OPTICAL ROTATION AND HELICAL POLYPEPTIDE CHAIN CONFIGURATION IN α-PROTEINS

Sir:

A theory of optical rotatory dispersion for helical macromolecules has recently been developed by Moffitt.[1] This has been applied successfully to the rotatory behavior of synthetic polypeptides in the α-configuration,[2,3,4,5] and of "globular" proteins.[6] Here we present the result of an examination of a series of "fibrous" proteins which yield in the condensed state the α-type wide-angle X-ray diffraction diagram.

In the native state the proteins examined have a rotatory behavior in aqueous solution similar to that of synthetic polypeptides in helix-inducing solvents. With the exception of fibrinogen, none of these proteins shows "simple" dispersion, *i.e.*, follows a one-term Drude equation. The data fit Moffitt's equation for helical systems

$$[\alpha]_\lambda = \left(\frac{100}{M}\right)\left(\frac{n^2+2}{3}\right)\left[\frac{a_0\lambda_0^2}{(\lambda^2-\lambda_0^2)} + \frac{b_0\lambda_0^4}{(\lambda^2-\lambda^2{}_0)^2}\right]$$

where M is the molecular weight per residue and n is the refractive index of the solvent. Within the experimental error of about ± 100Å., λ_0 equals 2100Å., in agreement with the λ_0 reported for poly-γ-benzyl-L-glutamate and poly-L-glutamic acid.[3] A value of near −600° for the constant b_0 characterizes a fully-coiled, right-handed α-helix of synthetic polypeptides.[3] Assuming that λ_0 for the helical and for the non-helical configurations are close, one can use b_0 as a measure of helical content. Table I lists the b_0's obtained on this assumption, using a value of λ_0 equal to 2100Å. The

TABLE I

	Native[a] $[\alpha]_{5780}$[c]	b_0[d]	Denatured[b] $[\alpha]_{5780}$	λ_0 (Å.)[e]
Light meromyosin fraction I[f]	−13.0°	−660°	−118°	2120
Tropomyosin	−16.0°	−620°	−118°	2130
Paramyosin	−11.1°	−600°	−63°	...
Light meromyosin	−20.4°	−490°	−107°	2150
Myosin	−28.7°	−370°	−108°	2180
Heavy meromyosin	−34.5°	−300°	−103°	2150
Fibrinogen	−58.2°	−210°	−110°	2130

[a] In 0.6*M* KCl, *p*H 7.0; fibrinogen in 0.3*M* NaCl, *p*H 6.2. [b] In 9.5*M* urea. [c] Measurements were made with a Rudolph High Precision Polarimeter with photoelectric attachment at four wave lengths: 3650, 4360, 5460, 5780Å., isolated by glass and interference filters from a mercury arc; room temperature, 20 ± 3°. [d] Obtained from plots of $[\alpha]\lambda(M/100)\ (3/(n^2+2)\ (\lambda^2-\lambda_0{}^2)$ *vs.* $1/(\lambda^2-\lambda_0{}^2)$, λ_0 = 2100Å. [e] Obtained from plots of $\lambda^2[\alpha]\lambda$ *vs.* $[\alpha]\lambda$.[6] [f] Part of light meromyosin resistant to ethanol treatment representing about half of light meromyosin.

negative sign indicates that the helices in the "fibrous" proteins have the same sense of twist as those in synthetic polypeptides and other proteins. Since the macromolecules of these α-proteins probably consist of cables of α-helices in a supercoiled configuration[7,8,9] it would appear that this association in aqueous solution, as well as superimposed backbone dissymmetry, do not markedly affect rotatory behavior.

In the denatured state one expects absence of helical backbone contribution to the rotation.[10] Indeed, we have found that b_0 is approximately zero for all the proteins except paramyosin whose b_0 is of the order of −190°. Moffitt's equation then reduces to a one-term Drude equation, and the λ_0's found from the latter are close to 2100Å. (see Table I). This fact supports our use of b_0 as a measure of helical content. There is independent evidence that paramyosin is not completely denatured by the urea treatment.[11] The values of the specific rotations listed in Table I are in agreement with those found for other denatured proteins[10] and synthetic polypeptides in the random coil configuration.[2]

The rotation of myosin is close to the proportional sum of the rotations of light and heavy meromyosin; hence it would appear that there is no large-scale unfolding of helical domains in the myosin molecule when split by trypsin.

We note that although there is a variation of helical content in "fibrous" proteins, the values are generally higher than those characterizing "globular" proteins. Asymmetry is thus an expression of high helical content.

The relationship of proline content to helical configuration in these α-proteins will be described in another communication.

We thank Professors Paul Doty and William Moffitt, and Dr. J. T. Yang, for discussions, and for allowing us to read their manuscripts before publication. We thank Professor Richard S. Bear for interest and advice, and Professor David Waugh for the fibrinogen sample.

(1) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).
(2) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 498 (1956).
(3) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. (Wash.)*, **42**, 597 (1956).
(4) W. Moffitt, *ibid.*, **42**, 736 (1956).
(5) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.*, in press.
(6) P. Doty ann J. T. Yang, THIS JOURNAL, in press.
(7) F. C. Crick, *Acta Cryst.*, **6**, 689 (1953).
(8) L. Pauling and R. J. Corey, *Nature*, **171**, 59 (1953).
(9) R. S. Bear and C. C. Selby, *J. Biophys. Biochem. Cytol.*, **2**, 55 (1956).
(10) C. Cohen, *Nature*, **175**, 129 (1955).
(11) A. G. Szent-Gyorgyi, unpublished data.
(12) The work in this laboratory was aided by a grant from the National Foundation for Muscular Dystrophy.
(13) Aided in part by Research Grant A-901 from the National Institutes of Arthritis and Metabolic Diseases of the National Institutes of Health, United States Public Health Service.
(14) This work done during the tenure of an Established Investigatorship of the American Heart Association.

CHILDREN'S CANCER RESEARCH FOUNDATION, AND
THE DEPARTMENT OF PATHOLOGY OF THE CHILDREN'S
MEDICAL CENTER,[12] BOSTON, MASSACHUSETTS
DEPARTMENT OF BIOLOGY[13] CAROLYN COHEN
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE, MASSACHUSETTS
INSTITUTE FOR MUSCLE RESEARCH[14]
MARINE BIOLOGICAL LABORATORY
WOODS HOLE, MASSACHUSETTS ANDREW G. SZENT-GYORGYI

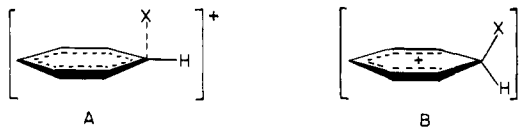
RECEIVED NOVEMBER 28, 1956

THE STRUCTURE OF THE TRANSITION STATE FOR ELECTROPHILIC AROMATIC SUBSTITUTION

Sirs:

Present theories of aromatic electrophilic substitution allow only an indefinite formulation of

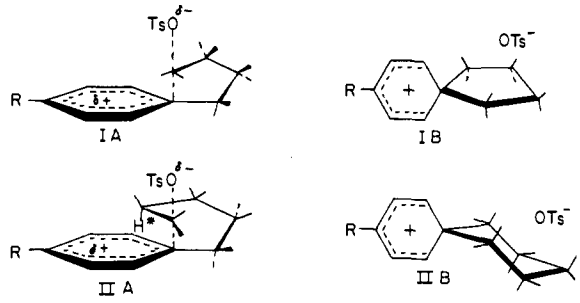
the transition state structure, somewhere between the extremes A and B. Most frequently the transition state is approximated by B,[1] although there are indications that in some systems B is a discrete intermediate.[2,3,4] We present herein kinetic and



A B

thermodynamic data which indicate that structure B is not a good representation of the transition state, at least for certain cases.

Under suitable conditions the ionization of arylsulfonate from 4-aryl-1-butyl and 5-aryl-1-pentyl arylsulfonates (I and II, resp.) may proceed with aryl participation (intramolecular electrophilic substitution) *via* IA, IB and IIA, IIB, resp. Comparison of the rate constants for these participation reactions (k^{I}_{par} and k^{II}_{par}) can be used to decide between A and B type transition states, since, as is derived below, for extreme A, $k^{I}_{par}/k^{II}_{par} > 1$ and for extreme B, $k^{I}_{par}/k^{II}_{par} \ll 1$. Accordingly, kinetic studies were carried out with I and II tosylates in formic acid,[5] pitting participation against direct formolysis.



IA IB

II A II B

The data on 5-aryl-1-pentyl tosylates (Table I)[6] fail to show significant aryl participation, except to a small degree for R equal to 2,4-dimethoxyphenyl, and contrast with the kinetic results indicating important participation for the 4-aryl-1-butyl brosylate series,[5] in which for aryl equal to *p*-methoxyphenyl and 2,4-dimethoxyphenyl, $k_{Ar}/k_{C_6H_5}$ is 1.8 and 10, resp.[5] Since the rate constant for direct formolysis should be about the same for I and II series, it follows that k^{I}_{par}/k^{II}_{par} must be considerably larger than unity.

TABLE I

FORMOLYSIS RATES OF 5-ARYL-1-PENTYL TOSYLATES

$R(CH_2)_4CHR^1OTs$ R	R^1	10^5k (sec.$^{-1}$)	$E^{\ddagger}$ (kcal./mole)
CH_3	H	2.13(80.50°)	25.4
C_6H_5	H	1.74(70.50°)	24.4
p-$CH_3OC_6H_4$	H	1.31(80.50°)	23.7
2,4-di-$CH_3OC_6H_3$	H	2.22(80.50°)	23.2
CH_3	CH_3	32.5 (41.90°)	
C_6H_5	CH_3	25.0 (41.90°)	
p-$CH_3OC_6H_4$	CH_3	21.9 (41.90°)	

The difference in free energy change for the processes $-(CH_2)_5- \rightarrow$ cyclopentane (ΔF_5) and $-(CH_2)_6- \rightarrow$ cyclohexane (ΔF_6), calculated accurately from thermodynamic data on cyclopentane,[7] cyclohexane,[7] $-(CH_2)_5-$[8] and $-(CH_2)_6-$,[8] *i.e.*, $\Delta F_5 - \Delta F_6$, is +4.3 kcal./mole (25°) and from this the difference in free energy, $\Delta F_{(I \rightarrow IB)} - \Delta F_{(II \rightarrow IIB)}$ is obtained[9] as +3.6 kcal./mole. On the basis of transition state B, therefore, $k^{I}_{par}/k^{II}_{par} \cong 2.5\text{-}(10)^{-3}$. For a transition state of type A the reverse is expected since the incipient ring in IA is strainless, with all hydrogens staggered and an optimum (linear) arrangement of C_{Ar}, C_{OTs} and OTs, whereas in IIA there is *strong* steric interaction between H* and the aromatic ring. The transition states for these reactions, therefore, are not correctly represented by B, but rather as having considerable A character.

If structure B is of relatively high energy and truly represents the transition for intermolecular electrophilic aromatic substitution (say by ROTs), it should also represent the transition state for the intramolecular substitutions of I and II, since additional energy terms peculiar to the cyclic intramolecular process (due to relatively small interactions between non-bonded atoms) would not be expected to be large enough to cause the appearance of higher energy species at an earlier stage. On the other hand, a transition state for intermolecular aromatic substitution having considerable A character accords with the facts above and would imply that B should be considered as an intermediate formed after the rate-determining step.

These conclusions agree not only with our own data, but also with molecular orbital calculations on the stability of B,[10] absence of secondary isotope effects[11] during aromatic substitution[12] and varia-

(1) See, *e.g.*, M. J. S. Dewar, "Electronic Theory of Organic Chemistry," Oxford University Press, London, 1949, p. 162.

(2) L. Melander, *Acta. Chem. Scand.*, **3**, 95 (1949).

(3) D. J. Cram, THIS JOURNAL, **74**, 2129, 2159 (1952).

(4) See, however, G. S. Hammond, *ibid.*, **77**, 334 (1955).

(5) While this work was in progress, a communication appeared describing the formolysis behavior of several 4-aryl-1-butyl brosylates corresponding to the 5-aryl-1-pentyl tosylates reported in Table I [S. Winstein, R. Heck, S. Lapporte and R. Baird, *Experientia*, **12**, 138 (1956)]. The data in that paper and in Table I suffice for present purposes.

(6) Internal-return complications should be absent from these systems. See S. Winstein and K. S. Schreiber, THIS JOURNAL, **74**, 2171 (1952). For these systems, as for the 4-aryl-1-butyl cases (ref. 5), only participation of the spiro type will occur.

(7) "Selected Values of Physical and Thermodynamic Properties of Hydrocarbons and Related Compounds," Carnegie Press, Pittsburgh, Pa., 1953.

(8) Calculated using $\Delta H_f^{298°}/CH_2 = 4.95$ kcal./mole (ref. 7) and S_0^{298} from the equation of K. Otozai, *et al.*, *Catalyst (Japan)*, **9**, 53 (1953); *C. A.*, **48**, 1742 (1954).

(9) Correcting for the presence of the spiro aromatic substituent from ΔF_f^{298} of cyclopentane, 1,1-dimethylcyclopentane, cyclohexane and 1,1-dimethylcyclohexane (ref. 7).

(10) N. Muller, L. W. Pickett and R. S. Mulliken, THIS JOURNAL, **76**, 4770 (1954), have calculated that the benzenium ion, $C_6H_7^+$, is stabilized by *Ca.* 15 kcal./mole by σ_{CH_2}–π delocalization in addition to a comparable amount for π-delocalization. These results lead to the far-reaching conclusion[11] that the nature of the group X has a strong influence on the stability of the intermediate B.

(11) See V. J. Shiner, *ibid.*, **75**, 2925 (1953).

(12) L. Melander, *Nature*, **163**, 599 (1949).

tion in *m/p* orientation ratio with the electrophilic reagent.[13,14]

(13) H. C. Brown and K. L. Nelson, THIS JOURNAL, **75**, 6292 (1953).

(14) H. C. Brown and C. W. McGary, Jr., *ibid.*, **77**, 2300 (1955).

(15) National Science Foundation Fellow, 1954–1955; Alfred P. Sloan Foundation Fellow, 1956.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF ILLINOIS ELIAS J. COREY
URBANA, ILLINOIS CAROL KILBOURNE SAUERS[15]

RECEIVED SEPTEMBER 24, 1956

RAUWOLFIA ALKALOIDS. XXIX. THE STRUCTURE OF RAUNESCINE AND ISORAUNESCINE[1]

Sir:

Two new alkaloids, raunescine and isoraunescine have been isolated by Hosansky and Smith[2] from *R. canescens*. Since raunescine possesses reserpine-like pharmacological activity, its structure is of some importance. On the basis of analytical data and spectral studies, Hosansky and Smith made the logical suggestion that these compounds are similar in structure to deserpidine, differing from it only in that they possess a C-17 hydroxyl rather than a methoxyl. We wish to report chemical proof for the formulation of raunescine as I and isoraunescine as II.

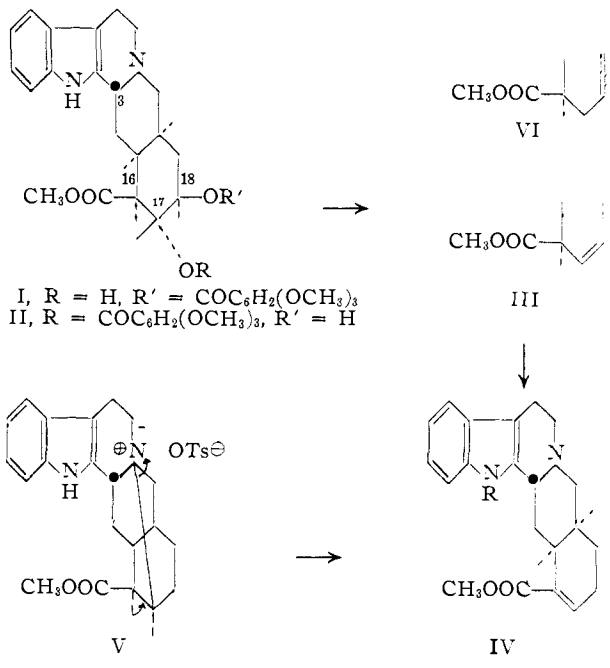
Raunescine and isoraunescine are both reduced with lithium aluminum hydride to the same triol (m.p. 235° with loss of water of crystallization at 150°; *Anal.* Calcd. for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.10. Found: C, 69.86; H, 8.05; N, 8.10) indicating that the isomerism of raunescine and isoraunescine resides only in the identity of the hydroxyl group esterified with 3,4,5-trimethoxybenzoic acid. The tosylate ester of isoraunescine (m.p. 227–230°; *Anal.* Calcd. for $C_{38}H_{42}N_2O_{10}S$: C, 63.49; H, 5.90; N, 3.89; S, 4.45. Found: C, 63.86; H, 6.23; N, 3.90; S, 3.97) suffers replacement of tosylate by bromide by heating in pyridine with lithium bromide to give the bromo derivative (m.p. 207°; *Anal.* Calcd. for $C_{31}H_{35}BrN_2O_7$: C, 59.29; H, 5.63; Br, 12.73; N, 4.46. Found: C, 58.78; H, 5.77; Br, 12.78; N, 4.56). This bromo compound readily eliminates bromide and trimethoxybenzoyloxy by short treatment with zinc in refluxing acetic acid to give the β,γ-unsaturated ester III (m.p. 223–225°; *Anal.* Calcd. for $C_{21}H_{24}N_2O_2$: N, 8.33. Found: N, 8.27, C=O absorption at 1726^{-1} cm. in Nujol mull). III is transformed by gentle treatment with sodium methoxide to the α,β unsaturated ester, apo-3-epi-α-yohimbine (IV) (crystallized as the hydrochloride) (m.p. 270–275°, *Anal.* Calcd. for $C_{21}H_{24}N_2O_2\cdot HCl$: C, 67.64; H, 6.76; N, 7.51; Cl, 9.51. Found: C, 67.67; H, 7.12; N, 7.47; Cl, 9.65, C=O and CH =CH absorption at 1714 and 1634^{-1} cm., in Nujol mull, $[\alpha]^{25}D$ −20° in 0.1*N* methanolic sodium hydroxide). IV is also prepared by refluxing 3-epi-α-yohimbine tosylate (V)[3] in collidine[4] thus fixing its structure. III is formulated as β,γ rather than the γ,δ unsaturated ester (VI) because of its ready conversion to IV.

(1) We are indebted to Dr. P. Ulshafer for a supply of raunescine and isoraunescine.

(2) N. Hosansky and E. Smith, *J. Am. Pharm. Assoc.*, **44**, 639 (1955).

(3) C. F. Huebner and D. F. Dickel, *Experientia*, **12**, 250 (1956).

(4) This reaction was carried out by Dr. D. F. Dickel.



Raunescine and isoraunescine are therefore 3-epi-α-yohimbanes substituted at C-16 with carbomethoxyl and at C-17 and C-18 with hydroxyl functions.

The stereochemistry at C-16, C-17 and C-18 is indicated as follows. Raunescic acid hydrochloride (amorphous) is transformed into raunescic acid lactone by the carbodiimide method[5] (m.p. 281–285°; *Anal.* Calcd. for $C_{20}H_{22}N_2O_3\cdot C_2H_5OH$: C, 68.72; H, 7.34; N, 7.29. Found: C, 68.79; H, 7.41; N, 7.51, C=O absorption at 1768^{-1} cm.). I and II are shown to have the less stable configuration at C-3 by the process of oxidation to tetradehydro compounds with lead tetraacetate and reduction with sodium borohydride to give the new C-3 epimers. Neither of them could be obtained crystalline but they are characterized and distinguished from the starting material by paper chromatographic R_f values (solvent A[6]: I-0.45, 3-iso-I-0.21; II-0.25, 3-iso-I-0.72. Conformational analysis shows that the substituents at C-16 and C-18 must be β-oriented on the 3-epialloyohimbane nucleus to account for this epimerization.[7] The same conclusion regarding the stereochemistry at C-16 and C-18 is reached through a study of the tosylation of raunescinetriol. As with reserpinol,[8] an inner quaternary tosylate salt is formed at 5°. Neither the tosylate nor any of its derivatives have been obtained crystalline but its quaternary nature is clearly demonstrated by chemical and infrared spectral evidence of the presence of tosylate ion (sharp absorption bands at 1015, 1037, 1124 and

(5) R. B. Woodward, F. E. Bader, H. Bickel, A. J. Frey and R. W. Kierstead, THIS JOURNAL, **78**, 2025 (1956).

(6) A. F. St. André, B. Korzun and F. Weinfeldt, *J. Org. Chem.*, **21**, 480 (1956).

(7) This already has been discussed in connection with the reserpine problem: C. F. Huebner, H. B. MacPhillamy, E. Schlittler and A. F St. André, *Experientia*, **11**, 303 (1955).

(8) C. F. Huebner and E. Wenkert, THIS JOURNAL, **77**, 4180 (1955); P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, *ibid.*, 4687 (1955); E. E. van Tamelen and P. D. Hance, *ibid.*, **77**, 4692 (1955).