hydrazine yielded a 2,4-dinitrophenylhydrazone which was identical with the $C_{21}H_2N_4O_7$ derivative isolated from the 2-cyclohexenone reaction.

Registry No.—**2**, 5216-84-2; **3** dinitrophenylhydrazone, 41008-55-3; diethyl methylmalonate, 609-08-5; 2-cyclohexenone, 930-68-7.

Photochemistry of Diazonium Salts. III. A New and Facile Synthesis of 4-Fluoroimidazoles

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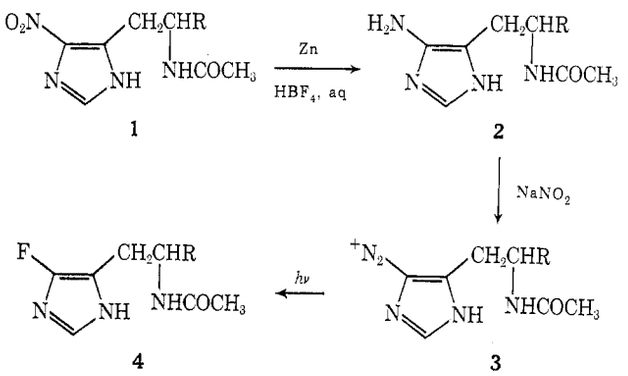
In paper I of this series,[1] we described the synthesis of a variety of 4-fluoroimidazoles, based on the irradiation of 4-diazonium imidazoles in aqueous tetrafluoroboric acid. Since the immediate precursors of the diazonium salts, 4-aminoimidazoles, are often too unstable for isolation, indirect and tedious synthetic routes were found necessary. Thus, 4-aminoimidazole itself was generated, *in situ*, by acid cleavage of its *tert*-butoxycarbonyl derivative; the latter compound was derived from ethyl imidazole-4-carboxylate by a three-step procedure. Other 4-fluoroimidazoles (*e.g.*, 4-fluoro-L-histidine, **4a**) were obtained by converting the stable 4-aminoimidazole-5-carboxylic esters to 4-fluoro analogs, followed by synthetic elaboration of the side chain, as necessary. Thus, the synthesis of 4-fluorohistamine required a sequence of nine steps from the commerically available 4-aminoimidazole-5-carboxamide. Subsequent to our announcement of these compounds,[1,2] extensive interest developed in the synthesis of polypeptide analogs containing 4-fluoro-L-histidine,[3] as well as in the pharmacological and enzymatic properties of the amino acid and the amine. This interest emphasized to us the fact that considerably larger quantities of these compounds would be required than could be obtained practically by our published procedures.

Initial attempts to exploit the readily available 4-nitroimidazoles as precursors were thwarted, repeatedly, by the instability of the amines. For example, catalytic hydrogenation of 4-nitroimidazole, followed by rapid work-up of the product under nitrogen and either in the presence or absence of tetrafluoroboric acid, resulted in extensive decomposition, as evidenced by insoluble dye formation. Diazotization and irradiation of the crude reduction products in tetrafluoroboric acid solution resulted in recoveries of only trace amounts of 4-fluoroimidazole. Catalytic reductions in other solvents, including 50% tetrafluoroboric acid itself, were similarly fruitless; however, the nitroimidazole was rapidly reduced with zinc dust in the same fluoroboric acid system and with little evidence of decomposition of the product. The aminoimidazole so formed can be diazotized and irradiated *in situ*. Thus, 4-nitroimidazole was rapidly reduced to 4-aminoimidazole at −10 to 0° (nitrogen atmosphere), progress of the reduction being monitored by disappearance of the chromophore at 298 nm. When reduction was complete, 1 equiv of sodium nitrite was added, generating a diazonium ion chromophore at 270 nm. The solution was subjected to irradiation at 0°, progress of the reaction being monitored by loss of the diazonium ion chromophore. From the reaction mixture, 4-fluoroimidazole was obtained in 17% yield. Considering the ready availability of 4-nitroimidazole and the fact that no intermediates were isolated, this overall yield is quite acceptable, and compares favorably with that reported previously.[1] With 4-nitro-5-methylimidazole, a 37% yield of the fluoro derivative was obtained by use of the same procedure.

In our earlier synthesis of 4-fluoro-L-histidine, an overall yield of 1.5% was obtained after eight steps, beginning with 4-aminoimidazole-5-carboxamide. 4-Fluorohistamine was obtained in 0.7% yield after nine steps from the same starting point. Recent development of the direct nitration of histidine and histamine (and their derivatives)[4,5] widened the range of accessible nitroimidazoles, and led us to explore the simplified synthetic approach with the more complex compounds. Indeed, α-*N*-acetyl-4-nitro-L-histidine methyl ester (**1a**) was converted into its fluoro analog **4a** in 10% yield, and α-*N*-acetyl-4-nitrohistamine (**1b**) was converted into its fluoro analog **4b** in 18% yield.



a, R = $COOCH_3$
b, R = H

Removal of the blocking groups from these derivatives incurs no losses.

This considerable facilitation of the syntheses of the histidine and histamine analogs removes the principal obstacle to expansion of a wide variety of biochemical and pharmacological studies, either already under way or being planned. A relatively broad spectrum of functional groups can be introduced into the imidazole ring (both at C-2 and at C-4) by photochemical decomposition of the respective diazonium ions.[6] These syntheses are also made more inviting and practical by the

(1) K. L. Kirk and L. A. Cohen, *J. Amer. Chem. Soc.*, **95**, 4619 (1973).

(2) A preliminary report was presented at the Symposium on Fluorine in Medicinal Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971; see also K. L. Kirk and L. A. Cohen, *J. Amer. Chem. Soc.*, **93**, 3060 (1971).

(3) See, *e.g.*, B. M. Dunn, C. DiBello, and I. M. Chaiken, *Fed. Proc.*, **32**, 541 (1973). Reports of other studies are in preparation.

(4) W. Tautz, S. Teitel, and A. Brossi, *J. Med. Chem.*, **16**, 705 (1973). We are indebted to these investigators for providing us with experimental details of the nitration procedure prior to publication.

(5) (a) Racemic 4-nitrohistidine has been obtained by synthetic elaboration of the side chain in 4-chloromethyl-5-nitroimidazole: G. E. Trout, *J. Med. Chem.*, **15**, 1259 (1972). (b) Nitration of α-*N*-acetyl-L-histidine, under more vigorous conditions, has been reported to yield a racemic, dinitrated product of unknown structure: N. P. Buu-Hoi and C. Lepoivre, *C. R. Acad. Sci.*, **257**, 3618 (1963).

(6) These studies are in progress and will be reported separately.

possibility of utilizing nitroimidazoles as precursors, at least in the 4-nitro case.

Experimental Section[7]

α-*N*-Acetyl-4-nitro-L-histidine Methyl Ester (1a).—α-*N*-Acetyl-4-nitro-L-histidine[4] (43 g, 0.18 mol) was added to 600 ml of methanol to which had previously been added 7 ml of thionyl chloride.[8] The solution was stirred at ambient temperature for 8 hr, after which time tlc showed almost complete esterification. The solvent was removed *in vacuo* (below 40°), 500 ml of cold water was added to the residue, and the aqueous solution was neutralized (pH 4) with solid sodium bicarbonate. The solution was chilled overnight and the colorless solid was collected by filtration. The product was recrystallized from water and then from ethanol to yield 29 g (63%) of **1a**, mp 202–205°.

Anal. Calcd for $C_9H_{12}N_4O_5$: C, 42.19; H, 4.72; N, 21.87. Found: C, 42.03; H, 4.92; N, 21.64.

The same compound was obtained in lower yield by introduction of hydrogen chloride gas into a cold, methanolic solution of the acid. Even at low temperature, N-deacylation occurred to a significant extent. Direct nitration of α-*N*-acetylhistidine methyl ester could not be effected without demethylation. Use of the free carboxylic acid in the irradiation step proved unsatisfactory.

α-*N*-Acetyl-4-nitrohistamine (1b).—This compound was prepared by nitration of α-*N*-acetylhistamine, following the published procedure for α-*N*-acetylhistidine.[4] The product was obtained in 44% yield following crystallization from water and ethanol–ethyl acetate, mp 225–229° dec (with gas evolution).

Anal. Calcd for $C_7H_{10}N_4O_3$: C, 42.42; H, 5.09; N, 28.27. Found: C, 41.90; H, 5.17; G, 28.42.

General Fluorination Procedure.—A solution of 20 mmol of the nitromidazole in 100 ml of 50% tetrafluoroboric acid was cooled to −10°. While the solution was stirred rapidly and was aerated with nitrogen, zinc dust was added in portions of *ca.* 25 mg, until a total of 4.25 g (65 mmol) had been added. Each addition was made only after the prior portion had dissolved and the temperature had fallen to at least −5°; the interval between additions ranged from 1 to 3 min, increasing toward the end of the reduction. Small aliquots were removed periodically and diluted with water, and the solutions were assayed at 298 nm. Total loss of the chromophore was taken to indicate complete reduction.[9] A solution of sodium nitrite (1.52 g, 22 mmol) in the minimum volume of water was then added dropwise over 15 min. The resulting solution was diluted to 185 ml with cold 50% tetrafluoroboric acid and was irradiated (Corex filter) as described previously.[10] Small aliquots were withdrawn periodically, diluted with water, and asayed at 270 nm; again, completion of the reaction was indicated by total loss of absorption at this wavelength. The solution was neutralized with cold, concentrated sodium hydroxide (the precipitate of zinc hydroxide was not removed), and the mixture was extracted continuously for 24 hr with ethyl acetate. The extract was dried (Na_2SO_4) and concentrated, and the residual solution was filtered through a short silica gel column to remove inorganic salts. The products were then purified by crystallization or sublimation, or both.

4-Fluoroimidazole was obtained in 17% yield, mp 101.5–104°; this material was identical with the product described previously.[1]

4-Fluoro-5-methylimidazole was obtained in 37% yield from 4-nitro-5-methylimidazole.[11] The product was purified by sublimation: mp 66–69°; nmr ($CDCl_3$) δ 2.20 (d, J = 1.5 Hz, 3 H, 5-CH_3)[12] and 7.11 ppm (d, J = 2 Hz, 1 H, 2-H).

Anal. Calcd for $C_4H_5N_2F$: C, 48.00; H, 5.03; N, 27.99. Found: C, 47.93; H, 5.03; N, 27.82.

α-*N*-Acetyl-4-fluoro-L-histidine Methyl Ester (4a).—The amino acid derivative was obtained in 10% yield from **1a**, mp 153.5–154.5° (ethanol–ethyl acetate).[13]

Anal. Calcd for $C_9H_{12}N_3O_3F$: C, 47.16; H, 5.28; N, 18.34. Found: C, 46.92; H, 5.42; N, 18.53.

Acid hydrolysis of **4a** gave a quantitative recovery of 4-fluoro-L-histidine, identical with the earlier product[1] with respect to spectral, chromatographic, and optical rotatory properties.

α-*N*-Acetyl-4-fluorohistamine (4b).—This compound was obtained in 18% yield from **1b**, mp 172–173° (ethanol–ethyl acetate).

Anal. Calcd for $C_7H_{10}N_3OF$: C, 49.12; H, 5.89; N, 24.55. Found: C, 48.72; H, 5.94; N, 25.15.

Acid hydrolysis of **4b** gave 4-fluorohistamine, characterized as its picrate, mp 202–204° dec.[1]

Registry No.—**1a**, 41429-88-3; **1b**, 41366-97-6; **4a**, 41366-98-7; **4b**, 41366-99-8; α-*N*-acetyl-4-nitro-L-histidine, 41367-00-4; tetrafluoroboric acid, 16872-11-0; 4-fluorimidazole, 30086-17-0; 4-nitroimidazole, 3034-38-6; 4-fluoro-5-methylimidazole, 41367-01-5; 4-nitro-5-methylimidazole, 14003-66-8.

(7) Analytical and spectral data were supplied by the Analytical Services and Instrumentation section of this laboratory, under the direction of Dr. D. F. Johnson. Melting points are uncorrected. Identity and homogeneity were confirmed, wherever feasible, by tlc and mass spectrum.

(8) E. Taschner and C. Wasielewski, *Justus Liebigs Ann. Chem.*, **640**, 136 (1961).

(9) Since a stoichiometry could be demonstrated between the per cent loss of absorption and the amount of zinc added, it was evident that intermediate stages were undergoing reduction at least as fast as the nitro group. The calculation is based on three atoms of zinc per mole of nitro compound.

(10) K. L. Kirk, W. Nagai, and L. A. Cohen, *J. Amer. Chem. Soc.*, in press.

(11) W. E. Allsebrook, J. M. Gulland, and L. F. Strong, *J. Chem. Soc.*, 232 (1942).

(12) Both the C-4 methyl group and the C-2 hydrogen are coupled to the fluorine atom.

(13) Undoubtedly, the low yield reflects some ester hydrolysis in the 50% tetrafluoroboric acid solvent.

Detection of Protonated Aldimine Group by Proton Magnetic Resonance Spectroscopy

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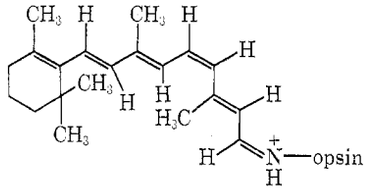
Rhodopsin, the visual pigment of the eye, is a Schiff base formed by the condensation of the aldehyde group of 11-*cis*-retinal with the ε-amino group of a lysine residue in the protein opsin.[1] Several models to explain the anomalous spectroscopic properties of rhodop-



rhodopsin

sin propose that the Schiff base linkage of this molecule is protonated.[2] However, except for the observation that the *N*-retinylidene moiety of rhodopsin shows λ_{max} in the same region where *N*-retinylidene-*n*-butylammonium ion (**8**) absorbs,[3] no direct experimental evidence for the presence of a protonated aldimine group (–HC=$^+$NH–) in the visual pigment has yet been obtained.

It occurred to us that the presence of a protonated aldimine group in rhodopsin may be directly observed by nmr spectroscopy. In principle, the resonance of the azomethine proton, H_a, of a protonated Schiff base

(1) D. Bownds, *Nature*, **216**, 1178 (1967).

(2) C. D. B. Bridges, "Comprehensive Biochemistry," Vol. 27, M. Florkin and E. H. Stolz, Ed., Elsevier, New York, N. Y., 1967, pp 31–78; J. Heller, *Biochemistry*, **7**, 2914 (1968); J. Toth and B. Rosenberg, *Vision Res.*, **8**, 1471 (1968).

(3) P. E. Blatz and J. H. Mohler, *Biochemistry*, **11**, 3240 (1972).