307. *The Constitution of Yeast Ribonucleic Acid. Part XIII. The Synthesis of Diuridine-2′ : 2′ Phosphate.*

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The first synthesis of a dinucleoside phosphate, diuridine-2′ : 2′ phosphate, is described. It is relatively stable towards alkali and acid, and it is concluded that the presence of phosphodiester linkages is unlikely to be the cause of the facile alkaline fission of yeast ribonucleic acid.

In previous papers (Gulland and Smith, *J.*, 1947, 338; previous paper) we have shown that the stabilities of nucleoside-2′ and -3′ phosphates towards alkali do not differ significantly, and hence that the facile alkaline fission of yeast ribonucleic acid is not the result of a much greater lability of phospho-ester groups in position 2′ as compared with those in 3′ as has long been postulated, but must be due to some as yet unknown property of the polynucleotide.

The further study of this question by synthetic methods requires the preparation of di-, tri-, and poly-nucleotides containing phosphoryl linkages in such positions, *e.g.*, 2′, 3′, 5′, and N, as might be expected in the nucleic acid molecule, and comparative studies of their hydrolyses with those of yeast ribonucleic acid and other natural polynucleotides. Factors of potential importance also are the relative stabilities of phospho-di- and -tri-esters and the mutual influence of adjacent phosphoryl groups.

The synthesis of *diuridine-2′ : 2′ phosphate* (II) now described indicates that the stabilities of phospho-diesters in this series are unlikely to differ markedly from the related monoesters.

Two mols. of 3′ : 5′-benzylidene uridine (Gulland and Smith, *J.*, 1947, 338) were condensed with one mol. of phenyl dichlorophosphinate in anhydrous pyridine to give a good yield of *di-(3′ : 5′-benzylidene uridine-2′ : 2′) phenyl phosphate* (I).

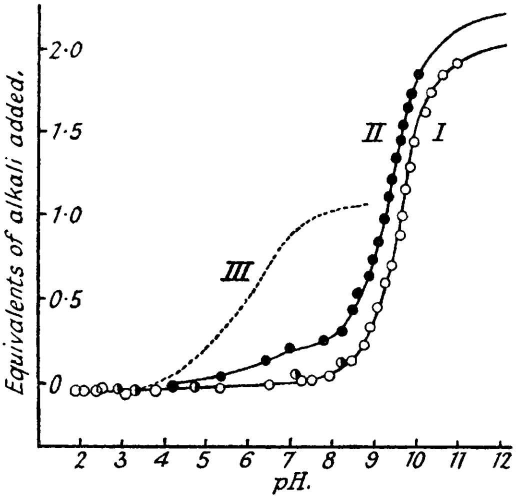
Attempts to remove the phenyl residue by hydrogenolysis with Adam's platinum catalyst resulted in a simultaneous hydrogenation of the benzylidene radicals which could not then be easily removed by acid hydrolysis (cf. Brigl and Müller, *Ber.*, 1939, **72**, 2121).

Hydrolysis of the compound (I) with N/2-sodium hydroxide at 100° for ½ hour removed the phenyl residue, and after elimination of the benzylidene radicals by means of hot N/4-sulphuric acid, *barium diuridine-2′ : 2′ phosphate* (as II) was isolated from the mixture in good yield. Purification from small amounts of uridine-2′ phosphate and uridine-2′ phenyl phosphate, inevitably produced in the hydrolysis, was effected by taking advantage of the facts that (i) the lead salt of (II) is not precipitated at pH 7 as is lead uridine-2′ phosphate, and (ii) the barium salt (as II) is soluble in 66% alcohol but not in 90% alcohol, whereas barium uridine-2′ phosphate is insoluble in the former solvent and barium uridine-2′ phenyl phosphate is soluble in the latter solvent. The barium salt was obtained analytically pure but could not be crystallised and was characterised by its optical rotation, decomposition point, and electrometric titration. Brucine, α-naphthylamine, acridine, *S*-benzylthiouronium, and sodium salts could not be crystallised, and attempts to crystallise the free nucleotide were also unsuccessful.

Electrometric titration of the barium salt (as II) demonstrated the presence of the dissociating groups required for diuridine phosphate, *i.e.*, two enolic hydroxyl groups (pK' 9·7) and no secondary phosphoric acid group.

The hot alkaline hydrolysis of di(benzylidene uridine) phenyl phosphate used in this preparation shows that the ester (II) conforms to the general pattern of phosphodiesters in approximating in stability to the monoesters. Electrometric titrations (see figure) (for details of the method used see Gulland, Jordan, and Taylor, *J.*, **1947**, 1131) gave quantitative evidence as to the stability of (II) under conditions which cause complete fission of yeast ribonucleic acid into a mixture of mononucleotides. Titration of the barium salt to pH 1, hydrolysis at 100° for $1\frac{1}{2}$ hours, and back-titration to pH 8 revealed no secondary phosphoryl dissociation, indicating that no significant degree of hydrolysis had occurred. Titration of the barium salt to pH 13, followed by preservation of the solution at room temperature for 24 days and back-titration to pH 4, showed the presence of a small amount (up to 0·2 equivalent) of secondary phosphoryl dissociation, indicating that even in those severe conditions hydrolysis was only very slight.

Electrometric titrations of barium salt of diuridine-2′ : 2′ phosphate.



Full curve I, theoretical curve for diuridine phosphate.

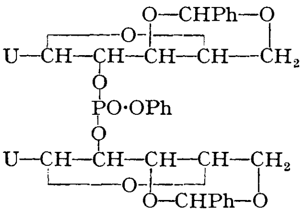
Full curve II, theoretical curve for diuridine phosphate which has undergone hydrolysis with liberation of 0·2 *equivalents of secondary phosphoric acid group.*

Dotted curve III, theoretical curve for a mixture of uridine phosphate and uridine formed by complete fission of diuridine phosphate.

○ *Titration of diuridine-*2′ : 2′ *phosphate.*

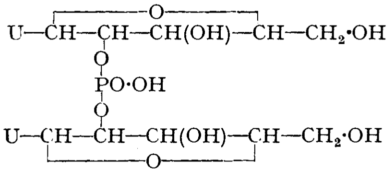
◑ *Back titration of diuridine-*2′ : 2′ *phosphate after hydrolysis at* pH 1 *for* 1·5 *hours at* 100°.

● *Back titration of diuridine-*2′ : 2′ *phosphate after hydrolysis at* pH 13 *for* 24 *days at room temperature.*



(I.)

(U = uracil radical)



(II.)

It is thus improbable that the presence of phospho-diester linkages as such is the cause of the facile fission of the nucleic acid. Further investigation is envisaged along the lines sketched above.

EXPERIMENTAL.

Di-(3′ : 5′*-benzylidene Uridine-*2′ : 2′) *Phenyl Phosphate.*—A solution of freshly distilled phenyl dichlorophosphinate (2·7 c.c.) (Brigl and Müller, *Ber.*, 1939, **72**, 2121) in dry pyridine (50 c.c.) was added during 15 minutes to a mechanically stirred solution of dry benzylidene uridine (10 g.) in dry pyridine (100 c.c.), cooled in ice-salt and protected from moisture. Stirring was continued for 3 hours in the freezing mixture and for 3 hours at room temperature; the reaction mixture attained a deep golden yellow colour. The solution was again cooled in a freezing mixture, 50% aqueous pyridine (50 c.c.) was added during 15 minutes, stirring was continued for 1 hour, and the reaction mixture poured into ice–water (1·5 l.). Pyridine was removed from the resulting yellow suspension by evaporation under reduced pressure to about 400 c.c., and, after the mixture had been cooled in ice, ice-cold N-sodium hydroxide (100 c.c.) was added; the precipitate dissolved to form a deep orange-red solution which was filtered, N-sulphuric acid was added until the solution was just acid to Congo-red, and the brown precipitate was collected by centrifugation and washed 5 times with ice-cold water. The process of solution and precipitation was then repeated. *Di(benzylidene uridine) phenyl phosphate* (I) (9·3 g.) was collected, washed 5 times with ice-cold water, and dried in a vacuum desiccator over phosphoric oxide; it formed a brown amorphous powder (Found: C, 56·9; H, 4·6; N, 7·0; P, 4·0. $C_{38}H_{35}O_{14}N_4P$ requires C, 56·9; H, 4·4; N, 7·0; P, 3·9%). It formed an intensely yellow solution in alkali which faded on removal of the phenyl radical (see below). It dissolved readily in the usual organic solvents but was practically insoluble in water, dilute acid, light petroleum, and benzene. Attempts to crystallise it have so far failed.

Diuridine-2′ : 2′ Phosphate.—Di(benzylidene uridine) phenyl phosphate (5 g.) was dissolved in N/2-sodium hydroxide (200 c.c.). The intensely orange-yellow solution was refluxed for 30 minutes; the colour faded leaving a faintly brown solution, and analysis for free phenol (Folin and Ciocalteu, *J. Biol. Chem.*, 1927, **73**, 627) showed that over 95% of the originally combined phenol had been liberated. N-Sulphuric acid (200 c.c.) was added, and the solution was refluxed gently for 1 hour, cooled, mixed with N-sodium hydroxide (98 c.c.), and extracted twice with ether (100 c.c.). Sodium sulphate was precipitated, and was removed by evaporation of the solution under reduced pressure to 100 c.c. and addition of absolute industrial alcohol (1 l.). The solution was concentrated and largely freed from alcohol by evaporation with water under reduced pressure. To the solution (50 c.c.), barium acetate (2 g.) was added to precipitate remaining sulphate ions, and then lead acetate (12 g.); the reaction was then adjusted to pH 7 with dilute ammonia, and barium sulphate and a small amount of lead uridine-2′ phosphate were removed and washed with water (20 c.c.). Ammonia was added to the combined filtrate and washings until the pH was 9·5—10·0, and the precipitate was collected by centrifugation and washed 4 times with water (100 c.c.) containing a little dilute ammonia. The lead salt was suspended in water (500 c.c.) and decomposed by passage of hydrogen sulphide for 4 hours. The solution was boiled, filtered from the coagulated lead sulphide, and aerated. After evaporation of the solution under reduced pressure to about 250 c.c., barium hydroxide solution was added until the mixture was alkaline to phenolphthalein, and the excess of barium hydroxide was removed by passing carbon dioxide, boiling, and filtration. Evaporation was then continued to 90 c.c., and absolute industrial alcohol (180 c.c.) was added. 8 Hours later a small precipitate was filtered off (kieselguhr) and washed with 66% alcohol (30 c.c.). Absolute industrial alcohol (800 c.c.) was added to the filtrate, and next day the precipitate was collected, and washed with 90% alcohol. It was redissolved in water (90 c.c.) and the above procedure repeated twice, the final product being washed with alcohol and ether and dried over phosphoric oxide in a vacuum. The *barium* salt (1·6 g.) formed a white amorphous powder (Found in samples of different batches dried at 100°/0·1 mm.: C, 34·8; H, 3·8; N, 9·0; P, 5·0, 5·0, 5·0, 5·0; Ba, 11·2, 11·3, 11·3, 11·0. $C_{36}H_{44}O_{28}N_8P_2Ba$ requires C, 35·0; H, 3·8; N, 9·1; P, 5·0; Ba, 11·1%). When the anhydrous material in a tube of 1 mm. diameter was placed in a bath at 240°, the rise of temperature being 5° per minute, shrinkage occurred at 242° and decomposition with vigorous effervescence at 249—252°. In water different batches of the dried salt had $[\alpha]_D^{13^\circ} + 12{\cdot}2^\circ$ (*c*, 3·4); $[\alpha]_D^{13^\circ} + 12{\cdot}5^\circ$ (*c*, 1·6); $[\alpha]_D^{27^\circ} + 12{\cdot}0^\circ$ (*c*, 2·8).

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