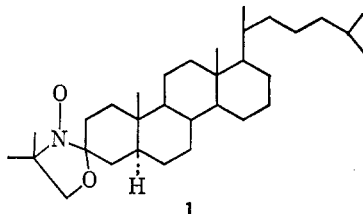
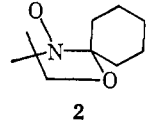
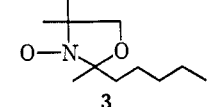


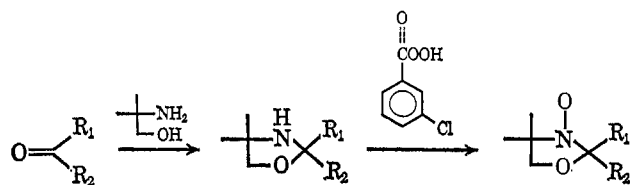
Table I. Physical and Spectral Data

Nitroxide	Mp or bp (mm), °C	$a_N,^a \pm 0.1$ gauss	$g$ value, $\pm 0.0002$	$\lambda_{max}, m\mu$ ( $\epsilon$ )	% yield
	175-176 (methanol- ether)	14.3	2.0058	443 (7.2) (ether)	70 <sup>b</sup>
	57-58 (pen- tane)	14.3	2.0058	420 (7.4) (ethanol)	42 <sup>c</sup>
	~70 (0.08)	14.4	2.0058	422 (7.0) (ethanol)	26 <sup>c,d</sup>

<sup>a</sup> X-band esr spectra were measured in ethyl acetate solution ( $ca. 5 \times 10^{-4} M$ ) and consisted of the usual three-line nitroxide spectra. All three nitroxides were found to have  $1.0 \pm 0.2$  spins per molecule. <sup>b</sup> This represents pure crystalline material. <sup>c</sup> Yields were determined by visible absorption spectroscopy. <sup>d</sup> The analytical specimen was obtained by preparative vapor phase chromatography.

paired electron spins. Elegant methods have recently been developed for the chemical attachment of relatively stable nitroxide free radicals to diamagnetic biomolecules, permitting study of the latter by esr techniques. Several workers have applied this spin-labeling method with notable success to the study of proteins such as bovine serum albumin.<sup>1</sup>

The continuing successful development of this technique depends to a large measure on the availability of a wide variety of spin-label molecules. We wish to report the first general method for converting ketones to stable nitroxide free radicals. The reaction sequence from the ketone to the nitroxide is outlined below.



For example, refluxing a toluene solution of cholestan-3-one containing an excess of 2-amino-2-methylpropan-1-ol<sup>2</sup> and a trace of *p*-toluenesulfonic acid monohydrate for several hours with continuous water removal by means of a Dean-Stark trap led to the corresponding oxazolidine, one epimer of which had mp 124-125°. The known<sup>4</sup> oxazolidines derived from cyclohexanone and heptan-2-one were similarly prepared.

In each instance oxidation of the oxazolidine to the corresponding nitroxide was effected by dropwise addition over 20 min of an ether solution of 1.50 equiv of *m*-chloroperbenzoic acid to an ice-cold, stirred ether

(1) T. J. Stone, T. Buckman, P. L. Nordio, and H. M. McConnell, *Proc. Natl. Acad. Sci. U. S. A.*, **54**, 1010 (1965); O. H. Griffith and H. M. McConnell, *ibid.*, **55**, 8 (1966); L. Stryer and O. H. Griffith, *ibid.*, **54**, 1785 (1965).

(2) Available from Aldrich Chemical Co.

(3) All new compounds gave carbon, hydrogen, and nitrogen elemental analyses within 0.3% of the theoretical values.

(4) E. M. Hancock and A. C. Cope, *J. Am. Chem. Soc.*, **66**, 1738 (1944).

solution of oxazolidine. The resulting solution was allowed to stand at 25° for 24 hr. The ether layer was washed with cold 5% sodium bicarbonate solution and dried over magnesium sulfate, and the solvent was removed under vacuum, affording the nitroxide. Table I summarizes pertinent physical and spectral data for the three representative nitroxides 1, 2, and 3.

The conformationally rather rigid nature of the oxazolidine ring system in these new spin labels should markedly facilitate the interpretation of rotational correlation times in terms of the local environment of the spin label.

**Acknowledgments.** We thank Professor O. H. Griffith for determination of the esr spectral data and the National Science Foundation (Grant GP5805) and the Office of Scientific and Scholarly Research of the Graduate School of the University of Oregon for financial support of this work.

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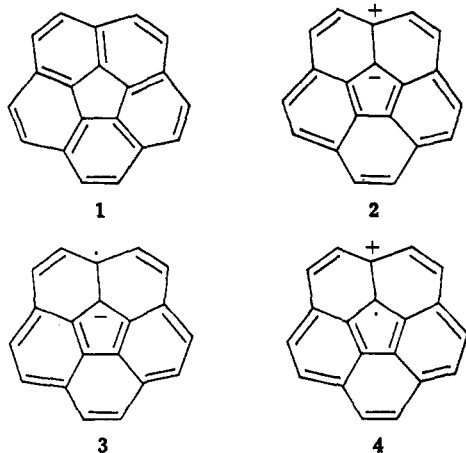
### Concerning the Anion and Cation Radicals of Corannulene

Sir:

The consideration of valence bond structures such as **2** for the recently synthesized strained, nonalternate benzoid hydrocarbon, corannulene (**1**),<sup>1</sup> intimated the theoretical accessibility of the corresponding radical anion and radical cation derivatives. The unusual polar form **2** implies that the radical cation and anion correspondingly will have large contributions of structures such as **3** and **4** to the resonance hybrid. Because of the importance of these species to more sophisticated computations on the corannulene molecule, as

(1) W. E. Barth and R. G. Lawton, *J. Am. Chem. Soc.*, **88**, 380 (1966).

well as to general interest in its chemistry, we were prompted to search for these derivatives.



Polarographic reduction of  $1 \times 10^{-3} M$  corannulene- $0.1 M$  *t*-butylammonium perchlorate solution in acetonitrile using either a stationary platinum microelectrode or dropping mercury electrode afforded two well-defined waves with half-wave potentials,  $E_{1/2}$ , of  $-1.88$  and  $-2.36$  v *vs.* the Ag-AgCl aqueous reference electrode. That the first wave on both electrodes represented a reversible, one-electron reduction was indicated by wave log analysis and controlled potential coulometry. The corannulene radical anion product of the first reduction wave was characterized by spectrophotometric and esr techniques in the following manner. Controlled potential electrolysis on a macro-scale at a mercury pool electrode and at a potential of  $-2.0$  v using a specially constructed flow cell<sup>2</sup> which was inserted in a Cary Model 14 spectrophotometer resulted in the formation of a dark emerald green solution having maxima in the visible at  $450$  and  $650$  m $\mu$  (Figure 1, curve 1). However, the ratio of intensity of the peaks at  $450$  and  $650$  m $\mu$  varied over several experiments, suggesting that these are possibly the result of more than a single species. Upon exposure to oxygen the green solution became yellow and the visible spectrum of this solution exhibited a single peak at  $450$  m $\mu$ . Thus, it is believed, the green solution is a result of the summation of the radical anion  $650$ -m $\mu$  maximum and a decay product  $450$ -m $\mu$  maximum caused by trace amounts of oxygen in the system.

Similar green solutions were observed upon reduction of  $5 \times 10^{-4} M$  solutions of corannulene in tetrahydrofuran by potassium or sodium metal. At room temperature these solutions exhibited esr spectra composed of 11 equally spaced lines (Figure 1B), the two outermost being observed only under conditions of high gain and resolution. The spectrum and its observed intensity distribution is exactly that expected for a radical with one set of ten equivalent protons. Confirmation that the chemically and electrolytically prepared species were identical was obtained by electrolytic reduction in the esr cavity of a  $10^{-3} M$  solution of corannulene in dimethylformamide at a potential of  $-1.9$  v *vs.* a Ag-AgCl reference electrode, giving an identical esr spectrum with that obtained chemically. The hyperfine splitting constant for the radical anion 3 in dimethoxyethane was  $a_H = 1.560 \pm 0.005$  gauss,

(2) J. Janata and H. B. Mark, Jr., to be published.

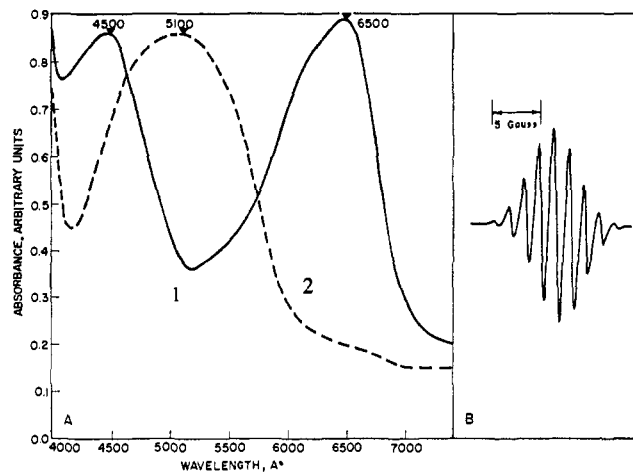


Figure 1. (A) Visible spectrum of electrolysis products produced at  $-2.0$  (curve 1) and  $-2.5$  v *vs.* a Ag-AgCl reference electrode. (B) ESR spectrum of corannulene anion radical.

and the  $g$  value was  $2.00270 \pm 0.00001$ . The splitting constant in dimethylformamide is identical within experimental error.

Controlled potential electrolysis at  $-2.5$  v *vs.* Ag-AgCl (diffusion plateau of second wave) produced a bright red species having a single absorbance maximum at  $500$  m $\mu$ . This same red substance could also be observed when corannulene solutions were subjected to long contact with alkali metals. The red species, which is not paramagnetic, is not believed to be the dianion, but rather some decay product of it. Further studies of this reaction are in progress.

Attempts to observe the radical cation (4) were uniformly unsuccessful; using a slow anodic sweep of a platinum electrode, a poorly defined oxidation wave was obtained with a half-wave potential of about  $1.46$  v *vs.* Ag-AgCl. The wave is actually peaked in shape. Apparently the oxidation product of corannulene undergoes rapid chemical reaction, producing a polymeric product which coats the electrode surface and blocks further oxidation. The passivation prevented any anodic coulometric study. Further, an attempt to observe an esr signal under similar conditions was also unsuccessful.

The neutral corannulene molecule is highly strained, and one would not expect it to be planar. A possible bowl-shaped configuration which preserves the five-fold symmetry of the planar structure was suggested by Barth and Lawton.<sup>1</sup> If the simple Hückel method is used to treat the  $\pi$  system, one possible model in which deviations from planarity can be approximately treated is by a reduction in the  $\beta_{io}$  for the five bonds between the inner and outer rings. The difference in  $\pi$  energy between planar and nonplanar forms for the radical is *less* than this difference for the neutral molecule. This indicates that no added stability for the planar configuration is obtained on going from the neutral species to the radical anion and hence, if the neutral molecule is nonplanar, the anion radical is likely to be also.<sup>3</sup>

A bowl-shaped configuration for the anion radical is consistent with the esr observation of ten equivalent protons. In any structure maintaining fivefold sym-

(3) Dr. Gerald J. Gleicher has carried out extensive SCFMO strain calculations, which are to be published.

metry, the unpaired electron occupies a degenerate orbital. Averaging over the ten hydrogen-bonded positions one can obtain average Hückel spin densities,  $\langle \zeta \rangle$ , and excess charge densities  $\langle \epsilon \rangle$ . For the radical anion,  $\langle \zeta \rangle$  is 0.0630 in the planar configuration ( $\beta_{i0} = 1.00$ ), decreases slightly to a minimum value of 0.0616 for  $\beta_{i0} = 0.72$ , and is 0.0667 for  $\beta_{i0}$  zero.

For corannulene, one is not sure of the  $\sigma$  framework for the carbon atoms with bonded hydrogen, but if we use the McConnell and Colpa-Bolton relationships,  $a^M = 27\zeta$  and  $a^{CB} = -27\zeta + 12.8\epsilon$ , as discussed by Snyder and Amos,<sup>4</sup> we find for the planar configuration  $a^M = -1.70$  and  $a^{CB} = -1.66$ ; if  $\beta_{i0} = 0.72$ ,  $a^M = -1.66$  and  $a^{CB} = -1.63$ . The calculation for the nonplanar configuration including the excess charge effect gives the best agreement with the experimental value  $|a| = 1.56$ . The agreement between the calculated and experimental values is somewhat surprising considering the nature of the approximations that have been made. However, in the case of coronene anion radical, which is alternate and planar, the calculated splitting using average Hückel spin and charge densities is  $-1.49$ , compared with an experimental value of  $-1.47$ .<sup>5</sup>

Segal, *et al.*,<sup>6</sup> have found, in agreement with Stone's theory,<sup>7</sup> that the  $g$  values of hydrocarbon radicals can be fitted very well by  $\Delta g = g - g_0 = (31.9 - 16.6\lambda) \times 10^{-5}$ . Here  $g_0$  is the free-electron value and  $\lambda$  is the Hückel energy level coefficient of the unpaired electron orbital. Segal found that the  $g$  value of radicals such as coronene with the unpaired electron in a degenerate orbital do not conform to this relationship, but corannulene does. For corannulene the experimental value of  $\Delta g$  is  $38 \times 10^{-5}$  and the calculated values for planar ( $\beta_{i0} = 1.00$ ) and nonplanar ( $\beta_{i0} = 0.72$ ) are  $40 \times 10^{-5}$  and  $38 \times 10^{-5}$ , respectively.

**Acknowledgment.** This research was supported in part by grants from the National Science Foundation (NSF GP-6425 and GP-4620), the Petroleum Research Fund (PRF-1941-AS), and the Western Electroanalytical Theoretical Society.

(4) L. C. Snyder and T. Amos, *J. Chem. Phys.*, **42**, 3670 (1965).

(5) I. C. Lewis and L. S. Singer, *ibid.*, **43**, 2722 (1965).

(6) B. G. Segal, M. Kaplan, and G. K. Fraenkel, *ibid.*, **43**, 4191 (1965).

(7) A. J. Stone, *Mol. Phys.*, **7**, 311 (1964).

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### On the Dissociation and Reassociation of the Polypeptide Chains of Tropomyosin and Paramyosin<sup>1</sup>

Sir:

The molecules of tropomyosin and paramyosin are similar. In benign media, both consist of two  $\alpha$ -helices arranged side by side and twisted slightly about one another.<sup>2-4</sup> Thus, although they differ significantly in amino acid composition, the only gross difference in molecular form is in the over-all molecular length.

(1) This investigation was supported by Research Grant RG-5488 from the Division of General Medical Sciences, Public Health Service.

(2) S. Lowey, J. Kucera, and A. Holtzer, *J. Mol. Biol.*, **7**, 234 (1963).

(3) A. Holtzer, R. Clark, and S. Lowey, *Biochemistry*, **4**, 2401 (1965).

(4) C. Cohen and K. C. Holmes, *J. Mol. Biol.*, **6**, 423 (1963).

Further questions immediately arise. Among these are: (1) How many individual polypeptide chains make up each molecule? Is there one long chain with a hairpin turn between long helical segments, or two completely helical chains held side by side, etc.? (2) Are there any covalent cross-linkages (in particular, disulfide bonds) between the two helical segments? (3) Are the covalent cross-linkages, if any, necessary to maintain the native conformation?

Two of these questions have already been answered for tropomyosin.<sup>3,5</sup> We report here experimental results which answer all three for both proteins. Except where otherwise noted, preparative methods and physical techniques were as reported earlier.<sup>2,3</sup>

**Tropomyosin.** A molecule of tropomyosin has a mass of 74,000 amu.<sup>3</sup> In a denaturing medium in which the helix content (measured by optical rotatory dispersion) is zero, the same mass is observed, and the other solution properties are not those of a linear, random coil of that mass.<sup>3</sup> Clearly, then, this molecule has one or more covalent cross-linkages. This agrees with chemical studies, which indicate one disulfide bond/molecule.<sup>6</sup> In a medium that would reduce disulfide linkages, the molecular mass is about one-half the value given above.<sup>5</sup> Thus, there are two polypeptide chains in tropomyosin, as it is usually prepared, held together by at least one disulfide linkage.

To investigate further, we prepared tropomyosin samples that had all the disulfide linkages reduced to sulfhydryl, and then all sulfhydryls were masked with acetyl groups. This was accomplished by dissolution of native tropomyosin in a denaturing medium (5 *M* guanidine hydrochloride), reduction with  $\beta$ -mercaptoethanol, acetylation with iodoacetic acid, dialysis, and lyophilization. This lyophilized material ("modified" tropomyosin) was dissolved in both benign and denaturing aqueous media, and several macromolecular properties of these solutions were determined. Specifically, measurements were made of intrinsic viscosity, intrinsic sedimentation coefficient, and (light scattering) molecular weight. In parallel, measurements of intrinsic viscosity and sedimentation coefficient were made on solutions of unmodified tropomyosin. Results for both kinds of material are summarized in Table I (rows labeled TM).

The data in Table I confirm our earlier experiments on unmodified tropomyosin in both media. We have also confirmed that the molecular weight of unmodified tropomyosin, in a medium that is both reducing and denaturing, is one-half that value. It is also plain from the table that the properties of modified and unmodified tropomyosin are quite different from one another in the denaturing medium, but are indistinguishable in the benign medium.

Most striking is the drop in molecular weight (by a factor of two) suffered by the modified protein when transferred from the benign to the denaturing medium and its complete recovery upon transfer back to the benign medium.

**Paramyosin.** A paramyosin molecule has a mass of 220,000 amu.<sup>2</sup> The molecule is extraordinarily stable; optical rotatory dispersion experiments show appreci-

(5) E. F. Woods, *Nature*, **207**, 82 (1965).

(6) A. G. Szent-Györgyi, R. E. Benesch, and R. Benesch in "Sulphur in Proteins," R. E. Benesch and R. Benesch, Ed., Academic Press Inc., New York, N. Y., 1959, p. 291.