butene rings are about 112 ± 2° with a *trans* configuration about the cyclobutane ring. The fluorine atoms are, of course, also *trans* with regard to the same ring.

Refinement continues and, when complete, full crystallographic details and data will be published elsewhere. We are indebted to Professor John D. Roberts for calling the problem to our attention and to Dr. Marjorie Caserio for providing the crystalline samples.

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RECEIVED MARCH 26, 1962

IDENTIFICATION OF A NEW TYPE OF MOLECULAR ASYMMETRY[1]

Sir:

Proton magnetic resonance has been used to show the non-equivalence of the two protons in a methylene group next to an asymmetric carbon atom.[2] Another type of molecular asymmetry has been found by this same technique. During an investigation of the oxidation of 2,6-di-*t*-butyl-4-methylphenol the stilbenequinone shown in Fig. 1 was

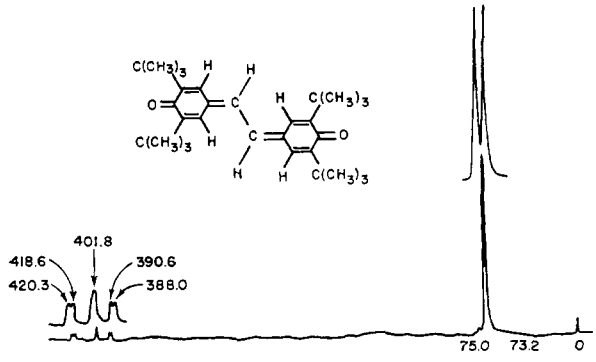


Fig. 1.—Proton resonance spectrum of stilbenequinone: peak positions are in cycles per second to low field of tetramethylsilane.

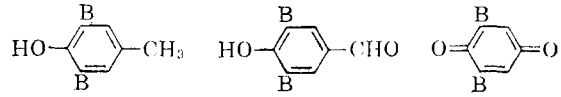
prepared and its proton resonance spectrum obtained. The peaks derive from the *t*-butyl group and the aromatic protons were found to be doublets, with a further spin coupling between the non-equivalent aromatic protons. These results may only be explained by a non-equivalence of the substituents on either side of the central axis of the aromatic rings. If rotation about the central carbon–carbon bond were strongly hindered or if the conformation shown were energetically the most favorable, it is obvious that one side of a given aromatic ring is closer to the second aromatic ring than is the other side. However, even with rapid rotation, the two sides are non-equivalent in the *cis* as well as the *trans* conformation and the non-equivalence is not averaged by the rotation. The observed difference in magnetic environment is therefore quite reasonable. It is 0.03 p.p.m. for the *t*-butyl group and 0.54 p.p.m. for the aromatic protons, which are considerably closer to the site of the non-equivalence. A similar result was obtained starting with the appropriate *t*-amyl phenol. In this case separate peaks could not be observed for the non-equivalent ethyl part of the *t*-amyl group but the lines were significantly broadened. For the methyl groups in the *t*-amyl radical the separation was 0.04 p.p.m. and for the aromatic protons 0.51 p.p.m. A variety of similar molecules were

(1) Published as N.R.C. No. 6860.

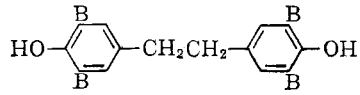
(2) P. M. Nair and J. D. Roberts, *J. Am. Chem. Soc.*, **79**, 4565 (1957).

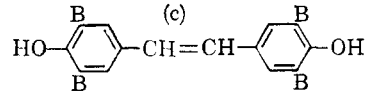
TABLE I

CHEMICAL SHIFTS OF SUBSTITUTED PHENOLS AND QUINONES[a]

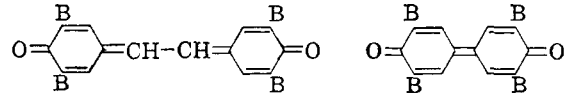


$C(CH_3)_3$	1.49	1.50	1.31
CH_3	2.23	...	...
CH_2	...	...	...
Olefin CH	...	...	...
Aromatic CH	6.94	7.66	6.41
OH	4.77	5.78	...
CHO	...	9.79	...

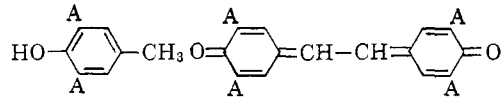




$C(CH_3)_3$	1.36	1.50
CH_3	...	...
CH_2	2.74	...
Olefin CH	...	6.79
Aromatic CH	6.80	7.26
OH	4.70	4.97
CHO	...	...



$C(CH_3)_3$	1.30	1.33	1.36
CH_3	...		...
CH_2	...		...
Olefin CH	7.12		...
Aromatic CH	6.90	7.44	7.69
	J = 2.3 cycles		...
OH	...		...
CHO	...		...



$C(CH_3)_3$	1.34		1.27	1.30
	Ethyl CH_3 = 0.63		Ethyl CH_3 = 0.64	
	Ethyl CH_2 = 1.84		Ethyl CH_2 = 1.81	
	J = 7.1 cycles		J = 7.3 cycles	
CH_3	2.22		...	
CH_2	...		...	
Olefin CH	...		7.14	
Aromatic CH	6.76		6.92	7.42
OH	4.75		...	
CHO	...		...	

[a] Listed in parts per million to low field of tetramethylsilane. [b] B represents the *tert*-butyl group and A the *tert*-amyl group. [c] This is assumed to be the *trans* isomer but the configuration is not proven.

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.

(3) S. L. Cosgrove and W. A. Waters, *J. Chem. Soc.*, 388 (1951).

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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 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]

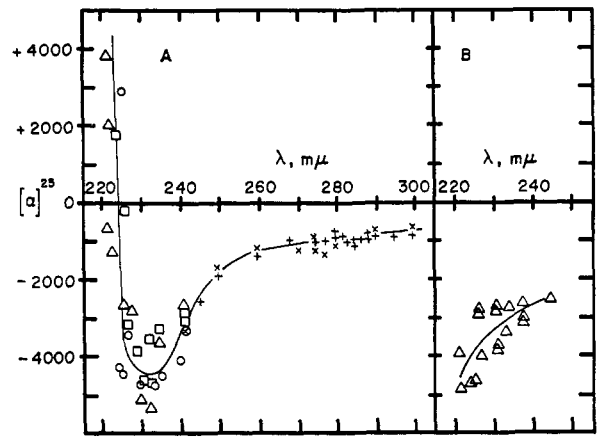

Fig. 1.—Rotatory dispersion of native and oxidized RNase: A, native RNase, in 0.5 cm. cell, the concentration in mg./ml. was X, 4.6; +, 2.3; □, 0.38; O, 0.37; △, 0.26; B, oxidized RNase, in 1.0 cm. cell, the concentration was 0.14 mg./ml. (the points are from three experiments).

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.

(2) P. Urness and P. Doty, *Advances in Protein Chem.*, **16**, 401 (1961).

(3) N. S. Simmons and E. R. Blout, *Biophys. J.*, **1**, 55 (1960).

(4) N. S. Simmons, C. Cohen, A. G. Szent-Gyorgyi, D. B. Wetlaufer and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 4766 (1961).

(5) S. Beychok and E. R. Blout, *J. Mol. Biol.*, **3**, 769 (1961).

(6) C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **235**, 633 (1960).

(7) W. F. Harrington and M. Sela, *Biochim. et Biophys. Acta*, **31**, 427 (1959).

(8) W. F. Harrington and J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim.*, **30**, 21 (1956).

(9) C. Tanford, J. D. Hauenstein and D. G. Rands, *J. Am. Chem. Soc.*, **77**, 6409 (1956).

(10) G. Taborsky, *J. Biol. Chem.*, **234**, 2652 (1959).