

In the acetylene cobalt hexacarbonyls, each acetylene molecule replaces two carbonyl groups,⁴ *i.e.*, contributes its four π -electrons to the π - d bonding of the complex; similarly the C—OH groups should each contribute three electrons.² In the present complex, however, the $[\text{RC}\equiv\text{CR}'2\text{C}(\text{OH})]$ grouping as a whole is required to contribute only eight or six (Fe—Fe bond assumed) electrons. It therefore seemed probable that four electrons are utilised in σ -bonds, presumably between the C—OH groups and the acetylene residue. This would also explain the steric effects described. Formula (II) was thus obtained. In conformity with this structure, the but-2-yne complex was found to be rapidly attacked by potassium ferricyanide, with the formation of dimethylmaleic anhydride; this evident *in itself* would not have been enough to establish the postulated σ -bonding, since these links might have been formed during the oxidative breakdown.

In formula (II) full bonds have been used for the π -electron framework, and dashed bonds to indicate the π - and d -electron bonding which confers upon the complex its aromatic character.² In view of the lack of agreement on the description of even so simple a compound as ferrocene,⁵ which similarly possesses the necessary number of electrons for an inert-gas completed shell and shows "aromatic" stability,⁶ the precise bonding in (II) is not specified, although a close relationship to $\text{Fe}_2(\text{CO})_9$ seems probable. It is implied in (II) that the two iron atoms lie on either side of the (approximate) plane of the four central carbon atoms. The steric hindrance observed when $\text{R} = \text{Me}$ requires an HO—C—CR angle of about 120° , rather than 180° , and suggests sp^2 hybridisation for the C—C and C—O bonds of the central grouping.

Formation of hydroquinone¹ by the action of a second molecule of an acetylene on (II) can readily be envisioned.

Acknowledgments.—We are indebted to the Commonwealth Scientific and Industrial Research Organisation (Australia) and to the British Oxygen Company, Ltd., for fellowships held by P. C. W., and R. C., respectively.

(4) R. A. Friedel, I. Wender, S. L. Shufler and H. W. Sternberg, *THIS JOURNAL*, **77**, 3951 (1955).

(5) See *inter alia* B. F. Hallam and P. L. Pauson, *J. Chem. Soc.*, 3030 (1956).

(6) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, *THIS JOURNAL*, **74**, 2125 (1952).

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THE PREPARATION AND PROPERTIES OF COMPLETELY DISPERSED THYMUS NUCLEOPROTEIN

Sir:

The preparation and characterization of deoxyribonucleic acids (DNA) from a variety of sources have now reached a level of development where there is semi-quantitative reproducibility and widespread agreement. In contrast the parent nucleoprotein particles have not been isolated in dispersed form. We wish to report on the prepara-

tion of such nucleoprotein particles and a study of their properties which shows that they consist of one DNA molecule and a protein complement of approximately equal weight.

The preparative procedures commonly used for the isolation of nucleoprotein material^{1,2,3} generally consist of mincing and multiple washings of tissue in cold 0.15 *M* NaCl, followed by repeated suspension and centrifugation in water and finally homogenization in water to produce a viscous solution of about 0.5 g./dl. concentration which exhibits pronounced gel-like character and is admittedly incompletely dispersed.¹ We have found that irreversible gel formation was caused by (1) maintaining nucleoprotein at concentrations above 0.08 g./dl., (2) precipitating nucleoprotein with 0.15 *M* NaCl or alcohol, or (3) allowing the salt-washed sediment to stand too long before homogenizing in water and by carrying out this homogenization too slowly. This led to the following procedure for isolating DNP in high yield free of gel.

Working in a cold room, about 20 g. of fresh, frozen thymus glands are chopped and homogenized in a Waring Blendor⁴ with 200 cc. of a saline-Versene (0.075 *M* NaCl and 0.024 *M* Versene, pH 8) solution containing a few drops of capryl alcohol. The homogenate is strained through cheesecloth and the filtrate is centrifuged at 2000 r.p.m. for ten minutes. The sediment is again homogenized in the Blendor (at slow speed for three minutes) in the same volume. The homogenate is centrifuged as above and the supernatant is decanted. This washing procedure is repeated six more times. The final sediment is dissolved by rapid mixing with water in the Blendor starting with 50 cc. of water and increasing this to 1 liter in a few seconds. The mixture is quickly transferred to a beaker and stirred for an hour and then dialyzed against 0.0007 *M* potassium phosphate buffer at pH 6.8. This solvent was employed for all the physical measurements.

Physical measurements on several samples of DNP are summarized in the table. From these data it appears that the nucleoprotein particle is an irregularly shaped coil somewhat less extended than DNA itself, that is, the average separation of the ends is about 4200 Å. for DNP compared with about 7100 Å. for DNA. DNA was prepared directly from the DNP and was found to have the same physical properties as samples carefully prepared by Simmons' method. These results are given in the table for comparison.

In making the physical measurements the concentration was determined by optical density measurements using $E_{269}^{1\%} = 106 \pm 5$. The amount of DNA in the nucleoprotein was found to be $49 \pm 2\%$. Other constants established for the DNP

(1) K. V. Shooter, P. F. Davidson and J. A. V. Butler, *Biochem. et Biophys. Acta*, **13**, 192 (1954).

(2) P. F. Davidson, B. E. Conway and J. A. V. Butler, in "Progress in Biophysics and Biophysical Chemistry," Vol. 4, Academic Press, New York, 1954, p. 148.

(3) C. F. Crampton, R. Lipshitz and E. Chargaff, *J. Biol. Chem.*, **206**, 499 (1954).

(4) The Blendor is operated at about half its maximum speed for 1 minute and then at a very slow speed, just sufficient to agitate the mixture, for 3 minutes.

include (1) a N/P ratio of 3.7 ± 0.3 , (2) a specific refractive index increment at $\lambda = 436 \text{ m}\mu$ of 0.193 ± 0.004 and (3) a partial specific volume of 0.68 ± 0.01 .

Measurement	DNP	DNA
Molecular weight (light scattering)	$19 \pm 4 \times 10^6$	$8 \pm 2 \times 10^6$
Radius of gyration (light scattering), \AA .	1700 ± 200	2900 ± 300
Intrinsic viscosity (100 cc./g.)	35 ± 2	70 ± 10
Sedimentation constant, S_{20}^w	50 ± 5	22 ± 2
Flow birefringence, $^\circ \text{\AA}$.	4800-3000	14,000-9,000

^a The values given are the range of apparent lengths calculated from the extinction angle assuming a rigid ellipsoid model. This provides a rough estimate of the polydispersity and the maximum dimension of the coiled particles.

Inasmuch as the yield of DNP accounts for the majority of DNA in the thymus, it appears that the particles isolated and studied here may be the principal structural units of chromosomes. A study of the organization of the DNA and protein in the DNP particle will be reported soon.

We wish to thank Dr. N. Simmons for helpful discussions during this investigation and to acknowledge support from U. S. Public Health Service Grant No. C2170 (C5) and the National Science Foundation.

(5) U. S. Public Health Service Predoctorate Research Fellow of the National Heart Institute.

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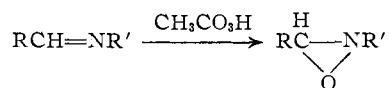
PAUL DOTY
GEOFFREY ZUBAY⁶

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THE SYNTHESIS OF OXAZIRANES


Sir:

We have observed recently that certain azomethines which are sluggish toward acid hydrolysis may be oxidized readily in good yield with anhydrous peracetic acid in methylene chloride to compounds whose structures are best formulated as oxaziranes. These materials appear to be the first obtained with a well-authenticated oxygen-nitrogen-carbon three-membered ring system.^{1,2}



They are active oxygen compounds comparable in many respects to organic peroxides and indeed may be assayed by iodometric procedures. Typical examples of azomethines which may be converted to oxaziranes are those derived from aldehydes and *t*-carbinamines³ and those obtained from *p*-nitrobenzaldehyde and most amines. The former compounds are resistant to acid hydrolysis because of steric factors and the latter because of electronic

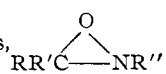
(1) Both nitrones and azoxy compounds have been written as three-membered rings in the older literature. However, no such ring apparently has ever been established with certainty, L. I. Smith, *Chem. Revs.*, **23**, 223 (1938).

(2) The numbering of the oxazirane system is 

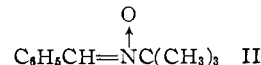
(3) M. D. Hurwitz, U. S. Patent 2,582,128, January 8, 1952.

effects. The physical properties and yields of some typical oxaziranes are summarized in Table I.

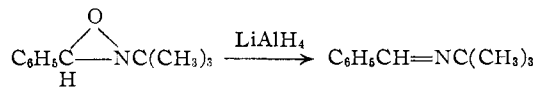
TABLE I

SYNTHESIS OF OXAZIRANES,							
R	R'	R''	$^\circ\text{C}$.	B. p. Mm.	Yield, %	n_{D}^{20}	
H	H	<i>t</i> -Bu	52	75	46	1.4150	
C_6H_5	H	<i>t</i> -Bu	63	0.5	71	1.5081	
H	H	<i>t</i> -Octyl	64	6	69	1.4445	
<i>p</i> - $\text{NO}_2\text{C}_6\text{H}_4$	H	Et	34-35	(m.p.)	95		
CH_3	<i>i</i> -Bu	<i>n</i> -Pr	64	7	63	1.4277	

The oxazirane structure of these compounds is based on a number of facts: (1) analytical data, (2) their quantitative hydrolysis to β -alkylhydroxylamines and aldehydes, (3) a comparison of their physical and chemical properties with those of the isomeric nitrones, (4) their conversion to the isomeric nitrones under anhydrous conditions, and finally (5) the partial resolution of 2-*n*-propyl-3-methyl-3-isobutyloxazirane. Most of the structural work was done with 2-*t*-butyl-3-phenyloxazirane (I) since its hydrolysis and reduction products could be conveniently characterized. This material on hydrolysis with sulfuric acid in aqueous methanol gave quantitative yields of benzaldehyde and β -*t*-butylhydroxylamine (m.p. 64° ; Found: C, 53.69; H, 12.64; N, 15.47). The availability of β -*t*-butylhydroxylamine permitted the synthesis of the isomeric nitron (II)



which may properly be regarded as an "electronic tautomer" of the oxazirane. This nitron melted at 76° (Found: C, 74.60; H, 8.55; N, 7.62), was not an active oxygen compound and had very different properties from the isomeric oxazirane. It absorbed strongly in the ultraviolet (λ_{max} , 225 $\text{m}\mu$, ϵ_{max} 6800; λ_{max} 295 $\text{m}\mu$, ϵ_{max} 16,700) whereas the corresponding oxazirane showed only that absorption associated with the benzene ring (λ_{max} 249 $\text{m}\mu$, ϵ_{max} 930). Reduction of the oxazirane (I) with lithium aluminum hydride in ether yielded only *N*-benzylidene *t*-butylamine while reduction of the corresponding nitron under comparable conditions gave benzyl-*t*-butylhydroxylamine (m.p. 72° . Found: C, 73.72; H, 9.77; N, 7.60).



The relationship between the oxazirane and the nitron also was indicated clearly by the observation that in boiling acetonitrile I was isomerized to II under completely anhydrous conditions. Final confirmation of the oxazirane structure was obtained by partial resolution ($\alpha_{\text{D}}^{20} -3.94^\circ$) of 2-*n*-propyl-3-methyl-3-isobutyloxazirane after incomplete degradation of the compound with