was distilled to give a light yellow oil, b.p. 80~100°/0.1 mm. (X+XI, 1.0 g.) and a colorless oil, b.p. 100~102°/0.1 mm. (X, 310 mg.), colorless needles (from petr. ether), m.p. 71~72°, $[\alpha]_D^{25}$ +71.0° (c=0.944), λ_{max} 286 mμ (ε 24.9), $\nu_{max}^{CHCl_3}$ 1720 cm^{-1}, ORD: $[\alpha]_{700}$ +34°, $[\alpha]_{316}$ +1626°, $[\alpha]_{262.5}$ −2448°, $[\alpha]_{250}$ −2187°; c=0.326 in MeOH; temperature 26°. *Anal.* Calcd. for $C_{12}H_{19}OCl$: C, 67.13; H, 8.86; Cl, 16.51. Found: C, 67.20; H, 8.95; Cl, 16.24. A mixture of the oil, b.p. 80~100°/0.1 mm., and zinc dust in HOAc was refluxed in an oil bath for 1~2 hr. to give ketone (XI).

b) A solution of crude VIII (4.56 g.) in AcOEt (45 ml.) was ozonized under the same conditions. A mixture of the ozonide and zinc dust (15 g.) in HOAc (30 ml.) was refluxed for 3 hr. in an oil bath and treated under the same conditions as described above to give a yellowish orange oil (3.7 g.), which was distilled at b.p. 84~85°/1 mm. as a colorless oil (XI, 1.85 g., in 67.5% yield calculated from VII), n_D^{20} 1.4880, d_4^{20} 1.0034, colorless needles, m.p. 24~25° (from petr. ether), λ_{max} 283 mμ (ε 29.3), $\nu_{max}^{CHCl_3}$ 1703 cm^{-1}, $[\alpha]_D^{20}$ +104° (c=1.154). ORD: $[\alpha]_{700}$ +74°, $[\alpha]_{308.5}$ +2740°, $[\alpha]_{266}$ −2551°, $[\alpha]_{250}$ −1958°; c=0.1078 in MeOH; temperature 25°. *Anal.* Calcd. for $C_{12}H_{20}O$: C, 79.94; H, 11.18. Found: C, 79.70; H, 11.12. 2,4-Dinitrophenylhyrazone, yellowish orange prisms, m.p. 142~143.5° (from EtOH). *Anal.* Calcd. for $C_{18}H_{24}O_4N_4$: C, 59.98; H, 6.71; N, 15.55. Found: C, 59.53; H, 6.89; N, 15.70.

Summary

levorotatory and *dextrorotatory* 6,10-Dimethylbicyclo[5.3.0]decan-3-ones (IIIa and IIIb) were derived from guaiol (I), and the former was converted into *dextrorotatory* 6,10-dimethylbicyclo[5.3.0]decan-4-one (XI).

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124. Morio Ikehara and Hitoshi Uno: Polynucleotides. I. Formation of Internucleotidic Linkage by Means of Dimethylformamide-Thionyl Chloride Complex.*1

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The synthesis of polynucleotides, either by chemical or biochemical means draw the attention of many investigators because of the important role of nucleic acid in the living cells. Activation of mononucleotides by dicyclohexylcarbodiimide (DCC) so as to polymerize them to polynucleotides was extensively studied by Khorana and colaborators.[1] The use of DCC as the condensing reagent of 5′-O-tritylthymidine and 3′-O-acetylthymidine 5′-monophosphate was studied and it proved to be the best among other reagents investigated so far.[2,3] But there seems to be a need for further search for new condensing agents to obtain much higher polymerizing potency.

During the course of our study of chlorination of inosine derivatives,[4,5] we found that, when inosine 5′-monophosphate was treated with DMF*3-thionyl chloride

*1 A preliminary account of this study has appeared in Chem. Pharm. Bull., **12**, 742 (1964).

*2 Nishi 5-chome, Kita 12-jo, Sapporo, Japan (池原森男, 宇野 準).

*3 N,N-Dimethylformamide.

1) H. G. Khorana: "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest.," John Wiley and Sons, Inc., New York, N. Y. (1961), Chapter 5 and following Series of papers of Studies on Polynucleotides.
2) H. G. Khorana, J. P. Viszolyi, R. K. Ralph: J. Am. Chem. Soc., 84, 414 (1962).
3) T. M. Jacob, H. G. Khorana: *Ibid.*, **86**, 1630 (1964).
4) M. Ikehara, H. Uno: This Bulletin, **12**, 267 (1964).
5) *Idem*: *Ibid.*, **13**, 221 (1965).

complex, oligonucleotidyl material presumably having 2′-5′, 3′-5′ internucleotide linkage was formed.*4 In order to obtain the information about condensing property of DMF-thionyl chloride complex, we have studied more details of the reaction of 5′-O-trityl-thymidine (I) and 3′-O-acetylthymidine 5′-monophosphate (II). The system proved to be suitable for estimating the internucleotidic linkage formation.[2] The reaction was investigating first using a system, in which molar ratio of the starting materials is 1:1. Both starting materials were dissolved in DMF and/or pyridine and DMF-thionyl chloride complex prepared previously was combined. In order to avoid an evolution of heat in the case of pyridine containing solution, the whole was cooled with dry ice bath. Despite of this cautious treatment a red colorization occurred. The reaction

Table I. Rf Values of Individual Nucleoside and Nucleotides

Substance	Paper chromatography		Paper electrophoresis (R_{pT})
	Solvent A	Solvent B	
Thymidine	0.67	0.77	0.31
TrT	0.80		
pT	0.14	0.19	1.00
pTAc	0.18	0.28	
TppT	0.24	0.37	
TpT	0.44	0.52	0.84

Table II. Reaction Condition and Yield of TpT Synthesis

TrT (mmole)	pTAc (mmole)	$SOCl_2$ (mmole)	DMF (ml.)	Pyridine (ml.)	Time (hr.)	TppT (%)	TpT (%)
0.1	0.1	0.3	2		4	24.8	
0.1	0.1	0.3	2		22	36.3	
0.1	0.1	0.5	2	2	22		38.4
0.1	0.1	0.5	2	2	81		48.3
0.1	0.1	0.5	0.5 mmole	2	6		33.6
0.1	0.1	0.5	0.5 mmole		22		44.5
0.1	0.1	0.5	2	2[a)]	22 (total)		43.2
0.1	0.2	0.5	0.5 mmole	2	120		43.8

a) Added after 6 hour's reaction, during which 38.0% of TppT was formed.

was carried out at room temperature under strict exclusion of moisture. After the treatment described in experimental, the extent of the reaction was estimated by paper chromatography and electrophoresis. Rf and R_{pT}*5 values of the individual products were listed in Table I. Results of the experiments were listed in Table II. When the reaction was carried out in DMF in the absence of acid acceptor, only P^1,P^2-dithymidine 5′-pyrophosphate (TppT) (IV) was obtained.*6

With the studies with DCC, in which the pyrophosphate derivative was assumed as the intermediary occurring product.[6] In the presence of pyridine, addition to the initial components, the reaction seems to proceed to the formation of end product, thymidinyl (3′-5′) thymidine (TpT) (III). The amount of TpT varied from 33 to 48%

*4 A nucleotidyl material, which migrates slowly on paper chromatogram in solvent c was formed. (Unpublished experiments by M. Ikehara).
*5 A value obtained from migration distance divided by that of thymidine 5′-phosphate.
*6 TppT was disappeared by the treatment of the reaction mixture with acetic anhydride in pyridine.[3]
6) G. Weimann, H. G. Khorana: J. Am. Chem. Soc., 84, 4329 (1962).

as the time of reaction and molar ratio of reactants were changed. Prolonged reaction seems to give higher reaction extent. In the case of the ratio of I to II equal to 1:2 and a prolonged reaction time (120 hr.), TpT was obtained in 43.8% yield. The cause with which the reaction proceeded in rather low yield is not clarified in the present stage.

The chlorination of 4-hydroxyl group of thymidine could be excluded by the comparison of ultraviolet absorption spectra of TpT with an authentic specimen and by the fact that inosine derivative was not chlorinated with DMF-thionyl chloride complex at room temperature.[5] Another possible reaction could be sulfinic acid ester formation of 3'-hydroxyl group of I. However the acid and alkaline treatment of the reaction mixture seems to remove this ester group as shown in the experiment of Anderson, *et al.*[7] Though the mechanism of the reaction was not clarified as yet, activation of phosphate by the formation of phosphorochloridate or phosphoric sulfinic anhydride[3] might be expected from the nature of DMF-thionyl chloride complex.

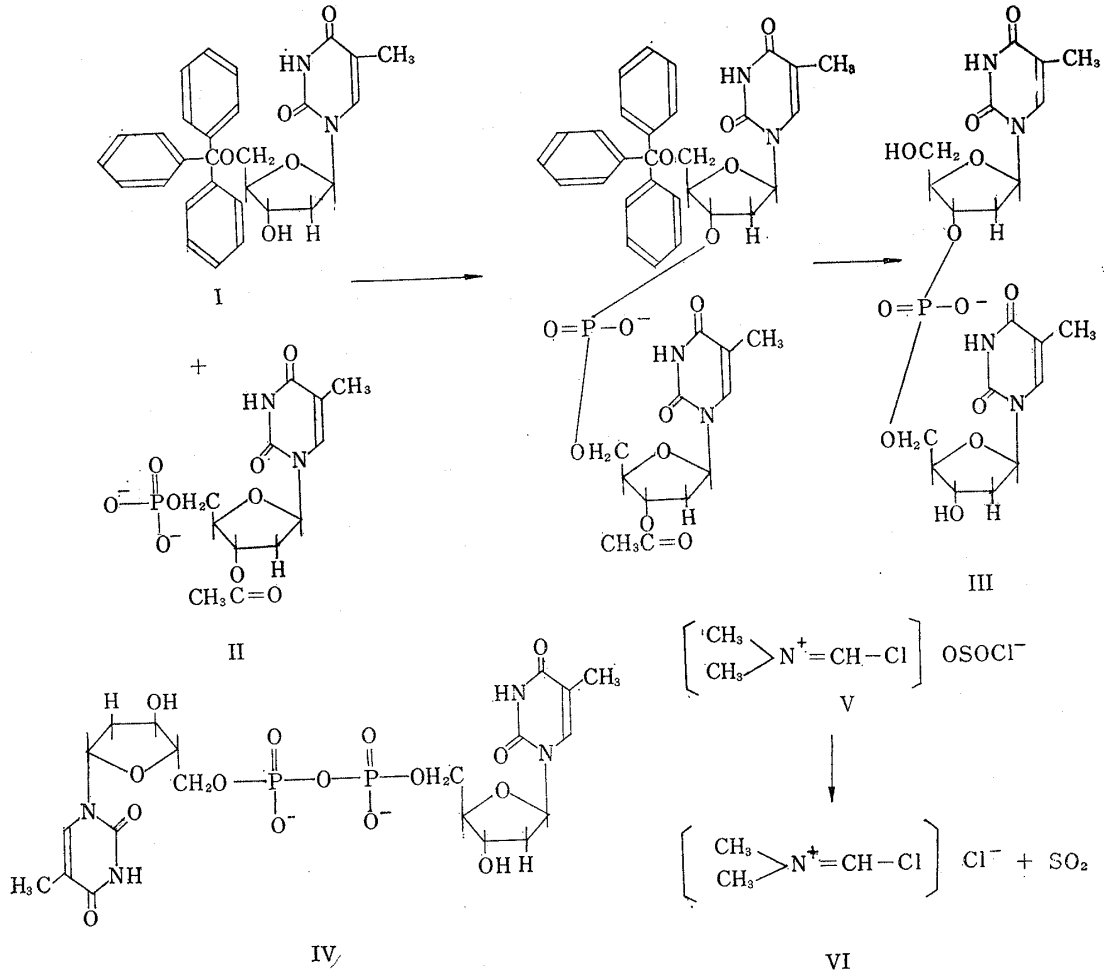

Chart 1.

On the structure of the complex, we investigated the nuclear magnetic resonance spectra of the freshly prepared reagent. As stated by Martin and Martin[8] in the case of complex formed from DMF and phosphoryl chloride, absorption band of CH_3-proton

7) C. D. Anderson, L. Goodman, B. R. Baker: J. Am. Chem. Soc., **81**, 3967 (1959).
8) G. Martin, M. Martin: Bull. soc. chim. France, **1963**, 637.

appeared at 6.30 τ and that of CH-proton appeared at 1.75 τ. This values correspond to the structure shown in V. However, the complex may be changed to have the structure (VI) at the elevated temperature with the loss of sulfur dioxide.

Experimental

Paper Chromatography——Solvent A: isopropanol-28% NH_3-water=7:1:2 (descending); solvent B: EtOH-1% ammonium acetate (pH 7.5)=7:3 (ascending). Solvent C: Isopropanol-1% ammonium sulfate 2:1 (ascending). Toyo filter paper No. 51 A was used.

Paper Electrophoresis——0.05*M* triethylammonium bicarbonate, pH 7.5, 20 V/cm., 1 hr. Toyo filter paper No. 51 A was used.

Nuclear Magnetic Resonance Spectroscopy——Taken with Varian V-4300 C high resolution spectrometer operating at 60 mc. Tetramethylsilane was used as internal reference.

Detection of Products on Paper Chromatograms——Ultraviolet absorbing spot was visualized by irradiation with the ultraviolet lamp. Phosphate was detected by spraying perchlorate-molibdate reagent.[9] With this reagent thymidine was detected after heating at 80° for 7 min. as an charcoal-brown spot.

General Procedure——5′-O-Tritylthymidine and 3′-O-acetylthymidine 5′-monophosphate (amounts tabulated in Table I) were dissolved in DMF (Dried over anhydrous cupric sulfate, decanted and freshly distilled). In some experiments pyridine (dried over potassium hydroxide) was added. A chloroform (30 ml.) solution of thionyl chloride (freshly distilled) and DMF (amount indicated in Table II) was refluxed for 45~60 min. and solvent was removed by oil pump. Into the residual crystalline complex was added the solution of tritylthymidine and acetyl-TMP. The whole reaction mixture was tightly stoppered and kept in a desiccator at room temperature. Aliquot (0.1 ml.) was extracted at intervals indicated in Table II and diluted with 0.5 ml. of aqueous pyridine (2:1, v/v). The mixture was kept at room temperature for over 6 hr. After the removal of the solvent *in vacuo*, residual oil was heated in 2 ml. of 80% acetic acid at 100 for 10 min. Acetic acid was evaporated under reduced pressure and the residue was taken up in 1 ml. of 0.5*N* NaOH solution. The reaction mixture was kept at room temperature for 1 hr. The aliquot of the mixture was examined by paper electrophoresis and paper chromatography in two solvents. Rf's and R_{pT} values are summarized in Table I. The reaction extent was estimated by ultraviolet absorption of the spot eluted from the paper of chromatogram. Each 3 ml. of 50% ethanol containing 1% ammonia was used for extraction. The spot of TMP, thymidine and TpT was confirmed by the comparison with authentic specimen. ε_{267} values used for calculation were 8.4×10^3 for one thymine chromophor.*7

Nuclear Magnetic Resonance Spectra Measurement——Into a solution of DMF (0.5 mmole) in 20 ml. of dry chloroform was added thionyl chloride (0.5 mmole). The mixture was refluxed on an oil bath for 45~60 min. under exclusion of moisture. Evaporation of the solvent by an oil pump gave a crystalline residue. This material was dissolved in chloroform (concentration, ca. 20%) and spectra was taken. Sharp absorption band at 6.30 τ (CH_3-proton) and 1.75 τ (CH-proton) were observed as singlets. Integration of these peaks showed a ratio of 3:1. Signals of CH_3 and CH in pure DMF were appeared at 7.05 τ (doublet) and 2.01 τ (singlet).

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Summary

Using a complex derived from thionyl chloride and dimethylformamide, a method of the formation of internucleotidic linkage was investigated. From 5′-O-tritylthymidine and 3′-O-acetylthymidine 5′-monophosphate, thymidinyl (3′-5′) thymidine was obtained in the yield around 40~50%.

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*7 ε_{267} for TppT and TpT was assumed as 16.8×10^3 regardless of hyperchromicity.

9) C. S. Hanes, F. A. Isherwood: Nature, **164**, 1107 (1949).