(*cis* addition), in accord with the stereochemical results for diimide reduction in other systems.[1]

Thermal decomposition of I at 100° *in vacuo* at the inlet of a mass spectrometer (Consolidated 21–103C, ionizing voltage 13 v., ionizing current 74 μ a.) caused the appearance of peaks at *m/e* 28, 29 and 30 (of intensity 1, 2 and 16, respectively) the last of which corresponds to the parent mass peak of diimide.[10] Since the *m/e* 30 peak is not produced from anthracene, hydrazine or nitrogen under our conditions and since this peak is shifted to *m/e* 32 in the mass spectrum from N^1N^1-dideuteriated I, it appears most probable that we are dealing with diimide as a thermal decomposition product of I.

The experiments described above provide additional evidence for the effective role of diimide in the reduction of multiple bonds and also for the interpretation advanced by Cohen and co-workers[11] to explain the anomalous products from saponification of the cyclopentadiene–diethyl azodiformate adduct.

(10) An *m/e* 30 peak has been observed previously in the mass spectrum of diimide produced by decomposition of hydrazine or hydrazoic acid in the gas phase: S. N. Foner and R. L. Hudson, *J. Chem. Phys.*, **28**, 719 (1958).

(11) S. G. Cohen, R. Zand and C. Steel, *J. Am. Chem. Soc.*, **83**, 2895 (1961).

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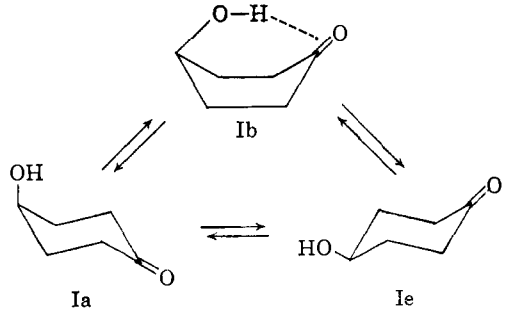
RECEIVED JANUARY 8, 1962

CONFORMATIONS OF 4-HYDROXYCYCLOHEXANONES

Sir:

The conformations of 4-hydroxycyclohexanone (I) include boat conformation Ib, in which intramolecular hydrogen bonding might be possible, as well as chair conformations Ia and Ie. This communication reports that *no* evidence of intramolecular hydrogen bonding in I was detected by infrared spectroscopy. It is concluded that the population of Ib is small.



Examination of a Stuart–Briegleb model of 4-hydroxycyclohexanone suggests that intramolecular hydrogen bonding might occur in conformation Ib between the hydroxyl group and the π electrons of the carbonyl group. The internuclear oxygen–oxygen distance of 2.95 Å., estimated for a Dreiding model of Ib, does not exceed the limits reported[1] for hydrogen bonds of the type O—H · · · O.[2]

(1) (a) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman and Co., San Francisco, California, 1960; (b) L. P. Kuhn, *J. Am. Chem. Soc.*, **74**, 2492 (1952), and subsequent publications.

Recently, it has been reported that 4-hydroxycyclohexanone is, perhaps, intramolecularly hydrogen bonded.[3] It is stated that observed infrared absorption at 3425 cm.$^{-1}$ is not inconsistent with an intramolecularly hydrogen bonded OH.[3] Such absorption might also be attributable to the first overtone of the carbonyl stretching vibration.[4] One of these two possibilities can be eliminated by observing the fate of the 3425 cm.$^{-1}$ absorption band when the OH group is transformed into an OD group by deuterium exchange.[4]

The infrared spectrum of a 0.0024 *M* solution of 4-hydroxycyclohexanone (I) in tetrachloroethylene[5] exhibits two absorption bands in the region 3000–4000 cm.$^{-1}$. The concentration dependence of the two bands is consistent with their assignment to monomeric I.[1,3] The first, a strong, sharp band at 3626 ± 2 cm.$^{-1}$, is replaced by a similar band at 2678 ± 4 cm.$^{-1}$ when the hydroxyl hydrogen of I is replaced by deuterium. The second, a weak, broad band at 3427 ± 5 cm.$^{-1}$, is not affected by deuterium exchange; no new band[7] appears near 2530 cm.$^{-1}$. Therefore, the band at 3427 cm.$^{-1}$ is not attributable to the hydroxyl group, but is most probably the first overtone of the carbonyl stretching vibration[8] (fundamental[5] at 1725.5 ± 1 cm.$^{-1}$). The band at 3626 cm.$^{-1}$ is attributable to the OH stretching vibrations of the hydroxyl groups *not* involved in hydrogen bonding.[1]

In the light of the results above for I, a reexamination of the evidence cited[3] for intramolecular hydrogen bonding in 4-hydroxy-4-phenylcyclohexanone (II) was undertaken. A 0.0060 *M* solution of II in tetrachloroethylene[5] exhibits two absorption bands in the region 3200–4000 cm.$^{-1}$. Upon replacing OH by OD in II, a strong band at

(2) As Ib is rotated slightly in either direction toward a twist conformation, eclipsed interactions are reduced, but the hydrogen bond is lengthened. Let Ib represent the small range of non-chair conformations, formed by twisting Ib, in which the oxygen-oxygen distance is less than 3.2 Å.

(3) W. von E. Doering and A. A.-R. Sayigh, *J. Org. Chem.*, **26**, 1365 (1961).

(4) See R. N. Jones and C. Sandorfy in "Chemical Applications of Spectroscopy," Vol. IX of A. Weissberger's "Technique of Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1956, p. 424.

(5) Mr. John Larsen prepared I and II by the reported procedures.[6] The tetrachloroethylene was Matheson, Coleman and Bell, spectroquality reagent, stored over Drierite. Spectra were recorded as previously described.[6] In addition, 1 mm. sodium chloride cells were used with 0.01 *M* solutions in the region 4000–680 cm.$^{-1}$. Calibration of the hydroxyl and carbonyl stretching bands was done by recording with these bands, the atmospheric water vapor absorption in the regions 3760–3560 and 1750–1690 cm.$^{-1}$ at scan speed 7 cm.$^{-1}$/min., plotting 5 or 10 cm.$^{-1}$/cm. with good resolution. Calibration required special attention because of the discrepancies between the frequencies reported here, and some of the values given in ref. 3.

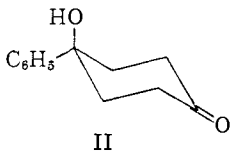
(6) R. D. Stolow, *J. Am. Chem. Soc.*, **83**, 2592 (1961).

(7) The 3626 cm.$^{-1}$ band is shifted by deuteration by the factor ν_{OH}/ν_{OD} = 1.354. If the 3427 cm.$^{-1}$ band were an O–H band, it would be expected to shift by a similar factor upon deuteration. See ref. 1a, p. 112, and ref. 6.

(8) Further evidence in support of this assignment is provided by the infrared spectrum of cyclohexanone in tetrachloroethylene.[9] Found: carbonyl overtone, 3420 ± 4 cm.$^{-1}$; fundamental, 1720 ± 2 cm.$^{-1}$. Reported:[9] fundamental 1720 ± 1 cm.$^{-1}$. Reported for carbon tetrachloride solution:[10] overtone, 3418 cm.$^{-1}$; fundamental, 1718 cm.$^{-1}$.

(9) L. J. Bellamy and R. L. Williams, *Trans. Faraday Soc.*, **55**, 14 (1959).

(10) H. W. Thompson and D. A. Jameson, *Spectrochim. Acta*, **13**, 236 (1958).



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).

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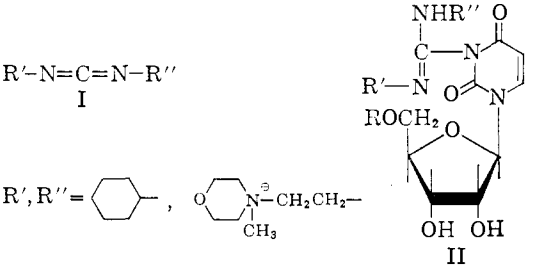
RECEIVED DECEMBER 18, 1961

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*.