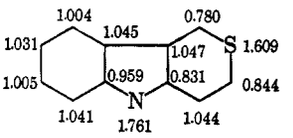
tern (summarized on formula 6)[11-13] further shows that the positive charge distribution is +0.391 on sulfur and +0.239 on nitrogen with the remaining charge distributed dominantly on the carbons of the sulfur-containing ring. The corroborative agreement of the charge density calculation with the observed nmr spectrum clearly characterizes this moiety as an indolo[3,2-*c*]-thiapyrylium cation.



6

Experimental Section[14]

3,4-Dihydroindolo[3,2-*c*]thiapyrylium Perchlorate (3).—In a three-necked flask equipped with a magnetic stirrer was placed 6.70 g (0.0354 mol) of 1,3,4,5-tetrahydrothiapyrano[4,3-*b*]-indole,[2,5] and 100 ml of glacial acetic acid, and the mixture was heated to 90° under a flow of dry nitrogen. Trityl perchlorate,[6] (12.0 g, 0.0351 mol) was then added slowly from a small erlenmeyer flask attached to the reaction flask with a piece of Gooch tubing. When all of the trityl perchlorate had been added, the solution temperature was held at 90° for 10 min, then refluxed for 10 min, the solution being deep orange at this point. The solution was then allowed to cool to room temperature with stirring as orange crystals separated. After standing at room temperature for 2 hr, the crystals were collected by filtration, washed with glacial acetic acid, then washed with ether, and air dried to give 9.56 g (94% yield) of orange crystals, mp 164–169° dec. Three recrystallizations from glacial acetic acid gave analytically pure **3**, mp 175–177° dec.

Anal. Calcd for $C_{11}H_{10}ClNO_4S$: C, 45.92; H, 3.50; N, 4.87; S, 11.14. Found: C, 46.11; H, 3.30; N, 4.80; S, 11.06.

Ultraviolet-visible spectrum gave $\lambda_{max}^{1\%\ HClO_4\ in\ MeCN}$ 257 mμ (log ε 4.15), 263 (4.10) sh, 294 (3.63), 414 (4.20).

The nmr spectrum in deuteriotrifluoroacetic acid showed H-1 at δ 9.48 (singlet integrating for one proton) and the methylene protons, H-3 and H-4, as a singlet at 3.65 (integration for four protons). The N–H and remaining aromatic protons at H-6, -7, -8, and -9 appeared as a low lying multiplet at δ 7.83 (one proton, not specifically assignable) and an intense multiplet at 7.58 (integration for four protons).

Indolo[3,2-*c*]thiapyrylium Perchlorate (4).—A stirred solution of 2.02 g (7.0 mmol) of **3**, 1.59 g (7.0 mmol) of 2,3-dichloro-5,6-dicyanoquinone, 1 ml of 70% perchloric acid, and 50 ml of glacial acetic acid was refluxed for 2 hr during which the solution became dark red. The mixture was then allowed to cool to room temperature, and the green-brown crystals which had formed were collected by filtration, washed with dry ether, and dried *in vacuo* over potassium hydroxide. The yield was 1.10 g of crude product, mp 158–165°. Recrystallization from acetic acid yielded 0.65 g (33%) of olive-drab crystals, mp 200–203° dec, with shrinking at 192°. Three more recrystallizations from acetic acid yielded analytically pure **4** as green-yellow crystals, mp 217–220° dec.

Anal. Calcd for $C_{11}H_8ClNO_4S$: C, 46.24; H, 2.82; S, 11.22. Found: C, 46.24; H, 3.09; S, 11.24.

Ultraviolet-visible spectrum showed $\lambda_{max}^{1\%\ HClO_4\ in\ MeCN}$ 240 mμ (log ε 4.04), 267 (4.41) sh, 277 (4.47), 297 (4.17) sh, 330 (3.69), 359 (3.60).

The nmr spectrum in deuteriotrifluoroacetic acid showed H-1 at δ 10.13 (doublet, J_{13} = 3.0 cps), H-3 at 9.16 (quartet, J_{13} = 3.0 cps, J_{34} = 9.5 cps), and H-4 at 8.85 (doublet, J_{34} = 9.5 cps), each integrating for one proton. The N–H and remaining aromatic protons at H-6, -7, -8, and -9 appeared as a doublet (J = 7.5 cps, integration for one proton) at δ 8.43 (not explicitly assignable) and an unresolved multiplet centered at 7.77 (integration for four protons).

Registry No.—**3**, 15816-29-2; **4**, 15816-28-1.

Acknowledgments.—We are grateful to the Warner-Lambert Research Institute for support of this work and to the National Science Foundation for a grant to purchase the nmr spectrometer.

(11) The electron densities of cation **4** were derived from Hückel molecular orbital calculations performed as previously described[2] using the parameters $\alpha_N = \alpha + 2\beta$ and $\beta_{CN} = \beta$ for pyridinium nitrogen[12] and $\alpha_S = \alpha + \beta$ and $\beta_{CS} = 0.6\beta$ for the sulfur atom.[13]

(12) R. Zahradník and J. Koutecký, *Advan. Heterocycl. Chem.*, **5**, 72 (1965).

(13) R. Zahradník, *ibid.*, **5**, 1 (1965).

(14) Melting points were determined on a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) and are corrected. Ultraviolet-visible spectra were recorded on a Beckman DK-2A spectrophotometer. Nmr spectra were determined on a Varian A-60 instrument using tetramethylsilane as an internal standard. Analyses were performed by Dr. V. B. Fish of Lehigh University and by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

Reinvestigation of the Orientation of Halogen Substitution in Imidazoles by Nuclear Magnetic Resonance Spectroscopy[1a]

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The chemistry of imidazole and the position of halogen substitution under different conditions of reaction have been studied in detail but hitherto the interpretation of some of the observations has proved difficult.[2] Pauly and his coworkers[3] iodinated imidazole and 4(5)-methylimidazole in alkaline conditions and concluded that the monoiodinated products obtained either by direct iodination or by deiodination were the C-2-substituted products. The diiodoimidazoles obtained either in alkaline media[3a] or at pH 7[4] were considered to be 2,4(5) substituted. It has also been shown that in alkaline media diazo coupling to imidazole occurs at the C-2 position.[5] Bromination of N-methylimidazole with cyanogen bromide also led to the exclusive formation of 2-bromoimidazole.[6] Monobromo and dibromo products obtained by bromination of 4(5)-methylimidazole[7] and imidazole in chloroform and by debromination of the fully halogenated derivatives were shown to be 4- and 5-substituted compounds.[8,9] Nitration and sulfonation in

(1) (a) Supported by the Research Grants AM-01825, HD-00669, and FR-5410 from the National Institutes of Health, U. S. Public Health Service. (b) Recipient of a Fulbright Travel Award through the U. S. Educational Foundation in India. Department of Chemistry, Sri Venkateswara University, Tirupati, A. P., India. (c) To whom correspondence should be addressed at the Benjamin Rose Hospital, 2073 Abington Road, Cleveland, Ohio 44106.

(2) (a) M. J. S. Dewar, "The Electronic Theory of Organic Chemistry," Oxford University Press, London, 1949, p 192; (b) K. Schofield, *Quart. Rev.* (London), **4**, 382 (1950); (c) K. Hofmann, "Imidazole and its Derivatives," Interscience Publishers, Inc., New York, N. Y., 1953, pp 111–124; (d) E. S. Schipper and A. R. Day, "Heterocyclic Compounds," Vol. 4, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., p 194; (e) R. D. Brown, "Current Trends in Heterocyclic Chemistry," A. Albert, G. M. Badger, and C. W. Shoppee, Ed., Academic Press Inc., New York, N. Y., 1958, p 13; (f) J. H. Ridd, "Physical Methods in Heterocyclic Chemistry," Vol. 1, A. R. Katritzky, Ed., Academic Press Inc., New York, N. Y., 1963, pp 109–160.

(3) (a) H. Pauly and K. Gundermann, *Ber.*, **41**, 3999 (1908); (b) H. Pauly, *ibid.*, **48**, 2243 (1910); (c) H. Pauly and E. Arauner, *J. Prakt. Chem.*, **118**, 33 (1928).

(4) J. H. Ridd, *J. Chem. Soc.*, **1955**, 1238.

(5) R. G. Fargher and F. L. Pyman, *ibid.*, **115**, 217 (1919).

(6) W. J. Langenbeck, *J. Prakt. Chem.*, (2) **119**, 77 (1928).

(7) F. L. Pyman, *J. Chem. Soc.*, **97**, 1815 (1910).

(8) F. L. Pyman and G. M. Timmis, *ibid.*, **123**, 494 (1923).

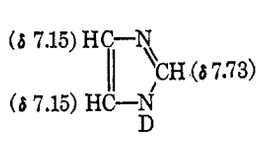
(9) I. E. Balaban and F. L. Pyman, *ibid.*, **121**, 947 (1922).

strong acid solutions also led to the exclusive formation of 4(5)-substituted products.[5,9] Studies of the deuteration[10] of imidazole indicated that a 4,5-disubstituted product was formed in neutral D_2O and the 2-deuterioimidazole was formed in alkaline D_2O solutions.

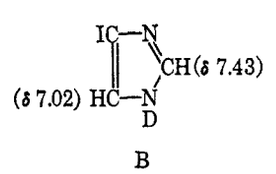
The monoiodo product obtained by iodination of histidine, considered to be 2-iodohistidine by Brunings,[11] has been shown by the present authors[12] by chemical reactions and nuclear magnetic resonance (nmr) spectroscopy to be 4(5)-iodohistidine. The same results were obtained by Holloway, *et al.*,[13] and Aonuma.[14] The 2-iodoimidazole reported by Pauly and Arauner[3c] was also demonstrated to be 4(5)-iodoimidazole by our studies.[12] Pauly and Arauner relied on the previously determined location of bromo substituents in the imidazole ring[8,9] in their identification of the iodo derivatives. Thus, the reported location of the bromine atoms is also open to question. The present study was undertaken to establish the configuration of bromo- and iodo-substituted derivatives of imidazole and 4(5)-methylimidazole.

Results and Discussion

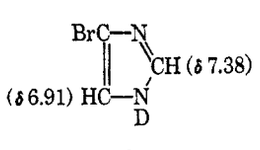
In the imidazole spectrum (A), the band at δ 7.15, which has twice the intensity of that at 7.73, clearly identifies the chemical shifts of the two symmetrical hydrogens attached to the C-4 and C-5 positions. We have shown[12] that the presence of the two bands of equal intensity at δ 7.02 and 7.43 in the spectrum of the monoiodoimidazole (B) proves that the isomer is 4(5)-iodoimidazole. The spectrum of the monobromoimidazole (C) shows two bands of equal intensity at δ 6.91 and 7.38 and confirms the identification by Balaban and Pyman[9] that the isomer is 4(5)-bromoimidazole since only this isomer could show two such bands.



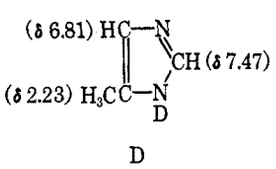
A



B



C



D

Preparation of the diiodoimidazoles by iodination of imidazole in alkaline media, at pH 7, and by the reduction of triiodoimidazoles with sodium sulfite yielded the same isomer. The nmr spectrum of each of the three isomers has a single band at δ 7.47. Since the position of this band is the same as the unequivocally identified C-2 hydrogen of 4(5)-iodoimidazole (B), it is assigned to the hydrogen at the C-2 position. These spectra confirm that the isomers formed in all the three different experimental conditions are only 4,5-diiodoimidazoles. The spectrum of dibromoimidazole shows a single band at δ 7.35 occupying the same position as the proton signal of the C-2 hydrogen of 4(5)-bromoimidazole (C). Hence, this spectrum confirms the identification by Balaban and Pyman[9] that the compound is 4,5-dibromoimidazole.

The nmr spectrum of 4(5)-methylimidazole shows two bands of equal intensity at δ 6.81 and 7.47 representing the hydrogen atoms at C-5(4) and C-2, respectively. A band at δ 2.23 is due to protons of the methyl group. The spectra of both monoiodo-4(5)-methylimidazole and monobromo-4(5)-methylimidazole show one single peak at δ 7.38 and 7.27, respectively. The band corresponding to the hydrogen atom at C-5(4) in 4(5)-methylimidazole is absent from both spectra. Pauly and Arauner[3c] considered the iodo derivative as C-2 substituted, but the spectrum shows that the compound is 4(5)-iodo-5(4)-methylimidazole. The result also establishes the conclusion of Pyman and Timmis[8] that the bromo derivative is 4(5)-bromo-5(4)-methylimidazole.

The present studies prove that during the iodination of imidazole in alkali or at pH 7 the initial attack occurs at the C-4(5) position of the imidazole ring rather than at the C-2 position. The sulfite reduction of both triiodo- and tribromoimidazole has been demonstrated to yield the corresponding 4(5)-mono- and 4,5-dihalogenated products. These results disprove the idea that the iodine substituent in the imidazole ring at the C-2 position is less reactive toward sulfite than is bromine.[2c,3c] The establishment of the configurations of these halogenoimidazoles indicates that the electrophilic substitution of bromine and iodine in the imidazole ring either in alkaline or neutral media (pH 7) preferentially occurs at the 4(5) position as with nitration and sulfonation.[5,9]

Since it now appears that electrophilic substitution occurs at the 4(5) position, we considered it important to investigate the reported electrophilic substitution into the 2 position by diazonium salts.[5] The monoazo derivative obtained by coupling 5-diazo-1H-tetrazole with imidazole was used in this study. The nmr spectrum of this compound shows a single band at δ 7.56. The tetrazole has no unexchangeable hydrogens and the spectrum should show only the chemical shifts of the hydrogens in the imidazole ring. The single peak in the spectrum indicates that either the compound is monosubstituted at the C-2 position or it is disubstituted at the C-2 and C-4(5) or at the C-4,5 positions. The chemical analysis of the compound indicates that it is monosubstituted and the absorption maximum at 376 mμ in bicarbonate buffer, pH 8.8, also confirms that it is monosubstituted, since monoazohistidine has a maximal absorption at 360 and the bisazo compound at 480 mμ.[15,16] Though there is a downfield shift of the proton signals at C-4,5 to δ 7.56 for this compound, the identification of the compound as a monosubstituted product establishes that it is C-2 substituted. This result also confirms[5] that the substitution in the imidazole occurs initially at the C-2 position during diazo-

(10) R. J. Gillespie, A. Grimison, J. H. Ridd, and R. F. M. White, *J. Chem. Soc.*, 3228 (1958).
(11) K. J. Brunings, *J. Amer. Chem. Soc.*, **69**, 205 (1947).
(12) H. B. Bensusan and M. S. R. Naidu, *Biochemistry*, **6**, 12 (1967).
(13) C. T. Holloway, R. P. M. Bond, I. G. Knight, and R. B. Beechey, *Biochemistry*, **6**, 19 (1967).
(14) S. Aonuma, Osaka University, Osaka, Japan, personal communication, 1967.
(15) B. N. Ames and H. K. Mitchell, *J. Amer. Chem. Soc.*, **74**, 252 (1952).
(16) M. Sokolovsky and B. L. Vallee, *Biochemistry*, **5**, 3574 (1966).

nium coupling in alkaline media. Although this is also an electrophilic substitution, this result is not contradictory since apparently the initial attack occurs at the nitrogen of the ring.[15]

Experimental Section

The nmr spectra were determined with a Varian Associates A-60 spectrometer with the samples dissolved in 1 *N* NaOD solution. Chemical shifts (δ) are given in parts per million relative to the internal reference, the sodium salt of 3-trimethylsilyl-1-propane sulfonic acid. Infrared spectra were obtained with the Beckman IR-8 spectrophotometer. Ultraviolet absorption spectra were measured with the Perkin-Elmer Model 202 spectrophotometer. All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Samples for analysis were dried under vacuum over phosphorus pentoxide at 62° for 12–18 hr and the elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Imidazole, obtained from Aldrich Chemical Co., Inc., was recrystallized from benzene (mp 89°).

4(5)-Iodoimidazole was prepared according to our procedure[12] by deiodinating triiodoimidazole with sodium sulfite, mp 137–138° (lit.[3a] 136°).

4(5)-Bromoimidazole was prepared by reducing tribromoimidazole with sodium sulfite in aqueous solution according to the method of Balaban and Pyman.[9] The compound was recrystallized from a mixture of chloroform and petroleum ether to give colorless leaflets, mp 130–131° (lit.[9] 131°).

Anal. Calcd for $C_3H_3N_2Br$: C, 24.51; H, 2.06; N, 19.06; Br, 54.38. Found: C, 24.59; H, 2.02; N, 18.90; Br, 54.32.

4,5-Diiodoimidazole was prepared in alkaline solution by iodinating imidazole with an equimolar amount of iodine. The imidazole (2 g, 29 mmol) was dissolved in 600 ml of 0.04 *N* sodium hydroxide. About 150 ml of pure hexane was added and the flask chilled to 0°. Iodine (7.44 g, 29 mmol) in 600 ml of hexane was added dropwise with vigorous stirring. Simultaneously, 600 ml of 0.2 *N* sodium hydroxide was added dropwise to prevent any precipitation of the reaction product. The addition took about 2 hr and the temperature of the reaction mixture rose to 10°. The reaction mixture was neutralized to pH 7 and left overnight in the refrigerator. The colorless, crystalline material which separated out was collected (3.4 g). The filtrate on evaporation gave 0.4 g of additional material. Recrystallization from dilute alcohol gave colorless, granular crystals, mp 197–198° (lit.[3a] 180°).

Anal. Calcd for $C_3H_2N_2I_2$: C, 11.30; H, 0.63; N, 8.76; I, 79.34. Found: C, 11.42; H, 0.71; N, 8.77; I, 79.40.

4,5-Diiodoimidazole was also prepared by iodinating imidazole at pH 7 according to Ridd[4] and by the removal of a single iodine from triiodoimidazole by refluxing with an equimolar amount of sodium sulfite, according to Brunings.[11] The products from these two preparations showed no depression of melting point with the analyzed preparation described above. The infrared spectra of the three diiodo compounds were identical.

4,5-Dibromoimidazole was prepared according to the procedure of Balaban and Pyman[9] by reducing tribromoimidazole with sodium sulfite in aqueous solution. The compound recrystallized from dilute ethanol and dilute acetic acid appeared as colorless, needle-shaped crystals, mp 229–230° (lit.[9] 225°).

Anal. Calcd for $C_3H_2N_2Br_2$: C, 15.95; H, 0.90; N, 12.41; Br, 70.80. Found: C, 16.12; H, 1.04; N, 12.44; Br, 70.86.

4(5)-Methylimidazole was prepared according to the procedure of Yabuta and Kambe.[17] The vacuum-distilled product has been further purified according to the procedure of Koessler and Hanke.[18] Fine, colorless, crystalline material, mp 56° (lit.[19] 56–56.5°).

Anal. Calcd for $C_4H_6N_2$: C, 58.51; H, 7.37; N, 34.13. Found: C, 58.28; H, 7.52; N, 33.90.

4(5)-Iodo-5(4)-methylimidazole was prepared by iodinating 4(5)-methylimidazole in 0.04 *N* NaOH with a 0.5 *M* amount of iodine and isolating the product from the solution at pH 7.4. Recrystallization from hot water gave colorless crystals, mp 176–177° (lit.[3c] 171°).

Anal. Calcd for $C_4H_5N_2I$: C, 23.10; H, 2.42; N, 13.47; I, 61.01. Found: C, 22.44; H, 2.13; N, 13.25; I, 62.09.

4(5)-Bromo-5(4)-Methylimidazole was prepared according to the procedure of Pyman.[7] The compound, recrystallized from ethyl acetate–*n*-hexane, gave fine, silky needles, mp 154–155° (lit.[7] 155°).

Anal. Calcd for $C_4H_5N_2Br$: C, 29.85; H, 3.13; N, 17.41; Br, 49.65. Found: C, 29.46; H, 3.08; N, 16.41; Br, 51.00.

2-(5-Tetrazolylazo)imidazole was prepared by the slow addition of 5-diazo-1-H-tetrazole (prepared from 2.6 g of 5-amino-1-H-tetrazole[16]) to a solution of 3.4 g of imidazole in 100 ml of 6% sodium carbonate at 0° with stirring. The dark orange material which separated was found to be the bisazo derivative with a maximal absorption at 475 mμ. The filtrate on concentration under vacuum yielded some bisazo derivative and then appreciable amounts of a crystalline, orange-yellow material. The orange-yellow material was recrystallized twice from water to yield light orange-yellow globules (3.5 g) which decomposed explosively at 240°. The absorption spectrum of the product in bicarbonate buffer, pH 8.8, showed a sharp absorption band at 376 mμ, indicating that it is a monoazo compound.

Anal. Calcd for $C_4H_4N_8 \cdot 2H_2O$: C, 24.00; H, 4.00; N, 56.00. Found: C, 24.34; H, 3.14; N, 57.39.

Registry No.—4(5)-Bromoimidazole, 6967-66-2; 4(5)-methylimidazole, 872-33-3; 4(5)-iodo-4(5)-methylimidazole, 15813-07-7; 4(5)-bromo-4(5)-methylimidazole, 15813-08-8; 4,5-diiodoimidazole, 15813-09-9; 2(5-tetrazolylazo)imidazole, 15813-10-2; 4,5-dibromoimidazole, 4150-74-5.

(17) Y. Yabuta and K. Kambe, *Bull. Agr. Chem. Soc. Jap.*, **4**, 74 (1928); *Chem. Abstr.*, **24**, 3509 (1930).

(18) K. K. Koessler and M. T. Hanke, *J. Biol. Chem.*, **39**, 497 (1919).

(19) A. Windaus and F. Knoop, *Ber.*, **38**, 1166 (1905).

Configuration of the Asparaginyl and Aspartyl Residues of Bacitracin[1a]

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Recently this laboratory reported a micromethod for identifying residues of asparagine and glutamine in *endo* position in peptides.[2] In this method, the peptide amides are dehydrated with ethylene chlorophosphite to the corresponding β-cyanoalanyl and γ-aminobutyryl derivatives, which are reduced with sodium–methanol–ammonia, then hydrolyzed to the easily recognizable 2,4-diaminobutyric acid (2,4-DAB) and ornithine. In a limited number of model compounds, residues of isoasparagine and isoglutamine were partly converted into β-alanine and γ-aminobutyric acid by reductive fission of the intermediate α-aminonitrile derivatives.[2,3]

When the dehydration–reduction procedure was applied to commerical bacitracin, approximately 0.7 mol of 2,4-DAB formed per mole of leucine or lysine, which identified the amide-bearing group as

(1) (a) This work was aided by Grant NB-04316 from U. S. Public Health Service and by Muscular Dystrophy Associations of America. (b) Recipient of a general research support summer fellowship, 1966.

(2) C. Ressler and D. V. Kashelikar, *J. Amer. Chem. Soc.*, **88**, 2025 (1966).

(3) Recently this technique proved useful in identifying, for the first time in a natural product, isoglutamine and isoasparagine residues as constituents of the cell wall peptidoglycans of certain bacteria: E. Muñoz, J.-M. Ghuysen, M. Leyh-Bouille, J.-F. Petit, H. Heymann, E. Bricas, and P. Lefrancier, *Biochemistry*, **5**, 3748 (1966); D. Jarvis and J. L. Strominger, *ibid.*, **6**, 2591 (1967); J.-M. Ghuysen, E. Bricas, M. Leyh-Bouille, M. Lache, and G. D. Shockman, *ibid.*, **6**, 2607 (1967).