studied and the results are shown in Table I. In no other cases was this behavior predicted or observed.

The proton resonance spectra were obtained on saturated solutions in carbon tetrachloride or deuteriochloroform containing tetramethylsilane as an internal reference. A Varian Associates high resolution spectrometer was used at an operating frequency of 56.4 Mc. Peak positions were obtained by audio side band modulation and are accurate to at least 2 cycles per sec. The dimeric products shown in Table I were isolated during the oxidation of the respective 2,6-di-*t*-alkylphenols by benzoyl peroxide[3] and *t*-butyl peroxide. The detailed preparation and identification of the products will be reported in a subsequent publication.

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DIVISION OF APPLIED CHEMISTRY
NATIONAL RESEARCH COUNCIL S. BROWNSTEIN
OTTAWA, CANADA K. U. INGOLD

RECEIVED APRIL 12, 1962

ROTATORY DISPERSION OF NATIVE AND OXIDIZED PANCREATIC RIBONUCLEASE IN THE FAR ULTRAVIOLET[1]

Sir:

Optical rotation measurements of proteins generally have been confined to the visible and near ultraviolet regions of the spectrum at wave lengths greater than those of known peptide absorption bands.[2] Recently, however, Simmons, Blout and their co-workers have measured the dispersions of several proteins and polypeptides down to 220 mμ, tracing a large portion of an apparent negative Cotton effect centered at about 225 mμ and having a trough at 233 mμ.[3,4,5] Upon disorientation of the helical structures by urea[3,4] or by the ionization of side groups,[4] the trough disappears and the levorotation at 233 mμ is greatly reduced. We wish to report a similar change upon performic acid oxidation of ribonuclease (RNase). The known primary structure of RNase[6] makes estimation of its helical content of particular interest. Also, with these systems comparison of the native and random coil form may be made with the same solvent, under almost identical conditions of absorption, thereby minimizing the spurious results which can arise from stray light.[2] Harrington and Sela[7] have summarized the evidence for a random coil conformation of oxidized RNase.

Rotations were estimated at 25° with a Rudolph Spectropolarimeter (Model 200, with a rocking polarizer). The maximum slit width was 1.0 mm. and the symmetrical angle was 5°. Blank rotations were checked frequently. High pressure d.c. xenon arcs (450-watt, Osram, Berlin) were

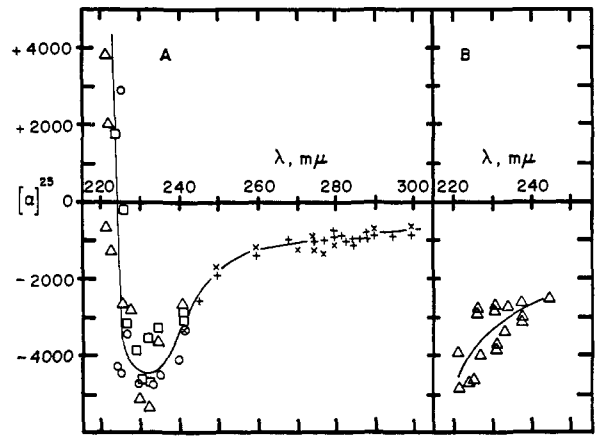

Fig. 1.—Rotatory dispersion of native and oxidized RNase: A, native RNase, in 0.5 cm. cell, the concentration in mg./ml. was X, 4.6; +, 2.3; □, 0.38; O, 0.37; △, 0.26; B, oxidized RNase, in 1.0 cm. cell, the concentration was 0.14 mg./ml. (the points are from three experiments).

selected for their intensity and stability. Bovine pancreatic RNase (Type II, lot R31B-204, Sigma Chemical Company, St. Louis) or oxidized RNase (prepared as sample 3 of reference 8) was dissolved in 0.1 *M* KCl (final pH 4.0) and their concentration determined spectrophotometrically.[8,9]

The results are shown in Fig. 1. The poor precision relative to measurements at longer wave lengths results from the compulsory low rotations and very low light intensity. Each point is the average of 5 to 15 determinations, with average deviations up to 30%. With the native protein an average specific rotation of about −4500° is obtained at the trough which is between 230 and 235 mμ. Similar curves were obtained with the native "D" fraction.[10] In contrast, oxidized RNase displays simple dispersion in the same wave length region.

Several facts indicate that the trough found with native RNase is not an artifact arising from stray light. It is absent with oxidized RNase under equivalent conditions of absorption. In the limited range of concentrations which were technically feasible, the specific rotation of RNase was constant at each wave length. Finally, the positive rotations would not arise from stray light.

It has been proposed that the amplitude of the residue rotation at 233 mμ relative to the rotation of the unfolded polypeptide chain provides a measure of the extent of helicity of a protein, and the validity of this procedure has been established in the case of myoglobin[5] assuming no change in structure in passing from the crystalline phase to solution. Using the rotation of the polyglutamic acid helix in water[4] as a reference, we calculate 13% net right-handed helix for RNase with this assumption. This is in reasonable agreement with the value obtained by comparing the b_0 of RNase with

(1) This research was supported by grants from the Sloan Foundation and National Cancer Institute, U. S. Public Health Service.

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that of a right-handed helix.[11] However, the interpretive basis for this type of calculation is that the protein consists of two kinds of conformational regions, one having the rotatory properties of a right-handed α-helix, the other having those of a random polypeptide. If this interpretation is correct, the same linear combination of rotations (13% helix, 87% random coil) should account for the dispersion at wave lengths other than 233 mμ. This is not the case with RNase. In particular the low helical content predicted from the amplitude of the Cotton effect at 233 mμ cannot account for the swing to positive rotations at lower wave lengths. It is our interpretation that the negative Cotton effect provides evidence for the presence of right-handed α-helices in RNase but that the simple two-state model does not lead to a consistent explanation of the observed dispersion, so that a meaningful helix content cannot be calculated. Experiments at lower wave lengths, which would help to clarify this situation, are not feasible with present apparatus.

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(12) Fellow of the National Foundation.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF OREGON — STEVEN B. ZIMMERMAN[12]
EUGENE, OREGON — JOHN A. SCHELLMAN

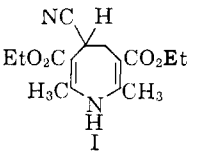
RECEIVED APRIL 28, 1962

2,7-DIMETHYLAZATROPONE-4

Sir:

We recently described the ring-expansion of II, R = CH_2Cl, to diethyl 2,7-dimethyl-4-cyano-4,5-dihydro-1H-azepine-3,6-dicarboxylate, I.[1] During dehydrogenation studies, we treated I, as well as several dihydropyridines, with sodium nitrite in glacial acetic acid.[2] Some dihydropyridines (*e.g.*, II, R = CH_3) gave the expected pyridine derivatives but II, R = CH_2Cl, yielded the oxime, III (analysis, ultraviolet, infrared and n.m.r. spectra and formation of benzoate) and I afforded a white crystalline solid (15.8%), $C_{14}H_{17}NO_5$, m.p. 119–120° (λ_{max}^{EtOH} 219 (ϵ 35,600), 249 (infl. ϵ 11,400) and 296 mμ (ϵ 9,400)) which had no NH stretching band in the infrared. The n.m.r. spectrum[3] of this compound (in $CHCl_3$ solution) showed that it had two non-equivalent ethyl esters, two methyl groups (7.07, 7.17 τ) and one other single proton (1.25 τ). These signals are all shifted to low field relative to starting material and together with the position of absorption of the single proton, suggest a ring current and hence aromaticity[4] of this product, which we believe, therefore, to be IV, R = COOEt. Compound IV, R = COOEt, now has been obtained directly from II, R = CH_2Cl, by prolonged treatment with aqueous ethanolic potassium cyanide (0.5 mole KCN/mole II, R = CH_2Cl), and chromatography of the oily product on alumina.

Hydrolysis of IV, R = COOEt, with caustic soda yielded a diacid (94%) which was decarboxylated with copper bronze at 320°[5] to give a colorless liquid (85%), C_8H_9NO, (λ_{max}^{EtOH} 217 (ϵ 13,370), 250 (ϵ 6,450), 257 (ϵ 5,920) and 290 mμ (ϵ 8,790)), showing no NH stretching band in the infrared but having strong absorption at 1613 cm.$^{-1}$ which we ascribe to the "carbonyl" frequency.[6] This product gave a crystalline picrate,[7] m.p. 128° (dec.). The n.m.r. spectrum (in CH_2Cl_2) of this decarboxylated material showed the presence of two methyl groups (7.41, 7.53 τ) and three other protons [2.36, 3.02 (doublet, J = 7.9 c.p.s.) and 3.69 τ] and this is consistent with structure IV, R = H. There appeared to be no "meta" or "para" interaction[8] between the ring protons, but the signal at 3.69 τ was a close quartet, J = 0.8 c.p.s., clearly by interaction with the higher field methyl group (a doublet, J = 0.8 c.p.s.).



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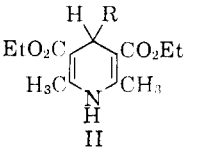
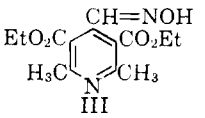
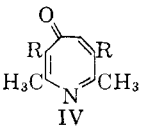


II



III



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(9) Department of Chemistry, University of California, Berkeley 4, California.

DEPARTMENT OF CHEMISTRY — E. BULLOCK
THE UNIVERSITY, NOTTINGHAM — B. GREGORY
ENGLAND — A. W. JOHNSON[9]

RECEIVED MARCH 19, 1962

NUCLEAR MAGNETIC RESONANCE SPECTRA OF SOME HYPOFLUORITES

Sir:

The n.m.r. spectra of F_2, OF_2, CF_3OF, SF_5OF, and SO_3F_2 were taken to see how the hypofluorite resonances of the hypofluorites compared with elemental fluorine and with each other. At the same time it was desirable to observe coupling constants between different types of fluorine atoms in the hypofluorites and to gain additional information concerning molecular structure.

In Table I is presented the order into which the n.m.r. resonances of the hypofluorite fluorine atoms of the hypofluorites fall along with the various coupling constants and chemical shifts observed. Sulfur hexafluoride, because of its ready availability and its ability to dissolve all substances mentioned here, was chosen as internal reference. The data suggest that the charge of fluorine in the –OF group is not positive, but instead, somewhat negative.