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THE EFFECT OF CONDITIONS OF HYDROLYSIS AND OF PROLONGED HEATING UPON THE OPTICAL ROTATION OF SULFURIC ACID HYDROLYSATES OF ZEIN*

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Some time ago we observed that a sulfuric acid hydrolysate of zein prepared in an autoclave at 165° did not support growth in young rats when it was supplemented with certain amino acids, even though the same amino acids, added to a hydrolysate prepared by refluxing, permitted moderate growth. Since the optical rotation of the autoclaved hydrolysate was lower than that of the refluxed, either destruction or racemization of essential constituents, or both, might have occurred. Loss of amino nitrogen and production of extra ammonia upon prolonged autoclaving of proteins with acids have been noted by Van Slyke (1912) and others, but simultaneous observations on optical rotation have apparently never been made. To determine whether racemization also takes place, hydrolysates of zein prepared under conditions differing widely with respect to sulfuric acid concentration, temperature, and time were examined for optical rotation, amino nitrogen content, and in some cases for ammonia content.

EXPERIMENTAL

Zein was ground to pass a 40 mesh sieve; it gave a clear, but faintly pigmented solution in 75 per cent alcohol. On the air-dried basis it contained 4.86 per cent of moisture, 0.22 per cent of ash, and 1.29 per cent of ether-extractable material. On an ash- and moisture-free basis the nitrogen content was 15.53 per

* A preliminary report of the data in this paper was presented before the American Society of Biological Chemists at New Orleans, March, 1940 (Borchers and Berg, 1940).

cent; Chittenden and Osborne (1892) found 16.12, and Hoffman and Gortner (1925), 15.33 per cent.

The sulfuric acid solutions (aqueous) were approximately 4, 8, 14, 20, 25, and 33 per cent by volume; normalities by titration were 1.6, 3.1, 5.3, 7.5, 9.5, and 12.6. 10 cc. of acid solution were used in each test together with 1.856 gm. of zein, mixed intimately with 1.86 gm. of pumice and 0.48 gm. of acid-washed charcoal to increase the porosity of the doughy mass formed upon heating and to clarify the hydrolysate. The heating was conducted in an oil-jacketed thermoregulated autoclave at temperatures ranging from 120–200°, as well as under a reflux, for 1 to 60 hours. The autoclave was preheated to 100°; the recorded time of heating included the period of temperature rise above that point (about 1 hour for each 80°), but not the period of cooling.

The hydrolysates were filtered by suction while hot. Polarimetric readings were made at room temperature in a Schmidt-Haensch polariscope with a 1 dm. tube and a sodium vapor lamp. Correction for variations in temperature was found unnecessary between 20–40° (note also Winnick and Greenberg (1941)). Amino nitrogen was estimated according to Van Slyke, ammonia by titration of the distillate from an aliquot of the hydrolysate made alkaline with magnesium oxide, and total nitrogen (as a basis for comparisons) by the Kjeldahl method.

Fig. 1 presents the data on optical rotation and amino nitrogen obtained on the several hydrolysates prepared with 20 per cent sulfuric acid. Complete hydrolysis was marked by values of 2.5° ± 0.1°, 71 ± 1 per cent, and 20 ± 1 per cent for optical rotation and amino and ammonia nitrogen, respectively. Calculated on the same basis, the data of Hoffman and Gortner (1925) gave 70.1 and 18.06 per cent for amino and ammonia nitrogen. For ammonia nitrogen Osborne and Harris (1903) report 18.41, and Gortner and Blish (1915) 20.75 per cent.

In all instances changes in rotation approximately paralleled changes in amino nitrogen until maximum values for both were attained. Refluxing for 60 hours (36 hours beyond completion of hydrolysis) caused no appreciable decrease from either maximum. The observations on hydrolysis under a reflux are consistent with data obtained on casein during 33 hours of refluxing with hydrochloric acid (Winnick and Greenberg, 1941). A period several

days longer would probably have induced some change (Gortner and Holm, 1917). Heating in the autoclave much beyond the time required for complete hydrolysis caused decreases from

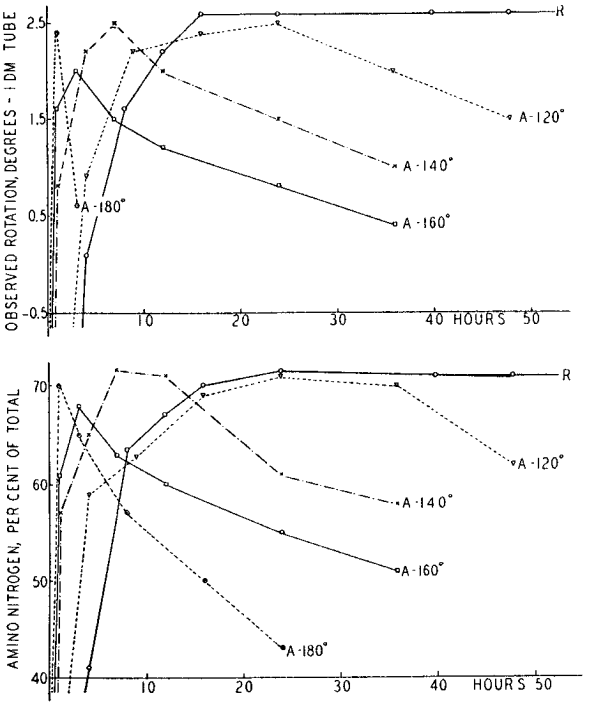

Fig. 1. Changes in optical rotation and amino nitrogen content upon heating zein with 20 per cent aqueous sulfuric acid solution. The curves connect points representing analytical results obtained on hydrolysates prepared by boiling under the reflux (*R*), or by heating in the autoclave (*A*) at the temperature specified, for various periods of time. "Observed rotations" are based on concentrations of 27.5 mg. of N per cc. The 1 hour hydrolysates prepared under a reflux and in the autoclave at 120° showed −5.3° and −3.6°; both yielded 29 per cent of amino nitrogen. Autoclaving at 200° for 2, 5, and 15 hours yielded 68, 58, and 50 per cent of amino nitrogen; the hydrolysates were too deeply pigmented for polarimetric observation, as were also those autoclaved at 180° for more than 3 hours.

both maxima. Increases in ammonia content accounted largely, though apparently not completely, for the decreases in amino nitrogen. Measured in relation to maximal values, the decreases

in rotation were proportionately greater than the decreases in amino nitrogen content. This divergence can logically be attributed to racemization.

The periods required for complete hydrolysis with 14, 25, and 33 per cent sulfuric acid under a reflux were approximately 30, 12, and 5 hours, respectively; in the autoclave at 120°, about the same; at 140°, 8, 6, and 3 hours; at 160°, 4, 2, and 1 hour. At 180° hydrolysis was complete in 1 hour with all concentrations of acid. In no instance did appreciable racemization or destruction occur in the 36 to 60 hours under the reflux or before completion of hydrolysis in the autoclave; continued autoclaving beyond that point induced both. The proportionate decreases in amino nitrogen and in optical rotation and the divergence between them were more marked at the higher temperatures; increase in acid concentration apparently had less influence than increase in temperature. Maximum amino nitrogen content and maximum optical rotation were never attained with concentrations of 4 and 8 per cent sulfuric acid; on prolonged heating at elevated temperatures excessive ammonia production occurred.

SUMMARY

During the course of the hydrolysis of zein with aqueous sulfuric acid, 14 to 33 per cent by volume, either under a reflux or in the autoclave at 120–180°, no appreciable racemization or destruction of amino acids occurred. Prolonging the refluxing to 36 to 60 hours had little or no effect, but autoclaving longer than necessary for hydrolysis induced both racemization and destruction, more markedly so at the higher temperatures. Concentrations of sulfuric acid as low as 8 per cent by volume are apparently not suitable for the complete and uncomplicated hydrolysis of zein.

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