This structure is reasonable biosynthetically[13]; triterpenoids with similar skeletons have been isolated from various plants.[14]

In accord with this tentative structural formulation, tetrahymanone has a positive carbonyl n → π* Cotton effect centered at 290 mμ with molecular amplitude[15] +18 (R.D. in dioxane (*c* 0.14), 27°; $[\alpha]_{450}$ +73°, $[\alpha]_{313}$ +389°, $[\alpha]_{308}$ +375°, $[\alpha]_{306}$ +376°, $[\alpha]_{273}$ −38.6°, $[\alpha]_{240}$ +236°).[16] Thus, the Cotton effect curves[17] for 4,4-dimethyl-19-nordihydrotestosterone and 4,4,17α-trimethyl-19-nordihydrotestosterone, two compounds whose configuration in the vicinity of the carbonyl group is essentially the mirror image of that we propose for tetrahymanone, have molecular amplitudes of −20 and −15, respectively.[18]

We have nearly completed the rigorous establishment of the structure of tetrahymanol by an X-ray crystallographic study[19] of its *p*-bromobenzoate ester, m.p. 297–298.5° (*Anal.* Calcd. for $C_{37}H_{55}BrO_2$: C, 72.64; H, 9.06; Br, 13.06. Found: C, 72.63, 72.49; H, 9.12, 8.95; Br, 13.34, 13.16). This crystal is orthorhombic with space group C_{222}; the unit cell contains four molecules and has dimensions a = 13.06, b = 6.33 and c = 38.06 Å. The shortness of the b dimension shows that the tetrahymanol skeleton is roughly flat and fully extended in accord with predictions made from consideration of a molecular model of the proposed structure in which non-chair conformations for the D and E rings appear to be favorable.

(13) A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).

(14) J. M. Beaton, F. S. Spring, R. Stevenson and J. L. Stewart, *Tetrahedron*, **2**, 246 (1958); K. Kimura, Y. Hashimoto and I. Agata, *Chem. Pharm. Bull.* (Tokyo), **8**, 1145 (1960); H. R. Arthur and W. H. Hui, *J. Chem. Soc.*, 551 (1961).

(15) This term is defined by N. L. Allinger and M. A. DaRooge, *J. Am. Chem. Soc.*, **84**, 4561 (1962), and by C. Djerassi and W. Klyne, *J. Chem. Soc.*, 4929 (1962).

(16) These rotatory dispersion measurements were made in the laboratories of Professor Kurt Mislow, New York University.

(17) C. Djerassi, O. Halpern, V. Halpern and B. Riniker, *J. Am. Chem. Soc.*, **80**, 4001 (1958).

(18) Since the testosterone derivatives were studied[17] in methanol solution the close agreement in absolute magnitude between these amplitudes and the amplitude for tetrahymanone[16] may be somewhat fortuitous.

(19) In collaboration with Professor T. H. Doyne, Villanova University.

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THE STEPWISE SYNTHESIS OF RIBO-OLIGONUCLEOTIDES CONTAINING C_3'–C_5' INTERNUCLEOTIDIC LINKAGES[1]

Sir:

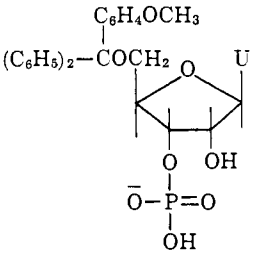
2′-*O*-Acetylribonucleoside-3′ phosphates are the key intermediates in the recently described approach to the synthesis of the C_3'–C_5' inter-ribonucleotidic linkage[2] and a method has been developed for the direct preparation of these derivatives in quantitative yield from the parent nucleotides.[2,3] In further work, this approach is being incorporated into schemes for the stepwise synthesis of ribopolynucleotides. The present communication records results of the initial phase of this work which has led to the syntheses of the two trinucleotides, adenylyl-(3′→5′)-uridylyl-(3′→5′)-uridine and uridylyl-(3′→5′)-uridylyl-(3′→5′)-uridine.

(1) This work has been supported by grants from the National Cancer Institute of the National Institutes of Health, the National Science Foundation and the Life Insurance Medical Research Fund, New York, N. Y.

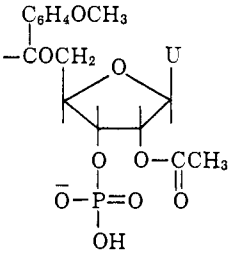
(2) D. H. Rammler and H. G. Khorana, *Biochem. Biophys. Res. Commun.*, **7**, 147 (1962); **8**, 61 (1962); D. H. Rammler, Y. Lapidot and H. G. Khorana, *J. Am. Chem. Soc.*, in press.

(3) Y. Lapidot and H. G. Khorana, *Chem. Ind.* (London), 166 (1963).

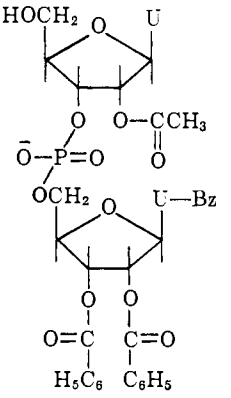
5′-*O*-monomethoxytrityluridine-3′ phosphate (I) was prepared by the reaction of pyridinium uridine-3′ phosphate with monomethoxytrityl[4] chloride (3 molar equiv.) in pyridine for 6 hr. ar room temperature and was purified by chromatography on a DEAE-cellulose (carbonate) column (isolated yield, 70%). Acetyla-



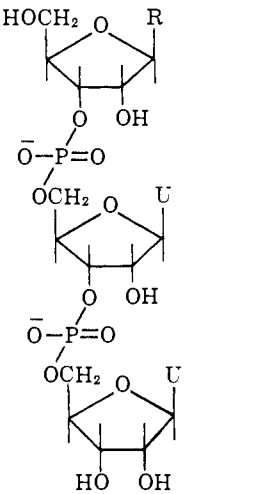
I



II



III; U-Bz = N-Benzoyluracil



IV; R = uracil or adenine
I-IV; U = uracil

tion of I with acetic anhydride in the presence of an excess of tetraethylammonium acetate[3] gave quantitatively the 2′-*O*-acetyl derivative (II) which was obtained as a white amorphous powder (pyridine salt) after precipitation from a mixture of pyridine and an excess of ether. The condensation of II (0.1 mmole) and *N*,2′,3′-tribenzoyluridine[5] (0.245 mmole) in dry pyridine in the presence of dicyclohexylcarbodiimide (DCC) followed by a work-up including an acidic treatment to remove the methoxytrityl group gave III as the major product which was purified by partition

TABLE I

PAPER CHROMATOGRAPHY OF DIFFERENT COMPOUNDS

Solvent A; isopropyl alcohol–concentrated ammonia–water (7:1:2)

Solvent B; *n*-butyl alcohol–acetic acid–water (5:2:3). Paper chromatography was performed using Whatman paper No. 1 by the descending technique.

Compound	R_f solvent A	R_f solvent B
Uridine-3′phosphate	0.12	0.23
5′-*O*-monomethoxytrityluridine-3′ phosphate	.52	
Uridylyl-(3′ → 5′)-uridine	.19	.09
2′-*O*-Acetyluridylyl-(3′→5′)-N,2′,3′-tribenzoyluridine (III)		.70
Uridylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-uridine	.065	.03
Adenylyl-(3′→5′)-uridylyl-(3′→5′)-uridine	.065	.027

(4) M. Smith, D. H. Rammler, I. H. Goldberg and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 430 (1962). Monomethoxytrityl is abbreviation for *p*-anisyldiphenylmethyl.

(5) M.p. 174–176°. Elemental analysis and other properties clearly show one benzoyl group on the uracil ring. This group is tentatively placed on N-1 position. D. H. Rammler, private communication, and R. Lohrmann, unpublished work from this Laboratory.

chromatography on a cellulose column using the solvent *n*-butyl alcohol–acetic acid–water (5:2:3) (isolated yield, 60%). This product (III), which was homogeneous by paper chromatography (R_f, Table I), was, as expected, resistant to the action of pancreatic ribonuclease and gave quantitatively uridylyl-(3′→5′)-uridine[4] after an ammoniacal treatment. The latter was degraded by the enzyme quantitatively to uridine-3′ phosphate and uridine.

The condensation of III (pyridine salt, 0.030 mmole) with 2′,5′-di-*O*-acetyluridine-3′ phosphate[5] (0.120 mmole) in dry pyridine in the presence of DCC followed by an ammoniacal treatment and separation by preparative paper chromatography gave uridylyl-(3′→5′)-uridylyl-(3′→5′)-uridine (IV; R = uracil) in 80% yield as based on the amount of III used. The product was degraded completely[6] by pancreatic ribonuclease to give uridine-3′ phosphate and uridine in the ratio 2:1. Analogous condensation of III with *N*,2′,5′-triacetyladenosine-3′ phosphate[7] (3-fold excess) gave adenylyl-(3′→5′)-uridylyl-(3′→5′)-uridine in 75% yield as based on III. This product was degraded quantitatively[6] to adenosine-3′ phosphate, uridine-3′ phosphate and uridine by the Lactobacillus acidophilus R26 phosphodiesterase.[8] The R_f values of different compounds are given in Table I.

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(6) Enzymatic degradation of IV (R = uracil) was performed using 7 optical density units (260 mμ) while that of IV (R = adenine) using 9.6 optical density units. By application of the total product on paper chromatograms, degradation was found to be complete. At the levels tested, therefore, the degradations by the specific enzymes provide conclusive proof of the exclusiveness of C_5'–C_5' internucleotidic linkages in the products.

(7) Prepared by the method used for acetylation of uridine-3′ phosphate (ref. 3) but using 3–5 days at room temperature for the N-acetylation.

(8) W. Fiers and H. G. Khorana, *J. Biol. Chem.*, in press.

BOOK REVIEWS

Gas–Liquid Chromatography. Theory and Practice. By STEPHEN DAL NOGARE, Senior Research Chemist, Plastics Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware, and RICHARD S. JUVET, JR., Associate Professor of Analytical Chemistry, Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois. Interscience Division, John Wiley and Sons, Inc., 440 Park Avenue South, New York 16, N. Y. 1962. xviii + 450 pp. 16 × 23.5 cm. Price, $13.95.

Dr. Dal Nogare is one of the top experts in gas chromatography in the U.S.A.; his name is particularly well known from his work in the practical introduction of temperature programming and from his bi-annual reviews on gas chromatography in *Analytical Chemistry*. The present book, coauthored by Dr. Juvet, is the result of a long and careful study and is highly welcomed; it can be expected that it will soon become the standard textbook for the practical gas chromatographers.

The book is composed of eighteen chapters. The introduction summarizes the different chromatographic techniques, and the next chapter gives the basic theoretical and practical information necessary for understanding the gas chromatographic process. The detailed discussion of the distribution theory is the subject of a separate chapter; continuously, the theoretical basis of retention and resolution is given with special emphasis on the conclusions important in practice. The discussion of packed column performance is based on the extended van Deemter–Jones equation.

Following these basic chapters, the book discusses the liquid, solid and mobile phases and the two basic instrumental components: sample introduction systems and detectors. Successively, the methods of qualitative and quantitative analysis and some individual techniques (capillary columns, high temperature and trace analysis, temperature programming and preparative chromatography). Finally, two chapters deal with non-analytical and special applications. Detailed relative retention tables (reproduction of the work of Scholly and Brenner[1]) and a list of U.S. commercial chromatographs and trade names close the book; a detailed and well prepared subject index helps when using the book as a reference book.

In my opinion, the book of Dal Nogare and Juvet is a very accurate summary of our present knowledge in gas chromatography, and its main strength is that the theoretical treatments are always closely related to practice. This and the many references after each chapter (totaling 883) make the book very valuable for every-day work.

I agree basically with the whole text of the book, and my remarks deal only with minor questions. The following detailed discussion of my remarks intends to serve as a contribution to future revisions of the text in consecutive editions.

The main question with similar textbooks dealing with subjects under rapid development is how up-to-date the compilation can be. In this respect, the authors did a remarkable job, because although the basic manuscript was evidently ready in the first part of 1961, they did include many of the most important newer publications up to 1962. Naturally, however, this could not be done in every part of the book; or, if it was done, the authors had no time in melting the additions together with the main part of the book. Thus, in some places, one feels that a sentence or paragraph was inserted later in the manuscript without logical transition from the previous paragraph, or that it was not possible any more to rewrite parts of the manuscript. I had, for example, the latter feeling at the part discussing the response of the flame ionization detector (pp. 218–221) where the material of the 1961 Lansing, Mich., meeting could not be included any more. Since a certain delay in the subject of a manuscript is always inevitable, one has to ask the question whether the publication of such important books could not be speeded up. At the present rapid development, publications of books 1–1.5 years after compilation of the manuscript is inadequate, since in this way, parts of the manuscript are more than 2 years old when the book is published. For example, in gas chromatography, a 2-year delay means 20% of the lifetime of the whole technique.

The second general problem with such textbooks concerns the symbols used. Particularly in gas chromatography, a very wide variety of symbols can be found for the same expression, and the authors decision to try to apply the symbols recommended by the I.U.P.A.C. Committee[2] is certainly welcome. I found only a very few cases where the application of the symbols is not accurate, *e.g.*, in the case of V_M, which means the *corrected* retention volume of the mobile phase in the book; although, according to the I.U.P.A.C. nomenclature, it is the *uncorrected* retention volume of a non-adsorbed sample.

It was a good idea to discuss at the beginning the principles of a general purpose laboratory apparatus; however, the given schematic (Fig. 2–5) is not typical, nor is its "commercial" version (Fig. 2–7) available. It is possible that it was built specially for the authors by the particular instrument manufacturer, but it is not available for others (it is not even included in the list of commercial instruments at the end of the book), nor is a *general* purpose instrument.

In my opinion, pyrolytic techniques are discussed in the wrong place (pp. 251–252). The pyrolysis–gas chromatographic technique is a special method similar to the microcatalytic–chromatographic technique (reaction kinetics studies), and if proper terms are used, the polymers which are pyrolyzed cannot be characterized as "the samples," for in this case, the mixture of the volatile pyrolysis products is the sample. It is unfortunate that later when discussing briefly the application of gas

(1) N. Brenner and P. R. Scholly, in "Gas Chromatography," ed. by H. J. Noebels, N. Brenner and R. F. Wall, Academic Press, Inc., New York, N. Y., 1961, pp. 263–309.

(2) D. Ambrose (Chairman), A. T. James, A. I. M. Keulemans, E. Kováts, H. Röck, C. Rouit and F. H. Stross, *Pure & Applied Chem.*, **1**, 177 (1960).