaction, 20 ml of ice-water was added to the reaction mixture under stirring and the white precipitate that occurred was taken by filtration, washed with small amount of MeOH and ether successively and dried to obtain 70 mg of a white powder. On recrystallization from MeOH, the product gave colorless needles (50 mg), mp 189.5—190.5°. The filtrate separated from the white precipitate, was extracted three times with the same volume of ether. The ethereal layer was washed two times with the same volume of

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[5] N.C. Yung and J.J. Fox, *J. Am. Chem. Soc.*, 83, 3060 (1961).

water to remove DMSO and dried over Na_2SO_4. Ether was evaporated and the residual needles were recrystallized from MeOH to give 20 mg of colorless needles, mp 189.5—190.5°.

The combined needles thus obtained in a yield of 33% were identified by mixed mp test and infrared spectra with authentic N_3, 2′–O–dibenzyluridine prepared by detritylation of the compound (IV) as described below. UV: $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (ε): 263 (8400), $\lambda_{\min}^{\mathrm{EtOH}}$ 235 (2400), $\lambda_{\max}^{\mathrm{EtOH+NaOH}}$[6] 264 (8080), $\lambda_{\min}^{\mathrm{EtOH+NaOH}}$ 235 (2200), *Rf* 0.84, *Rf*t_2 0.35, $[\alpha]_D^{30}$:+61.5° (*c*=0.4 in EtOH). *Anal.* Calcd. for $C_{23}H_{24}O_6N_2$: C, 65.08; H, 5.69; N, 6.60. Found: C, 65.03; H, 5.78; N, 6.47.

This product did not show positive test with periodate–benzidine reagent[7] on paper chromatogram.

On catalytic hydrogenation with Pd–charcoal (10%) in EtOH, the compound (I) was converted into a monobenzyluridine which was identified with the product (II) by paper chromatography and periodate–benzidine reaction.

B) The commercial NaH (1 mmole) was washed three times with 3 ml of petroleum ether and suspended in 5 ml of DMF (dimethylformamide). To the suspension a solution of 0.5 mmole of uridine in 2 ml of DMF was added. The suspension, which evolved H_2 gas, was stirred for 20 min at room temperature and for successive 10 min at 50°, and followed by dropwise addition of 1.16 mmoles of benzyl bromide under ice–cooling. After the addition, the stirring was continued for 10 min at 0° and for successive 30 min at 45—50°. To the mixture was added under cooling 40 ml of ice–water and the mixture was extracted with the same volume of ether for three times. The ethereal layer was washed two times with the same volume of water and dried over Na_2SO_4. On evaporation of ether, the residue solidified to needles which were recrystallized from EtOH to give 45 mg of colorless needles, mp 187.5—189° (yield, 21%).

This product did not show depression of mp on admixture with the product (I) obtained in A).

N_3-Benzyluridine (II)——The aqueous layer which was obtained after the ether extraction in the procedure A) was carefully neutralized with 2 N HCl and deionized by addition of the ion–exchange resin MB–3. The deionized solution was evaporated *in vacuo* and the trace of DMSO was removed by azeotropical distillation with water. To the residue was added a small amount of aqueous EtOH and the mixture was set aside at room temperature for 3 months. The needles that appeared were recrystallized from EtOH to furnish 50 mg of colorless needles, mp 175.5—176.5°, yield 30.5%. UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (ε): 263 (8580); $\lambda_{\min}^{\mathrm{EtOH}}$ 234 (2400), $\lambda_{\max}^{\mathrm{EtOH+NaOH}}$ 264 (8250), $\lambda_{\min}^{\mathrm{EtOH+NaOH}}$ 235 (2520), $\lambda_{\max}^{\mathrm{pH}>13}$ 263, $\lambda_{\min}^{\mathrm{pH}>13}$ 239. *Rf* 0.69, *Rf*t_2 0.15. $[\alpha]_D^{30}$ +12.9° (*c*=0.15 in EtOH). *Anal.* Calcd. for $C_{16}H_{18}O_6N_2$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.62; H, 5.62; N, 8.30.

The spot of this product on paper chromatogram gave positive reaction with periodate–benzidine reagent.

N_3, 2′-O-Dibenzyl-3′,5′-O-trityluridine (IV)——To a suspension of 200 mg (*ca.* 4 mmoles) of NaH, which was previously washed with each 6 ml of petroleum ether for three times, in 10 ml of DMF, was added 729 mg (1 mmole) of 3′,5′–di–O–trityluridine (III)[4] dissolved in 10 ml of DMF. The mixture was stirred for 10 min at room temperature and to the mixture was added dropwise 685 mg (4 mmoles) of benzyl bromide under ice–cooling. The stirring was continued for 10 min under ice–cooling and for additional 10 min at room temperature and finally for 80 min at 50—53°. To the reaction mixture which was concentated to *ca.* 5 ml *in vacuo*, was added 40 ml of ice–water and the mixture was extracted two times with each 60 ml of $CHCl_3$. The chloroform layer was washed three times with each 50 ml of water and dried. From the solution, the solvent was removed under reduced pressure and the residual orange syrup was dissolved in a minimum amount of EtOH under warming. The white crystals that appeared were filtered, washed with EtOH and dried to furnish 420 mg of fine crystals (yield, 46%). Recrystallization from EtOH gave pale yellow fine neeldes which melted at 110—117°. UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (ε): 263 (8480), $\lambda_{\min}^{\mathrm{EtOH}}$ 264 (5150). *Rf*t_1 0.50, *Rf*t_2 0.63. $[\alpha]_D^{30}$ +14.7 (*c*=0.5 in EtOH). *Anal.* Calcd. for $C_{61}H_{52}O_6N_2$: C, 80.59; H, 5.77; N, 3.08. Found: C, 80.59; H, 5.73; N, 3.31.

Detritylation of the Compound (IV)——A suspension in 5 ml of 80% AcOH of 200 mg (0.22 mmole) of N_3, 2′–O–dibenzyl–3′,5′–di–O–trityluridine (IV) prepared as above was heated for 25 min on a boiling water bath under stirring. The supernatant was taken by decantation and evaporated *in vacuo*. The residual amount of AcOH was removed exclusively by codistillation with small amount of EtOH. From the final residue, on extraction of triphenyl carbinol with hot cyclohexane was obtained 56 mg of pale yellow needles which were recrystallized from EtOH to give 30 mg of colorless needles, yield 32%, mp 189.5—190.5°. UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (ε): 263 (8480), $\lambda_{\min}^{\mathrm{EtOH}}$ 236 (2600), $\lambda_{\max}^{\mathrm{EtOH+NaOH}}$ 263 (8200), $\lambda_{\min}^{\mathrm{EtOH+NaOH}}$ 237 (2800). *Rf* 0.84, *Rf*t_2 0.35. *Anal.* Calcd. for $C_{23}H_{24}O_6N_2$: C, 65.08; H, 5.69; N, 6.60. Found: C, 64.96; H, 5.76; H, 6.58. The spot of this product on the paper chromatogram did not show positive test with periodate–benzidine reagent.

N_3, 3′-O-Dibenzyl-2′,5′-di-O-trityluridine (VI)——To a solution of methylsulfinyl carbanion, which was prepared as described above from 200 mg (*ca.* 4 mmoles) of NaH and 10 ml of DMSO, was added 729 mg (1 mmole) of 2′,5′–di–O–trityluridine (V)[5] dissolved in 10 ml of DMSO. The mixture was stirred at room temperature for 15 min and then added dropwise with 684 mg (4 mmoles) of benzyl bromide under cooling. Three minutes after the addition, the cooling was stopped and the stirring was continued at room temperature

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6) EtOH+NaOH indicates that solvent was a mixture of EtOH and 10 N NaOH solution in a ratio of 49:1 (v/v).

7) J.A. Cifonelli and F. Smith, *Anal. Chem.*, 26, 1132 (1954).

for 5 min. The temperature was then raised to 55° and the stirring continued for additional 15 min. To the reaction mixture under cooling, 100 ml of ice–water was added and the mixture was extracted two times with 50 ml of $CHCl_3$. The $CHCl_3$ layer was washed with the same volume of water and dried over Na_2SO_4. On removal of the $CHCl_3$ *in vacuo* a yellowish brown oil was obtained, which was treated with 5 ml EtOH to furnish white solid. The solid was repeatedly triturated with 10 ml of ether, the ether soluble parts were combined and evaporated *in vacuo* to furnish orange gum. On setting aside overnight of a solution of the gum in hot ethanol, crystals appeared which were separated by filtration and dried to give 450 mg of colorless needles. Recrystallization from 100 ml of EtOH, gave 300 mg of colorless needles, mp 198—204° (yield 33%). UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (ε): 261 (9370), $\lambda_{\min}^{\mathrm{EtOH}}$ 245 (6000). $[\alpha]_D^{30}$ +7.44° (c=0.5 in EtOH). Rft_1 0.50. *Anal.* Calcd. for $C_{61}H_{52}O_6N_2$: C, 80.59; H, 5.77; N, 3.08. Found: C, 80.37; H, 5.73; N, 2.96.

The products of detritylation of this compound with 80% AcOH, HCl-satulated $CHCl_3$, EtOH, or ether were separated by thin-layer chromatography to obtain a spot of the main product having Rft_2 0.45. The spot did not consume periodate–benzidine reagent and the ethanolic extract of the spot showed the characteristic ultraviolet absorption spectra of N_1, N_3-dialkyluracil.

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