Model Systems for Sulfate Transfer. Sulfur Analogs of 1-Phosphoimidazole

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Abstract: Several sulfur analogs (I, II, and III) of 1-phosphoimidazole have been synthesized and their reactions have been investigated. When the imidazolium salt (II) of imidazole-N-sulfonic acid was allowed to react with the acceptor compounds methanol, diethylamine, hydroquinone, p-nitrophenol, and water, good yields of the sulfonated products were obtained. Analogous results were obtained using the sulfonating agent N-methylimidazole-N'-sulfonate (III). In the case of acetic acid, however, reaction with either II or III led to formation of acetic anhydride. Our findings show that imidazole species can participate in high yield sulfate transfer reactions with a variety of acceptors and suggest the possibility that intermediates related to compounds I, II, and III may be involved in similar reactions of biological systems.

1-Phosphoimidazole has been proposed to be a possible intermediate in the coupling of oxidation to phosphorylation during the process of oxidative phosphorylation.[2a-c] In an earlier communication we reported studies on the oxidative cleavage of hydroquinone monosulfate as a model reaction for sulfate transfer.[2d] In the present article we wish to record the synthesis and chemical properties of some sulfur analogs of 1-phosphoimidazole—imidazole-N-sulfonic acid (I), the imidazolium salt of the acid (II), and N-methylimidazole-N'-sulfonate (III).
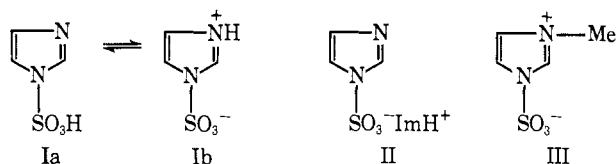
Experimental Section

Synthesis of II. Since the imidazolium salt II was easy to obtain in large quantities in a pure state, most of our work was done with it. Imidazole (27.2 g, 0.4 mol) was dissolved in 350 ml of purified chloroform. This solution was stirred using a magnetic stirrer and maintained at a temperature of 0 to −10° with an ice-salt bath. Sulfur trioxide (11.7 g, 0.195 mol) was distilled into the solution, and after the distillation was completed the reaction mixture was kept cold for 1 additional hr. Then the mixture was allowed to warm slowly to room temperature (over a period of ca. 2 hr), and the precipitate (II) was isolated by filtration and used without further purification (mp 88.5–91°). The nmr and ir spectra of the material obtained were consistent with the assigned structure.[3] The preparation of the acid I was entirely analogous except that 0.41 mol of sulfur trioxide was employed.

Anal. Calcd for $C_6H_8N_4O_3S$: C, 33.33; H, 3.73; N, 25.91; S, 14.83. Found: C, 33.35; H, 3.72; N, 25.94; S, 14.71.

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(2) (a) J. H. Wang, Proc. Nat. Acad. Sci. U. S., 58, 37 (1967); (b) W. S. Brinigar, D. B. Knaff, and J. H. Wang, Biochemistry, 6, 36 (1967); (c) for a review of current knowledge on the process of mitochondrial oxidative phosphorylation, see G. Schatz, Angew. Chem. Intern. Ed. Engl., 6, 1035 (1967); (d) S. W. Weidman, D. F. Mayers, O. R. Zaborsky, and E. T. Kaiser, J. Amer. Chem. Soc., 89, 4555 (1967).

(3) On heating II at 170° for 1.5 hr the compound was converted cleanly and quantitatively to the imidazolium salt of 4(5)-imidazolesulfonic acid. The free acid corresponding to this latter compound which softened at 293° and melted at 307–308° (starts to soften at 290° and is entirely molten at 307°⁴) was obtained by passing a solution of the imidazolium salt through a Dowex 50 cation-exchange column which was in the hydrogen ion form.

(4) F. L. Pyman and L. A. Ravald, J. Chem. Soc., 1429 (1920).

Anal. Calcd for $C_3H_4N_2O_3S$: C, 24.32; H, 2.72; N, 18.91; Found: C, 24.12; H, 3.09; N, 18.64.[5]

N-Methylimidazole-N'-sulfonate (III) was prepared from N-methylimidazole and chlorosulfonic acid by a slight modification of the method used by Sisler and Audrieth for the preparation of the pyridine–sulfur trioxide adduct.[6] The ir spectrum of III was consistent with the assigned structure. The 60-Mcps nmr spectrum displayed the following signals: three-proton singlet at δ 2.67, one-proton multiplet at 7.42, one-proton multiplet at 7.65, and one-proton multiplet at 8.80. The melting point of material recrystallized from water was 188–190°.

Anal. Calcd for $C_4H_6N_2O_3S$: C, 29.62; H, 3.73; N, 17.29; S, 19.77. Found: C, 29.74; H, 3.71; N, 17.32; S, 19.70.

Results and Discussion

Compound II underwent reaction with many different types of reagents to give sulfonated products in high yield.[7] In aqueous solution over the pH range 1.8–11.5, II is stable for periods of at least 1 week. However, II could be converted to sulfuric acid and imidazole (imidazolium sulfate) by refluxing its aqueous solution for 1 hr. II was decomposed quantitatively in 12 hr by (a) methanol, giving monomethyl sulfate; (b) diethylamine, giving N,N-diethylsulfamic acid; and (c) acetic acid, giving acetic anhydride.[8] Hydroquinone and II undergo a very slow reaction in dimethylformamide (DMF) solution at room temperature. This reaction can be accelerated considerably by raising the temperature, and it was complete in 24 hr at 100°. At either room temperature or 100° the reaction of II and hydroquinone yields only hydroquinone monosulfate. In DMF at room temperature II and p-nitrophenol react to give the sulfonated product, p-nitrophenyl sulfate. With the exception of acetic anhydride the products of all of the above reactions were obtained as the imidazolium salts.

Compound III also reacted with methanol, acetic acid, diethylamine, hydroquinone, p-nitrophenol, and

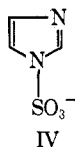(5) Analyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill.

(6) H. H. Sisler and L. F. Audrieth in "Inorganic Syntheses," Vol. 2, W. C. Fernelius, Ed., McGraw-Hill Publishing Co., Inc., New York, N. Y., 1946, p 173.

(7) The high yields obtained in these reactions contrast with the results found for phosphorylation by 1-phosphoimidazole.[2b] For example, the yield of ATP synthesized by the model reaction of ADP with 1-phosphoimidazole was found to be only about 5%. See W. S. Brinigar and D. B. Knaff, Biochemistry, 4, 406 (1965).

(8) The mode of formation of the acetic anhydride is not certain. One possibility is that acetyl sulfate is formed first in the reaction of acetic acid with II and then reacts further to give acetic anhydride.

water to give products similar to those obtained from the reactions of II.

A sample of II was dissolved in water and brought to pH 11 by the addition of sodium hydroxide solution. Back-titration of the aqueous solution with 0.1 N hydrochloric acid gave results which indicated the presence of two ionizable groups in II with pK values of 5.12 and 6.86. The pK of 6.86 can best be assigned to the protonation of the imidazole which is present in II (lit.[9] pK = 6.91). The pK of 5.12 can then be assigned to the protonation of the anion IV. Protonation of IV would be expected to occur at the nitrogen at position 3 of the ring (see formula Ib). The pK value for the

IV

protonation of this nitrogen might have a lower value than that for the protonation of nitrogen in imidazole because of the influence of the partial positive charge on the sulfur attached to the nitrogen in position 1.[10]

Whether sulfate transfer in biological systems occurs by way of intermediates related to compounds I, II, and III is not established.[11] However, our results do show that the imidazole species can participate in high-yield sulfate transfer reactions with a variety of acceptors. Further studies on these systems are in progress.

(9) The Merck Index of Chemicals and Drugs, 7th ed, Merck and Co., Inc., Rahway, N. J., 1960, p 551.

(10) A downfield deshielding shift in the nmr spectra for given types of protons was noted for all the compounds studied when one goes from the unsulfonated to sulfonated material, i.e., imidazole to I, methanol to monomethyl sulfate, and hydroquinone to hydroquinone monosulfate. This effect can best be explained as due to the influence of the partial positive charge on the sulfur atoms in the sulfonated systems.

(11) Sulfate transfer from 3'-phosphoadenosine 5'-phosphosulfate to acceptor compounds is catalyzed by the sulfokinases. See F. Lipmann, Science, 128, 575 (1958).

Stereospecificity in the Hydrolysis of Conformationally Homogeneous Substrates by α-Chymotrypsin[1]

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Contribution from the Department of Chemistry, Amherst College, Amherst, Massachusetts 01002. Received April 26, 1968

Abstract: The relative rates of hydrolysis by α-chymotrypsin of the p-nitrophenyl esters of trans-dl-, cis-(R)-, and cis-(S)-3-t-butylcyclohexanecarboxylic acids are 1:6.5:210. The enzyme here preferentially cleaves equatorial ester groups and, furthermore, exhibits considerable stereospecificity in the hydrolysis of the equatorial ester groups of the pair of cis enantiomers. These and other experiments generally support the view that the reactive conformation of D-3-carbomethoxydihydroisocarbostyril possesses an equatorial ester group.

A large number of rotational conformations are available to methyl acetyl-L-phenylalaninate (L-APME) and other specific substrates of α-chymotrypsin (ChT). Attempts[2–7] to use the less flexible substrate D-3-carbomethoxydihydroisocarbostyril (D-CDIC) as a model for defining that conformation of L-APME (the "reactive" conformation) most susceptible to hydrolysis by the enzyme require knowing whether D-CDIC undergoes hydrolysis by ChT with its ester group in the axial[8a] or equatorial[8b] position. In the place of direct evidence on this point, which is not

readily obtainable, there have appeared conjectures based on studies of a second generation of model compounds, substances whose geometry is even less ambiguous than that of D-CDIC and whose behavior hopefully will answer the D-CDIC conformational question. The results of these studies have been interpreted as supporting either the axial[7] or the equatorial[4b,9,10] ester hypothesis.

The observation[2] that ChT hydrolyzes D-CDIC 200 (k_0) to 4000 (k_0/K_0) times more rapidly than L-CDIC places a further demand on the reactive conformation of D-CDIC, namely that it be sufficiently more reactive than both axial and equatorial L-CDIC. This is readily imagined for the axial ester hypothesis if the relative rates of hydrolysis of the various conformations of the enantiomers are equated to the relative spatial positions of the carbonyl carbon atoms of their ester groups when the aromatic and amide functions are superimposed upon each other.[2,3,11] In contrast, the requirement of the equatorial ester hypothesis that ChT distinguish

(1) Supported by Grant AM 08005 of the U. S. Public Health Service.
(2) G. Hein and C. Niemann: (a) Proc. Natl. Acad. Sci. U. S., 47, 1341 (1961); (b) J. Am. Chem. Soc., 84, 4487, 4495 (1962).
(3) E. S. Awad, H. Neurath, and B. S. Hartley, J. Biol. Chem., 235, PC35 (1960).
(4) (a) I. B. Wilson and B. F. Erlanger, J. Am. Chem. Soc., 82, 6422 (1960); (b) B. F. Erlanger, Proc. Natl. Acad. Sci. U. S., 58, 703 (1967).
(5) M. S. Silver and T. Sone, J. Am. Chem. Soc., 89, 457 (1967).
(6) S. C. Cohen and R. M. Schultz, Proc. Natl. Acad. Sci. U. S., 57, 243 (1967).
(7) W. B. Lawson, J. Biol. Chem., 242, 3397 (1967).
(8) The following shorthand notations have been used: (a) the conformation with an axial ester group = axial conformation, and the proposition that this is the reactive conformation = axial ester hypothesis; (b) the terms equatorial conformation and equatorial ester hypothesis are similarly defined.

(9) M. S. Silver, J. Am. Chem. Soc., 88, 4247 (1966).
(10) S. G. Cohen, L. H. Klee, and S. Y. Weinstein, ibid., 88, 5302 (1966).