Although the nmr spectral transitions for I (Figure 1) do not show clear-cut changes corresponding to cyclohexane flip and tetrathiane twist pseudorotation, the data do provide evidence for slow twist pseudorotation in a 6 ring. A lower limit of about 10 kcal/mol can be assigned to the barrier ($\Delta G^{\pm}$) to pseudorotation in the twist form of an *s*-tetrathiane. This value is dramatically higher than the calculated barrier to pseudorotation in the cyclohexane twist (0.8 kcal/mol)[5] and attests to the significance of vicinal lone pair–lone pair repulsions and a large prow–prow nonbonded repulsion in the transition state for pseudorotation (boat form).[5] Since the pseudorotatory process involves passing vicinal lone pairs on adjacent sulfur atoms, the high barrier to pseudorotation in the twist form of I is consistent with a relatively high calculated cis barrier in H_2S_2 (9.33 kcal/mol)[7] and experimental (dnmr) cis barriers in dialkyl disulfides (8–9 kcal/mol).[8]

Acknowledgment. We are grateful to the National Science Foundation (Grant No. GP-18197) and The Society of Sigma Xi for support.

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(8) R. R. Fraser and G. Boussard, Abstracts, 161st National Meeting of the American Chemical Society, Los Angeles, Calif., March 28–April 2, 1971, No. ORGN 082.
(9) Alfred P. Sloan Research Fellow, 1971–1973.

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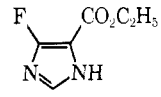
Photochemical Decomposition of Diazonium Fluoroborates. Application to the Synthesis of Ring-Fluorinated Imidazoles

Sir:

Of all possible replacements for hydrogen in carbon–hydrogen bonds, fluorine is unique in effecting the least increase in size while, at the same time, introducing a markedly enhanced electronegativity and a strong hydrogen-bonding potential. Recognition of these properties has induced extensive activity in the synthesis and testing of fluorinated analogs of biologically significant compounds. Such research has provided the biochemist with useful enzyme inhibitors and the pharmacologist with valuable drugs.[1] Despite the key role of the imidazole ring in biological structure and function, no example of a ring-fluorinated imidazole has yet been reported. Accordingly, the development of a synthetic route to such compounds has been a concern of this laboratory for a number of years.

The various procedures in general use for the formation of carbon–fluorine bonds[2] failed, in our hands and undoubtedly in others', to provide a single example of a ring-fluorinated imidazole. We can now report that the photochemical decomposition of diazonium fluoroborates in aqueous fluoroboric acid offers a general route to this series of compounds, as well as to other ring-fluorinated aromatics. A significant feature of the technique is the fact that the diazonium fluoroborate can be generated *in situ* and be subjected, without isolation, to photolysis at room temperature or below; furthermore, the method is readily amenable to moderate scale batches. In the absence of ultraviolet light, none of the diazonium fluoroborates examined provided detectable quantities of fluorinated imidazoles, either in solution or under pyrolytic conditions. The general procedure can be illustrated by several examples.



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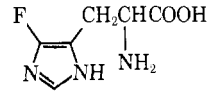

2

2-Fluoroimidazole. A solution of 2-aminoimidazole in aqueous fluoroboric acid (50%) was diazotized by addition of an equivalent amount of sodium nitrite. The resulting diazonium fluoroborate solution was subjected to photolysis (Hanovia mercury vapor lamp, medium pressure, 450 W) at 10–25° until evolution of nitrogen ceased. The solution was neutralized and subjected to continuous ether extraction. The product was separated from a small amount of 2-azidoimidazole[3] by silica gel chromatography and crystallized as the hydrochloride,[4] mp 215–226°; yield 30%.[5]

4-Fluoroimidazole. Because of the instability of 4-aminoimidazole,[6] this compound was generated, *in situ*, from an N-protected derivative. Curtius rearrangement of imidazole-4-carbonyl azide[7] in boiling *tert*-butyl alcohol[8] provided *tert*-butyl imidazole-4-carbamate, mp 142–152° dec. Upon solution of the latter compound in aqueous fluoroboric acid, the blocking group was rapidly removed and the resulting 4-aminoimidazole fluoroborate was treated as described above. Following work-up and purification by sublimation, 4-fluoroimidazole, mp 101–104°, was obtained in 41% yield.

Ethyl 4-Fluoroimidazole-5-carboxylate (1). This compound, mp 155–157.5°, 38% yield, was obtained from ethyl 4-aminoimidazole-5-carboxylate by use of the procedure described for 2 aminoimidazole. Ammonolysis of the fluoro ester provided 4-fluoroimidazole-5-carboxamide, mp 257–260°, of interest with respect to purine biosynthesis.[9]

4-Fluoro-DL-histidine (2). The fluoro ester **1** was reduced with lithium aluminum hydride to 4-fluoroimidazole-5-methanol, mp 136–138°. Reaction of the alcohol with thionyl chloride provided the 5-chloromethyl compound which, without isolation, was used to alkylate ethyl formamidomalonate in the usual manner.[10]

Acid hydrolysis of the alkylation product, mp 120–122°, provided 4-fluorohistidine dihydrochloride (hy-

(1) P. Goldman, *Science*, **164**, 1123 (1969); F. Weygand and W. Oettmeier, *Usp. Khim.*, **39**, 622 (1970); *Russ. Chem. Rev.*, 290 (1970).
(2) M. Hudlicky, "Organic Fluorine Chemistry," Plenum Press, New York, N. Y., 1970; A. E. Pavlath and A. J. Leffler, "Aromatic Fluorine Compounds," Reinhold, New York, N. Y., 1962.
(3) Thermal decomposition of imidazole-2-diazonium fluoroborate in fluoroboric acid leads to 2-azidoimidazole exclusively. The mechanism of formation of the azido derivative is under investigation.
(4) This compound was stored as the hydrochloride since the free base was found to undergo slow decomposition.
(5) Identities and purities of all compounds were confirmed by elemental analysis, nmr and mass spectroscopy, and by tlc.
(6) K. Hofmann, "Imidazole and Its Derivatives," Interscience, New York, N. Y., 1953, p 142.
(7) I. E. Balaban, *J. Chem. Soc.*, 268 (1930).
(8) *Cf.* K. L. Kirk and L. A. Cohen, *J. Org. Chem.*, **34**, 395 (1969).
(9) The preparation of another analog, 2-fluoro-4-aminoimidazole-5-carboxamide, is in progress.
(10) N. F. Albertson and S. Archer, *J. Amer. Chem. Soc.*, **67**, 308 (1945).

groscopic). The free amino acid was obtained by ion-exchange chromatography and crystallized from water–acetone.

In preliminary studies with histidine ammonia lyase,[11] 4-fluoro-DL-histidine appears to have a higher affinity for the enzyme ($K_m = 0.9 \times 10^{-3}$ *M*) than does DL-histidine ($K_m = 2.7 \times 10^{-3}$ *M*); on the other hand, the fluoroamino acid is deaminated (to 4-fluorourocanic acid) much more slowly ($V_{max} = 0.09$ unit/mg of protein)[11] than is DL-histidine ($V_{max} = 14.3$ units/mg of protein) and serves as a competitive inhibitor ($K_i = 1.27 \times 10^{-3}$ *M*) for the normal substrate. The pharmacological testing of these and other ring-fluorinated imidazoles is in progress. The scope of the method, with respect to the preparation of other difficultly accessible fluoro organic compounds, is also being explored.

(11) C. B. Klee, *J. Biol. Chem.*, **245**, 3143 (1970); we are indebted to Dr. Klee for performing the enzymatic studies.

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Chitotriose, a "Tritium Exchange Probe" of the Active Cleft of Lysozyme

Sir:

We should like to report a "tritium exchange probe" technique for the study of protein active sites. A molecule which binds to the active site is labeled with tritium by means of exchange with tritiated water followed by freeze drying. The rate of exchange of the tritium back into water is measured in the presence and in the absence of the protein by means of a freeze–drying technique.[1] Comparison of these rates gives information about interactions between the molecule and the active site. As an example, we have studied the exchange of chitotriose in the presence and absence of hen egg-white lysozyme.

Samples of chitotriose in tritiated water (10^{-3} *M*, 10^{-3} Ci/ml) were frozen and dried to high vacuum. Addition of unlabeled water, followed by liquid scintillation counting, indicated a value of 11.8 ± 0.7 for the apparent number of hydrogens/molecule exchanged. This is consistent with complete exchange of all OH and NH groups. A buffer solution of succinate (0.01 *M*, pH 5.0) at 0° was added to such samples for varying periods of time followed by freezing and freeze–drying during which the ice temperature was maintained at −30°. Unlabeled water was then added and the samples counted. In experiments with lysozyme the buffer solution was replaced by lysozyme solutions (0°, pH 5.0) containing amounts of enzyme approximately equivalent to the chitotriose present. Exchange out was measured in the same way.

The results of these experiments are shown in Figure 1. In all cases exchange out of the tritium-labeled OH groups is too fast to measure. In the absence of lysozyme there appears to be some exchange too rapid for accurate measurement (see insert of Figure 1) and one hydrogen per molecule which exchanges fairly

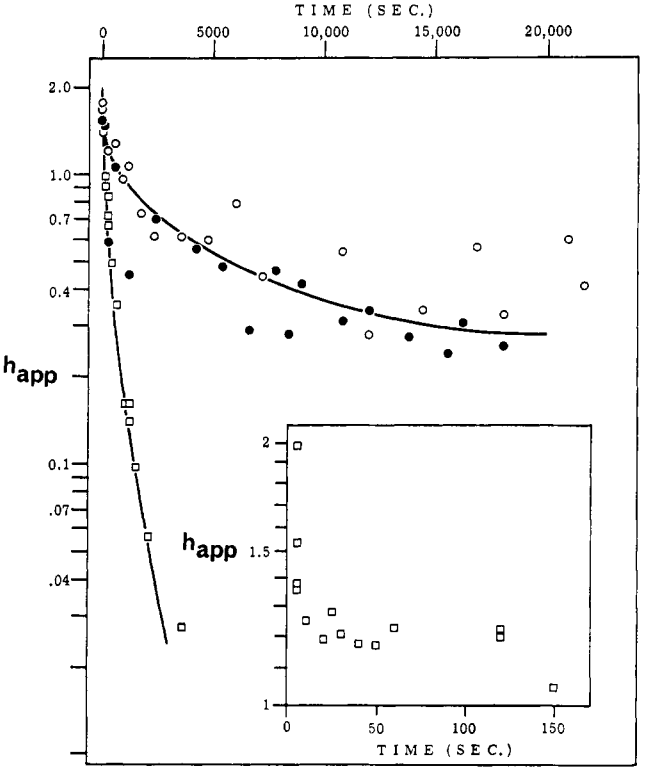

Figure 1. Semilogarithmic plot of the apparent number of unexchanged hydrogen atoms per molecule of chitotriose, h_{app}, *vs.* time, at pH 5.0 and 0°: □, 1.0×10^{-3} *M* chitotriose, 1.0×10^{-2} *M* succinate buffer; points at short times are shown on insert; ○, 0.98×10^{-3} *M* chitotriose, 1.54×10^{-3} *M* lysozyme; ●, 1.04×10^{-3} *M* chitotriose, 0.90×10^{-3} *M* lysozyme.

slowly. We can tentatively identify this hydrogen as the amide hydrogen of the reducing saccharide unit of chitotriose. The other two amide hydrogens appear to exchange more rapidly at pH 5.0. Control experiments indicate that points near the beginning of this exchange may be systematically low due to experimental artifacts[1] but that points defining the slower exchange are fairly accurate.

Results of exchange at two different lysozyme concentrations are also shown. There is a dramatic difference due to marked slowing of exchange. Data for the different lysozyme concentrations are badly scattered but seem in approximate agreement. One of the amide hydrogens of chitotriose apparently exchanges at a rate too fast to measure. A second hydrogen exchanges more slowly and can be observed. The third hydrogen exchanges quite slowly. It should be noted that the portion of the exchange curve representing this slow exchange is not linear. This portion of the curve should be linear if all the hydrogens involved in this slow exchange are chemically equivalent.

The interactions between chitotriose and the active cleft of lysozyme have been studied by Phillips and coworkers[2] by means of X-ray crystallography. They divide the cleft into six sites, A–F. Chitotriose occupies sites A, B, and C. The reducing terminus is in site C. The amide hydrogen in site A is thought to form a hydrogen bond with the carboxylate group of aspartic acid 101, the amide hydrogen in site B does not interact with the enzyme, and the amide hydrogen

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(2) C. C. F. Blake, *et al.*, *Proc. Roy. Soc., Ser. B*, **167**, 378 (1967).