kinetic isotope effect of 2.7 was found for the photobleaching of 9-(2′-hydroxyethyl-2′,2′-$d_2$)isoalloxazine, the $\alpha$-deuterio analog of IIb. In addition, it was found that the rate of photobleaching of riboflavin was unaffected by deuterium oxide.[6] Also, 6,7-dimethyl-9-(formylmethyl)isoalloxazine has been isolated from photobleached riboflavin solutions; this confirmed that attack occurred at the 2′ position in polyhydroxy flavins.[7] Although several other products resulted from the riboflavin photobleaching, Ib photobleached by a simple pathway which produced only one heterocyclic product, alloxazine (III), and acetaldehyde. This resulted from the collapse of the diradical produced by the hydrogen abstraction reaction.

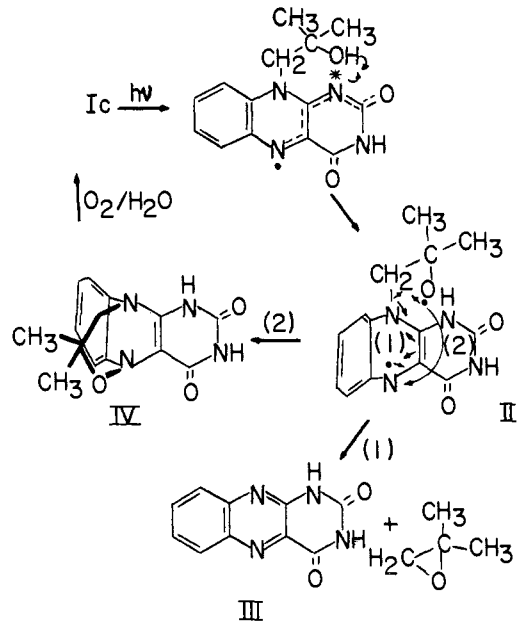
Kendall and Leermakers[8] have reported that pyruvic acid was photoreduced with *t*-butyl alcohol although this alcohol has been shown to be a poor hydrogen donor[3] due to the absence of hydrogen on the $\alpha$ carbon. They speculated that the aliphatic hydrogens on the methyl groups were donated, as had Cohen and Aktipis[9] for another system.

The synthesis of 9-(2′-hydroxy-2′-methylpropyl)-isoalloxazine was accomplished by the method of Karrer and Meerwein.[10] Irradiation of $1.5 \times 10^{-4}$ *M* Ic in neutral aqueous solution *in vacuo* produced alloxazine (III) and a reduced flavin in a 55:45 ratio. Air oxidation converted the reduced flavin to starting material Ic. Nmr analysis and thin-layer chromatography were used for identification. The rate of photobleaching was followed spectrophotometrically at 430 m$\mu$, which is the long wavelength absorption maximum for Ic. The photochemical apparatus and procedure were the same as described previously[6] and the light intensity was $1.2 \times 10^{16}$ quanta $sec^{-1}$ $cc^{-1}$. The rate followed pseudo-first-order kinetics in the absorbance range 2 to 1. Simultaneous irradiation of Ic in water and deuterium oxide gave first-order rate constants of $2.7 \times 10^{-2}$ and $5.5 \times 10^{-3}$ $min^{-1}$, respectively. This represents a kinetic isotope effect of 4.9.

Polarographic studies on the photobleaching of Ic in 0.1 *M* potassium chloride confirmed the other results. As measured *vs.* a silver–silver chloride electrode, half-wave potentials of −0.58, −0.42, and −0.22 v were obtained for alloxazine, Ic, and the reduced flavin, respectively. These data show that the reduced flavin is not the 1,10-dihydro form of Ic, but possibly a cyclic reduced flavin since the potential is shifted. Photoreduction of Ic with EDTA confirmed that the 1,10-dihydro form of Ic did indeed have the same half-wave potential as the oxidized flavin Ic.

These experimental results can be explained by the mechanism shown in Scheme I. The isoalloxazine nucleus abstracts the hydroxyl hydrogen atom and produces the diradical II. Although the electron density of semiquinone radical is somewhat delocalized, it is conveniently shown localized at N-10. The diradical can collapse by two pathways which have almost equal potentials. Pathway 1 permits an electronic reorganization to form an epoxide and alloxazine (III). A direct cyclization to form a reduced flavin (IV) can be visualized for pathway 2. Although reduced cyclic flavins have previously been illustrated as coupling at N-1, the almost equal distribution of products does not permit tautomerization of II with another form containing a free radical site at N-1. However, the exact structure of IV cannot be experimentally verified at present.

**Scheme I**




The product distribution and the cyclic reduced flavin could be explained in terms of hydrogen abstraction of aliphatic hydrogens, but no *kinetic isotope effect would be expected.* No exchange of deuterium at the aliphatic positions was observed during nmr analysis which was performed in trifluoroacetic acid–deuterium oxide solutions. Also we have found that 9-(2′-keto propyl)isoalloxazine is stable to light,[11] which indicates that methyl groups are resistant to attack in this type of system. Thermochemical calculations on *t*-butyl alcohol indicate that homolytic abstraction of the hydroxyl hydrogen is comparable to abstraction of an aliphatic hydrogen. The sum of the evidence indicates that it is the hydroxyl hydrogen which participates in the anaerobic photoreaction of 9-(2′-hydroxy-2′-methylpropyl)isoalloxazine. Although the mechanism involving the participation of the hydroxyl group may only be applicable to diradical systems, other atypical hydrogen abstraction reactions should be examined more closely.

**Acknowledgment.** This investigation was supported by Grant GM11401 from the National Institutes of Health, U. S. Public Health Service.

(11) W. M. Moore and C. Baylor, Jr., unpublished results.

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## Dihedral Angle and Bond Angle Dependence of Vicinal Proton–Fluorine Spin–Spin Coupling

*Sir:*

**Dihedral Angle Dependence.** The assumption has been widely made that $J_{HF(vic)}$ depends on the dihedral angle between the coupling nuclei in the same way

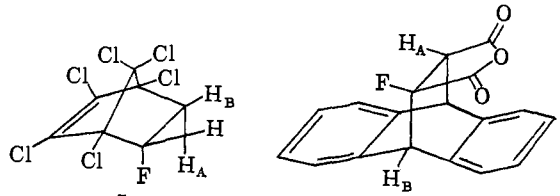
(8) D. S. Kendall and P. A. Leermakers, *J. Am. Chem. Soc.*, **88**, 2766 (1966).
(9) S. G. Cohen and S. Aktipis, *Tetrahedron Letters*, 579 (1965).
(10) P. Karrer and H. F. Meerwein, *Helv. Chim. Acta*, **18**, 480, 1126 (1935).

Table I. Vicinal Proton–Fluorine Coupling Constants (Hz)

| | Approximate dihedral angles | | | | |
|---|---|---|---|---|---|
| | 0° ($J_{AF}$) | 60° ($J_{BF}$) | 90° ($J_{BF}$) | 120° ($J_{BF}$) | 132° ($J_{BF}$) |
| I | 24.7 | | | 12.2 | |
| II | 22.5 | 6.0 | | | |
| III | 30.8 | 3.8 | | | |
| IV | 10.55 | | 2.3 | | |
| V | 19.8 | | *Ca.* 2.0 | | |
| VI | +17.7 | | | | +6.3 |

that $J_{HH'(vic)}$ does,[1] *i.e.*, that $J_{HF(vic)}$ should have a maximum value when the dihedral angle, $\phi_{HF}$, is 0 and 180° and a minimum value when $\phi_{HF}$ is 90°. However, it has recently been reported that $J_{HF}$ is a *linear* function of dihedral angle, being 5.8, 11.4, 14.6, and 17.8 Hz for 0, 60, 90, and120°, respectively.[2]

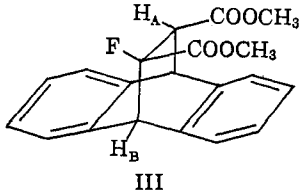
This conclusion[2] and the assumptions of previous workers[1] regarding the dihedral angle dependence of $J_{HF}$ suffer from the fact that, without exception, the studies have been made on conformationally flexible molecules, *i.e.*, highly halogenated alkanes and fluorinated cyclohexanes and cyclobutanes. The object of the present work was to synthesize a series of conformationally rigid molecules in which the vicinal proton and fluorine atoms are held in fixed and known conformations. The results for compounds I–VI are presented in Table I.



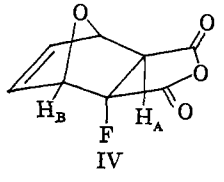

I



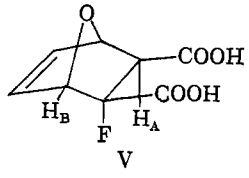

II



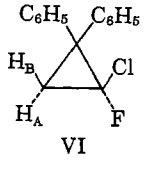

III




IV




V




VI

It is clear from the present work that the dihedral angle dependence of $J_{HF(vic)}$ is very similar to that of $J_{HH'(vic)}$ and is *not* a linear function of dihedral angle. With the accumulation of more data on unstrained systems it will undoubtedly be found that $J_{HF(vic)}$ follows a curve of the type

$$J_{HF(vic)} = A + B\cos\phi + C\cos 2\phi$$

just as $J_{HH'(vic)}$ does.[1] The vicinal proton–fluorine coupling constant is probably dependent on the electronegativity of adjacent substituents, as $J_{HH'(vic)}$ has been shown to be,[3] and is also probably a function of bond length,[4] which would account for the larger value of $J_{AF}$ in the cyclopropane compound, VI.

**Bond Angle Dependence.** Karplus,[5] utilizing a nonionic six-electron, six-orbital fragment in a valence bond $\sigma$-electron calculation has predicted that $J_{HH'}$ in the fragment HCC'H' should decrease as $\theta$ and $\theta'$ increase [$\theta = \angle HCC'$, $\theta' = \angle CC'H'$]. This prediction has been confirmed for $J_{HH'(cis)}$ in cyclic ethylenic systems.[6] In the present work we have adduced evidence that $J_{HF(vic)}$ in saturated systems is markedly dependent on the angles $\theta$ and $\theta'$ in the manner predicted by Karplus for $J_{HH'}$.

Because $J_{HF(vic)}$ is very dependent on dihedral angles, as shown above, and probably on substituents as well, the variation of $J_{HF}$ with bond angle is most convincingly demonstrated in a series of compounds having the same dihedral angles and approximately the same substituents. In compounds II, III, IV, and V, the dihedral angle between fluorine and an adjacent proton is 0° and the carbons bearing the proton and fluorine each bear a carboxyl residue. It will be noted that $J_{H_AF}$ in compound IV is 10.5 Hz and that this increases to 19.8 Hz on hydrolysis to the diacid V. Then the anhydride II exhibits $J_{H_AF}$ of 22.5 Hz, which increases to 30.8 Hz on methanolysis. We attribute these increases in $J_{HF(vic)}$ to slight decreases in the bond angles HCC' and CC'F as the angle strain is relieved in going from IV, where the HCC'F residue is part of two five-membered ring systems, to III, where it is part of an unstrained six-membered ring system. The observed changes in $J_{HF(vic)}$ parallel analogous $J_{HH'}$ couplings and are in the direction predicted by Karplus[5] for $J_{HH'(vic)}$. From these observations as well as the dependence of $J_{HF(vic)}$ on dihedral angle, and probably bond length as well, it appears that $J_{HF}$ over three saturated bonds is almost completely analogous in behavior to $J_{HH'(vic)}$.

Compound I, mp 70–71°, was prepared by Diels–Alder condensation of vinyl fluoride with hexachlorocyclopentadiene. The three proton–proton coupling constants and the internal chemical shifts are in excellent agreement with those expected from a previous study of 5-*endo*-substituted hexachlorobicyclo[2.2.1]-heptenes.[3] Compound II, mp 198–199°, was prepared by allowing anthracene to react with fluoromaleic anhydride.[7] Esterification (to confer solubility) gave III, mp 159–160°. Diels–Alder condensation of fluoromaleic anhydride with furan gave the adduct IV, mp 105–106°, which on hydrolysis gave the *exo* diacid V, mp 141–142°. Both IV and V were shown to be *exo* because $J_{H_iH_A} = 0$ Hz. Compound VI, mp 76–77°, was prepared by the addition of fluorochlorocarbene[8] to 1,1-diphenylethylene. If $J_{AB}$ is negative in sign in compound VI, as it is in other cyclopropanes,[9] then $J_{AF}$ and $J_{BF}$ must be positive in order

(1) R. J. Abraham and H. J. Bernstein, *Can. J. Chem.*, **39**, 39 (1961); H. S. Gutowsky, G. G. Belford, and P. E. McMahon, *J. Chem. Phys.*, **36**, 3353 (1962); J. B. Stothers, J. D. Talman, and R. R. Fraser, *Can. J. Chem.*, **42**, 1530 (1964); F. A. Bovey, E. W. Anderson, F. P. Hood, and R. L. Kornegay, *J. Chem. Phys.*, **40**, 3099 (1964); L. D. Hall and J. F. Manville, *Chem. Ind.* (London), 991 (1965); M. Takahashi, D. R. Davis, and J. D. Roberts, *J. Am. Chem. Soc.*, **84**, 2935 (1962); J. B. Lambert and J. D. Roberts, *ibid.*, **87**, 3891 (1965).

(2) H. F. White, *Anal. Chem.*, **37**, 403 (1965).

(3) K. L. Williamson, *J. Am. Chem. Soc.*, **85**, 516 (1963).

(4) $J_{HH'(vic)}$ is expected to increase as the bond length decreases.[5]

(5) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).

(6) (a) O. L. Chapman, *ibid.*, **85**, 2014 (1963); G. V. Smith and H. Kriloff, *ibid.*, **85**, 2016 (1963); P. Laszlo and P. von R. Schleyer, *ibid.*, **85**, 2017 (1963). (b) See also J. D. Graham and M. T. Rogers, *ibid.*, **84**, 2249 (1962).

(7) M. S. Raasch, R. E. Miegel, and J. E. Castle, *ibid.*, **81**, 2678 (1959).

(8) B. Farah and S. Horensky, *J. Org. Chem.*, **28**, 2494 (1963).

(9) H. M. Hutton and T. Schaefer, *Can. J. Chem.*, **41**, 684, 1623 (1963); C. N. Banwell and N. Sheppard, *Discussions Faraday Soc.*, **34**,

to give the observed $F^{19}$ and $H^1$ line positions and intensities.[10] On this basis we assume that, with the possible exception of $J_{BF(90°)}$ in IV and V, all the coupling constants in Table I are positive in sign.[11] All six compounds had elemental analyses and infrared, ultraviolet, and nmr[12] spectra in accord with the assigned structures.

**Acknowledgment.** The hospitality of Cornell University during the tenure of a visiting professorship is gratefully acknowledged. We wish to thank the Petroleum Research Fund and the Public Health Service (Grant GM-10224-04) for financial support and the Cornell and University of Massachusetts Computation Centers for the use of CDC 1604 and 3600 computers.

115 (1962); D. J. Patel, M. E. H. Howden, and J. D. Roberts, *J. Am. Chem. Soc.*, **85**, 3218 (1963).

(10) This compound has been independently synthesized and its spectra were analyzed by S. Terabe and W. Funasaka with the same results (private communication). The assumption is made for this analysis that $J_{AF(cis)} > J_{BF(trans)}$.

(11) From calculated line positions and intensities, using the LAOCOON II computer program of A. A. Bothner-By, it is not possible to ascertain whether $J_{BF}$ in IV and V is positive or negative in sign.

(12) Fluorine and proton nmr spectra were determined on A-60, DP-60, HR-60, and HA-100 spectrometers located at the University of Massachusetts and Cornell University. Compounds I, III, and VI were run as 10% (w/v) solutions in $CDCl_3$, compound II as a 10% (w/v) solution in dimethylformamide, and compounds IV and V as 10 and 20% (w/v) solutions in $CD_3COCD_3$ and $CD_3CN$. The aromatic protons in II and III were decoupled from $H_B$ to facilitate analysis. The 100-MHz proton spectrum of IV was completely decoupled in the frequency sweep mode.

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## Stable Conformations of Polyamino Acid Helices[1]

*Sir:*

Recently Scott and Scheraga[2] have computed energy contour diagrams for helical polyglycine and poly-L-alanine, including torsional energies, nonbonded interactions, hydrogen bonding, and electrostatic interactions. By carrying out energy minimization calculations, they found that the right-handed $\alpha$-helix of poly-L-alanine was more stable than the left-handed one by a few tenths of a kilocalorie per residue; this energy difference is large enough to favor the right-handed form if the chain is more than 10–20 residues long.

We report here the results of similar calculations for several homopolymer polyamino acid helices (poly-L-valine, poly-$\beta$-methyl-L-aspartate, poly-$\gamma$-methyl-L-glutamate, and poly-L-tyrosine) of considerable interest in the field of biopolymers. In contrast to poly-L-alanine, for these helices one has to take into account the rotations about the single bonds in the side chains, *i.e.*, the energy must be minimized not only with respect to the dihedral angles of the backbone but also with respect to those of the side chains. The expressions used for the various energies are essentially the same as those used earlier for poly-L-alanine.[2]

In the case of poly-L-valine, the right-handed $\alpha$-helix was found to be the most stable helical structure, being more stable than the left-handed $\alpha$-helix by 0.6 kcal/residue. This prediction may, at first sight, seem surprising since calculations based only on a hard-sphere potential,[3] or on other nonbonded potentials,[4] and experimental results of Blout[5] and Bloom, *et al.*,[6] all seem to suggest that the $\alpha$-helical form of poly-L-valine would not be the most stable one. However, it has been shown[3,7,8] that the $\alpha$-helical backbone conformation can accommodate the L-valyl side chain if the latter is rotated about 10° away from a position of the torsional potential minimum. Indeed, we have found that the torsional energy required for such a rotation is more than compensated by the resulting decrease in energy from favorable nonbonded interactions including the side chain. In addition, we have obtained experimental proof that poly-L-valine can exist in the $\alpha$-helical conformation. To circumvent the problem that poly-L-valine is insoluble in most solvents, we have prepared a block copolymer of the type $(DL\text{-Lys})_x$-$(L\text{-Val}_x)$-$(DL\text{-Lys})_x$ where $x \sim 40$, by the procedure reported previously.[9] This polymer is water soluble. Using ORD data as a criterion, the poly-L-valine portion appears to be $\alpha$-helical in 98% aqueous methanol at room temperature. The evidence for the helical conformation is based on the location (233 and 198 m$\mu$) and magnitude ($m'$ = mean residue rotation = $-13{,}500 \pm 500°$ and $+ 57{,}000 \pm 6000°$) of the trough and peak, respectively, of the Cotton effect.

In the case of poly-$\beta$-methyl-L-aspartate, the left-handed $\alpha$-helix was found to be more stable than the right-handed $\alpha$-helix by 0.1 kcal/residue but, for poly-$\gamma$-methyl-L-glutamate, the right-handed $\alpha$-helix was found to be more stable than the left-handed $\alpha$-helix by 0.4 kcal/residue. The origin of the effect on screw sense lies in the interaction between the dipole of the ester group of the side chain and that of the amide group of the backbone (using a dielectric constant of 3 D.). In the absence of this dipole interaction, both poly-$\beta$-methyl-L-aspartate and poly-$\gamma$-methyl-L-glutamate would be right-handed $\alpha$-helices. The dipole interaction destabilizes the right-handed form of the aspartate polymer, thereby favoring the left-handed form. The addition of another $CH_2$ group in the side chain, in the case of the glutamate polymer, leads to a different orientation and distance of the side-chain ester group with respect to the backbone; in this case, the dipole–dipole interaction favors the right-handed $\alpha$-helix. These calculations provide the explanation for the well-known difference in screw sense between the aspartate and glutamate polymers.[10,11]

(1) This work was supported by a research grant (GB-4766) from the National Science Foundation and by a research grant (AI-01473) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

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(11) M. Goodman, E. E. Schmitt, and D. A. Yphantis, *ibid.*, **84**, 1288 (1962).