[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE NEW YORK STATE EXPERIMENT STATION]

The Reaction of Formaldehyde with *l*(−)-Histidine[1]

BY D. C. CARPENTER

Information on the combination of formaldehyde and *l*(−)-histidine as its sodium salt, has been obtained by the polariscopic method. Similar studies in this Laboratory with asparagine, aspartic and glutamic acids[2] have shown that the amino acids react with formaldehyde (1:1 mole) giving fairly stable compounds. On increasing the aldehyde content of the solution these compounds react further, combining reversibly with a second mole of aldehyde and forming compounds that are unstable and which cannot be isolated.

The present study reports the reaction of formaldehyde with *l*(−)-histidine to form a relatively stable 1:1 mole compound and the further reaction of two moles of this compound with one mole of additional aldehyde. The latter reaction is reversible and the reaction product unstable.

Preparation of Materials

***l*(−)-Histidine.**—A commercial sample of *l*(−)-histidine monohydrochloride monohydrate was dissolved in hot water and converted into the free base by addition of ammonium hydroxide and crystallized by the addition of ethyl alcohol.[3] Repetition of this procedure four times, each time reconverting to monohydrochloride and decomposing by ammonium hydroxide, gave a product of constant rotation. Final drying was over phosphorus pentoxide *in vacuo*. Amino acid nitrogen determination (Van Slyke) gave 8.97% amino nitrogen (calcd. 9.02%).

TABLE I

REACTION OF FORMALDEHYDE WITH *l*(−)-HISTIDINE AT 20°

0.01 Mole of histidine and one equivalent of sodium hydroxide per 25 ml. of solution

Soln.	Formaldehyde added, mole	Angular rotation, degrees (2-dm.)	Concn. of H ion
1	0	−1.19	2.0×10^{-11}
2	0.00274	7.49	3.8
3	.00539	13.70	4.2
4	.00810	19.65	4.8
5	.01099	19.91	7.6
6	.01218	15.91	1.5×10^{-10}
7	.01360	11.95	2.1
8	.01485	9.92	2.8
9	.01636	8.51	3.0
10	.01889	7.06	4.0
11	.02175	6.07	4.9
12	.03205	4.65	1.0×10^{-9}
13	.04360	5.21	1.6
14	.06540	3.30	3.2
15	.0877	3.38	5.7
16	.1096	3.56	8.0
17	.1304	3.72	1.0×10^{-8}
18	.1537	3.85	1.2
19	.1775	3.95	1.3
20	.1972	4.06	1.3

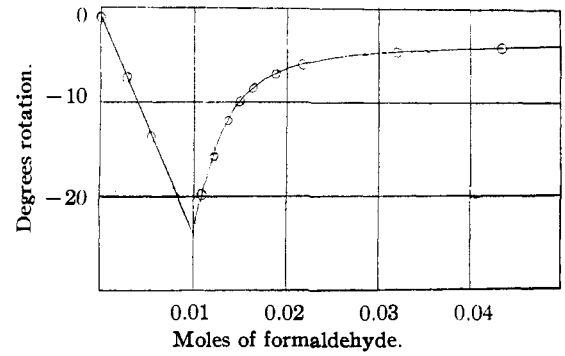

Fig. 1.—Effect of added formaldehyde on angular rotation (2 dm.) of 0.01 mole of *l*(−)-histidine containing one equivalent of sodium hydroxide in 25 ml. of solution.

Melting point was 287° (cor.) with decomposition. A solution containing 0.6420 g. of *l*(−)-histidine made up to exactly 25 ml. gave a rotation of −2.40° (2-dm.) at 20°; $[\alpha]^{20}D$ −39.72°. Pyman[4] records +39.3° (at 23°) for the antipode separated from *dl*-histidine and Dunn,[5] from interpolation, states that −39.7° (at 20°) is a very accurate value.

Formaldehyde.—A very pure concentrated formaldehyde solution was brought to exactly *p*H 7.0, measured against the glass electrode, by the addition of sodium hydroxide solution, and the aldehyde content of this stock solution determined by the sodium bisulfite method of Kleber.[6]

Experimental

Into each of a series of 25-ml. volumetric flasks, exactly 0.01 mole of *l*(−)-histidine was weighed out, 0.01 mole of carefully standardized sodium hydroxide solution added and the mixture shaken until the amino acid was dissolved. Various amounts of the stock formaldehyde solution were added from a micro-buret to each flask and the volume of each solution made up to the 25-ml. mark with water and well shaken. Part of each solution was placed in a 2-dm. polarizing tube and the reserve solutions and those in the polarizing tubes were kept at 20° in a constant temperature bath and the rotation read periodically in the polariscope with the sodium arc as light source. In the more

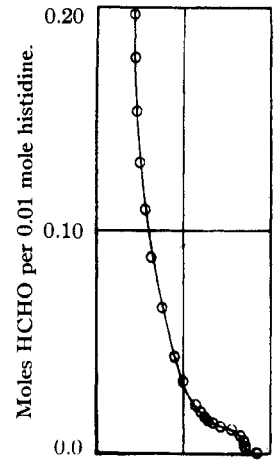

Fig. 2.—Effect of added formaldehyde on *p*H of 0.01 mole of *l*(−)-histidine containing one equivalent of sodium hydroxide in 25 ml. of solution.

(1) Journal Paper No. 629 of the New York State Experiment Station.

(2) D. C. Carpenter and F. E. Lovelace, THIS JOURNAL, **64**, 2899 (1942); **65**, 1161 (1943).

(3) M. S. Dunn, E. H. Frieden, M. P. Stoddard and H. V. Brown, *J. Biol. Chem.*, **144**, 487 (1942).

(4) F. L. Pyman, *J. Chem. Soc.*, **99**, 1386 (1911).

(5) M. S. Dunn, personal communication.

(6) C. Kleber, *Pharm. Rev.*, **22**, 94 (1904).

TABLE II

CALCULATION OF EQUILIBRIUM CONSTANT FOR HISTIDINE-FORMALDEHYDE REACTION

Completion of reaction	Rotation, degrees	Concentration, moles per liter: Total formaldehyde	Total formaldehyde minus 0.40 mole	AF	A_2F_3	Free formaldehyde	k	log $\overline{AF}^2/A_2F_3$	log F
0.20	−19.94	0.4426	0.0426	0.32	0.04	0.0026	0.00665	+0.408	−2.585
.30	17.86	.4648	.0648	.28	.06	.0048	.00627	.116	2.319
.40	15.78	.4890	.0890	.24	.08	.0090	.00648	− .143	2.046
.50	13.70	.5164	.1164	.20	.10	.0164	.00656	.398	1.785
.60	11.62	.5512	.1512	.16	.12	.0312	.00665	.672	1.506
.70	9.54	.6078	.2078	.12	.14	.0678	.00697	.987	1.169
.80	7.46	.7320	.3320	.08	.16	.1720	.00688	1.398	0.765
						Mean	.00658		

dilute aldehyde solutions, equilibrium was reached in a few days but in the solutions containing more aldehyde than 0.01 mole, nineteen to twenty-five days were required. The 1:1 mole reaction goes to completion rapidly while the second reaction is very slow. The angular rotations are recorded in Table I. After reaching equilibrium, the hydrogen-ion concentrations of the reserve solutions were measured with a standardized glass electrode against a saturated calomel half-cell and these data are likewise recorded in Table I. In Fig. 1, the relation between optical rotation and aldehyde concentration and, in Fig. 2, that between pH and aldehyde concentration are shown. The stable 1:1 mole histidine-aldehyde compound was neutralized with hydrochloric acid and precipitated by adding ethyl alcohol to the concentrated aqueous solution and subsequently recrystallized from warm ethyl alcohol as colorless octahedra, m. p. 196.5° (cor.) with decomposition.

Discussion

From the optical rotation data it is clear that a definite compound having a maximal levo rotation occurs when $l(-)$-histidine solutions containing an equivalent of sodium hydroxide react with formaldehyde in a 1:1 mole ratio. Greater amounts of aldehyde react to form a second compound which is unstable and in which two moles of the first compound have reacted with one mole of additional aldehyde.

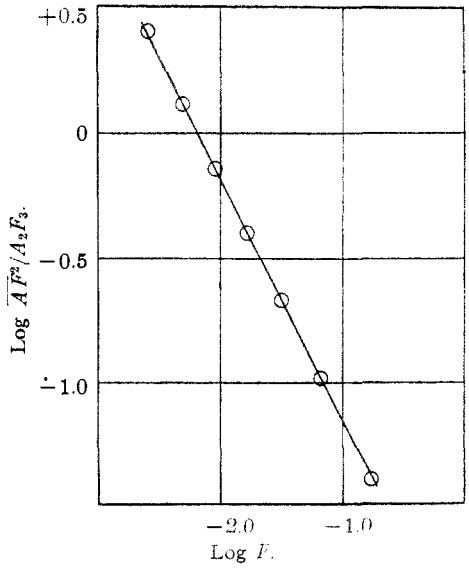

Fig. 3.—Graph of log $\overline{AF}^2/A_2F_3$ against log F for unstable histidine-formaldehyde compound.

$$A + F \longrightarrow AF \quad (1)$$
$$2AF + F \longrightarrow A_2F_3 \quad (2)$$

In Table II are set forth data and computations of the equilibrium constant of the second reaction in which $C^2_{AF}C_F/C_{A_2F_3} = k$. The average value of k for this reaction is 0.00658.

The equilibrium constant may be obtained also by the graphical method shown in Fig. 3 in which the logarithmic form of the equilibrium equation log $C^2_{AF}/C_{A_2F_3}$ + log F = log k is employed. In Fig. 3 log $\overline{AF}^2/A_2F_3$ is plotted against log F. This method yields the value k = 0.00652 in good agreement with the foregoing treatment. The angular rotations and the corresponding concentrations employed in the calculations of k have been read from the experimental curve in which the rotation of the AF compound was −24.10° and that for the A_2F_3 compound was −3.30°.

Beyond the rotation of −3.30°, at a total aldehyde addition of 0.065 mole, the rotation increases slowly but there is no indication of any further compound formation. The aldehyde content in this region is quite high and it seems likely that the small increase in rotation observed is due to change of solvent.

Frieden, Dunn and Coryell[7] employing an optical rotation method somewhat similar to ours but adding a series of increments of aldehyde directly to the observation tube and reading the rotation before equilibrium could have been established, concluded that histidine and aldehyde formed two compounds, one of the AF type and the other of the AF_2 type as is common with most amino acids.

Neuberger[8] has examined the reaction of $l(-)$-histidine and formaldehyde in neutral and acid solutions at 37° and concluded that the first mole of aldehyde reacting with histidine formed $l(-)$-1,2,5,6-tetrahydropyrido-3,4-imidazole-6-carboxylic acid having a melting point of 277° (uncor.). The second aldehyde group reacted either at the imide group of the pyridine or of the imida-

(7) E. H. Frieden, M. S. Dunn and C. D. Coryell, *J. Phys. Chem.*, **47**, 85 (1943).

(8) Neuberger, *Biochem. J.*, **38**, 309 (1944).

zole ring, forming a methylol derivative with a melting point of 210–215° (uncor.). As shown by the melting point, neither of Neuberger's compounds is the 1:1 mole compound we have isolated. The conditions of the reaction between amino acids and aldehyde no doubt determine whether ring closure takes place resulting in pyridine compounds or whether simple methylol derivatives are formed.

In our work we expect that the 1:1 mole compound is the usual methylol type of derivative of the amino acid. The second mole of aldehyde must link together two molecules of the 1:1 mole compound. This could be accomplished by reaction with two methylol groups— $2R—NHCH_2OH + HCHO \rightarrow R—NHCH_2O—CH_2—OCH_2NH—R + H_2O$ or by reaction of the aldehyde with the imino groups of two imidazole rings. Inasmuch as the second compound is not stable, we are inclined to favor the first alternative as the more probable.

Summary

1. The reaction between solutions of *l*(−)-histidine containing one equivalent of sodium hydroxide and various amounts of formaldehyde has been followed by polariscopic and hydrogen-ion measurements.

2. Under the experimental conditions, histidine forms two compounds, one of the AF type and one of the A_2F_3 type as the aldehyde concentration is increased.

3. The equilibrium constant of the latter reaction has been calculated.

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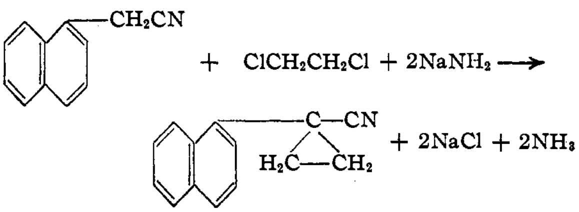
[CONTRIBUTION FROM THE WALKER LABORATORY OF THE RENSSELAER POLYTECHNIC INSTITUTE]

1-α-Naphthylcyclopropanecarbonitrile and Some of its Derivatives[1]

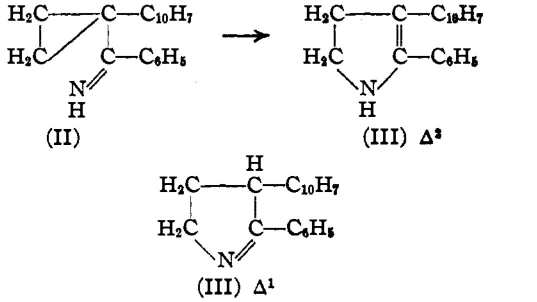
BY JOHN B. CLOKE AND THOMAS S. LEARY

1-α-Naphthylcyclopropanecarbonitrile (I) and some of its derivatives have been made as a part of a general study of compounds containing the cyclopropane ring. Compound (I) was prepared from α-naphthylacetonitrile, ethylene dichloride and sodium amide in liquid ammonia



by methods already reported for similar compounds.[2,3]

The presence of a cyclopropane ring in (I) is supported by its stability in the presence of potassium permanganate, which has been used for the differentiation of cyclopropane from ethylenic nitriles,[3,4] and also by the fact that it will give a ketimine, 1-α-naphthylcyclopropyl phenyl ketimine (II), whose cyclopropane structure is demonstrated by its rearrangement to give the isomeric 1-phenyl-2-α-naphthylpyrroline (III). Whether (III) has the conventional Δ^2 pyrroline structure or the Δ^1 structure or whether these two structures may be in tautomeric equilibrium has not been ascertained.



The use of the cyclopropyl ketimine–pyrroline rearrangement as a test for the presence of the cyclopropane ring in such compounds is based upon considerable work, some of which has already been reported.[2,4,5] The effect of heating on the rearrangement of the ketimine base (II) may be seen in the top curve of Fig. 1, which was obtained by the differential thermocouple method already described.[5] It will be noted that the decomposition of the ketimine (II) is evident at about 144°, becomes more rapid as the temperature rises, and appears to be complete around 196°. The effect of heating on the 1-α-naphthylcyclopropylphenylketimmonium chloride (IV), $CH_2CH_2C(C_{10}H_7)—C(=NH_2Cl)C_6H_5$, may be seen in the middle and lowest curves of the figure. A comparison of these curves with those obtained with the simpler phenyl cyclopropyl ketimine, $CH_2CH_2C(H)—C(=NH)C_6H_5$,[5] would indicate that the replacement of the hydrogen (H) in the cyclopropane ring by the α-naphthyl group increases the resistance of the compound to rearrangement. The

(1) The data reported in this paper have been taken from a thesis presented by Thomas Samuel Leary to the Rensselaer Polytechnic Institute in partial fulfillment of the requirements for the degree of Master of Science in 1939.

(2) Knowles and Cloke, THIS JOURNAL, **54**, 2028 (1932).

(3) Murray and Cloke, *ibid.*, **58**, 2014 (1936).

(4) Gotkis and Cloke, *ibid.*, **56**, 2710 (1934).

(5) Cloke, *ibid.*, **51**, 1174 (1929).