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Tyunosin Ukita, Yasushi Takeda, and Hikoya Hayatsu : On the Synthesis of Uridylyl-(3′→5′)-uridine.

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The chemical synthesis of oligoribonucleotidic compounds specifically having 3′→5′ internucleotidic linkages as involved in the natural ribonucleic acids has been attained not so easily on account of the presence of a vicinal *cis*-diol group at the 2′- and 3′-position of the nucleoside; either the 2′- or 3′-hydroxyl group has a similar reactivity to the phosphorylation reactions.

Two synthetic routes can be designed to synthesize the 3′→5′ internucleotidic phosphodiester bond, the one is to condense a protected ribonucleoside having a free hydroxyl group at 3′-position with a suitably protected ribonucleoside 5′-phosphate (Route A), and the other is to condense a nucleoside derivative having a free hydroxyl group at 5′-position with a suitably protected ribonucleoside 3′-phosphate (Route B). All of the recent communications concerning with this type of synthesis were originally based on the

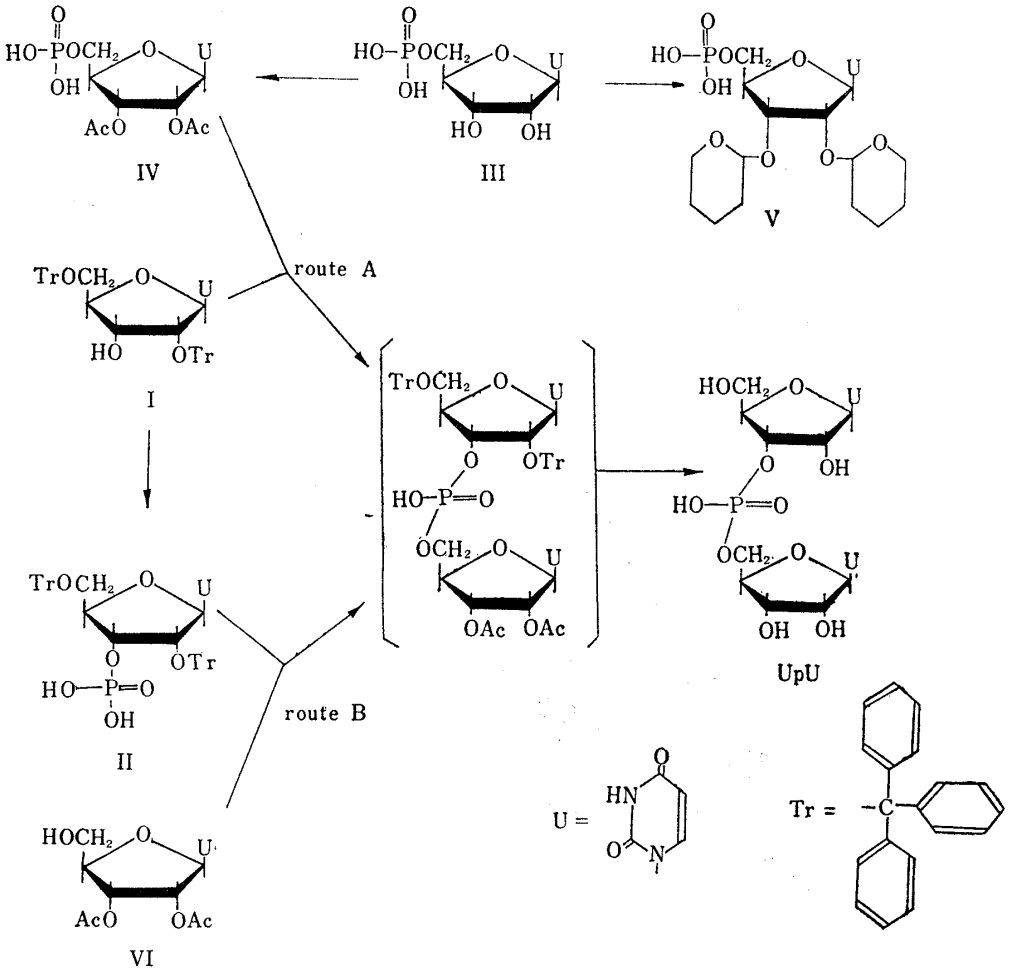

Chart 1.

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rout B.[1,2] But the preparations of the protected nucleoside 3′-phosphates have been tedious because of the multiple steps involved in the synthetic procedures or of the possible difficulties in the purification of the starting nucleoside 3′-phosphates.

This paper communicates several observations obtained on the synthesis of uridylyl-(3′→5′)-uridine *via* both route A and B using 2′,5′-di-O-trityluridine (I)[3,4] as one of the starting materials.

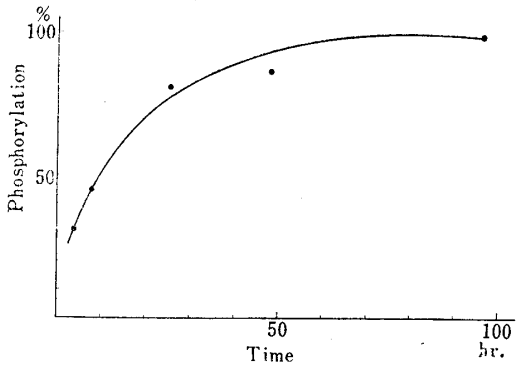

Fig. 1. Phosphorylation of 2′,5′-Di-O-trityluridine

In order to have a precise information on the reactivity of 2′,5′-di-O-trityluridine (I) to the phosphorylating agent,*2 compound (I) was phosphorylated by use of cyanoethylphosphate[5] at 37°. After removal of the cyanoethyl and trityl groups, the reaction mixture was submitted to paper chromatography (PPC). The yield of uridine 2′(3′)-phosphate was estimated spectrophotometrically and was plotted against time (Fig. 1). Figure 1 indicates that the reaction completed after 4 days. Thus, in this reaction the phosphorylation of 3′-hydroxyl group of I does not seem to suffer large steric hindrance anticipated to be caued by the neighboring bulky trityl group.*3

From the product obtained by cyanoethylphosphorylation of I, the cyanoethyl group was removed by mild alkaline treatment and 2′,5′-di-O-trityluridine 3′-phosphate (II) was isolated by using cellulose column chromatography in a yield of 78%. On detritylation with 80% acetic acid at room temperature for 18 hours or at 100° for 5 minutes, the compound (II) gave uridine 2′(3′)-phosphate which was identified by PPC with an authentic specimen.*4

In the synthesis of uridylyl-uridine (UpU) *via* route A, compound (I) was condensed, in the presence of dicyclohexylcarbodiimide (DCC), with 2′,3′-di-O-acetyluridine 5′-phosphate (IV), which in turn was prepared from uridine 5′-phosphate (III) by treatment with acetic anhydride and pyridine according to the method of preparation of 2′,3′-di-O-acetyladenosine 5′-phosphate reported by Rammler, *et al.*[*5,1b] The product was deacetylated with aqueous ammonia followed by detritylation with 80% acetic acid to give UpU.

In the above detritylation reaction with 80% acetic acid at 37°, aliquots were withdrawn at intervals and the yields of UpU were estimated. Since the UpU contained a small amount of a side product (probably uridylyl-(2′→5′)-uridine, a migration product of the phosphoryl linkage[1]), the UpU was digested with bovine pancreatic RNase-A and

*2 Yung and Fox[4] have observed that the 3′-hydroxyl group of I resisted to tosylation and have suggested the participation of a steric hindrance of 2′-O-trityl group in this reaction. The phosphorylation of 5′-O-tritylthymidine at room temperature has been reported to complete within two days.[5]

*3 See reference 9).

*4 Purchased from Sigma Chemical Co.

*5 Another protected uridine 5′-phosphate derivative, 2′,3′-di-O-tetrahydropyranyluridine 5′-phosphate (V), was synthesized. But it was found difficult to completely remove the tetrahydropyranyl groups of this compound with a reaction condition mild enough to avoid the migration of phosphoryl linkage of the condensation product of V with I (see Experimental).

1) *a*) M. Smith, D. H. Rammler, I. H. Goldberg, H. G. Khorana : J. Am. Chem. Soc., **84**. 430 (1962); *b*) D. H. Rammler, H. G. Khorana : *Ibid.*, **84**, 3112 (1962).
2) *a*) J. Smrt, F. Šorm : Coll. Czech. Chem. Commun., **27**, 73 (1962); *b*) *Idem* : *Ibid.*, **28**, 61 (1963); *c*) *Idem* : *Ibid.*, **28**, 887 (1963).
3) P. A. Levene, R. T. Tipson : J. Biol. Chem., **104**, 385 (1934).
4) N. C. Yung, J. J. Fox : J. Am. Chem. Soc., **83**, 3060 (1961).
5) G. M. Tener : *Ibid.*, **83**, 159 (1961).

the amount of the RNase-A-resistant material (RRM) was estimated (Table I). Table I indicates an increase in amounts of the RRM and a simultaneous decrease in the yield of total UpU with prolonged acid treatment.

Table I. Yields of UpU and RRM Contents in the UpU obtained by Acid Treatment with 80% Acetic Acid at 37° (Route A)

Time of acid treatment (hr.)	14.5	24	49
Yield of UpU (%)	25	24	20
RRM content (%)	10	16	23

The detritylation reaction was performed with 80% acetic acid at 37° for 16 hours and UpU was obtained in a yield of 30% and the product contained 8% of RRM.

The UpU thus obtained was identified by PPC as well as by paper electrophoresis (PEP) with an authentic specimen of uridylyl-(3′→5′)-uridine which was synthesized from uridine 2′,3′-cyclic phosphate and uridine by enzymatic condensation.[6]

In order to characterize the RRM contained in the synthetic UpU, the RRM was isolated from the RNase digest of UpU by preparative paper chromatography. The isolated RRM was indistinguishable from UpU as regards the behaviors in acid hydrolysis, PPC and PEP. The only difference found between the RRM and uridylyl-(3′→5′)-uridine was the complete inertness of the former to the action of RNase. These results indicated that the RRM must be uridylyl-(2′→5′)-uridine[7] as has been suggested by Smith, *et al.*[1a]

In the synthesis of UpU *via* route B, 2′,5′-di-O-trityluridine 3′-phosphate (Ⅱ) was reacted with 2′,3′-di-O-acetyluridine (Ⅵ)[8] in the presence of DCC. The condensation product, after the removal of acetyl groups, was treated with 80% acetic acid at 37° for 14 hours. The product, UpU, was obtained in a yield of 40%, which contained 4.4% of RRM.

The results of the experiments described above showed that there is no large difference between route A and B as regards the yield of UpU. The RRM content in the UpU synthesized also does not differ significantly.

Recently, Hall and Thedford[9] independently of our research reported a synthesis of UpU in a yield of 29%, which contained 1.3% of RRM, by condensation of Ⅱ with 2′,3′,-O-isopropylideneuridine followed by subsequent removal of the protecting groups with 80% acetic acid at 100° for 30 minutes. In order to know if the detritylation conditions reported by Hall is actually more excellent to avoid the migration of phosphoryl linkage than those used in our experiment, the present authors tested the stability of UpU to the treatment with 80% acetic acid at both 37° and 100°. The amount of the remaining UpU and the increase of RRM were plotted against time and the results are summarized in Fig. 2. The figure indicates that both the decrease in amount of UpU and the increase of RRM are larger at 100° than at 37°, that is to say, the migration of internucleotidic linkage of UpU from 3′→5′ to 2′→5′ is more favourable at higher temperature. In the work up in route B, the detritylation was performed with 80% acetic acid at 100° and the yields of UpU, as well as the RRM contents in the UpU were examined as a function of time of the acidic treatment. Figure 3 indicates that in detritylation at 100°, the reaction time at which the maximum amount of UpU accumulates is 10 to 15 minutes, and after that time a gradual decomposition of UpU takes place associating

6) L. A. Heppel, P. R. Whitfeld, R. Markham : Biochem. J., **60**, 8 (1955).
7) cf., D. M. Brown, D. I. Magrath, A. H. Neilson, A. R. Todd : Nature, **177**, 1124 (1956).
8) G. W. Kenner, A. R. Todd, R. F. Webb, F. J. Weymouth : J. Chem. Soc., **1954**, 2288.
9) R. H. Hall, R. Thedford : J. Org. Chem., **28**, 1506 (1963).

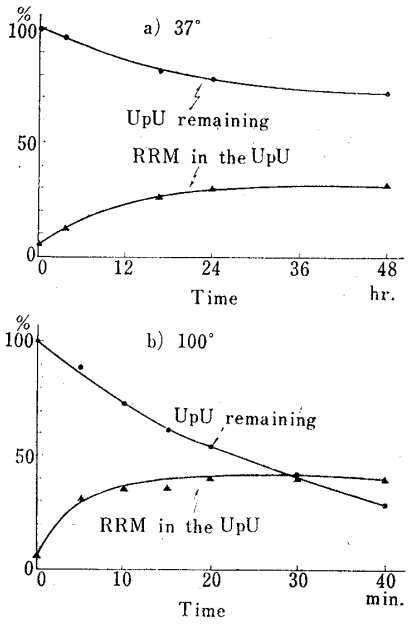

Fig. 2. Stability of UpU in 80% Acetic Acid at 37° and 100°

with a rapid increase in RRM. The UpU isolated from the reaction mixture at that time contained 5 to 8% of RRM.

As was described above, the both routes, A and B, in the synthesis of UpU

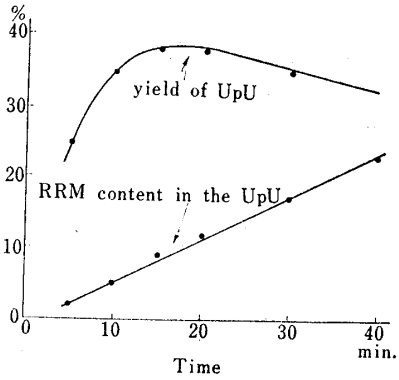

Fig. 3. Yields of UpU and RRM Contents in the UpU obtained by Acid Treatment with 80% Acetic Acid at 100° (Route B)

gave the desired product in a similar yield. The route A, however, could serve as a convenient method because of the ready availability of 2′,5′-di-O-trityluridine (I) and uridine 5′-phosphate (III), the former compound being obtainable by one step reaction from uridine and the latter being recently conveniently obtainable from the industrial sources.

Experimental

General Method——Paper chromatography (PPC) was performed ascendingly on Toyo Roshi No. 51 paper. The following solvent systems were employed: (1) iso-PrOH–conc. NH_4OH–H_2O (7:1:2); (2) BuOH–AcOH–H_2O (5:2:3); (3) EtOH–0.5*M* $AcONH_4$ (pH 3.8) (5:2); (4) EtOH–1*M* $AcONH_4$ (pH 7.5) (5:2); (5) BuOH–EtOH–H_2O (4:1:5). The Rf values obtained for these solvent systems are represented by Rf_1, Rf_2, Rf_3, Rf_4 and Rf_5, respectively. Paper electrophoresis (PEP) was performed on Toyo Roshi No. 51 paper by a method described previously.[10] The buffers used were: (1) 0.02*M* Tris–HCl (pH 8.2); (2) 0.02*M* ammonium formate (pH 4.0). The mobilities of the compounds tested in each buffer, (1) and (2), are represented by M_1 and M_2, respectively, taking uridine 5′-phosphate as a standard compound. The average potential was ca. 20 v./cm. The detection of the spots was carried out as follows: UV absorbing compounds were detected by UV-lamp. Phosphorus-containing compounds were detected by the molybdate–perchloric acid spray[11] and subsequent irradiation of UV ray.[12] The same spray with trityl compounds gives a yellow color on heating.[1a] Compounds having *cis*-diol group were located with periodate–benzidine reagent.[13] The quantitative determination of phosphorus was performed by Allen's method.[14]

Quantitative analysis using paper chromatography was performed by eluting the UV absorbing spot with 3~5 ml. of H_2O at room temperature for 15~20 hr. and determining optical density at 260 mμ of the eluate taking a blank from the similar space of the paper. In the calculation, the molecular extinction coefficients 10,000 for uridine and uridine phosphate and 20,000 for uridylyluridine were used.

2′,5′-Di-O-trityluridine 3′-Phosphate (II)——a) To a solution of 364 mg. (0.5 mmoles) of 2′,5′-di-O-trityluridine (I)[4] in 5 ml. pyridine, was added 1 ml. of a pyridine solution of 2-cyanoethylphosphate[6]

10) T. Ukita, M. Irie: This Bulletin, **9**, 217 (1961).
11) C. S. Hanes, F. A. Isherwood: Nature, **164**, 1107 (1949).
12) R. S. Bandurski, B. Axelrod: J. Biol. Chem., **193**, 405 (1951).
13) J. A. Cifonelli, F. Smith: Anal. Chem., **26**, 1132 (1954).
14) R. J. L. Allen: Biochem. J., **34**, 858 (1940).

(1 mmole), and the solvent was evaporated.*6 The residual syrup was dissolved in 5 ml. of anhyd. pyridine, and the solution was applied to azeotropical distillation. The process was repeated twice, and to a solution of the residue in 5 ml. anhyd. pyridine, 600 mg. of DCC was added. The tightly stoppered mixture was kept standing at 37° for 5 days. After addition of 0.5 ml. of H_2O, the reaction mixture was set aside at room temperature overnight, and concentrated to a syrup. The trace of pyridine in the syrup was removed by repeating evaporation of added water. To the residue 20 ml. of 0.5*N* NaOH was added and refluxed for 40 min. to remove cyanoethyl group. The cooled reaction mixture was filtered to remove dicyclohexylurea, which was washed with 20 ml. of 0.5*N* NaOH. The filtrate was combined with the washings and applied on a column of Dowex 50 W (NH_4^+ form) and the column was eluted with 80% aq. MeOH. The combined effluent was evaporated to dryness to furnish a slightly yellow powder which was dissolved in solvent 1. The solution was centrifuged and the supernatant was applied onto a column of cellulose powder (Toyo Roshi cellulose powder A, 5.5×28 cm.), and eluted with the same solvent. Fractions (500~700 ml.), which on spot test gave positive coloration for phosphorus and trityl groups, were combined and concentrated to dryness to give slightly yellowish powder. After drying *in vacuo* over P_2O_5 the product weighed 349 mg. (yield 78%). The product gave a single spot in PPC (Rf_1 0.79).

For elemental analysis, the product was further purified by preparative paper chromatography (Toyo Roshi No. 26, solvent 1), and the amorphous ammonium salt obtained was dried *in vacuo* over P_2O_5 and analyzed. *Anal.* Calcd. for $C_{47}H_{41}N_2O_9P\cdot(NH_3)_2\cdot 3H_2O$: C, 62.9; H, 5.96; N, 6.25; P. 3.45. Found : C, 63.14; H, 6.40; N, 6.62; P, 3.42. UV mμ (ε) : λ_{max}^{EtOH} 263 (7300), λ_{min}^{EtOH} 247 (5000).

After removal of trityl groups by treatment of the product with 80% AcOH at room temperature for 18 hr. or at 100° for 5 min., the reaction mixture gave no other product than uridine 2′(3′)-phosphate when tested by paper chromatography (Rf_1 0.10, Rf_3 0.58, Rf_4 0.12).

b) The determination of the rate of phosphorylation of I : Seventy three milligrams of I was phosphorylated as described above and during the reaction, aliquots were removed at intervals, added with equal volume of H_2O and after standing at room temperature for 12 hr. evaporated to dryness. To the residue 80% AcOH was added and heated in boiling water bath for 40 min.*7 The cooled reaction mixture was neutralized with conc. NH_4OH and applied to PPC in solvent 1. The rate of phosphorylation of I was calculated from the ratio of optical density at 260 mμ of aqueous extract of the spots of uridine 2′(3′)-phosphate and uridine found. The results are presented in Fig. 1.

2′,3′-Di-O-acetyluridine 5′-Phosphate (IV)——Well dried pyridinium salt of uridine 5′-phosphate (III) (1 mmole) was dissolved in 10 ml. of anhyd. pyridine. To the solution, 10 ml. of Ac_2O was added and shaken in a tightly stoppered vessel to effect the solution. The mixture was set aside in a dark for 3 days. Five milliliters of EtOH was added dropwise to the darkred reaction mixture under stirring and ice-cooling, and kept standing for 30 min. To the mixture, 5 ml. of H_2O was added and set aside at room temperature overnight. Solvents were evaporated from the mixture and remaining AcOH was removed by repeated coevaporation with pyridine. To the final 5 ml. of pyridine solution 200 ml. of H_2O was added and lyophilized. The light brown powder thus obtained was dissolved in 10 ml. of anhyd. pyridine under exclusion of moisture, and stored in a refrigerater to use as the stock solution (containing 0.1 mmole of IV per ml.). PPC run in solvent 2 of the solution gave only one phosphorus-containing spot (Rf_2 0.58) and the aqueous extract of the spot showed UV absorption of $\lambda_{max}^{H_2O}$ 258 mμ.*8 On incubation of IV with intestinal alkaline phosphomonoesterase in 0.2*M* Tris-HCl buffer (pH 8) at 37° for 1 hr. and subsequent PPC (solvent 5) of the hydrolyzate, the spot of 2′,3′-di-O-acetyluridine (Rf_5 0.76, major product) and 2′(3′)-monoacetyluridine (Rf_5 0.56, trace) were detected.

The compound (IV) was deacetylated with a mixture of pyridine-dioxane-conc. NH_4OH (1:1:1) at room temperature for 1 hr. and subsequent PPC (solvent 2) of the reaction product revealed only uridine 5′-phosphate as phosphorus-containing product.

Uridylyl-(3′→5′)-uridine (UpU)——a) Route A (Removal of trityl groups at 37°) : To 2.5 ml. of the stock solution of 2′,3′-di-O-acetyluridine 5′-phosphate (IV) (250 μmoles), were added 91 mg. of 2′,5′-di-O-trityluridine (I) and 260 mg. of DCC and the well stoppered mixture was set aside at 37° for 5 days. After addition of 0.5 ml. of H_2O, the reaction mixture was kept standing at room temperature overnight. To the mixture, 3 ml. each of dioxane and conc. NH_4OH were added and set aside at room temperature for 5 hr. in a tightly stoppered vessel. Dicyclohexylurea precipitated was filtered and the filtrate was concentrated to remove pyridine. The oily brown residue, the deacetylated intermediate, was suspended in 3 ml. of 80% AcOH and after standing at 37° for 16 hr., the mixture was centrifuged to remove triphenylcarbinol separated. The supernatant was neutralized with pyridine and concentrated to a small

*6 All evaporations were carried out *in vacuo* at bath temperature below 40°.

*7 The cyanoethyl group of the nucleoside 2′(3′)-cyanoethylphosphate is removable with 80% AcOH at 100° for 40 min. (T. Ukita, R. Funakoshi : unpublished data).

*8 Another UV absorbing spot (Rf_2 0.86, $\lambda_{max}^{H_2O}$ 292 mμ), which was negative to the molybdate-perchloric acid spray, was always detected.[1b]

volume. The condensate was streaked on Toyo Roshi No. 514 paper and chromatographed in solvent 1 run about 13 hr. The paper was dried and developed again in the same solvent, and this process was performed once more. The dinucleoside phosphate band, which was located between uridine and uridine 5′-phosphate, was eluted with H_2O. Lyophilization of the eluate gave a slightly yellowish powder. The yield of this product as determined spectrophotometrically was 30.1% calculated from I. The product was found homogeneous and was identified by PPC and PEP with the authentic UpU, synthesized enzymatically according to Heppel, *et al.*[7] Rf_1 0.15, Rf_2 0.30, Rf_3 0.51, Rf_4 0.28, M_1 0.46, M_2 0.64. UV mμ: $\lambda_{max}^{pH\,4}$ 261, $\lambda_{min}^{pH\,4}$ 230, $\lambda_{max}^{pH\,12}$ 261, $\lambda_{min}^{pH\,12}$ 243.

Acid hydrolysis of the product with *N* HCl at 100° for 1 hr. yielded uridine 2′(3′)-phosphate (Up) and uridine (U) in a ratio of 1.00:1.02. On incubation of the UpU with RNase (RNase A 50 μg./0.1 ml.) in 0.2*M* Tris-HCl buffer (pH 7.5) at 37° for 3 hr., the enzyme digested 92% of this product yielding Up and U in 1.00:1.04, thus UpU contained 8% of ribonuclease-resistant material (RRM).

In another experiment, 100 μmoles of I and 200 μmoles of Ⅳ were treated as above and detritylated with 5 ml. of 80% AcOH at 37°. Aliquots were withdrawn at appropriate intervals, neutralized and analyzed by PPC. Results are shown in Table I.

b) Route A (Removal of trityl groups at 100°): The deacetylated intermediate, which was obtained as described above, was suspended in 3 ml. of 80% AcOH and heated in a boiling water bath for 10 min. After working up as described in a), UpU was obtained in 33.3% yield. The RRM content in this product estimated by digestion with RNase A was found to be 5.6%.

c) Route B (Removal of trityl groups at 37°): From a 5 ml. of anhyd. pyridine solution containing 50 μmoles of 2′,5′-di-O-trityluridine 3′-phosphate (Ⅱ) obtained by a usual mothod, trace of H_2O was removed by repeating azeotropical distillation. To a solution of the gummy residue in 1 ml. of anhyd. pyridine were added 100 μmoles of 2′,3′-di-O-acetyluridine (Ⅵ) and 100 mg. of DCC. The tightly stoppered mixture was set aside at 37° for 5 days. Removal of the protecting groups was carried out by the same procedure as described in a) except that the trityl groups were removed by the treatment with 80% AcOH at 37° for 14 hr. The final product (UpU) was obtained in a yield of 39.9%, which contained 4.4% of RRM.

d) Route B (Removal of trityl group at 100°): The deacetylated intermediate was detritylated by its keeping in 3 ml. of 80% AcOH in a boiling water bath for 15 min., other procedures were similar to that described in c). Yield of the final product was 36.7% and it contained 6.9% of RRM.

In another experiment, 60 μmoles of Ⅱ and 120 μmoles of Ⅵ were treated as above and detritylated with 1.2 ml. of 80% AcOH at 100°. Aliquots were taken up at suitable intervals, neutralized and analyzed by PPC and by RNase digestion. Results are shown in Fig. 2.

Properties of RRM——The final product (UpU) which contained 5~8% of RRM was digested with RNase A and RRM was separated and isolated by PPC (solvent 1). The RRM was homogeneous on PPC and PEP and not further hydrolyzed with RNase A (50 μg./0.1 ml.). Acid hydrolysis of RRM (*N* HCl, 100°, 1 hr.) yielded Up and U in a ratio of 1.00:1.06. Other properties are as follows: Rf_1 0.16, Rf_2 0.29, Rf_3 0.50, Rf_4 0.28, M_1 0.45, M_2 0.64. UV: $\lambda_{max}^{pH\,4\ or\ 12}$ 261 mμ.

Stability of UpU in Acid Solution——About 20 mg. of UpU (containing 5.8% of RRM) was dissolved in 0.6 ml. of 80% AcOH and heated at 37° or 100°. Aliquots were taken up at suitable intervals and neutralized with pyridine under ice-cooling. A portion of the solution was applied to the quantitative determination of remaining UpU by PPC (solvent 1) and the other was used for isolation of the UpU by PPC. Thus, the spot of UpU was eluted with H_2O and lyophilized. The isolated UpU was digested with RNase A and the content of RRM was determined by PPC. Results are shown in Fig. 2.

2′,3′-Di-O-tetrahydropyranyluridine 5′-Phosphate (Ⅴ)——An aqueous solution containing 1 mmole of disodium uridine 5′-phosphate was passed through a column of Dowex 50 W (H^+ form) and the eluate was concentrated to dryness and the free uridine 5′-phosphate thus obtained was dissolved in 4 ml. of dimethylsulfoxide. To the solution was added 4 ml. of dihydropyran and 1 drop of conc. HCl, and the mixture was set aside at room temperature for 9 hr. The reaction was stopped by addition of 5 ml. of conc. NH_4OH and excess of dihydropyran and dimethylsulfoxide were extracted with Et_2O. An oily residue obtained on evaporation of the aqueous layer was dissolved in a small volume of solvent 1 and applied on a column of cellulose powder (2.5×35 cm.). The column was eluted with the same solvent and effluents fractionated. The fractions, which on PPC gave the spot of the product having Rf_1 0.34, were combined and evaporated to dryness and kept *in vacuo* over P_2O_5 to give 505 mg. (yield 87.6%) of a white powder. The product was homogeneous both in PPC and PEP. *Anal.* Calcd. for $C_{19}H_{29}N_2O_{11}P \cdot NH_3 \cdot 1/2\text{-}H_2O$: C, 44.0; H, 6.42; N, 8.11; P, 5.97. Found: C, 43.82; H, 6.83; N, 8.57; P, 6.19. UV mμ (ε)· $\lambda_{max}^{pH\,4}$ 261 (9600), $\lambda_{min}^{pH\,4}$ 230 (1900), $\lambda_{max}^{pH\,12}$ 261 (7000), $\lambda_{min}^{pH\,12}$ 244 (5300). Rf_1 0.34, M_1 0.90, M_2 0.78.

The compound (Ⅴ) was treated with 80% AcOH at 37° for 24 hr. to remove tetrahydropyranyl groups and the products were analyzed by PPC (solvent 1). The result was that although uridine 5′-phosphate was produced in 93% yield, 7% of the total UV absorbing material of the hydrolysis product still remained as the starting material (Ⅴ) and presumably the partially hydrolyzed material (Rf_1 0.24).

The authers are indebted to Takeda Chemical Industries, Ltd. and Ebios Pharmaceutical Co., Ltd. for their gifts of the starting materials.

Summary

Uridylyl-(3′→5′)-uridine (UpU) was synthesized *via* two different routes, *i.e.*, by condensation of 2′,5′-di-O-trityluridine (I) with 2′,3′-di-O-acetyluridine 5′-phosphate (IV) (Route A) and by condensation of 2′,3′-di-O-acetyluridine (VI) with 2′,5′-di-O-trityluridine 3′-phosphate (II) (Route B). The acid treatments to remove trityl groups were performed with 80% acetic acid at both 37° and 100° in several time intervals. The yields of the final product, UpU, and the amounts of uridylyl-(2′→5′)-uridine contained in the UpU were compared.

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