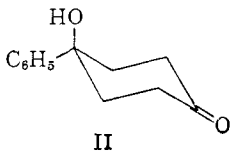

II

3603 ± 2 cm.$^{-1}$ (OH str.) is replaced by one at 2661 ± 4 cm.$^{-1}$ (OD str., ν_{OH}/ν_{OD} = 1.354) while a weak band at 3423 ± 4 cm.$^{-1}$ is not affected and no new band appears near 2530 cm.$^{-1}$. The band at 3423 cm.$^{-1}$ cannot be assigned to the hydroxyl group, but is most probably attributable to the first overtone of the carbonyl stretching vibration[4,8] (fundamental at 1722.5 ± 1 cm.$^{-1}$). The band at 3603 cm.$^{-1}$ is comparable to that found for 1-phenylcyclohexanol (ν_{OH}, 3603 ± 2 cm.$^{-1}$; ν_{OD}, 2662 ± 4 cm.$^{-1}$). Therefore no evidence of intramolecular hydrogen bonding between the hydroxyl and carbonyl groups in II was detected by infrared spectroscopy. In the demonstrated absence of strong, transannular, intramolecular hydrogen bonding, application of conformational principles[11] leads to the prediction that the chair conformation illustrated above, with the 4-phenyl group equatorial, is the most stable conformation of II.

Acknowledgment.—This work was aided by a grant from the National Science Foundation.

(11) N. L. Allinger and H. M. Blatter, *J. Am. Chem. Soc.*, **83**, 994 (1961); N. L. Allinger, *ibid.*, **81**, 5727 (1959); E. L. Eliel, *J. Chem. Educ.*, **37**, 126 (1960).

CONTRIBUTION NO. 276
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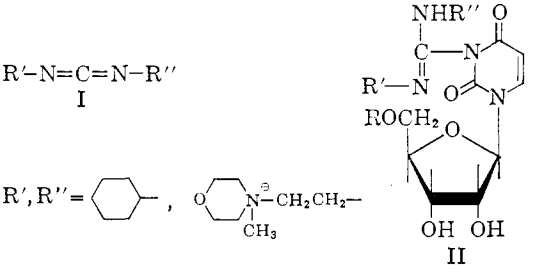
RECEIVED DECEMBER 18, 1961

AN ADDITION REACTION SPECIFIC FOR URIDINE AND GUANOSINE NUCLEOTIDES AND ITS APPLICATION TO THE MODIFICATION OF RIBONUCLEASE ACTION

Sir:

Uridine-5′ phosphate and guanosine-5′ phosphate react under mild conditions with compounds of the type I where one of the R groups contains a closely situated quaternary ammonium group. The reaction of uridine-5′ phosphate (0.01 mmole) with the *p*-toluenesulfonate salt of I (0.1 mmole) in water (1 ml.) at 30° and pH 8 is complete in 4 hours. Guanosine-5′ phosphate requires 10 hours reaction time, while the 5′-phosphates of adenosine and cytidine are unaffected under these conditions. The derivatives can be converted quantitatively back to the nucleotides by hydrolysis at pH 10.5 for 20 hours at 20°.

On a preparative scale the product from uridine-5′ phosphate can be isolated by chromatography on cellulose powder with 95% ethanol as solvent. It is tentatively assigned the structure II (R = phosphate) on the basis of these observations. The product was chromatographically homogeneous in three solvent systems and its electrophoretic mobility at pH 7.1 compared with that of uridine-5′ phosphate was 0.32, indicating one net negative charge. Analysis supported a formula of $C_{23}H_{38}O_{10}N_5P$ (calcd: C, 48.0; H, 6.6; N, 12.2. Found: C, 47.9; H, 6.7; N, 12.2). In water, the compound had λ_{max} 265 mμ (neutral), 269 mμ (pH 2). The double bond stretching region of the infrared spectrum[1] taken in D_2O favors the N-substituted rather than an O-substituted structure.



I

II

In view of the specificity and mild conditions of this reaction, it is conceivable that reagents of this type could be used as chemical mutagens or as blocking groups to limit the action of those nucleases which are specific only for the two pyrimidines or the two purines in nucleic acids. For example, pancreatic ribonuclease is known[2] to hydrolyze uridine-3′ phosphoryl and cytidine-3′ phosphoryl bonds in ribonucleic acids and one application of the present work has resulted in the limitation of this activity to the cleavage of cytidine-3′ phosphoryl bonds by modification of the uridine bases. On reaction with I, cytidylyl-(3′-5′)-uridine (CpU)[3] and uridylyl-(3′-5′)-cytidine (UpC) gave monosubstituted derivatives while uridylyl-(3′,5′)-uridine (UpU) gave a disubstituted derivative as shown by electrophoresis at pH 7.1. The product from UpU on hydrolysis gave UpU which could be completely degraded by ribonuclease to uridine-3′ phosphate and uridine, a result which indicated that the 3′–5′ internucleotide linkage had not been affected during reaction. The product from UpC was completely resistant to the action of the enzyme. However, after hydrolysis, the reformed UpC was completely hydrolyzed to uridine-2′(3′) phosphate and cytidine. The product from CpU (II, R = cytidine-3′ phosphoryl) was hydrolyzed by ribonuclease to cytidine-2′(3′) phosphate and a product chromatographically indistinguishable from II (R = H) prepared by the reaction of uridine with I.

In order to test further this modification of ribonuclease activity yeast s-RNA was treated with I, then pancreatic ribonuclease and, after the removal of the enzyme, the products were treated at pH 10.5 to remove the blocking groups. The mixtures of mono- and oligo-nucleotides obtained were compared with those obtained by the direct treatment of s-RNA with the enzyme. Preliminary experiments showed that for the s-RNA treated with I, the cytidine-2′(3′) phosphate present in the hydrolysate was reduced by 56% while the uridine-2′(3′) phosphate was reduced by 93%, the result to be expected if the majority of the uridine bases had reacted with the reagent and were thus not subject to attack by the enzyme. This approach is now

(1) The author is indebted to Dr. H. T. Miles, National Institutes of Health, for the infrared spectrum and its comparison with those of substituted uracils.

(2) G. Schmidt in "The Nucleic Acids," Vol. I, pp. 555, Academic Press, Inc., New York, N. Y., 1955.

(3) Nomenclature as used by the *Journal of Biological Chemistry*.

being applied to sequence analysis studies where the limitation of enzyme action in this way is expected to permit the isolation of larger fragments from the enzyme degradation of nucleic acids.

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RECEIVED JANUARY 6, 1962

DIRECT NEF REACTION BY ACID-CATALYZED HYDROLYSIS OF 2-NITROÖCTANE TO 2-OCTANONE

Sir:

We wish to report the first example of a Nef reaction proceeding directly from a nitroalkane in acid solution. Ordinarily the nitronic acid intermediate required in this reaction is generated from a nitronate salt. Although the reaction of nitroalkanes with hot mineral acids has been examined,[1,2] no instance is known of the direct conversion of these substances in acidic medium to an aldehyde or ketone having the same carbon content.

2-Nitroöctane (I)[3] (1.0 g.) was refluxed with *N* hydrochloric acid (500 ml.) for 335 hr. (heterogeneous mixture). The product was isolated by extraction with methylene chloride to yield 0.9 g. of a mixture of 2-octanone (II) and I b.p. 160–210° (690 mm.) (infrared spectrum identical with a mixture of authentic I and II containing 65% I.) The extinction coefficient of the product mixture in "90%" ethanolic 0.05 *N* sodium hydroxide solution at 230 mμ (λ max.) indicated 62% I; ϵ_{max}^{230} 12,000 for authentic I in this medium. The 2-octanone was separated by distillation, b.p. 160–170° (690 mm.); semicarbazone m.p. 122–123°; *p*-nitrophenylhydrazone, m.p. 85–87° (no depression of melting point of these derivatives when mixed with authentic samples).

The hydrolysis reaction was conducted with *d*-2-nitroöctane[3] $[\alpha]^{25}D$ + 4.55 (c, 27 in chloroform) in refluxing *N* hydrochloric acid. After 87.5 hr. 13.6% II had formed and the recovered (distilled) I had retained its optical activity $[\alpha]_D^{25}$ + 4.53 (*c*, 27 in chloroform). A sample of *d,l*-2-nitroöctane refluxed with *N* deuteriosulfuric acid in deuterium oxide for 124 hr. produced 4.5% II[4]; the recovered I was found to contain no deuterium (absence of CD stretching absorption near 2100 cm.$^{-1}$ in 0.5 mm. thick sample; spectrum identical with I); comparison of the n.m.r. spectrum of this product with authentic I revealed no differences.

The rate of disappearance of 2-nitroöctane dissolved in "50%" ethanolic *N* hydrochloric acid at 100° was measured. Samples were placed in ethanolic sodium hydroxide and the extinction coefficient at 230 mμ determined at intervals. The rate was first order in 2-nitroöctane (k = 1.2 × 10^{-5} min.$^{-1}$); excellent first-order plots of log *c versus* time passing through the origin were obtained through two reaction half-lives. The rate of tautomerization of octane-2-nitronic acid (generated from the sodium salt by neutralization with hydrochloric acid) to I at 25° in "85%" ethanol was found to be much faster. Excellent first order plots of log *c versus* time through three reaction half-lives were obtained (k = 3.1 × 10^{-3} min.$^{-1}$).

The above observations are consistent with a slow rate-determining proton removal from a protonated intermediate (III) by a base such as water or chloride ion, leading to a nitronic acid (IV). Hydrolysis of IV to II (Step 3, Nef reaction)[5] evidently occurs more rapidly than tautomerization to I,[6] although the rate has not been measured.

$$\underset{\text{I}}{C_6H_{13}(CH_3)CHNO_2} + H^+ \rightleftharpoons \underset{\text{III}}{C_6H_{13}(CH_3)CHNO_2H^+} \quad (1)$$

$$\underset{\text{III}}{C_6H_{13}(CH_3)CHNO_2H^+} + B \overset{\text{slow}}{\rightleftharpoons} \underset{\text{IV}}{C_6H_{13}(CH_3)C{=}NO_2H} + BH^+ \quad (2)$$

$$2\ \underset{\text{IV}}{C_6H_{13}(CH_3)C{=}NO_2H} \longrightarrow 2\ \underset{\text{II}}{C_6H_{13}(CH_3)C{=}O} + N_2O + H_2O \quad (3)$$

The kinetic data on acid-catalyzed bromination of nitroalkanes[7] are also in agreement with a slow proton removal step (2), then halogenation of a nitronic acid intermediate (rate independent of halogen or halogen concentration).

(1) W. E. Noland, *Chem. Revs.*, **55**, 137 (1955).

(2) M. J. Kamlet, A. Kaplan, and J. C. Dacons, *J. Org. Chem.*, **26**, 4371 (1961).

(3) We wish to thank Prof. N. Kornblum for supplying generous samples of *d,l*- and *d*-2-nitroöctane.

(4) A parallel experiment with *N* sulfuric acid in water produced 5.5% II in 124 hr.

(5) M. F. Hawthorne, *J. Am. Chem. Soc.*, **79**, 2510 (1957).

(6) For nitronic acids derived from β-hydroxynitroalkanes, such as 2,5-dinitro-1,6-hexanediol, acid-catalyzed tautomerization proceeds more rapidly than the competing Nef reaction (H. Feuer and A. T. Nielsen, forthcoming publication).

(7) R. Junell, *Z. physik. Chem.*, **141A**, 71 (1929).

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RECEIVED DECEMBER 26, 1961

NEW BORON HETEROCYCLES. 5-, 6- AND 7-MEMBERED SYSTEMS CONTAINING NITROGEN, OXYGEN AND SULFUR

Sir:

We have for some time been interested in the synthesis of new boron heterocycles for the purpose of studying their chemical and pharmacological properties. Recent publications by Dewar and his collaborators[1] prompt us to communicate recent work, which has led to stable and biologically active boron–nitrogen heterocycles.

Condensation of a variety of N-substituted anthranilamides with aryleneboronic acids in boiling toluene using a Dean–Stark separator for the continuous removal of water furnished compounds of

(1) S. S. Chissick, M. J. S. Dewar and P. M. Maitlis, *J. Am. Chem. Soc.*, **83**, 2708 (1961), and earlier papers cited therein.