[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY TULANE UNIVERSITY SCHOOL OF MEDICINE]

Rearrangements in the Displacement of Chloride from 2-Chloro-7-methyladenine

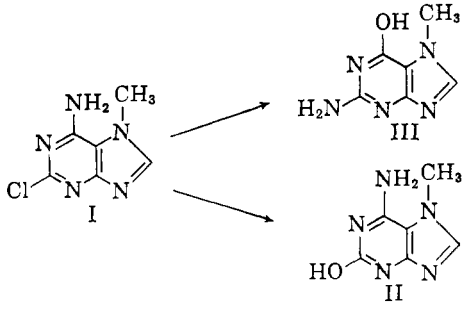
ELLIOTT SHAW

Received July 24, 1961

2-Chloro-7-methyladenine was shown by Fischer[1] to give a rearranged product on treatment with sodium hydroxide, namely, 2-amino-6-hydroxy-7-methylpurine. It has been found that a second hydrolytic pathway is also followed, leading to the expected 6-amino-2-hydroxy-7-methylpurine, however, by way of 1-methyl-4-ureido-5-cyanoimidazole. The latter was isolated from the incomplete reaction and prepared by an independent synthesis.

Halogen atoms are more or less readily displaced from the 2, 6, and 8 positions of purines by hydroxide ion and a variety of other nucleophilic reagents, a type of reaction which historically played a major role in clarifying the structures of purines, is widely used in purine syntheses, and which generally proceeds without rearrangement. However, in the behavior of 2-chloro-7-methyladenine (I) toward hot alkali, Fischer[1] observed that an anomalous reaction took place as, instead of the expected 2-hydroxy-7-methyladenine (II), there was obtained as the sole product a 60% yield of the isomeric 2-amino-6-hydroxy-7-methylpurine (7-methylguanine, III).[2] The structure of the product was proved by an alternate synthesis and by degradation to guanidine.

It seemed very likely that a hydrolytic cleavage of the pyrimidine ring of 2-chloro-7-methyladenine to an imidazole derivative was taking place in this conversion and that a subsequent recyclization accounted for the product. Because of interest in this laboratory in the ring-opening reaction of



purines to imidazoles[3] some observations were made of this reaction. It was hoped that by interrupting the reaction before it went to completion, an intermediate might be detected in the event that recyclization proceeded at a slower rate than the ring cleavage. In a preliminary experiment 2-chloro-7-methyladenine was heated in alkali; samples were withdrawn periodically and analyzed by paper chromatography. It was apparent that when less than one-third of the time specified by Fischer for complete reaction (three hours) had passed, a number of transient intermediates were present which ultimately diminished in number and amount. In order to isolate intermediates in milligram quantities for identification, column separation on a cation exchange resin was undertaken and conditions were eventually found for resolving the mixture. In addition, the reproducibility of elution patterns with an ion-exchange column is useful in characterization of substances. In Table I is shown the elution pattern of an interrupted reaction mixture. Paper chromatography showed that peak A was a mixture but peaks B through E were homogeneous. Peak D was identified as the known end product, 7-methylguanine, by ultraviolet and infrared spectroscopy. Similarly, peak E was identified as unchanged starting material. Peak B, on removal of the solvent, left a crystalline hydrochloride which gave a sharp band at 4.5 μ in the infrared and analytical data satisfactory for the empirical formula $C_6H_8N_5OCl$. It seemed certain that this was a nitrile, for which IV was a probable structure if the substance was an intermediate in the formation of 7-methylguanine. However, when the material was treated with alkali and the mixture was analyzed on the same column, it was found that the unknown did not provide 7-methylguanine but instead led mainly to the substance in peak C. The nitrile was eventually identified as 1-methyl-4-ureido-5-cyanoimidazole (V) and its cyclization product as 2-hydroxy-7-methyladenine (7-methylisoguanine, II) on the basis of synthesis and various interconversions. For example, the alkaline cyclization product had an absorption maximum at 287 mμ similar to the spectrum of isoguanine[4] and, like the latter[5] was resistant to nitrous acid but could be deaminated by prolonged refluxing with hydrochloric acid to 7-methylxanthine (VI) which was obtained for comparison, from 7-methylguanine.

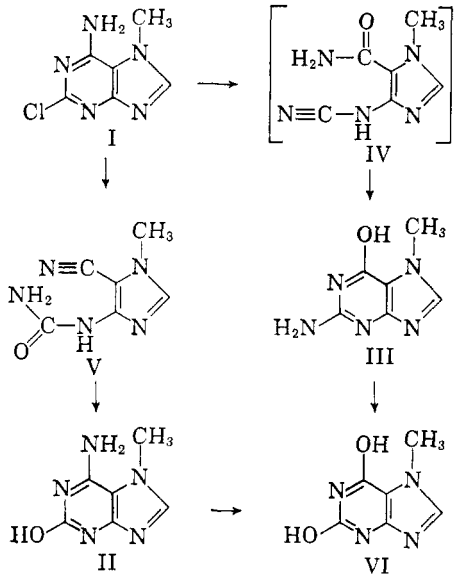
(1) E. Fischer, *Ber.*, **31**, 542 (1898).

(2) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **24**, 1570 (1959), report a similar rearrangement in the pyrazolo-[3,4-*d*]pyrimidine series.

(3) E. Shaw, *J. Am. Chem. Soc.*, **80**, 3899 (1958); **81**, 6021 (1959).

(4) L. F. Cavalieri, A. Bendich, J. F. Tinker, and G. B. Brown, *J. Am. Chem. Soc.*, **70**, 3875 (1948).

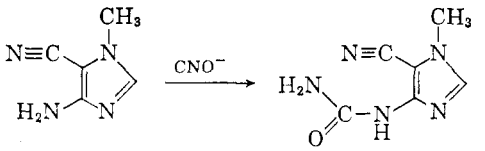
(5) R. Purrmann, *Ann.*, **544**, 182 (1940).



Thus, 7-methylisoguanine, the product expected by Fischer from the alkaline hydrolysis of 2-chloro-7-methyladenine was shown to be formed in the reaction along with the abnormal product, 7-methylguanine. About 30% of the starting material was following a course of hydrolysis through the nitrile (V).

For the synthesis of 4-ureido-1-methyl-5-cyanoimidazole (V) from the amine, the reaction of the latter with cyanate was undertaken. In previous experiments with 4(5)-amino-5(4)imidazolecarboxamide and cyanate, it had been learned that, as hot cyanate solutions become increasingly alkaline with time, any intermediate ureido compound formed was in danger of cyclizing to a purine. Consequently, in the synthesis of V, the *p*H was maintained at 6.0 to 6.4 to avoid secondary changes.

The reaction mixture from the treatment of 4-amino-1-methyl-5-cyanoimidazole with cyanate on column chromatography gave a low yield of a



product emerging at the expected volume of eluent which was identical with the substance obtained by the action of alkali on 2-chloro-7-methyladenine (agreement of infrared spectra of crystalline picrates, ultraviolet shift with *p*H, and paper chromatograms). The reversible shift of the maximum absorption of V from 246 mμ in acid to 288 mμ in alkali is unusual but not without precedent.[6]

Although this investigation did not directly shed light on Fischer's observed rearrangement of 2-chloro-7-methyladenine to 2-amino-6-hydroxy-7-methylpurine (7-methylguanine, III), it did reveal that the expected product of simple displacement of chloride by hydroxyl ion was actually formed in the reaction to a minor extent. However, this "normal" product (II) does not form because of a simple displacement reaction at the carbon atom to which the halogen is attached, but as a result of a series of events including ring-opening, hydrolytic removal of halogen and recyclization. It is very likely that the sequence of events leading to Fischer's rearranged product is in some ways similar, proceeding through an intermediate such as IV, and that there are two abnormal displacements of chloride in this unusual chloropurine. In another instance, the site of attack of hydroxide ion on a chloropurine derivative was not even in the same ring.[7] It may be that many ostensibly simple group replacements in pyrimidine and its bicyclic derivatives (purines, pteridines) have taken a more complicated course than expected or have involved rearrangement. Examples of ring-opening reactions of the pyrimidine ring in pyrimidines,[8] purines,[3,9] and pteridines[10] are increasing in number and variety. The example given in this work does not appear to have been observed previously.[11]

EXPERIMENTAL[12]

A solution of 2-chloro-7-methyladenine[13] (75 mg.) in 0.5 *N* sodium hydroxide (7.5 ml.) was refluxed for 45 min., cooled, and acidified with 6 *N* hydrochloric acid (1 ml.). The mixture was applied to a column (1 × 80 cm.) of Dowex-50-H$^+$ (200–400 mesh, 8% crosslinked), washed with water (20 ml.), and eluted with *N* hydrochloric acid. Five-milliliter fractions were collected and analyzed spectroscopically. The elution diagram is summarized in Table I. Peak D on concentration provided 23 mg. of 7-methylguanine hydrochloride and peak E, 20 mg. of starting material (confirmation by paper chromatography, ultraviolet, and infrared spectra).

Peak B was concentrated under reduced pressure. The residue, 16 mg., was recrystallized by addition of 6 *N* hy-

(6) J. C. Rabinowitz and W. E. Pricer, *J. Biol. Chem.*, 218, 189 (1956), observed, in the case of 4-ureido-5-imidazolecarboxylic acid, a shift of maximum from 242 to 270 mμ accompanying a *p*H change from 3 to strong alkali.

(7) B. R. Baker and K. Hewson, *J. Org. Chem.*, 22, 959 (1957).

(8) D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1298 (1961).

(9) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 539 (1960); and papers by G. B. Elion and by A. Albert in *The Chemistry and Biology of the Purines*, G. E. W. Wolstenholme and M. P. Cameron, ed., Little, Brown, and Co., Boston, 1957.

(10) *Cf.* summary by E. C. Taylor in *Chemistry and Biology of Pteridines*, G. E. W. Wolstenholme and M. P. Cameron, Ed., Little, Brown, and Co., Boston, 1954.

(11) The formation of a nitrile as a byproduct of the displacement of chloro by methoxy in 1*H*-3-chloro-4-phenylpyrido[1,2-*c*]pyrimidone-1 was reported by A. Hunger and K. Hoffman, *Helv. Chim. Acta*, 40, 1319 (1957).

(12) Melting points are uncorrected and were observed on a heated block. Microanalyses were carried out by S. Theodore Bella of The Rockefeller Institute, The Scandinavian Microanalytical Laboratory, Copenhagen, Denmark, and Alfred Bernhardt, Muhlheim, Germany.

(13) E. Fischer, *Ber.*, 31, 117 (1898).

drochloric acid to a concentrated aqueous solution and gave a hydrochloride, m.p. 273–274°.

Anal. Calcd. for $C_6H_8N_5OCl$: C, 35.75; H, 4.00; N, 34.74. Found: C, 35.67; H, 4.05; N, 35.21.

In 0.1 *N* sodium hydroxide, the nitrile exhibited a single λ_{max} 288 mμ, ϵ = 6,150. In 0.1 *N* hydrochloric acid a peak at 246 mμ, ϵ = 9,900 was found.

The product in peak B, 1-methyl-4-ureido-5-cyanoimidazole, when left at 4° with aqueous picric acid, slowly formed a characteristic picrate, cubes, m.p. 150–153°.

Anal. Calcd. for $C_{12}H_{10}N_8O_8$: C, 36.55; H, 2.56. Found: C, 35.88; H, 2.90.

Action of alkali on peak B. A sample of the nitrile obtained from a rearrangement as described above was divided into two parts (14 mg. each), one of which was rechromatographed to check on its homogeneity and stability (Table I) while the other was refluxed for 2 hr. in 0.5 *N* sodium hydroxide (5 ml.). After acidification, the alkali-treated nitrile was also chromatographed in the standard way and gave two bands (Table I) identified as 7-methylxanthine (530 ml.) and 7-methylisoguanine (1350 ml.) by comparison with authentic samples prepared as described below.

TABLE I

ION EXCHANGE SEPARATION OF MIXTURE RESULTING FROM PARTIAL ALKALINE HYDROLYSIS OF 2-CHLORO-7-METHYLADENINE

(Dowex-50-H$^+$ column (1 × 80 cms.) eluted with *N* HCl; spectra in *N* HCl)

	Chromatographic Peaks				
	A	B	C	D	E
Ml. Eluate to peak	550	750	1350	1700	>2500
λ_{max} (mμ)	270	246	287	248, 275	268–270
Rechromatography of B		750			
λ_{max} (mμ)		246			
B, alkali boiled	530		1350		
λ_{max} (mμ)	270		287		

TABLE II

PAPER CHROMATOGRAPHY OF COMPONENTS FROM PARTIAL ALKALINE HYDROLYSIS OF 2-CHLORO-7-METHYLADENINE (R_f VALUES)

Solvent[a]	Fractions				
	A	B	C	D	E
n-Propyl alcohol–water (4:1)	0.36	0.63	0.16	0.36	0.69
Diethylene glycol–*n*-butyl alcohol–water (1:4:1)	0.29	0.25		0.32	0.81
s-Butyl alcohol–90% formic acid–water (5:1:1)	0.42	0.78	0.19	0.30	0.82

[a] Ratios given are for solvent volumes.

7-Methylisoguanine. A. From 2-chloro-7-methyladenine. The residue from peak C, 10 mg., when redissolved in boiling water (5 mg./ml.) and neutralized with ammonium hydroxide, crystallized on cooling. It was dried at 100° *in vacuo* for 1 hr. and appeared to be a dihydrate.

Anal. Calcd. for $C_6H_7N_5O \cdot 2H_2O$: C, 35.83; H, 5.51; N, 34.83. Found: C, 35.95; H, 5.35; N, 34.83.

B. By synthesis. 1-Methyl-4-amino-5-cyanoimidazole hydrochloride[14] (80 mg.) and urea (100 mg.) were kept at 165° for 5 hr. The melt was broken up with water, filtered, washed, and dried, yielding 45 mg. of residue. Twenty milligrams of crude product was dissolved in boiling 0.03 *N* hydrochloric acid (40 ml.), cooled, and applied to the Dowex 50-column described above for elution with *N* hydrochloric acid. Two peaks were found: at 500 ml. (λ_{max} 266 mμ) and 1300 ml. (λ_{max} 287 mμ). The material in the latter peak, 9 mg., provided an infrared spectrum identical with that obtained from the rearrangement of 2-chloro-7-methyladenine.[15]

7-Methylxanthine. A. From 7-methylguanine. 7-Methylguanine hydrochloride (22 mg.) was dissolved in water (10 ml.); sodium nitrite (20 mg.) and *N* hydrochloric acid (0.5 ml.) were added. After standing 2 days at room temperature, the solution was applied to Dowex-50-H$^+$ (0.8 × 50 cms.) and eluted with *N* hydrochloric acid. 7-Methylxanthine emerged at about 200 ml. of eluate; 8 mg. was obtained, on concentration of this pool, with λ_{max} at 270 mμ. Starting material (10.9 mg.) was recovered at 450 ml.

B. From 7-methylisoguanine. 7-Methylisoguanine (28 mg.) in water (28 ml.) was treated with sodium nitrite (50 mg.) and 6 *N* hydrochloric acid (0.5 ml.). After 24 hr., the maximum of the solution was still at 286 mμ. Fresh nitrite and acid were added and the solution was held at 50° for 4 hr. There was still only a slight change in the wave length of maximum absorption. Consequently, the solution was taken to dryness and the residue was refluxed with concentrated hydrochloric acid (15 ml.) for 60 hr. A shift of λ_{max} to 270 mμ was found. The residue was chromatographed on Dowex-50-H$^+$ with *N* hydrochloric acid and the main band pooled and concentrated to yield 24 mg. of 7-methylxanthine.[16] A sample was sublimed *in vacuo* for analysis.

Anal. Calcd. for $C_6H_6N_4O_2$: C, 43.38; H, 3.67. Found: C, 43.23; H, 3.86.

1-Methyl-4-ureido-5-cyanoimidazole. 1-Methyl-4-amino-5-cyanoimidazole[14] (75.3 mg.) and potassium cyanate (75 mg.) were dissolved in water (7.5 ml.) and the solution was placed in a boiling water bath. The pH was kept in the range of 6.0–6.4 by the dropwise addition of 20% acetic acid as needed. After 10 min., a second portion of potassium cyanate was added and the heating with *p*H control continued for an additional 15 min. The cooled mixture was acidified, brought to a volume of 15 ml., one-third of which was applied to the Dowex-50-H$^+$ column (1 × 80 cm.) for analysis as described above. There were a number of products. However, starting material was the major band, emerging at 900 ml. and was readily identifiable by the diazotizable amino group and ultraviolet spectrum. A product was eluted at around 710 ml., in the region found for the nitrile isolated in the rearrangement; the location of the peak together with the shift of 42 mμ to longer wave length in absorption maximum with increasing *p*H as described above suggested that the desired material was at hand. From optical density measurements it was calculated that 3.6 mg. of product was obtained, a 10% conversion.

A crystalline picrate prepared from a concentrated aqueous solution of the product was identical in infrared spectrum with that obtained by rearrangement.

Acknowledgment. The author is grateful to Inge Kohelik and Helen McIntosh for technical assistance and to the U.S. Public Health Service (Research Grant C-3871) for support of this work.

NEW ORLEANS 18, LA.

(14) R. N. Prasad and R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6401 (1957). We are grateful to Dr. Robins for a sample of this material.

(15) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **23**, 855 (1958), effected a similar ring-closure in the pyrazolopyrimidine series.

(16) Prepared previously by various methods: E. Fischer, *Ber.*, **30**, 2400 (1897); **31**, 104 (1898); E. Sarasin and E. Wegmann, *Helv. Chim. Acta*, **7**, 713 (1924); also, ref. 14.