TABLE IV. Kinetic Characteristics of "Inverse Substrate" Ia-*para* with Trypsin and Pseudotrypsin

Enzyme	K_S (M)	k_2 (s^{-1})	k_3 (s^{-1})	k_2/K_S ($s^{-1}M^{-1}$)
Trypsin[a]	$3.87 \pm 0.29 \times 10^{-5}$	$1.70 \pm 0.34 \times 10$	$9.26 \pm 0.97 \times 10^{-3}$	4.39×10^5
Pseudotrypsin[b]	$1.82 \pm 0.37 \times 10^{-3}$	$2.87 \pm 0.33 \times 10^{-3}$ [c]	—	1.57

The reaction was carried out in 0.05 M Tris, 0.02 M $CaCl_2$ (pH 8.0) at 25°.
a) See reference 3*a*).
b) Assay was carried out at $[E]_0$: 5.45×10^{-6} M, $[S]_0$: 2.08×10^{-3}—5.53×10^{-4} M.
c) Overall reaction rate.

than 2.8×10^5 times between trypsin and pseudotrypsin. Comparative kinetic properties of pseudotrypsin and trypsin were reported by Foucault *et al.* in the hydrolysis of normal-type substrates.[12] In this study, kinetic parameters in the pseudotrypsin-catalyzed hydrolysis of NPGB were: k_2, 0.14 s^{-1}; k_3, 6.4×10^{-5} s^{-1}; K_s, 5.7×10^{-3} M, k_2/K_s; 24.6. The k_2/K_s values can thus be calculated to differ by 1.5—3.0×10^5 times between trypsin and pseudotrypsin (*cf.* Table III). This difference is comparable to that for the "inverse substrate" IIa.

Our present observations show that not only amidinium, but also guanidinium and ammonium, which are related to the side chains of arginine and lysine, can provide the site-specific positive charge at the leaving portion of an "inverse substrate," and that loss of catalytic activity followed modification in a way that eliminated the charge in either the substrate or the enzyme. This study provides further detailed evidence for the concept of "inverse substrates."

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Electron Spin Resonance Study of the Conformations of Anion Radicals derived from Some Thermochromic Ethylenes[1]

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Electron spin resonance (ESR) spectra of the anion radicals of 10(9*H*)-xanthene-9-ylidene-9(10*H*)-anthracenone, 10-diphenylmethylene-9(10*H*)-anthracenone, 9-diphenylmethylene-9*H*-xanthene, 9,9′-bixanthene and diphenylfulvene were measured. These compounds, except for diphenylfulvene, are thermochromic ethylenes. On the basis of calculation of the spin densities by McLachlan's method and h.f.s. analysis of the ESR spectra, the anion radicals were found to be substantially twisted about the central double bond.

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Keywords——ESR; conformation; anion radical; thermochromic ethylene; twist angle; spin density

Introduction

Some anthrone derivatives, termed "thermochromic ethylenes," of which a typical compound is bianthrone, show a reversible change of color when they are heated in solution.[3] From the viewpoint of organic chemistry, the conformational distorition arising from the double-bond rotation which causes this color change is very interesting.

In this paper, the conformational structures of the anion radicals of 10(9*H*)-xanthene-9-ylidene-9(10*H*)-anthracenone (I), 10-diphenylmethylene-9(10*H*)-anthracenone (II), 9-diphenylmethylene-9*H*-xanthene (III), 9,9′-bixanthene (IV), and diphenylfulvene (V). all of which, except for V, are thermochromic ethylenes, are described on the basis of electron spin resonance (ESR) studies of the anion radicals generated by metal reduction.

Experimental

I, III and IV were prepared according to the methods given in the literature and purified by repeated recrystallization.[4] II and V were commercial products (Aldrich Co., U.S.A.). The identity of the compounds was confirmed by elemental analysis and mass spectrometry.

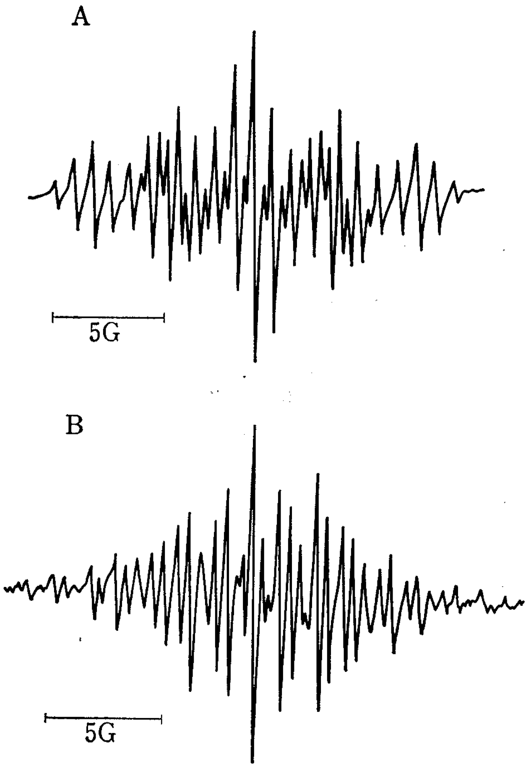

Fig. 1. ESR Spectra of the Anion Radicals of I and II

A: ESR spectrum of the anion radical of I.
B: ESR spectrum of the anion radical of II.

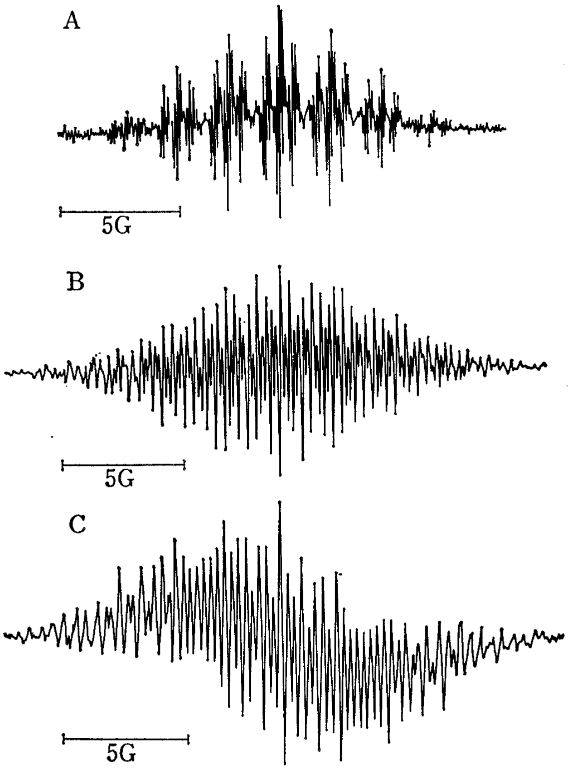

Fig. 2. ESR Spectra of the Anion Radicals of III, IV and V

A: ESR spectrum of the anion radical of IV.
B: ESR spectrum of the anion radical of V.
C: ESR spectrum of the anion radical of III.

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Tetrahydrofuran (THF) was purified by distillation of the commercial product (E. Merck Co., Darmstadt, Germany) and dried by refluxing with CaH_2. It was degassed and stored in the presence of Na-K alloy in a flask equipped with a stopcock.

The compounds were dissolved in THF and degassed under a vacuum of 10^{-5} Torr in a glass tube, then the tube was sealed. The anion radicals were prepared in a glass tube at room temperature by reduction with a potassium mirror preformed in the tube.

ESR measurements were carried out with a JEOL JES-3BSX spectrometer, and the temperature of the sample was controlled at $-70°$ to $+80°\pm 1°$ with a JEOL JES-VT-2 thermoregulator.

Spin densities were calculated by McLachlan's method,[5] the details of which have been described in our previous paper.[6] The parameters for molecular orbital calculations are taken in part from a report on bianthrone derivatives by Agranat *et al.*[7]

Results and Discussion

The anion radicals prepared by metal reduction displayed finely resolved spectra. The observed spectra are shown in Figs. 1 and 2. The calculated spin densities *vs.* resonance integral for the central double bond, which changes as a function of twist angle about the bond, are shown in Fig. 3.

In the case of compound I, although all of the protons are capable of coupling with delocalized unpaired electrons if the molecule is coplanar, only 8 protons were found to couple with unpaired electrons in fact. Spin densities calculated by McLachlan's method indicated that the planes of the two aromatic moieties of the molecule are twisted about the central double bond. In the case of compound II, the coupled protons amounted to only 10 out of 18 in the molecule, and the calculated spin densities indicate that the molecule is twisted almost 90° about the axis of the central double bond and that the two phenyl groups

TABLE I. Spin Densities of I, II, III, IV, and V

	Position	obs.	calc.
I	1′, 8′	0.163	0.1655
	2′, 7′	0.037	−0.0500
	3′, 6′	0.142	0.1226
	4′, 5′	0.037	0.0292
II	2′, 6′, 2″, 6″	0.125	0.1240
	3′, 5′, 3″, 5″	0.051	−0.0432
	4′, 4″	0.143	0.1458
III	4′, 4″	0.176	0.1760
	2′, 6′, 2″, 6″	0.094	0.1414
	1, 8	0.057	−0.0639
	3, 6	0.057	−0.0539
	3′, 5′, 3″, 5″	0.035	−0.0292
	4, 5	0.023	−0.0253
	2, 7	0.023	−0.0155
IV	1, 8, 1′, 8′	0.089	0.0820
	3, 6, 3′, 6′	0.089	0.0821
	2, 7, 2′, 7′	0.022	−0.0259
	4, 5, 4′, 5′	0.005	0.0049
V	2′, 6′, 2″, 6″	0.108	0.1084
	4′, 4″	0.091	0.0980
	3, 4	0.068	−0.0628
	3′, 5′, 3″, 5″	0.038	−0.0343
	2, 5,	0.015	0.0133

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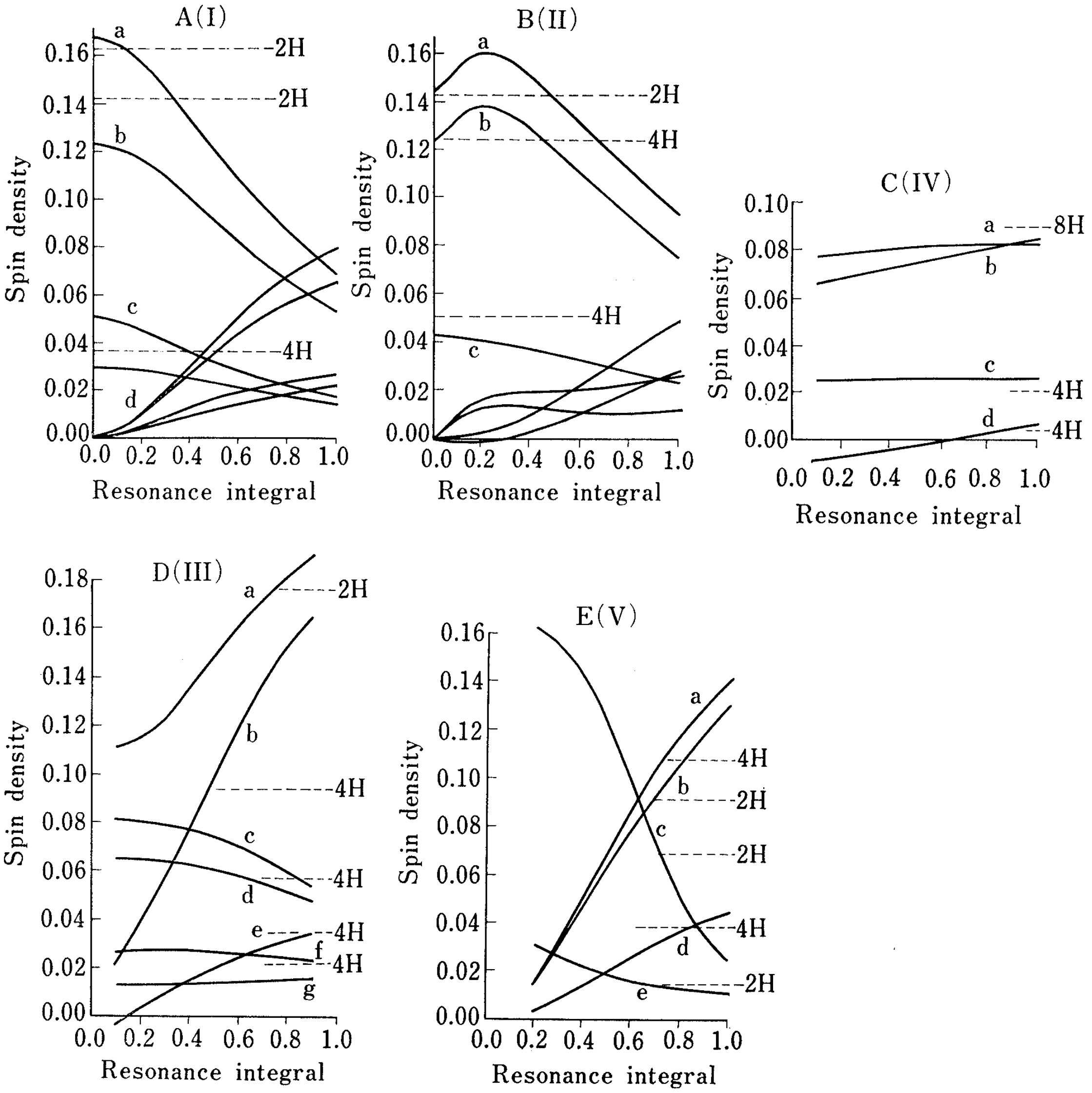
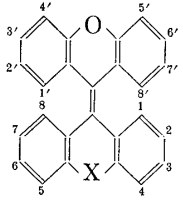
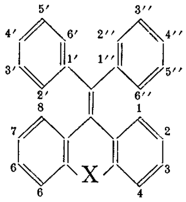
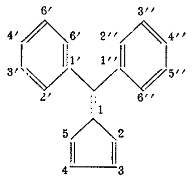




I: X=CO
IV: X=O



II: X=CO
III: X=O



V

Fig 3. Calculated Spin Densities at Ring Carbon Atoms *vs.* Resonance Integral about the Central Double Bond

The dotted lines indicate observed spin densities. The symbols 2H, 4H, *etc.* indicate the number of protons which are sources of ESR h.f.s. The solid lines indicate the calculated spin densities on each carbon atom: In Fig. 3–A, the curves indicate the spin densities, (a) on position 1′, 8′, (b) 3′, 6′ (c) 2′, 7′ (negative sign), (d) 4′, 5′. In Fig. 3-B, (a) 4′, 4″, (b) 2′, 6′, 2″, 6″, (c) 3′, 5′ 3″, 5″ (negative sign). In Fig. 3-C, (a) 1, 8, 1′, 8′ (b) 3, 5, 3′, 5′, (c) 2, 7, 2’, 7′ (negative sign), (d) 4, 5, 4′, 5′. In Fig. 3-D, (a) 4′, 4″, (b) 2′, 6′, 2″, 6″, (c) 1, 8, (d) 3, 6, (e) 3′, 5′, 3″, 5″, (f) 4, 5 (negative sign), (g) 2, 7 (negative sign). In Fig. 3-E, (a) 2′, 6′, 2″, 6″, (b) 4′, 4″, (c) 3, 4, (d) 3′, 5′, 3″, 5″ (negative sign), (e) 2, 5.

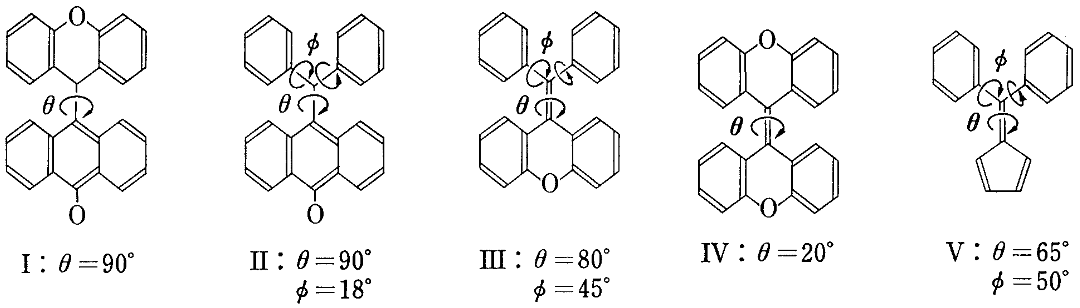
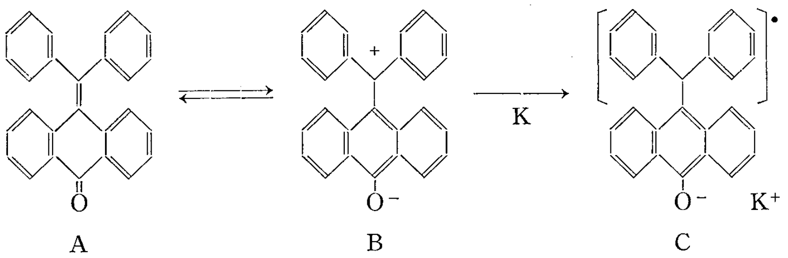


Fig. 4. The Twist Angles about the Central Double (or Single) Bond

are twisted *ca.* 18° about the plane which includes the central double bond and ring carbon $C_{1'}$. In the cases of compounds III, IV and V, the central double bonds are also twisted, but not by 90°. The conformations and the spin densities of these compounds are listed in Fig. 4 and Table I.

The two types of twist angles of the central double bonds may be rationalized as follows. In the case of compounds I and II, the molecules have a polar structure, as shown by (B) in the following scheme, so that the central double bond changes to a single bond when an electron is added to the molecule.



In the case of the other compounds, III, IV and V, which have no carbonyl group, the double bond character remains because the molecular polarity is small compared with the first case. The twisting might arise from steric hindrance of hydrogen atoms. We are at present calculating the energies involved in changes of conformation of these molecules by the semi-empirical MO method.

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