

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

## The Reaction of Formaldehyde with 1(-)-Asparagine<sup>1</sup>

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The action of formaldehyde on various amino acids has been investigated by numerous workers. Much of the older work was concerned with the isolation and analysis of compounds.<sup>1a</sup> More recently the reactions have been followed by measurements of the unreacted amino groups and hydrogen-ion concentrations and various constants evaluated.<sup>2</sup> Lately measurements of the optical rotation have been used in a study of the reaction between formaldehyde and 1(-)-proline.<sup>3</sup>

In the present paper studies of the latter sort have been employed in indicating the progress of the reaction and the compounds formed. Under the experimental conditions we have used, methylene-1(-)-asparagine is formed which reacts further with a second mole of formaldehyde. The latter compound has not been isolated because of its ease of decomposition. The existence of these compounds is a valuable clue in interpreting the reaction of formaldehyde with proteins in the manufacture of plastics, etc.

### Preparation of Materials

**1(-)-Asparagine.**—A commercial sample of 1(-)-asparagine was recrystallized twice from hot water as the monohydrate, m. p. 225° (cor.), and its moisture content determined by drying to constant weight *in vacuo* at 61° over phosphorus pentoxide (H<sub>2</sub>O, 12.18; calcd., 11.99%). The amino nitrogen was determined by the Van Slyke method (amino N, 9.30; calcd., 9.33%).

**Formaldehyde.**—A very pure concentrated formaldehyde solution was brought to exactly pH 7.0 against the glass electrode by the addition of sodium hydroxide solution and the formaldehyde content of this stock solution determined by the sodium bisulfite method of Kleber.<sup>4</sup>

### Experimental

Into each of a series of 50-ml. volumetric flasks exactly 0.02 mole of 1(-)-asparagine was weighed out (corrected for water content) and 0.02 mole of carefully standardized sodium hydroxide solution added and the mixture shaken until all the 1(-)-asparagine was in solution. 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 50-ml. mark with water and well shaken. Part of each solution was transferred to 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 of the latter read periodically in the polariscope with the sodium arc as a light source. In solutions containing small amounts of formaldehyde, equilibrium was attained very slowly (thirty-four days for solution 5 containing 0.02 mole of formaldehyde per 0.02 mole of 1-asparagine); however, equilibrium was reached in a much shorter period when higher aldehyde concentrations were employed. The angular rotations at equilibrium for a 2-dm. tube are recorded in Table I. When equilibrium was attained, the hydrogen-ion concentrations of the reserve solutions were measured with a standardized glass electrode against a saturated calomel half-cell.

TABLE I

OPTICAL ROTATION, DENSITY AND HYDROGEN ION CONCENTRATION OF SODIUM 1-ASPARAGINATE (0.02 MOLE) AND FORMALDEHYDE SYSTEMS AT 20°

Soln.	Total moles HCHO present	Rotation, <sup>a</sup> <i>levo</i> degrees	Density	C <sub>H</sub>	Remarks
1	0.00000	0.90	1.0308	1.0 × 10 <sup>-10</sup>	
2	.00500	5.424	1.0320	1.55	
3	.01001	9.33	1.0327	2.10	
4	.01501	13.19	1.0334	3.5	
5	.02002	15.41	1.0339	3.1 × 10 <sup>-9</sup>	HCHO odor very faint
6	.02503	14.61	1.0346	2.0 × 10 <sup>-8</sup>	HCHO odor faint
7	.03004	13.06	1.0353	4.2	
8	.04005	10.14	1.0378	7.8	HCHO odor very noticeable
9	.06008	6.56	1.0408	1.3 × 10 <sup>-7</sup>	
10	.09012	5.00	1.0460	1.8	
11	.12016	4.55	1.0509	2.6	
12	.15020	4.38	1.0556	3.0	
13	.18024	4.28	1.0603	4.0	
14	.21028	4.23	1.0649	4.3	
15	.23834	4.20	1.0692	5.5	
16	.30040	4.19	1.0785	7.0	

<sup>a</sup> In this column the angular rotation of solutions containing 0.02 mole of 1-asparagine with equivalent of base (Na) and designated moles of formaldehyde are reported for 2-dm. tube length after reaching equilibrium.

For estimating the uncombined formaldehyde in the solutions, appropriate aliquots of the reserve solutions were taken and caused to react with dimethyldihydroresorcinol as described by Vorländer.<sup>5</sup> The crystalline methylene derivative was filtered off after three hours of interaction at room temperature onto fritted glass crucibles, washed several times with water, dried at 110° and weighed.

The solubility of methylene-(bis)dimethyldihydroresorcinol in a saturated aqueous solution of dimethyl

(1) Published by permission of the Director, New York State Experiment Station, as Journal Paper No. 521, August 12, 1942.

(1a) H. Schiff, *Ann.*, **310**, 25 (1899).

(2) M. Levy, *J. Biol. Chem.*, **99**, 767 (1932); M. Levy and D. E. Silberman, *ibid.*, **118**, 723 (1937); T. Tomiyama, *ibid.*, **111**, 51 (1935); A. Wadsworth and M. C. Pangborn, *ibid.*, **116**, 423 (1936); E. W. Balson and A. Lawson, *Biochem. J.*, **30**, 1257 (1936).

(3) E. H. Frieden, M. S. Dunn and C. D. Coryell, *J. Phys. Chem.*, **46**, 215 (1942).

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

(5) D. Vorländer, *Z. anal. Chem.*, **77**, 241 (1929).

TABLE II  
ESTIMATION OF COMBINED FORMALDEHYDE IN SODIUM 1-ASPARAGINATE SERIES (0.02 MOLE)

Soln.	Moles HCHO originally present	Free aldehyde analysis				
		Aliquot factor	Dimedon compd., g.	Free HCHO present, g.	Moles free HCHO present	Moles HCHO combined
2	0.00500	5	0.0000			0.00500
3	.01001	5	.0000			.01001
4	.01501	5	.0000			.01501
5	.02002	5	.0000			.02002
6	.02503	5	.2922	0.1504	0.00501	.02002
7	.03004	16.66	.1749	.2992	.00997	.02007
8	.04005	16.66	.3503	.600	.02003	.02002
9	.06008	16.66	.6980	1.195	.0398	.02028
10	.09012	25	.8200	2.102	.07005	.02007
11	.12016	100	.2922	3.004	.10012	.02004
12	.15020	100	.3810	3.91	.1302	.02000
13	.18024	100	.4703	4.840	.1603	.01994
14	.21028	166.6	.3342	5.71	.1903	.01998
15	.23834	166.6	.3842	6.55	.2183	.02000
16	.30040	166.6	.4905	8.406	.2802	.02020

dihydroresorcinol was found to be 0.0025 g. per liter and 0.0050 g. per liter in water at 20°. The weights of methylene-(bis)dimethylidihydroresorcinol reported have been corrected for the above respective solubilities. One gram of methylene-(bis)dimethylidihydroresorcinol is equivalent to 0.1027 g. of formaldehyde.

aldehyde is so unstable that, in the presence of an excess of dimethylidihydroresorcinol as is used in the precipitation, it decomposes giving formaldehyde and methylene-1-asparagine. The aldehyde so liberated reacts with dimethylidihydroresorcinol in addition to any free aldehyde remaining in the solution at equilibrium.

The results of the analyses for free formaldehyde are given in Table II. The optical rotation and hydrogen-ion concentrations are shown graphically in Figs. 1 and 2.

### Discussion

It is clear from the results of the optical rotation work (Fig. 1) that a definite compound having a maximum levo rotation is formed when the mole ratios of asparagine and formaldehyde are 0.02 and 0.02, respectively. The compound formed is methylene-1-asparagine, inasmuch as we have obtained 6-oxy-5-bromopyrimidine 4-carbonic acid (m. p. 206-7° (cor.) with decomposition) therefrom by the action of sodium hypobromite, the same compound previously described by Cherbuliez and Starvitch.<sup>5</sup> From Fig. 2 it is clear that the maximum rate of change of hydrogen-ion concentration produced

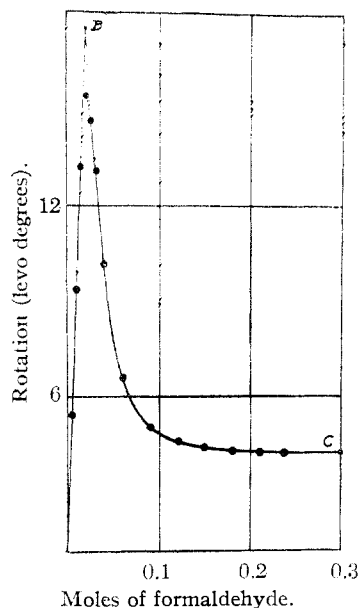


Fig. 1.—Effect of added formaldehyde on angular rotation (2 dm.) of 0.02 mole of sodium 1(-)-asparaginate in 50 ml. of solution: B, sodium salt of methylene-1(-)-asparagine; C, same with additional mole of formaldehyde forming unstable compound.

We have found it unnecessary to add acetic acid to effect the precipitation of the resorcinol derivative as was recommended by Wadsworth and Pangborn.<sup>2</sup> The addition of acid produces decomposition of methylene-1-asparagine and consequent liberation of formaldehyde and is to be avoided. In our experience the methylene-1-asparagine compound with one additional mole of form-

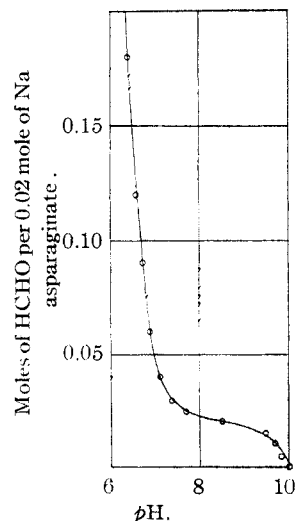


Fig. 2.—Effect of added formaldehyde on pH of 0.02 moles of sodium 1-asparaginate in 50 ml. of solution.

<sup>5</sup> E. Cherbuliez and K. N. Starvitch, *Helv. Chim. Acta*, **5**, 267 (1922).

TABLE III  
 CALCULATION OF EQUILIBRIUM CONSTANT

Completion of react.	Rotation, levo degrees	Formaldehyde concn. moles per liter					A/AF	log A/AF	log F
		Total	Bound (AF)	Free (F)	$k$ ( $n = 1$ )				
0.20	14.83	0.094	0.080	0.014	0.056	4.00	+0.602	-1.854	
.30	13.49	.171	.120	.051	.119	2.33	.368	-1.292	
.40	12.16	.259	.160	.099	.149	1.50	.176	-1.004	
.50	10.82	.342	.200	.142	.142	1.00	0	-0.848	
.60	9.49	.455	.240	.215	.143	0.67	-0.176	.667	
.70	8.16	.590	.280	.310	.133	.43	.368	.508	
.80	6.82	.761	.320	.441	.110	.25	.602	.355	

by adding formaldehyde is achieved in the region where 0.02 mole of formaldehyde reacts with 0.02 mole of asparagine. To this maximum we give the usual interpretation of compound formation in equimolecular proportions. That compound formation has been with the  $\alpha$ -amino group rather than the amide group is confirmed from Fig. 2 by the rapid increase in acidic properties.

With regard to the further reaction of methylene-1-asparagine with additional formaldehyde (B-C section of Fig. 1), we have approached the problem from the basis of the equilibrium constant. If the concentrations of formaldehyde, methylene-1-asparagine and the reaction product be represented by  $C_F$ ,  $C_A$  and  $C_{AF_n}$ , respectively, and  $n$  moles of formaldehyde take part in the reaction, then at equilibrium  $(C_A \times C_F^n)/C_{AF_n} = k$ , where  $k$  is the equilibrium constant. On the assumption that the optical rotation of substances A and  $AF_n$  are of different magnitude and each proportional to its concentration and further that the presence of neither species has an influence on the rotation of the other, we may calculate  $k$  from the rotation data. From examination of the first few experimental points it is clear that  $n$  cannot be greater than unity. In evaluating  $k$  we have employed the extrapolated values  $-17.50$  and  $-4.15^\circ$  for the maximum and minimum ends, respectively, of the curve. Points where the reaction is 0.20, 0.30, 0.40, etc., complete have been chosen and the respective formaldehyde concentrations read off from the curve. It is difficult to estimate the aldehyde concentrations near the beginning of the curve on account of its steepness and likewise near the end on account of its flatness, and partly for this reason only the central section has been employed in the calculations, which latter are carried out on a moles per liter basis. These data are given in Table III. In the column headed Total Formaldehyde, the 0.40 mole of formaldehyde required to form 0.40 mole of methyleneasparagine has been subtracted

from the total formaldehyde in the system as read from the graph. The equilibrium constant  $k$  shows a fair constancy in the region 0.14.

The equilibrium constant may also be evaluated from the logarithmic form of the equilibrium equation  $\log (C_A/C_{AF}) + \log C_F = \log k$  as employed by Frieden, Dunn and Coryell,<sup>3</sup> in which a graph of  $\log (A/AF)$  plotted against  $\log F$  should give a straight line. This relation is given in Fig. 3. A straight line can be drawn through several of the points, which gives a graphical solution for  $k$  of 0.142 in reasonable agreement with the foregoing treatment.

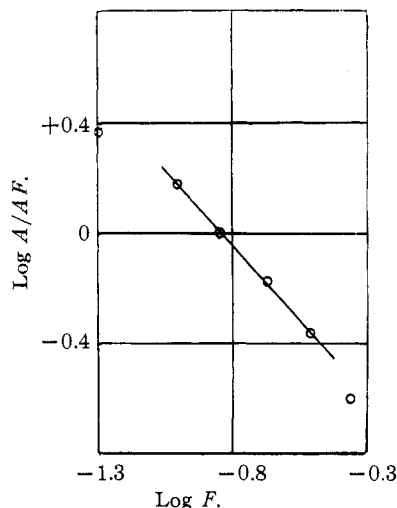


Fig. 3.—Graph of  $\log A/AF$  against  $\log F$  for unstable compound of methylene-1(-)-asparagine with formaldehyde.

While the data show that methylene-1-asparagine binds an additional mole of formaldehyde to form an unstable compound, evaluation of the data depends on the validity of the assumptions mentioned earlier, of which the lack of constancy of rotation of the two species with respect to concentration seems to be the main disturbing feature for the compounds under consideration.

There can be little doubt that the first mole of formaldehyde reacts with the  $\alpha$ -amino group of

asparagine to give a methylene derivative. One might expect that the second mole of formaldehyde reacts with the amide group forming a methylol derivative. In support of this view it may be said that many methylol compounds of acid amides and formaldehyde have been reported in the literature, and in common with our second asparagine compound all are unstable and give off formaldehyde. Further work is in progress in this Laboratory to ascertain the formaldehyde binding of 1(+)-aspartic and 1(+)-glutamic acids, in an effort to elucidate the point of attachment of the second mole of formaldehyde.

In 6.64% aqueous solution  $[\alpha]^{20D}$  is  $-116.04^\circ$  for the sodium salt of methylene-1(-)-asparagine and  $-23.06^\circ$  for the sodium salt of the methylene-1(-)-asparagine compound with one additional mole of formaldehyde, the latter concentration being 9.04% and dissolved in 14.4% formaldehyde solution. For sodium 1(-)-asparaginate  $[\alpha]^{20D}$  is  $-7.28^\circ$  for a 6.16% solution in water.

Clough<sup>7</sup> records for the latter a value of  $-7.53^\circ$  for a 14.51% solution at  $25^\circ$  and Becker<sup>8</sup> a value of  $-7.42^\circ$  for a 11.68% solution at  $20^\circ$ . Clough notes that the rotation becomes more negative as the temperature is raised and hence our value compares well with the older data.

### Summary

1. The reaction between solutions of 1(-)-asparagine containing an equivalent of sodium hydroxide and various amounts of formaldehyde was followed by polariscopic and hydrogenion measurements, and estimation of unreacted aldehyde.

2. 1(-)-Asparagine reacts with formaldehyde, mole per mole, to form methylene-1(-)-asparagine. The latter reacts further with one additional mole of formaldehyde to form an unstable compound of unestablished constitution which readily loses aldehyde.

(7) G. W. Clough, *J. Chem. Soc.*, **107**, 1509 (1915).

(8) A. Becker, *Ber.*, **14**, 1028 (1881).

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## Studies in Chemotherapy. VI. Sulfanilamido Heterocycles<sup>1</sup>

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On the basis of our present knowledge, N<sup>1</sup>-heterocyclic substituted sulfanilamide derivatives still appear to offer the greatest possibilities for therapeutically effective sulfanilamide type compounds. Continuing our investigations in this field,<sup>2</sup> we have prepared a number of new derivatives of this type. These compounds, together with pertinent data concerning them, are listed in Table I. Several of the amino heterocycles required as intermediates have not been reported previously. The syntheses and properties of these substances are described in the Experimental part.

Most of the sulfanilamide derivatives were prepared by the standard procedure. In a few cases, such as the conversion of 2-aminoöxazole to the corresponding sulfanilamido compound, it was necessary to use *p*-nitrobenzenesulfonyl chloride. Even this method was hardly satisfactory, since

in this particular case, the over-all yield, including the synthesis of the amino heterocycle, was only about 0.2%. Numerous attempts to prepare an unsubstituted sulfanilamido triazine from 2-amino-1,3,5-triazine were unsuccessful. Under all of the conditions employed, this intermediate appeared to be unstable in the presence of sulfonyl chlorides. Both sulfanilamide and sulfaguanidine were isolated as final products of the various reactions.

Some of the sulfanilamido derivatives are of interest because of their chemical relationship to well-known sulfonamides. For example, 2-sulfanilamidoöxazole is the oxygen analog of sulfathiazole, and 3-sulfanilamidopyridazine is an isomer of sulfadiazine and sulfapyrazine. The imidazole derivative corresponds to sulfadiazine in the five-membered ring series. 4-Sulfanilamido-1,2,4-triazole represents a somewhat different type of heterocyclic derivative, in that the sulfanilamido group is joined to the ring through one of the hetero-atoms rather than through a carbon atom.

(1) Presented in part before the Division of Medicinal Chemistry, Buffalo meeting of the American Chemical Society, Sept. 9, 1942.

(2) Roblin, Williams, Winnek and English, *THIS JOURNAL*, **62**, 2002 (1940).