Oxidative Degradation of *N*-Sulfonylimidazoles*

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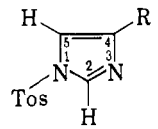
ABSTRACT: The reactivity of a series of 4-substituted-1-*p*-tolylsulfonylimidazoles toward *N*-bromosuccinimide has been studied. Oxidative degradation of the imidazole ring leads to a ketoaldehyde, formamide, and *p*-toluenesulfonamide. Reactivity toward the oxidant is shown to be dependent on the electron-releasing ability of the 4- substituent.

In the case of a propionic acid side chain, arguments are presented for the intermediate formation of a labile lactone. Location of the sulfonyl residue with respect to the side chain was determined by nuclear magnetic resonance spectroscopy.

Imidazole and a number of its 4(5)-substituted derivatives undergo rapid ring degradation upon reaction with bromine or with *N*-bromosuccinimide (NBS)[1] in aqueous media (Schmir and Cohen, 1961, 1965). In earlier papers in this series (Schmir and Cohen, 1961; Wilson and Cohen, 1963), we have described, as part of our studies on the oxidative cleavage of tyrosylpeptide bonds, the difficulties arising from competitive consumption of oxidant by the imidazole ring of histidine. In the hope of deactivating the ring toward oxidation, we have explored the reactivity of various *N*-*p*-tolylsulfonylimidazoles toward NBS. The sulfonyl derivative was selected for several reasons: (1) The imidazole ring in polypeptides can be sulfonylated selectively at *p*H values in the vicinity of 5.5;[2] (2) the sulfonyl residue may be removed readily by mild acid or alkaline treatment (Staab and Wendel, 1960) or by hydroxylamine or cyanide ion at neutral *p*H;[2] (3) the strong electron-withdrawing tendency of the sulfonyl group should be effective in decreasing the π-electron density required for electrophilic attack on the imidazole ring by positive halogen.



I, R = H
II, R = CH_2CN
III, R = CH_2CH_2COOH
IV, R = $CH_2CH_2COOCH_3$
V, R = $CH_2CH_2CONHCH_2C_6H_5$
VI, R = $CH_2CH_2CONHCH_2COOC_2H_5$
VII, R = CH_3

Studies with 1-*p*-tolylsulfonylimidazole (compound I) fully confirmed our expectations (Figure 1, curve A). Under comparable reaction conditions, imidazole consumed three equivalents of NBS within 2 minutes, whereas compound I had consumed only 0.1 equivalent after 2 hours.[3] A similar resistance to oxidation was observed with 1-*p*-tolylsulfonyl-4-cyanomethylimidazole (compound II).[4] However, 1-*p*-tolylsulfonylimidazole-4-propionic acid (compound III), as well as its methyl ester (compound IV) and amides (compounds V and VI), did consume NBS rapidly,[3] leading to degradation of the imidazole ring (Figure 1, curves B–D). That increased reactivity was not due to hydrolytic removal of the sulfonyl residue was demonstrated by the fact that the reaction mixtures, following oxidation, contained high yields of *p*-toluenesulfonamide (assayed by gas chromatography); the latter could arise only by degradation of the imidazole ring. The other principal cleavage product, RCOCHO, was identified (for compound III) by isolation of its bisthiosemicarbazone (Schmir and Cohen, 1965).

At the time, this requirement of a three-carbon side chain for reactivity implied not only the existence of carboxyl participation (compound XI) of the type found in the reaction of NBS with other γ,δ-olefinic acids (and their esters and amides), as in compounds VIII–X (Cohen and Witkop, 1961, 1964; Witkop, 1961), but even more significantly, that the reaction represented a rare example of anchimeric assistance to olefin addition by a carboxyl group.[5] *Only* with the demonstration that 1-*p*-tolylsulfonyl-4-methylimidazole (compound VII) also consumes NBS readily

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[1] Abbreviations used in this work: NBS, *N*-bromosuccinimide; NMR, nuclear magnetic resonance.

[2] L. A. Cohen, unpublished observations.

[3] Similar results were obtained using bromine in place of NBS.

[4] The structural assignments of compounds II–VII are based on NMR spectral evidence, as described later.

[5] Although there are numerous examples of participation by carboxyl in addition to olefins (Cohen and Witkop, 1961, 1964; Witkop, 1961), only one decisive case of participation *with rate acceleration* is known to us for the formation of γ-lactones (Arnold and Lindsay, 1953; see also Johnson and Bell, 1960). Acceleration has also been observed in the formation of β-lactones (Tarbell and Bartlett, 1937).

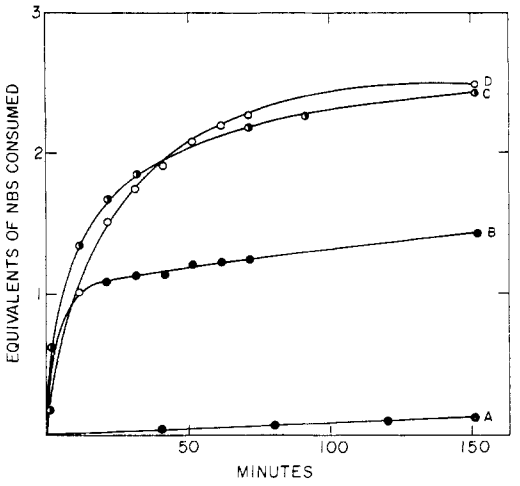


FIGURE 1: Rates of consumption of NBS by sulfonylimidazoles. Three equivalents per mole of substrate; acetonitrile-acetate buffer, 3:1; apparent *p*H, 6.0. (A) 1-*p*-Tolylsulfonylimidazole (compound I); (B) 1-*p*-tolylsulfonylimidazole-4-propionic acid (compound III); (C) compound V; (D) 1-*p*-tolylsulfonyl-4-methylimidazole (compound VII).

did it become apparent that the reactivity of compounds III–VI is largely a consequence of the electron-releasing ability of the side chain substituents.

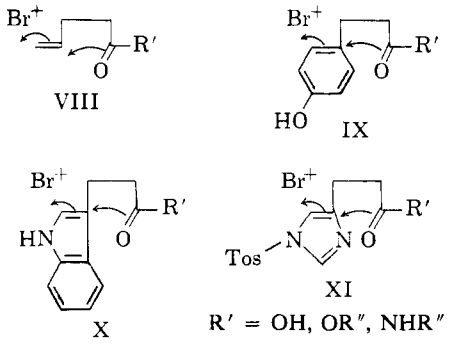
It is instructive to compare the relative reactivities of the sulfonylimidazoles toward NBS with the σ_p values of 4- substituents derived from aromatic systems (Table I). Indeed, within the limits of the available data, the relationships of Table I follow a Hammett

TABLE I: Electronic Effect of 4- Substituents on the Reactivity of 1-Sulfonylimidazoles.

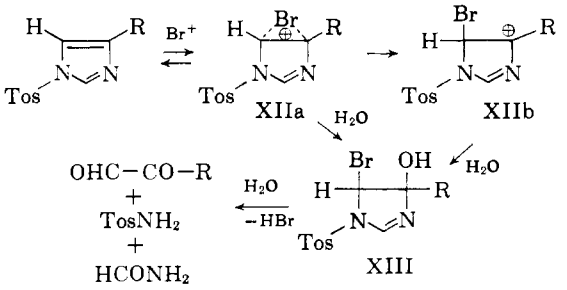
R	Relative Rate[a]	σ_p
H	1	0
CH_2CN	1	0.007[b]
$CH_2CH_2COO^-$	30	−0.12[c]
CH_3	35	−0.15[d]

[a] Based on 10-minute consumption of NBS in 50% acetonitrile-acetate buffer (*p*H 4.5) containing three equivalents of NBS. [b] Jaffé (1953). [c] Based on the phenolic *pK* of *p*-hydroxyphenylpropionic acid. [d] Based on the phenolic *pK* of *p*-cresol.

free-energy plot with reasonable linearity. Since the reaction involves electrophilic attack, it is not entirely clear whether the major effect of the side chain is one of altering electron density at the 4,5- double bond or



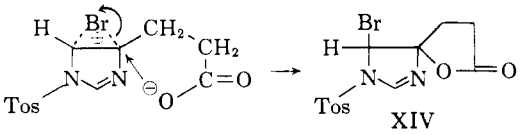
of stabilizing a carbonium ion at C-4 (compound XIIb) by hyperconjugation (Hine, 1962).[6] We have indicated a role for the latter effect by formulating compound XIII as the product of nucleophilic attack. At present, the only basis for excluding the alternate formulation of compound XIII, with hydroxyl at C-5, is the decreased possibility for resonance stabilization in the isomeric carbonium ion. By similar reasoning, we do not consider nucleophilic attack at C-2 likely.



Initially, one equivalent of NBS is consumed by the sulfonylimidazole in a rapid reaction (Figure 1, curves B-D), followed by further uptake of oxidant by formamide released in the course of degradation. The release of formamide, rather than of formic acid and ammonia, is suggested by the relatively slow secondary consumption of NBS.[7] Furthermore, it could be demonstrated that reaction of the sulfonylimidazoles (compounds IV, V, and VII) with one equivalent of NBS led to 60–80% disappearance of starting material without the formation of significant quantities of ammonia (ninhydrin assay). It is evident from Figure 1 that the rate of secondary oxidation for the propionic acid compound (III) is lower than that for compounds V or VII. We consider this difference to be the result of the formation of a spirolactone (compound XIV), followed by its rate-limiting breakdown; however, the lability of compound XIV is too great to permit isolation or characterization. Although amides VIII–X readily release their amine components in coupled oxidation-cleavage reactions with NBS (Cohen and Witkop, 1961, 1964; Witkop, 1961), compounds V and VI fail to do so. In the former cases, cleavage is

[6] Because such data are not available (except for R = CH_3), we were unable to utilize σ^+ values in a rate-substituent correlation.

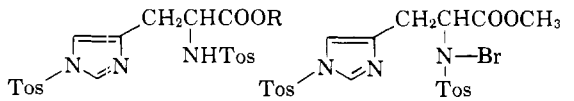
[7] See rate data in preceding paper (Schmir and Cohen, 1965).



XIV

due to the ability of the amide to compete successfully with solvent as a nucleophile toward a bromonium ion or carbonium ion intermediate (as in compounds XIIa or XIIb). In the indole case, we have proposed a nucleophilic attack by amide oxygen on the bromonium ion (Cohen and Witkop, 1961, 1964). It appears from the sulfonylimidazole data that the bromonium ion (compound XIIa) collapses to a carbonium ion (compound XIIb) more rapidly than in the indole system and that such collapse leads to a loss of partiality for the intramolecular nucleophile.[8]

Early studies with *N*,1-di-*p*-tolylsulfonylhistidine (compound XV) and its methyl ester (compound XVI) provided rather puzzling results. Although the acid



XV, R = H
XVI, R = CH_3

XVII

consumed one equivalent of NBS rapidly, the ester was almost inert. Once again, the evidence appeared strong for anchimeric assistance by the carboxylate ion; however, when two equivalents of NBS were added to the ester (compound XVI), one equivalent was consumed rapidly and the second, although capable of liberating iodine from acidified potassium iodide, had become unreactive, even toward imidazole itself. We consider NBS to have exchanged with compound XVI to form the less reactive *N*-bromosulfonamide (compound XVII). Qualitatively, the stability of an N-halo bond appears to vary directly with the acidity of the corresponding NH bond. The difference in stability between compound XVII and the corresponding *N*-bromo derivative of compound XV may, then, be attributed to the greater electron-withdrawing effect of the carbomethoxy group.[9] As in the case of compound III, titration data suggested that compound XV, prior to ring degradation, forms a lactone intermediate which, once again, defied isolation.

The reactivities of compounds I and II (and to a lesser extent, those of compounds III–VII) toward NBS are markedly enhanced by increasing the acidity of the medium (e.g., Figure 2). The effect is not due to acid-catalyzed removal of the sulfonyl residue since high yields of *p*-toluenesulfonamide could be demonstrated by gas-chromatographic assay. The effect may be due either to an increase in the reactivity of NBS by protonation or to an increase in the reactivity of

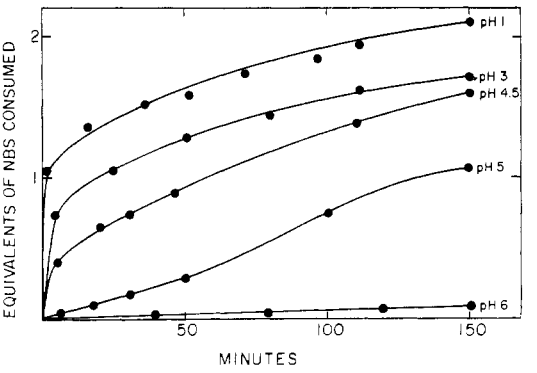


FIGURE 2: Effect of *p*H on rate of consumption of NBS by 1-*p*-tolylsulfonylimidazole (compound I). In acetonitrile-acetate buffer (3:1) or acetonitrile-water (3:1); *p*H values adjusted with acid prior to addition of three equivalents of NBS.

the sulfonylimidazole following protonation of the unsubstituted ring nitrogen.[10,11]

The reactivities of compounds I and II are further dependent on the ratio of acetonitrile to buffer in the medium (Figure 3). Since the apparent *p*H of acetate buffer is markedly increased by addition of acetonitrile (Table II), the rate differences are probably the result

TABLE II: Effect of Acetonitrile on *p*H of Acetate Buffer (0.2 M).

CH_3CN (%)	*p*H[a]
0	4.5
25	5.0
50	5.5
75	6.0

[a] Measurements were made following immersion of glass electrodes for 2 hours in solvent-buffer mixture, using a Beckman *p*H-meter, Model G.

of changes in the hydrogen ion concentration and in the concentration of the solvent nucleophile, water. Indeed, a satisfactory correlation was obtained by comparing relative reaction rates with ratios of the product $[H_2O][H^+]$ at varying acetonitrile concentra-

[8] The argument is analogous to that for the SN2-SN1 case. Whereas acetate is a more potent nucleophile than water in an SN2 reaction, differences in nucleophilicity are minimized in an SN1 reaction (Hine, 1962).

[9] Cf. the *pK* values for glycine (9.72) and for glycine ethyl ester (7.75) (Edsall and Wyman, 1958).

[10] Although the latter explanation appears less acceptable, its plausibility is currently under examination.

[11] Although the presence of an *N*-sulfonyl group depresses the basicity of the imidazole ring considerably, compound I is soluble in 1 M hydrochloric acid and a crystalline salt may be obtained with ether–hydrogen chloride. Just as a 4-alkyl substituent increases the basicity of the imidazole nitrogen, it would have a comparable effect on the sulfonylated imidazole.

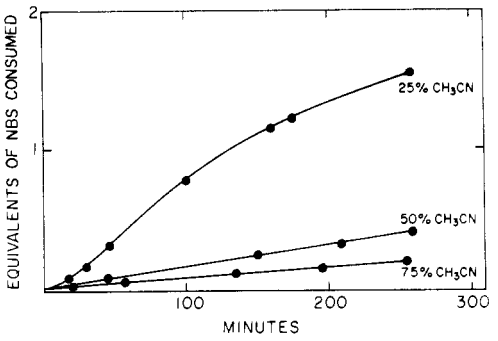


FIGURE 3: Effect of composition of acetonitrile-acetate buffer medium on rate of consumption of NBS (three equivalents per mole of substrate) by 1-*p*-tolylsulfonylimidazole (compound I).

tions. We have represented the initial formation of the bromonium species (compound XIIa) as a reversible reaction. When stabilization of a carbonium ion is difficult, as in compound XIIb (R = H or CH_2CN), the equilibrium between the sulfonylimidazole and its bromonium ion adduct will be shifted by nucleophilic attack of the solvent. Such a mechanism would account for the marked dependence of rate on water concentration. Increasing the acetonitrile content of the medium depresses the reactivities of compounds IV and VII toward NBS to a lesser extent, while that of compound III is not altered. As might be expected, the acetonitrile content of the medium has relatively little effect on rates in acidic media.

From our studies on the reactivities of these model compounds toward NBS, in aqueous media, it appears that sulfonylation of imidazole rings in a polypeptide can provide neither protection from attack by NBS nor a basis for selective oxidative cleavage of histidyl-peptide bonds (Witkop, 1961).

In the preparation of sulfonylimidazoles II–VII, there was no indication for the formation of mixtures of isomers (i.e., both N-1 and N-3 derivatives). By analysis of NMR spectra, it was possible to assign a position to the sulfonyl group, in each case, with respect to the side chain at C-4. The symmetrical quartet resulting from the four aromatic protons of the *p*-tolylsulfonyl group[12] overlaps the proton positions of the imidazole ring, but analysis of spin-spin coupling patterns permitted an assignment of chemical shifts (Figure 4). The protons at C-4 and C-5 of imidazole have, of course, identical chemical shifts (Reddy *et al.*, 1962; Gillespie *et al.*, 1958) while that at C-2 occurs at lower field as the result of decreased shielding by two adjacent nitrogen atoms. Sulfonylation of imidazole results in a separation of the chemical shifts of the protons at C-4 and C-5. It is quite reasonable to consider that the proton closer to the electron-withdrawing sulfonyl group will be the less shielded (Figure 4B).[13] Assignments for other sulfonylated imidazoles (Figure 4C–F) are based on such a premise.[14] It is evident that the entire aromatic spectrum is shifted progressively to higher field as the electron-releasing character or shielding ability of the side chain increases. Thus the electron density at the 4,5-double bond of a sulfonylimidazole varies with the side chain approximately in the same order as its electrophilic reactivity toward positive halogen.[15] The absence of sulfonylation at N-3 is probably the result of steric interference by the side chain.[16,17]

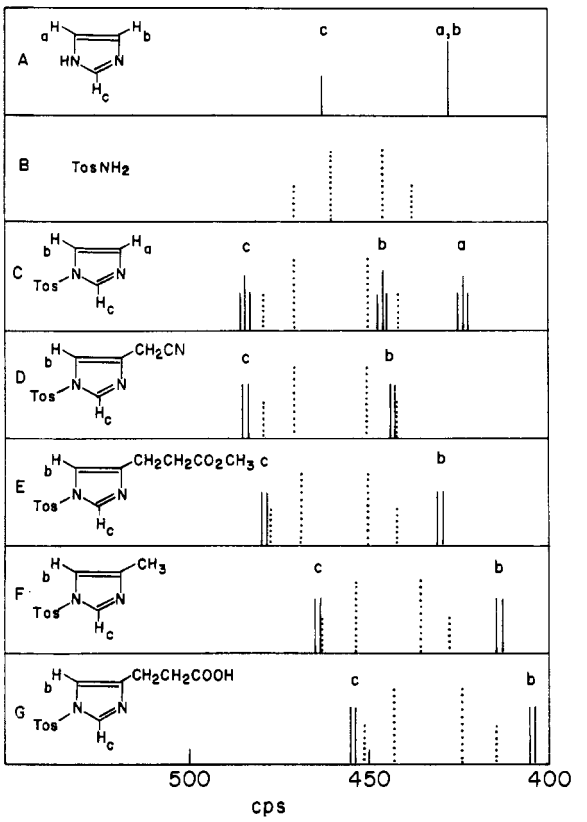


FIGURE 4: Partial NMR spectra of 1-*p*-tolylsulfonylimidazoles in acetonitrile at 60 Mc (relative to tetramethylsilane).

[12] The quartet is considered to be of the A_2B_2 type, since $\Delta\delta/J \cong 3$.

[13] A similar analysis for the structure of 1-acetyl-4-methylimidazole has recently been reported (Reddy *et al.*, 1963).

[14] Structures for compounds V and VI are assumed to correspond to those for compounds II–IV and VII.

[15] Whether the spectrum of the propionic acid derivative (Figure 4G) must be considered an anomaly in the series depends on the applicability of the σ constant (Table I) for the type of reaction in question.

[16] If the imidazolate anion is the active nucleophile in sulfonylation, an electron-releasing side chain would also encourage preferential anion formation on the far side, at N-1.

[17] The reaction of *N*-α-benzoylhistidine with iodoacetic acid leads to the formation of an *N*-carboxymethyl derivative; assignment of the substituent to N-1 is based largely on steric considerations (personal communication, Dr. William H. Stein).

Experimental[18]

1-p-Tolylsulfonylimidazole (Compound I). To a slightly warm solution of 2.72 g (0.04 mole) of imidazole in 150 ml of benzene and 75 ml of ether was added 3.80 g (0.02 mole) of solid *p*-toluenesulfonyl chloride. The reaction mixture was stirred for 24 hours at room temperature, then chilled in ice. The precipitated imidazole hydrochloride was removed by filtration; the filtrate was concentrated to about one-third volume, washed with cold 1% sodium bicarbonate, with cold 0.01 N hydrochloric acid, and, finally, with 30% aqueous sodium chloride. After drying over magnesium sulfate, the solvent was removed *in vacuo*. The crystalline residue was triturated under cyclohexane and collected by filtration, giving compound I (3.97 g, 90%), mp 75–77°. Following recrystallization from 15 ml of benzene and 120 ml of cyclohexane, the product melted at 75–77°.[19] For analysis, it was dried at 56° for 11 hours.

Anal. Calcd for $C_{10}H_{10}N_2O_2S$ (mw 222.26): C, 54.01; H, 4.53; N, 12.60; S, 14.42. Found: C, 53.75; H, 4.46; N, 12.84; S, 14.26.

The compound could also be prepared by reaction of equimolar amounts of imidazole and *p*-toluenesulfonyl chloride in the presence of triethylamine in ethyl acetate. The product was obtained in high yield but proved to be rather more difficult to purify than that obtained by the foregoing procedure.

1-p-Tolylsulfonyl-4-cyanomethylimidazole (Compound II). To a solution of 1.07 g (0.01 mole) of 4-cyanomethylimidazole (Bauer and Tabor, 1957)[20] in 20 ml of warm tetrahydrofuran was added a solution of 1.90 g (0.01 mole) of *p*-toluenesulfonyl chloride in 15 ml of tetrahydrofuran, followed immediately by 1.41 ml of triethylamine. Crystals of triethylamine hydrochloride began to separate at once. After the reaction mixture had been stored for 2.5 hours at room temperature, the triethylamine hydrochloride (1.14 g, 83%) was collected. The filtrate was concentrated to dryness, the residue was taken up in benzene, and the solution was filtered to remove 0.155 g (14%) of unreacted starting material. The benzene solution was washed with 0.01 N hydrochloric acid and with 30% sodium chloride, dried over magnesium sulfate, and concentrated to a small volume. Upon addition of cyclohexane, there was obtained 2.22 g (85%) of crystalline material, mp 85–89°. After two recrystallizations from benzene-cyclohexane, the product melted at 89–90°. Crystallization may also be effected from ether-cyclohexane.

Anal. Calcd for $C_{12}H_{11}N_3O_2S$ (mw 261.29): C, 55.15; H, 4.24; N, 16.09; S, 12.27. Found: C, 55.28; H, 4.25; N, 15.86; S, 12.39.

1-p-Tolylsulfonylimidazole-4-propionic Acid (Compound III). To a solution of 1.40 g (0.01 mole) of imidazole-4(5)-propionic acid[21] in 40 ml of 0.25 N sodium hydroxide was added 2.09 g (0.011 mole) of solid *p*-toluenesulfonyl chloride. With vigorous magnetic stirring, the *p*H of the reaction mixture was maintained at 8.3–8.6 by addition of 2 N sodium hydroxide either manually or automatically using a Radiometer TTT1 *p*H-stat equipped with magnetic valve. After 75 minutes, 5.4 ml of sodium hydroxide had been consumed and the almost clear reaction mixture was extracted with ether, chilled in ice, and acidified to *p*H 2 with 6 N hydrochloric acid. The crystalline product (2.37 g, 81%), mp 118–120°, was recrystallized twice from ethyl acetate to give compound III, mp 126–127°. For analysis, the compound was dried at 25° *in vacuo* for 24 hours.[22]

Anal. Calcd for $C_{13}H_{14}N_2O_4S$ (mw 294.32): C, 53.06; H, 4.80; N, 9.52; S, 10.90. Found: C, 53.06; H, 4.88; N, 9.49; S, 10.78. $\lambda_{max}^{CHCl_3}$ 5.80 μ (–COOH).

Methyl 1-p-Tolylsulfonylimidazole-4-propionate (Compound IV). The ester was prepared from a solution of the acid (compound III) in tetrahydrofuran and diazomethane in ether. Following removal of the solvent, the crystalline residue was triturated with cyclohexane to yield 0.54 g of crude compound IV (87%), mp 82–87°. Recrystallization from 40 ml of cyclohexane (discarding a small amount of oily residue) afforded 0.40 g of compound IV, mp 88–90°. For analysis, the compound was dried *in vacuo* at 25°.

Anal. Calcd for $C_{14}H_{16}N_2O_4S$ (mw 308.35): C, 54.51; H, 5.23; N, 9.04; S, 10.40. Found: C, 54.73; H, 5.49; N, 8.90; S, 10.31. $\lambda_{max}^{CHCl_3}$ 5.74 μ (ester).

1-p-Tolylsulfonylimidazole-4-propionyl-N-benzylamide (Compound V). To a solution of 0.88 g (3 mmoles) of compound III in 15 ml of tetrahydrofuran, at −10°, was added 0.42 ml (3 mmoles) of triethylamine followed by 0.33 g (3 mmoles) of ethyl chlorocarbonate in 2 ml of tetrahydrofuran. The mixture was kept at −10° for 45 minutes and a solution of 0.32 g (3 mmoles) of benzylamine in 2 ml of tetrahydrofuran was added. After the reaction mixture had been stirred for 20 hours at room temperature, the precipitated triethylamine hydrochloride was removed by filtration, the solvent was evaporated *in vacuo*, and the crystalline residue was triturated with ether, yielding 0.98 g (85%) of compound V, mp 125°. After two recrystallizations from benzene-cyclohexane, the product melted at 128–129°. For analysis, it was dried at room temperature *in vacuo*.

Anal. Calcd for $C_{20}H_{21}N_3O_3S$ (mw 383.45): C, 62.63;

[18] Melting points are uncorrected; NMR spectra were measured on a Varian A-60 spectrometer by Dr. E. D. Becker and Mr. R. Bradley of this Institute. Microanalyses were performed by the Analytical Service Laboratory of this Institute under the direction of Mr. H. G. McCann.

[19] By a somewhat different procedure, the preparation of compound I in quantitative yield, mp 78–78.5°, has been described (Staab and Wendel, 1960).

[20] We are indebted to Dr. H. Bauer of this Institute for a generous sample of the compound.

[21] Prepared by hydrogenolysis of α-chloroimidazolepropionic acid with 5% rhodium on alumina (T. W. Beiler, unpublished experiments).

[22] When dried at elevated temperatures, or when stored at 25° for several months, the compound undergoes a transformation to a lower-melting material, currently under investigation.

H, 5.52; N, 10.95; S, 8.36. Found: C, 62.58; H, 5.44; N, 11.07; S, 8.42. $\lambda_{max}^{CHCl_3}$ 5.97 μ (amide).

Ethyl 1-p-Tolylsulfonylimidazole-4-propionylglycinate (Compound VI). To a solution of 0.52 g (1.78 mmoles) of compound III in 10 ml of tetrahydrofuran, at −10°, was added 0.28 ml (2 mmoles) of triethylamine followed by a solution of 0.28 ml (2 mmoles) of ethyl chlorocarbonate in 3 ml of tetrahydrofuran. The mixture was stored at −10° for 15 minutes. To a suspension of 0.28 g (2 mmoles) of glycine ethyl ester hydrochloride in 10 ml of cold tetrahydrofuran was added 0.28 ml (2 mmoles) of triethylamine. The mixture was shaken briefly in the cold, then added to the previously prepared solution of the mixed anhydride. After the mixture had remained 13 hours at room temperature, the precipitated triethylamine hydrochloride was removed by filtration and the filtrate was evaporated to dryness. The crystalline residue was triturated with ether and collected to yield 0.62 g (92%) of compound VI, mp 125–127°. After two recrystallizations from benzene-cyclohexane, the product melted at 127–128°. The compound also crystallizes readily from acetonitrile-water. For analysis, the material was dried at 80° *in vacuo* for 14 hours.

Anal. Calcd for $C_{17}H_{21}N_3O_3S$ (mw 379.42): C, 53.81; H, 5.58; N, 11.08; S, 8.45. Found: C, 53.58; H, 5.52; N, 10.87; S, 8.35. λ_{max}^{CHCl} 5.72 μ (ester); 5.95 μ (amide I).

1-p-Tolylsulfonyl-4-methylimidazole (Compound VII). 4-Methylimidazole (Chemicals Procurement Co.) was sulfonylated using a procedure analogous to that for the preparation of compound I. The product was obtained in 85% yield and recrystallized from cyclohexane, mp 89–90°.

Anal. Calcd for $C_{11}H_{12}N_2O_2S$ (mw 236.29): C, 55.92; H, 5.12; N, 11.85; S, 13.57. Found: C, 55.90; H, 5.14; N, 11.69; S, 13.35.

N,1-Di-p-tolylsulfonyl-L-histidine (Compound XV). To a solution of 9.55 g (0.05 mole) of L-histidine hydrochloride in 100 ml of 1 N sodium hydroxide was added 19 g (0.1 mole) of finely powdered *p*-toluenesulfonyl chloride. With magnetic stirring, the reaction mixture was maintained at *p*H 9 by addition of 2 N sodium hydroxide, either manually or by use of a *p*H-stat. After 1 hour the solid had disappeared and the reaction mixture was diluted with 150 ml of water. Ethyl acetate was added and the *p*H of the aqueous phase was adjusted to 2 with 6 N hydrochloric acid. The precipitated mass was collected, yielding 20.3 g (88%) of the product. The crude product was readily soluble in dioxane, from which hard granules, mp 116–118°, rapidly separated. After recrystallization of the product from hot dioxane, its mp remained 116–118°. The compound was dried at 25° *in vacuo*. Analytical data indicated the presence of a dioxane solvate, as did characteristic bands in the fingerprint region of the infrared spectrum.

Anal. Calcd for $C_{20}H_{21}N_3O_6S_2 \cdot C_4H_8O_2$ (mw 551.61): C, 52.25; H, 5.30; N, 7.62; S, 11.62. Found: C, 52.09; H, 5.33; N, 7.60; S, 11.58. $\lambda_{max}^{CHCl_3}$ 5.76 μ (COOH); sharp bands at 8.92 μ, 11.26 μ, and 11.45 μ.

When dried at 80° for 20 hours the solvate darkened without loss of dioxane, as judged by melting point and infrared spectrum. The crude product crystallized from hot ethyl acetate, yielding silky needles, mp 133–145°. Once again a solvate was obtained, containing 0.5 mole of ethyl acetate. The infrared spectrum did not show the dioxane bands present in the material of mp 116–118°, but the carbonyl band at 5.76 μ was of much greater intensity.

Anal. Calcd for $C_{20}H_{21}N_3O_6S_2 \cdot C_2H_4O$: C, 52.06; H, 4.97; N, 8.28; S, 12.63. Found: C, 52.18; H, 5.37; N, 8.09; S, 12.48.

Drying at 80° effected an incomplete loss of ethyl acetate. The crude product could be recrystallized from warm ethanol-water, depositing needles which were free of solvent, mp 138–143°. The compound was dried for analysis at 80°.

Anal. Calcd for $C_{20}H_{21}N_3O_6S_2$: C, 51.82; H, 4.57; N, 9.07; S, 13.83. Found: C, 51.68; H, 4.69; N, 8.74; S, 13.78.

Any one of the three products could be converted into any other of the three by recrystallization from the appropriate solvent. All three led to the identical methyl ester following reaction with diazomethane.

Methyl N,1-Di-p-tolylsulfonyl-L-histidinate (Compound XVI). The methyl ester was prepared by treatment of compound XV in tetrahydrofuran with diazomethane in ether. After further dilution of the solution with ether, the ester, mp 183–185°, was obtained as a crystalline precipitate. Following recrystallization from acetone-water, the product melted at 186–187°.[23]

Anal. Calcd for $C_{21}H_{23}N_3O_6S_2$ (mw 477.5): C, 52.82; H, 4.85; N, 8.80; S, 13.43. Found: C, 53.12; H, 5.02; N, 8.70; S, 13.28.

Oxidation Experiments. Depending on their solubility, compounds were dissolved in acetonitrile or in acetate buffer (0.2 M, *p*H 4.5)–acetonitrile. Solutions of NBS in acetonitrile were added, containing from one to six equivalents. Final concentrations of substrate were 5×10^{-3} M and acetonitrile content of the medium varied from 25 to 75% by volume. Aliquots were withdrawn and mixed with 10% potassium iodide–1 N hydrochloric acid. The liberated iodine was titrated with 0.005 N sodium thiosulfate. For reactions in acidic media, acetate buffer was replaced by water or 0.1 N hydrochloric acid, and the *p*H was adjusted to the desired value.

Assay of p-Toluenesulfonamide. After reaction times of 24 hours, aliquots of the mixtures were concentrated to dryness and the residues were triturated with measured volumes of ethyl acetate. Without separation of the insoluble inorganic material, the ethyl acetate solution was drawn into a micropipet and injected into a Chromalab gas chromatograph, fitted with a β ionization detector. The column consisted of 2% QF-1 on Gas-Chrom A packing. Injection temperature was maintained at 250° and column temperature at 150°. Elution time for *p*-toluenesulfonamide was 3.4 minutes. Other than the solvent, no additional material, including

[23] Synthesis by sulfonylation of histidine methyl ester hydrochloride led to a product, mp 188–190° (Helferich and Böshagen, 1959).

succinimide, was eluted from the column. By comparison with a calibration curve, yields of the sulfonamide were found to range from 60 to 95%, according to the NBS/substrate ratio and the rate of oxidation.

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Studies on the Oxidation-Reduction Potentials of Heme Proteins. IV. The Kinetics of Oxidation of Hemoglobin and Myoglobin by Ferricyanide*

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ABSTRACT: The kinetics of oxidation of horse myoglobin (Mb) and human hemoglobin (Hb) by ferricyanide have been studied in a stopped-flow apparatus. The oxidation of Mb corresponds to a bimolecular reaction; at 19° the second-order rate constant (o') is $\sim 2 \times 10^6$ M^{-1} sec^{-1} at *p*H 6.0 and $\sim 1.4 \times 10^6$ M^{-1} sec^{-1} at *p*H 9.2.

The results on the rate of the reaction of ferricyanide with MbO_2, measured as a function of O_2 concentration, are similar to those on the displacement of O_2 by CO and indicate a complex situation. The reaction of Hb with ferricyanide does not correspond to a simple bimolecular reaction; at alkaline *p*H the rate tends to increase as the reaction proceeds, while at acid *p*H values it tends to decrease. This phenomenon corresponds to the change of shape of the equilibrium curve with *p*H. The apparent initial second-order rate constant for the oxidation of Hb obtained by extrapolation is, at 19.5°, $\sim 7 \times 10^4$ M^{-1} sec^{-1} at *p*H 6.0 and $\sim 0.8 \times 10^4$ M^{-1} sec^{-1} at *p*H 9.2.

Since the introduction by Hartridge and Roughton (1923) of methods for studying fast reactions in solution, the kinetics of the reaction of hemoglobin and myoglobin[1] with ligands has been extensively investigated. The results obtained up to 1959 have been reviewed and discussed in detail by Gibson (1959); later work has been summarized in some recent reviews (Antonini, 1963, 1965; Rossi Fanelli *et al.*, 1964). As a first approximation, the reactions of ferromyoglobin with various ligands (O_2, CO, isocyanides, nitroso-aromatic com-

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[1] Abbreviations used in this work: Hb, ferrohemoglobin; HbO_2, oxyhemoglobin; Hb^+, ferrihemoglobin; Mb, ferromyoglobin; MbO_2, oxymyoglobin; Mb^+, ferrimyoglobin; k', rate constant for the combination with oxygen; k, rate constant for the dissociation of HbO_2; o', rate constant for the oxidation (by ferricyanide); l', rate constant for combination with carbon monoxide.