des Lösungsmittels ein braunes Öl (500 mg). Dieses wurde an 20 g Kieselgel mit Hexan–Äther (98:2) chromatographisch gereinigt, wobei farblose Nadeln vom Schmp. 53° (Petroläther) erhalten wurden: Ausbeute 210 mg.

Ginkgol (III)——Die Fraktion II (5.5 mg) wurde an 300 g Kiesegel (Säule: 50×2.5 cm) mit $CHCl_3$ chromatographisch gereinigt, wobei ein farbloses Öl vom Sdp_1 180° erhalten wurde. Ausbeute 1.8 g (0.06%). IR ν_{max}^{Film} cm^{-1}: 3400 (OH), 1600 (aromat. Ring). $C_{21}H_{34}O$ (mol. Gew., 302.48)——Ber.: C, 83.38; H, 11.33. Gef.: C, 83.27; H, 11.27.

(+)-10-Nonacosanol (IV)——Die Fraktion, aus der bei der oben erwähnten Chromatographie der Fraktion II mit $CHCl_3$ vorher die Substanz III eluiert war, ergab farblose Nadeln vom Schmp. 84° (Äthanol). Ausbeute 180 mg (0.006%). $[\alpha]_D$ ±0° (c=1.0, $CHCl_3$). IR ν_{max}^{Film} cm^{-1}: 3400 (OH). $C_{29}H_{60}O$ (mol. Gew., 424.77)——Ber.: C, 81.99; H, 14.24. Gef.: C, 81.65; H, 14.30.

Anerkennung Die Autoren möchten den Herren des botanischen Gartens dieser Firma, besonders Herrn R. Hatta, für die Beschaffung der Früchte von *Ginkgo biloba* L., sowie den Damen und Herren in der physikochemischen Abteilung, die die Elementaranalysen und die Aufnahmen der Spektren durchgeführt haben, herzlichst danken.

[Chem. Pharm. Bull. 16(11) 2286—2288 (1968)]

UDC 547.963.32.07 : 547.854.4.07

A Direct Synthesis of 3′,5′-Di-O-acetyl-O^2,2′-cyclouridine

Yoshiyasu Furukawa and Mikio Honjo

Chemical Research Laboratories, Research and Development Division, Takeda Chemical Industries, Ltd.[1]

(Received March 27, 1968)

O^2,2′-Cyclouridine (I) has received considerable attention as an intermediate in the chemical modification of the ribose or uracil moiety of uridine.[2] Walwick, *et al.* synthesized I from uridine by heating it with polyphosphoric acid, followed by dephosphorylation of the resulting O^2,2′-cyclouridine-3′,5′-di-O-polyphosphate (II) with prostatic phosphatase.[3] The method, however, has some drawbacks in that the purification of II is troublesome and that the enzymatic dephosphorylation of II to I does not give a satisfactory yield.[4]

We attempted to find a phosphorylating reagent superior to polyphosphoric acid used in the above-mentioned Walwick's method. A solution or suspension of uridine in dimethylformamide, toluene or ethyl acetate was heated under various conditions with one of the phosphoric acid derivatives, such as *ortho*-phosphoric acid, phosphorus oxychloride, phosphorus trichloride, phosphorus pentachloride, monophenyl phosphorodichloridate and diphenyl phosphorochloridate. No shift of the absorption maximum (at pH 2) of the resulting reaction mixture was observed in all experiments with only one exception where a suspension of uridine in ethyl acetate was heated with partially hydrolyzed phosphorus oxychloride.[5–7] The reaction mixture, after desalting with activated charcoal treatment, was subjected to paper

---

[1] Location: *Juso, Higashiyodogawa-ku, Osaka.*

[2] A.M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York and London, 1963, p. 15.

[3] E.R. Walwick, W.K. Roberts and C.A. Dekker, *Proc. Chem. Soc.*, **1959**, 84.

[4] We used a phosphatase of *Phytophthora infestans* IFO 4872 available in quantities.

[5] Formation of I from uridine results in shift of absorption maximum, because the maxima of uridine and I are 262 mμ and 250 mμ, respectively.

[6] A.F. Leo and G.F. Walter, U.S. Patent 2610177 (1952).

[7] No shift was observed when partially hydrolyzed phosphrous trichloride was used.

electrophoresis (borate, pH 9.2), which showed unexpectedly the absence of phosphorylated products, but showed the presence of a substance which did not travel in the electrophoresis at the same pH. The ultraviolet absorption spectrum of this substance was indistinguishable from that of I.[8] Increase in the amount of the partially hydrolyzed phosphorus oxychloride resulted in higher yields (up to 95%) (Table I).

Table I. Effect of the Amount of Partially Hydrolyzed Phosphorus Oxychloride on the Yield of O^2,2′-Cyclouridine Derivative

uridine 100 mg, ethylacetate 10 ml

No.	$H_2O/POCl_3$[a] ml	Reflux time hr	$\lambda_{max}^{pH\,2}$ mμ	Yield[b] %
1	0.1	2	258	20
2	0.2	2	256	36
3	0.4	2	252	70
4	0.6	1	250	94
5	0.8	1	250	95

a) reaction mixture of $POCl_3$ (5 g) and H_2O (0.47 ml) at 0° for 30 min
b) Determined by paper electrophoresis (borate, pH 9.2).

The reaction mixture (Table I, No. 4), after desalting with activated charcoal treatment, was concentrated to afford colorless needles, mp 178—179°. This compound was found identical with 3′,5′-di-O-acetyl-O^2,2′-cyclouridine (III)[9] by comparison of the melting points, ultraviolet, infrared absorption and nuclear magnetic resonance spectra as well as by elementary analysis. Compound (III) was thus prepared by a single step in high yields when uridine was heated with partially hydrolyzed phosphorus oxychloride in ethyl acetate.[10] The reaction of 2′,3′,5′-tri-O-acetyluridine with partially hydrolyzed phosphorus oxychloride, however, did not give III under similar conditions as described above. This fact indicates that O^2,2′-cyclization of uridine to III does not proceed *via* 2′-O-acetate. When ethyl formate, methyl acetate, butyl acetate, ethyl propionate, ethyl benzoate, acetic acid, ethanol, acetone or acetonitrile was used in place of ethyl acetate, the shift of the ultraviolet absorption maximum was not observed. Only a small shift of the absorption maximum was observed in the absence

Table II. Effect of Molar Ratio ($H_2O/POCl_3$) on the Yield of 3′,5′-Di-O-acetyl-O^2,2′-cyclouridine

uridine 100 mg, ethylacetate 10 ml, partially hydrolyzed phosphorus oxychloride 0.6 ml

No.	Molar ratio $H_2O/POCl_3$	Reflux time hr	$\lambda_{max}^{pH\,2}$ mμ	Yield[a] %
6	0.1	2	259	15
7	0.5	1	252	80
8	1.0	1	250	94
9	1.5	1	252	88
10	2.0	2	258	38

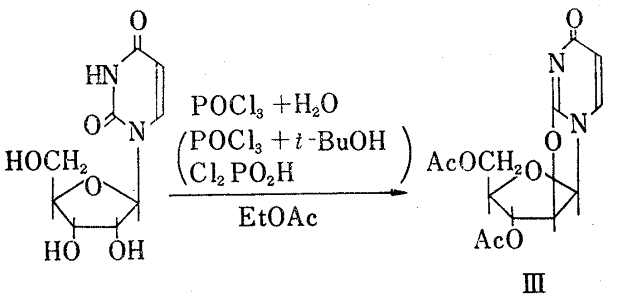
a) See Table I.

---

8) D.M. Brown, A. Todd and S. Varadarajan, *J. Chem. Soc.*, **1956**, 2388.
9) D.M. Brown, D.B. Parihar and A. Todd, *J. Chem. Soc.*, **1958**, 4242.
10) Recently, Hampton and Nichol reported a convenient method for the synthesis of I, which comprises treatment of uridine with diphenylcarbonate in dimethylformamide (A. Hampton and A.W. Nichol, *Biochem.*, **5**, 2076 (1966)). Our method is more convenient for the synthesis of III on a laboratory scale, since it does not require diphenylcarbonate.

of solvent where the phosphorylation had also taken place. The phosphorylated product, however, was not feasible to dephosphorylation with phosphatase.

The molar ratio of water to phosphorus oxychloride was significant: III was given in high yields when the molar ratio was 0.5—1.5 (see Table II). When the reaction was carried out in a molar ratio of 0.1, the paper electrophoresis (borate, pH 9.2) of the reaction mixture gave a strong ultraviolet absorbing spot at a position of the same *Rf* value as that for uridine. When the ratio was 2.0, or the reaction period was longer at the ratio of 1.0, a strong ultraviolet absorbing spot was located at the position for 1-*β*-D-arabinofuranosyluracil. The use of dichlorophosphoric acid[11] or a mixture of phosphorus oxychloride and *tert*-butanol (1:1), in place of partially hydrolyzed phosphorus oxychloride also gave III in yields of 96% and 86%, respectively. Cytidine was heated with partially hydrolyzed phosphorus oxychloride in ethyl acetate as in the case of uridine, but no cyclized products were obtained.



III

Chart 1

Experimental[12]

3′,5′-Di-O-acetyl-O^2,2′-cyclouridine (III)——1) Partially hydrolyzed $POCl_3$ was prepared by adding H_2O (2.35 ml, 0.13 mole) to ice-cooled $POCl_3$ (20 g, 0.13 mole) and stirring the mixture for 30 min. This agent was poured to a suspension of uridine (2 g) in AcOEt (200 ml). The mixture was refluxed for 40 min and poured into H_2O (800 ml) containing $NaHCO_3$ (25 g). AcOEt was distilled off *in vacuo*. The solution was run through a column of activated charcoal (20 g). The column was washed with H_2O and eluted with EtOH-pyridine (4:1 v/v).[13] The eluate (400 ml) was evaporated to dryness. The residue was dissolved in hot EtOH (30 ml) and left standing in a cold room to afford colorless needles, mp 178—179°, 1.89 g (75%). No depression of the melting point was shown upon the admixture with an authentic sample prepared by acetylation of I.[5] *Anal.* Calcd. for $C_{13}H_{14}O_7N_2$: C, 50.30; H, 4.55; N, 9.03. Found: C, 50.35; H, 4.54; N, 9.11. UV λ_{max}^{EtOH} mμ: 224, 249; λ_{min}^{EtOH} mμ: 235. NMR (D_2O) δ: 2.03 (singlet, CH_3-CO-), 2.27 (singlet, CH_3-CO-), 6.30 (doublet, $J=7.5$ cps, H_5), 6.67 (doublet, $J=6.0$ cps, H_1'), 8.02 (doublet, $J=7.5$ cps, H_6).

2) Partially hydrolyzed $POCl_3$ (2 ml) was added to a suspension of 5′-O-acetyluridine[4] (572 mg) in AcOEt (50 ml). The mixture was refluxed for 2 hr, and treated in a similar manner as described in 1) to give III (480 mg, 80%).

Acknowledgement The authors are grateful to Drs. Y. Abe and K. Tanaka for their continuing interest and encouragement and to Mr. Y. Yoshioka for his helpful advice. Thanks are also due to Mr. M. Kan and his associates for elementary analyses and to Mr. K. Shinozaki for NMR measurement.

---

[11] H. Grunze, *Z. Anorg. Allgem. Chem.*, **298**, 152 (1959).

[12] Melting point is uncorrected. NMR was measured using $(CH_3)_4Si$ as an external reference. Chemical shifts were recorded in ppm.

[13] Ethanol-aqueous ammonia is generally used for elution of nucleic acid related compounds from activated charcoal. However, we used ethanol-pyridine for elution of I, because I is labile to alkali.