[Chem. Pharm. Bull. 12 (6) 742 ~ 744]

UDC 612.398.145

Synthesis of 3′-5′-Internucleotidic Linkage by the Use of Dimethylformamide-Thionyl Chloride Complex as Condensing Agent

Recently, we have reported the use of Vilsmeier complex prepared from dimethylformamide (DMF) and thionyl chloride for chlorination of 6-hydroxyl group of inosine derivatives.[1] In order to extend this reaction to the nucleotide, inosine 5′-monophosphate was treated with DMF-thionyl chloride complex. In this instance, besides some 3′,5′-cyclic monophosphate, slower moving nucleotidic materials were detected on paper chromatogram.*1 This suggested the polymerization of mononucleotide to the higher polynucleotide having 2′-5′- or 3′-5′-phosphodiester linkage.[2] In this communication, more detailed study of formation of internucleotidic linkage between 5′-O-trytylthymidine (TrTh) (I) and 3′-O-acetylthymidine 5′-monophosphate (pTAc) (II) was described. As was reported by Khorana,[3] the system using TrTh (I) and pTAc (II) to afford 5′-O-tritylthymidilyl-(3′→5′)-3′-O-acetylthymidine (TpT) (III) will be suitable for the estimation of the ability of condensing agent to form the internucleotidic linkage.

Compound (I) (0.1 mmole) and (II) (0.1 mmole) were dissolved in 2 ml. of DMF (dried over anhydrous cupric sulfate and freshly distilled). Freshly distilled thionyl chloride (0.3~0.5 mmole) was added to dry DMF and the mixture was combined with the solution obtained above. In some experiments dry pyridine (2 ml.) was added. The reaction was carried out at room temperature under strict exclusion of moisture. 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 standing over 6 hours at room temperature and solvent was removed *in vacuo*. Residual oil was heated in 2 ml. of 80% acetic acid at 100° for 10 minutes and evaporated under reduced pressure. Residue was taken up in 1 ml. of 0.5*N* sodium hydroxide solution and kept standing at room temperature for 1 hour. The aliquot of reaction mixture was examined by paper chromatography and paper electrophoresis. Rf in two solvent systems and R_{pT} values were listed in Table I. Reaction extent was estimated by ultraviolet absorption of the spot of thymidine and TpT or P^1,P^2-di-5′-thymidylpyrophosphate (TppT) (IV) and was tabulated in Table II.

Table I.

Substance	Paper chromatography		Paper electrophoresis[c] (R_{pT})
	Solvent A[a]	Solvent B[b]	
Thymidine	0.67	0.77	0.31
TrTh	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

a) iso-PrOH-NH_3-H_2O=7:1:2 (descending).
b) EtOH-1*M* $AcONH_4$=7:3, pH 7.5 (ascending),
c) 0.005*M* triethylammonium hydrogencarbonate, pH 7.5, 20 v/cm., 1 hr.

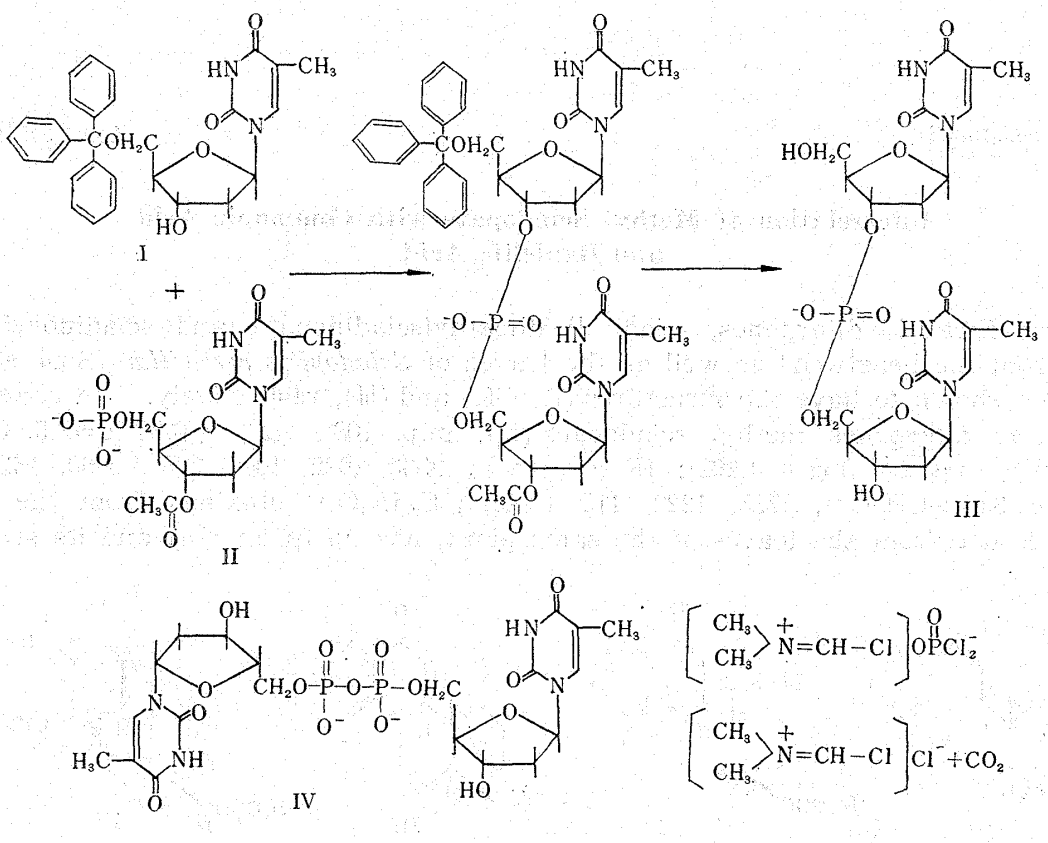
*1 Although some chlorination of 6-hydroxyl group would be expected, treatment of 2′,3′-O-isopropylidene-5′-O-benzoylinosine with the complex at room temperature gave essentially no chlorinated nucleoside (unpublished experiment by H. Uno).

1) M. Ikehara, H. Uno, F. Ishikawa : This Bulletin, **12**, 267 (1964).
2) M. Smith, J. G. Moffatt, H. G. Khorana : J. Am. Chem. Soc., **80**, 6204 (1959).
3) H. G. Khorana, J. P. Vizsolyi, R. K. Ralph : *Ibid.*, **84**, 414 (1962).

Table II.

TrTh (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	2	22		44.5
0.1	0.1	0.5	2	2[a)]	22 (total)		43.2

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



In the experiment using 3 equivalents of thionyl chloride in DMF solution without the addition of pyridine, 36.3%*[2] of TppT was obtained.*[3] When 5 equivalents of thionyl chloride was used in DMF and pyridine as solvent, TpT was obtained in 38.4% at 22 hours period. Use of an equivalent amount of DMF increased the yield of TpT to 44.5%. In the case of the addition of pyridine to initially formed TppT, equivalent amount of TpT was obtained.

Although deffinite structure of DMF–thionyl chloride complex was elucidated as yet, study of Martin[4] suggested the structure of DMF–phosphoryl chloride and DMF–phosgen as shown in the chart. Consequently, the complex could activate the phosphate ester by the formation of phosphorochloridate or by affording anhydride with sulfinic

*[2] Absorption of TpT or TppT was calculated as having two times as much as pT without any care of hyperchromicity.

*[3] TppT disappeared after hydrolysis in 1*N* hydrochloric acid at 100° for 15 minutes.

4) G. Martin, M. Martin: Bull. soc. chim. France, **1963**, 637.

acid derivative. Both mechanisms have the analogy in the experiment of internucleotidic linkage formation achieved by Todd[5] and Khorana.[3]

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Received March 16, 1964

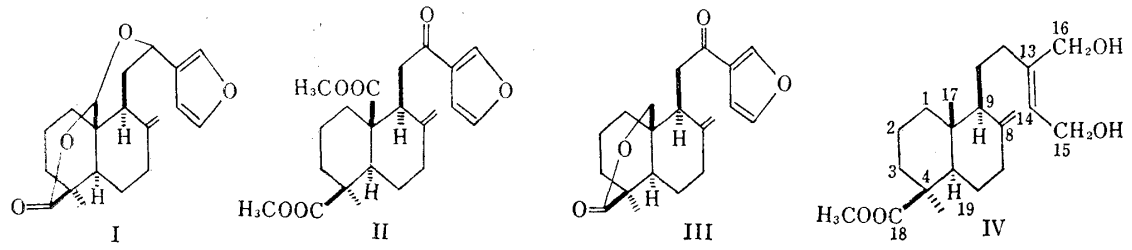
5) A. M. Michelson, A. R. Todd: J. Chem. Soc., **1955**, 2632.

[Chem. Pharm. Bull. 12 (6) 744 ~ 747]

UDC 547.913.6.07

Interrelation of Methyl Sciadopate with Communic Acid and Daniellic Acid

Three furanoid diterpenes, sciadin,[1] dimethylsciadinonate, and sciadinone[2,3] obtained from the heartwood as well as the leaves of *Sciadopitys verticillata* SIEB. et ZUCC. have been shown to have the structures (I), (II), and (III), respectively. In contrast to these three diterpenes methyl sciadopate (IV), m.p. 108°, $[\alpha]_D$ +0.36 (c=2.5, $CHCl_3$), UV: $\lambda_{max}^{95\%EtOH}$ mμ 202 (log ε 4.202); IR ν_{max}^{KBr} cm^{-1}: 3330, 1022, 1000 (OH), 3060, 1639, 884 (>C=CH_2), 847 (-CH=C<), 1735, 1222, 1150 (ester); $C_{21}H_{34}O_4$*1 obtained from the heartwood, but not from the leaves of the same plant, has no furan ring and its structure



(including absolute configuration) has been determined independently by Sumimoto, *et al.*[4] and the author.[5] Not only do these four diterpenes closely resemble each other structurally, but they all have so-called normal absolute configurations. The common carbon skeleton strongly suggests that they are biogenetically related quite closely.

A literature survey of diterpenoid chemistry indicates that methyl sciadopate (IV) is seemingly situated biogenetically at the mid-point between communic or agathadienedicarboxylic acid and the furanoid diterpenes mentioned above, since the *cis*-2-butene-

*1 All the analytical values were in good agreement with molecular formula shown.

1) M. Sumimoto: Tetrahedron, **19**, 643 (1963).
2) C. Kaneko, T. Tsuchiya, M. Ishikawa: This Bulletin, **11**, 271 (1963).
3) *Idem*: *Ibid.*, **11**, 1346 (1963).
4) M. Sumimoto, *et al.*: Abstract of papers, Symposium on the organic chemistry of natural products, Kyushu, Japan (1963), p. 243.
5) T. Miyasaka: *Ibid.*, p. 238.