amylose and amylopectin is in accord with the differences in their chemical constitutions: the amylose, which is completely hydrolyzed to maltose by this enzyme possesses a non-branched long chain structure, while the incompletely hydrolyzed amylopectin is identified with a branched-chain structure.

BERKELEY, CALIFORNIA RECEIVED OCTOBER 6, 1942

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

The Reaction of Formaldehyde with $l(+)$-Aspartic and $l(+)$-Glutamic Acids[1]

BY D. C. CARPENTER AND F. E. LOVELACE

The polariscopic method has been employed in the work herein described to give information concerning the reaction of formaldehyde with $l(+)$-aspartic and $l(+)$-glutamic acids as their respective di-sodium salts. A similar study has shown[2a] that $l(-)$-asparagine with an equivalent of base reacts with one mole of formaldehyde at the α-amino group to give a fairly stable compound designated by Schiff[2b] as methylene asparagine. At higher aldehyde concentrations, a second compound of highly unstable character was produced which could not be isolated or identified. It was suggested before that the second mole of formaldehyde perhaps reacted with the amide group of asparagine inasmuch as many acid amides are known to react with aldehyde giving comparatively unstable derivatives. From the work here reported it is clear that α-amino acids react with formaldehyde at the amino group, binding first one mole and then a second mole as the concentration of the latter is increased. The first mole of aldehyde reacts readily but the second one unites with difficulty and the latter product readily decomposes. From the equilibrium constants found for the latter reaction, it is clear that for any arbitrarily selected ratio between the concentrations of methylol amino acid and its final reaction product, the free aldehyde concentration required for methylol $l(+)$-aspartic acid is about 3.5 times and for methylol $l(+)$-glutamic acid about 7.7 times that required for methylol $l(-)$-asparagine. Under the experimental conditions the amide group of the α-amino acid previously examined ($l(-)$-asparagine) does not react with formaldehyde and the reaction with aldehyde is confined to the α-amino group.

We have employed melting point and amino nitrogen content of our acids as criteria of purity as well as specific rotation. Whether an impurity may be a foreign amino acid or the *dl*-form of the acid, the above criteria should give a reliable clue as to purity. In comparisons of specific rotations we have referred only to rotations reported on preparations in which the *d*- and *l*-isomers were actually separated from one another as crystalline salts of benzoyl derivatives of the respective acids. The above criteria indicate a high degree of purity of our preparations.

Preparation of Materials

$l(+)$-Aspartic Acid.—This was prepared from a sample of recrystallized $l(-)$-asparagine that we have used before, by boiling for six hours under reflux with two moles of normal hydrochloric acid solution. The solution was then cooled and the calculated amount of normal sodium hydroxide added with good stirring and then set away in the cold for the aspartic acid to crystallize. It was recrystallized four times from hot water and twice from fifty per cent. ethyl alcohol; m. p. 283° (cor.). Amino nitrogen by the Van Slyke method 10.43% (found); 10.52% (calcd.). A solution of $l(+)$-aspartic acid, 5.320 g. containing two equivalents of sodium hydroxide and diluted to 100 ml., gave a rotation of $-0.17°$ (2 dm.); $[a]^{20}D$ $-1.60°$. Fischer[3] reports $[a]^{20}D$ -1.90 for the di-sodium salt (c = 6.0).

A solution (d = 1.032) of our $l(+)$-aspartic acid weighing 7.8993 g. and containing 0.3311 g. of aspartic acid dissolved in three equivalents of hydrochloric acid solution gave a rotation of $+1.10°$ (1 dm.); $[a]^{20}D$ $+25.43°$. This is in close agreement with $[a]^{20}D$ $-25.35°$ reported by Fischer[3] for a similar solution of the antipode (c = 4.15).

$l(+)$-Glutamic Acid.—This was prepared from a commercial sample, precipitating as the hydrochloride and decomposing the hydrochloride with the calculated amount of aniline[4] and allowing to crystallize overnight in the cold. The crystals were filtered off and washed with 95% ethyl alcohol until free from chloride and dried. The separation and decomposition of the hydrochloride was re-

(1) Journal Paper No. 544 of the New York State Experiment Station.

(2a) Carpenter and Lovelace, THIS JOURNAL, **64**, 2899 (1942).

(2b) Schiff, *Ann.*, **310**, 25 (1899).

(3) Fischer, *Ber.*, **32**, 2451 (1899).

(4) Gilman, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1932.

peated. This method gives a product free from pyrrolidonecarboxylic acid; m. p. 213° (cor.). Amino nitrogen by the Van Slyke method 9.49% (found); 9.51% (calcd.). A solution of *l*(+)-glutamic acid, 1.006 g. diluted to 100 ml., gave a rotation of +0.25° (2 dm.); $[a]^{20}D$ +12.45°. A solution of the above glutamic acid, 5.880 g. containing two equivalents of sodium hydroxide and diluted to 100 ml., gave a rotation of +1.24° (2 dm.); $[a]^{20}D$ +10.52°.

A further solution (d = 1.0237) of our *l*(+)-glutamic acid having 0.4147 g. in 7.7493 g. of solution containing one equivalent of hydrochloric acid gave a rotation of +1.66° (1 dm.); $[a]^{20}D$ +30.30°. For a similar solution of the antipode Fischer[3] reports $[a]^{20}D$ −30.05°.

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.[5]

Experimental

Into each of a series of 50-ml. volumetric flasks, exactly 0.02 mole of the amino acid was weighed out (corrected for moisture content), 0.04 mole of carefully standardized sodium hydroxide solution added and the mixture shaken until all of 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 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 read periodically in the polariscope with the sodium arc as a light source. Equilibrium was attained in five to seven days in the case of *l*(+)-glutamic acid solutions but forty-five days were required for final equilibrium with *l*(+)-aspartic acid solutions, the behavior of the latter being closely paralleled by that of *l*(−)-asparagine previously described. The reaction between *l*(+)-glutamic acid and formaldehyde goes a great deal more rapidly than the reaction with *l*(+)-aspartic acid and at equilibrium about twice as much free formaldehyde is required for forming the second aldehyde compound of *l*(+)-glutamic acid than is the case with *l*(+)-aspartic acid. The angular rotations at equilibrium for a 2-dm. tube are recorded in Tables I and II. After attaining 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 Tables I and II. The optical rotation and the hydrogen-ion concentration are shown graphically in Figs. 1 and 2.

TABLE I

REACTION OF FORMALDEHYDE WITH *l*(+)-ASPARTIC ACID AT 20°

0.02 Mole aspartic acid and two equivalents of sodium hydroxide per 50 ml. solution

Soln.	Formaldehyde added, moles	Angular rotation degrees (2 dm.)	Concn. of H ion
1	0	−0.17	3.5×10^{-12}
2	0.005	0.92	6.0
3	.010	1.74	7.0
4	.015	2.39	9.0
5	.020	2.70	1.4×10^{-11}
6	.025	2.81	3.0
7	.030	2.66	3.6
8	.045	2.17	6.5
9	.060	1.88	1.1×10^{-10}
10	.075	1.68	1.6
11	.090	1.52	2.5
12	.120	1.32	4.7
13	.150	1.20	7.0
14	.180	1.13	1.1×10^{-9}
15	.210	1.06	1.6
16	.240	1.03	2.4
17	.300	1.00	6.2

TABLE II

REACTION OF FORMALDEHYDE WITH *l*(+)-GLUTAMIC ACID AT 20°

0.02 Mole glutamic acid and two equivalents of sodium hydroxide per 50 ml. solution

Soln.	Formaldehyde added, moles	Angular rotation degrees (2 dm.)	Concn. of H ion
1	0	+1.24	7.0×10^{-12}
2	0.005	0.63	1.0×10^{-11}
3	.010	− .10	1.4
4	.015	.72	1.6
5	.020	1.03	2.2
6	.025	1.07	4.5
7	.030	1.04	6.0
8	.060	0.36	2.0×10^{-10}
9	.090	+ .07	6.0
10	.120	.33	1.3×10^{-9}
11	.150	.53	2.2
12	.180	.64	3.0
13	.210	.75	4.5
14	.240	.81	6.0
15	.300	.89	8.0

Discussion

It is clear from the results of the optical rotation work (Fig. 1) that definite compounds having maximal *levo* rotations are formed between *l*(+)-aspartic and *l*(+)-glutamic acids and formaldehyde when the mole ratios are 0.02 and 0.02, respectively. A similar result was found in the case of *l*(−)-asparagine. In our previous paper we converted the asparagine-formaldehyde (mole:mole) compound into 6-oxy-5-bromopyrimidine-4-carbonic acid (previously prepared by Cherbuliez and Starvitch[6]) by the action of sodium hypobromite and concluded that the asparagine-formaldehyde compound was methylene-l-asparagine in keeping with the nomenclature of Schiff which had been adopted also by Cherbuliez and Starvitch.

In view of the results with *l*(+)-aspartic and *l*(+)-glutamic acids, we are forced to conclude that Schiff's compound was methylol-*l*(−)-

(5) Kleber, *Pharm. Rev.*, **22**, 94 (1904).

(6) Cherbuliez and Starvitch, *Helv. chim. acta*, **5**, 267 (1922).

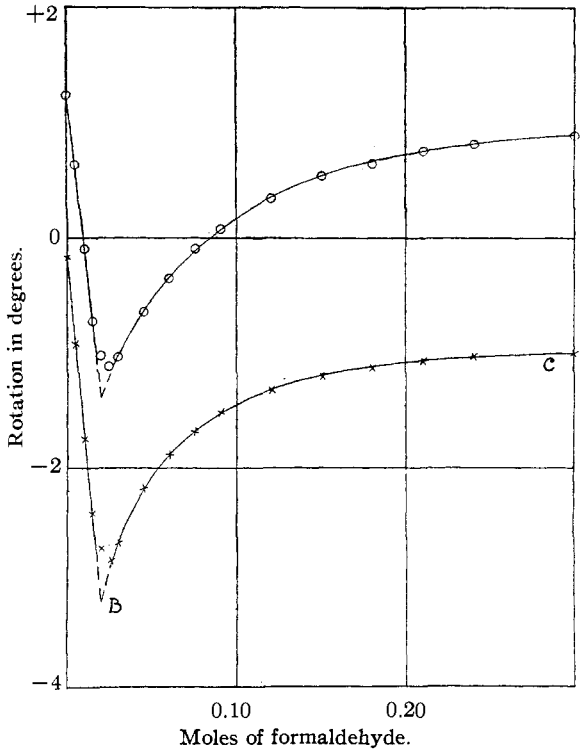

Fig. 1.—Effect of added formaldehyde on angular rotation (2 dm.) of 0.02 mole of $l(+)$-aspartic and $l(+)$-glutamic acids containing 2 equivalents of sodium hydroxide in 50 ml. of solution: ×, aspartic acid; O, glutamic acid; B, methylol compound of acid; C, higher aldehyde compound of same.

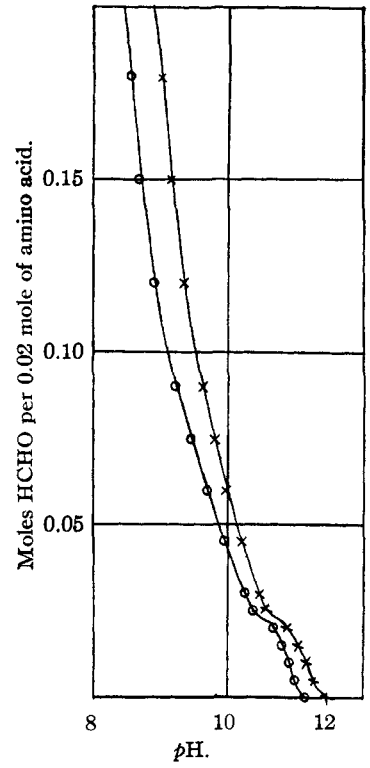

Fig. 2.—Effect of added formaldehyde on pH of 0.02 mole of $l(+)$-aspartic and $l(+)$-glutamic acids containing 2 equivalents of sodium hydroxide in 50 ml. of solution: ×, aspartic acid; O, glutamic acid.

asparagine and not methylene-$l(-)$-asparagine. Either of these compounds would be oxidized by hypobromite to the pyrimidine compound isolated but the methylene compound could not bind a second mole of formaldehyde on the nitrogen, while if the first compound were a methylol derivative, an additional mole of aldehyde could react with the imino group.

With the further reaction of methylol-$l(+)$-aspartic and methylol-$(+)$-glutamic acid with formaldehyde (B-C section of Fig. 1), we have approached the problem from the basis of the equilibrium constant. If the concentrations of formaldehyde, methylolamino acid 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. Assuming that the optical rotation of species 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 evaluate k from the rotation data. Examination of the first few experimental points in each case reveals that n cannot be greater than unity. In evaluating k we have employed for $l(+)$-aspartic acid the extrapolated values -3.17 and $-0.77°$ for the two ends of the curve and for $l(+)$-glutamic acid the corresponding values -1.30 and $+1.10°$. Points on the equilibrium curves where the respective reactions were 0.20, 0.30, 0.40, etc., complete were chosen, the respective formaldehyde concentrations read off and the equilibrium constants calculated. These data are given in Tables III and IV. In the columns headed Total Formaldehyde, the aldehyde concentration of the system is reported as read from the graph. From this value, 0.40 mole has been subtracted as the amount of formaldehyde already utilized in forming the methylol amino acid and the next column containing these values is headed Total Formaldehyde minus 0.40 mole. The equilibrium constant for the reaction of methylol-$l(+)$-aspartic acid with formaldehyde is 0.493 and the corresponding constant for the methylol-$l(+)$-glutamic acid is 1.085.

TABLE III

CALCULATION OF EQUILIBRIUM CONSTANT FOR ASPARTIC ACID–FORMALDEHYDE REACTION

Completion of reaction	Rotation in degrees	Concentration, moles per liter: Total formaldehyde	Total formaldehyde minus 0.40 mole	A	AF	Free formaldehyde	k (n = 1)	log A/AF	log F
0.20	−2.69	0.6025	0.2025	0.32	0.08	0.1225	0.490	+0.602	−0.912
.30	2.45	.7310	.3310	.28	.12	.2110	.492	.368	.676
.40	2.21	.8892	.4892	.24	.16	.3292	.494	.176	.483
.50	1.97	1.0932	.6932	.20	.20	.4932	.493	.000	.307
.60	1.73	1.3784	.9784	.16	.24	.7384	.492	− .176	.132
.70	1.49	1.8300	1.4200	.12	.28	1.1500	.493	.368	+ .061
.80	1.25	2.6960	2.2960	.08	.32	1.9760	.494	.602	.296

TABLE IV

CALCULATION OF EQUILIBRIUM CONSTANT FOR GLUTAMIC ACID–FORMALDEHYDE REACTION

Completion of reaction	Rotation in degrees	Concentration, moles per liter: Total formaldehyde	Total formaldehyde minus 0.40 mole	A	AF	Free formaldehyde	k (n = 1)	log A/AF	log F
0.20	−0.84	0.752	0.352	0.32	0.08	0.272	1.088	+0.602	−0.566
.30	.56	0.984	.584	.28	.12	.464	1.081	.368	.334
.40	.28	1.283	.883	.24	.16	.723	1.085	.176	.140
.50	0	1.686	1.286	.20	.20	1.086	1.086	.000	+ .036
.60	+ .28	2.270	1.870	.16	.24	1.630	1.086	− 176	.212
.70	.56	3.210	2.810	.12	.28	2.530	1.085	.368	.401
.80	.84	5.070	4.670	.08	.32	4.350	1.087	.602	.638

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 which has been employed by Frieden, Dunn and Coryell[7] in similar work on $l(-)$-proline and by us on $l(-)$-asparagine. By this method a graph of log A/AF plotted against log F should give a straight line. This relation is shown in Fig. 3 for the reaction between methylol $l(+)$-aspartic and methylol $l(+)$-glutamic acid and formaldehyde. This method gives values for k of 0.490 and 1.086, respectively, in close agreement with the treatment given above.

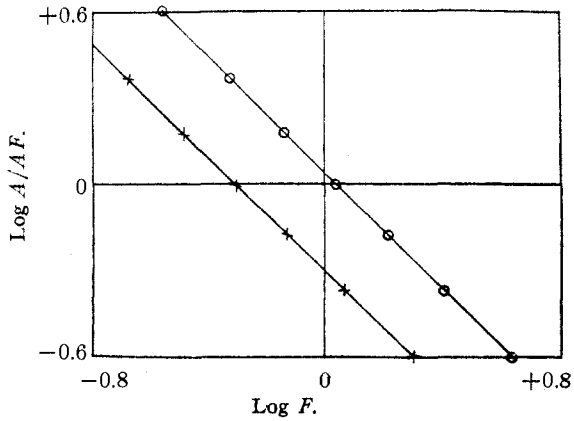

Fig. 3.—Graph of log A/AF against log F for unstable dimethylol compounds: ×, aspartic acid; O, glutamic acid.

(7) Frieden, Dunn and Coryell, *J. Phys. Chem.*, **46**, 215 (1942).

It is clear that not only the length of the side-chain of the amino acid greatly influences the extent of binding of the second mole of formaldehyde but the introduction of certain substituted groups on the side-chain has even a greater effect on the binding. Thus the introduction of a CH_2 group in the side-chain of methylol $l(+)$-aspartic acid (forming the corresponding glutamic acid compound), for any empirically chosen ratio between the concentrations of methylol amino acid and the final reaction product, requires the presence of about 2.2 times as much free aldehyde in the system. In the same way the introduction of the acid amide group on the side-chain (forming the corresponding asparagine compound) requires a concentration of free aldehyde less than one-third that required by methylol $l(+)$-aspartic acid.

It is not yet established whether the second mole of formaldehyde unites with the imino group giving di-methylol derivatives or whether the additional aldehyde reacts at some other point, for instance with the methylol group giving a $-CHOHCH_2OH$ linkage.

In Fig. 2 we give the customary interpretation of compound formation to the rapid break in hydrogen-ion concentration at the point where the mole ratio of amino acid to formaldehyde is one to one.

From our results it may be expected that in the manufacture of protein-aldehyde plastics

the amount of aldehyde bound by the protein will be largely influenced by the character of the side-chains of the various amino acids present in the protein, although the side-chains as such may not react directly with formaldehyde. It seems likely that the order of sequence of the amino acids of the peptide chain also would exert an influence on the amount of aldehyde binding. Further work is in progress in this laboratory to ascertain the effect of other substituted groups on the binding of formaldehyde.

Summary

1. The reactions between solutions of *l*(+)-aspartic and *l*(+)-glutamic acid containing two equivalents of sodium hydroxide and various amounts of formaldehyde have been followed by polariscopic and hydrogen-ion measurements.

2. Each of these amino acids reacts with formaldehyde, mole per mole, to form definite compounds. Each of these latter compounds combines with further amounts of formaldehyde when the aldehyde concentration is increased, giving unstable compounds that cannot be isolated.

3. The equilibrium constants of the above reactions have been calculated.

GENEVA, N. Y. RECEIVED FEBRUARY 10, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING AND THE LIGNIN AND CELLULOSE RESEARCH LABORATORIES, UNIVERSITY OF WASHINGTON]

Volatile Hydrogenation Derivatives of Lignin

BY A. BAILEY

A qualitative survey of the effects of hydrogenation of lignin involving the variables of solvent, catalyst and source of lignin was completed. This study indicated the most favorable point of attack for a quantitative study and served as a development and proving ground for apparatus able to effect the extremely difficult separation of compounds in a condition of purity sufficient to permit identification. This report covers the qualitative survey and the first portion of a quantitative study of the hydrogenation products of butanol lignin. The compounds thus far identified were separated by more exacting technique than that reported in any previous investigation. These compounds are largely new hydrogenation products and would not have been separated in the distilling columns used by other workers. As new products, they have constitutional interest.

Catalytic hydrogenation of Willstätter lignin was effected in 1925 by Fierz-David and Hannig.[1] Klason (sulfuric acid) lignin was hydrogenated in 1935 by Moldavskii and Vainshtein[2] and in 1938 by Harris, D'Ianni and Adkins.[3] Many research reports have been published in subsequent years. The hydrogenation reaction has high intrinsic interest and worth since yields are higher than by other reactions, the reaction is inherently one of stabilization rather than disintegration, and the products have been of great constitutional interest and value.

Eight compounds were identified in the present experiments in the fraction of the reaction mixture which boiled below butanol. The yields of these are shown in Table I.

TABLE I

HYDROGENATION PRODUCTS AND YIELDS

Compound	Yield, %
Water	17.9
2,3-Dimethylbutane	0.6
Methyl alcohol	0.2
Tetrahydrofurfuryl alcohol	1.2
Ethyl alcohol	0.3
2,2-Dimethylbutanol	1.0
Secondary butanol	1.2
Methylisopropylcarbinol	0.3

The yield of water was not reported by Harris, D'Ianni and Adkins but 7.5–8.7% was reported by Cooke, *et al*[4]. This value is undoubtedly too low since a Widmer column was used to effect the separation. Cooke did not report efficiency data but according to the data of Baker, Barkenbus and Roswell[5] the H. E. T. P. of a Widmer column

(1) H. E. Fierz-David and M. Hannig, *Helv. Chim. Acta*, **8**, 900 (1925).

(2) B. L. Moldavskii and S. M. Vainshtein, *Khim. Tverdogo Topliva*, **6**, 656 (1935).

(3) E. E. Harris, J. D'Ianni and H. Adkins, THIS JOURNAL, **60**, 1467 (1938).

(4) L. M. Cooke, *et al.*, *ibid.*, **63**, 3056 (1941).

(5) R. H. Baker, C. Barkenbus and C. A. Roswell, *Ind. Eng. Chem., Anal. Ed.*, **12**, 468 (1940).