THE REACTION OF NINHYDRIN WITH KETO-ACIDS*

E. D. Schilling, P. I. Burchill, J. R. Coffman and R. A. Clayton

General Mills, Inc., Golden Valley Research Center
Minneapolis 27, Minnesota

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In connection with our studies of the amino acid composition of cereal grains, our elution patterns, as obtained with an automatic amino acid analyzer[1], have repeatedly suggested that these hydrolysates contain several previously unreported amino acids. These peaks have been found in chromatograms of acid hydrolysates of lactalbumin, soybean meal, and oat and wheat flours. Of particular interest was the observation that of these anomalies, the major component exhibited an absorption maximum at 440 mμ, - a maximum generally reserved for proline and hydroxyproline. Since these peaks appear before aspartic acid in the Spackman, Moore and Stein elution sequence (1958), it was reasoned that the putative compounds would be less basic in nature than aspartic acid.

In a personal communication[2], we were advised that levulinic acid might react "positively" to ninhydrin. Subsequent study showed that levulinic acid, which could be detected in micromole quantities, was present as an artifact in our hydrolysates and represented the major anomaly in these elution patterns. (Presumably, levulinic acid was derived from starch

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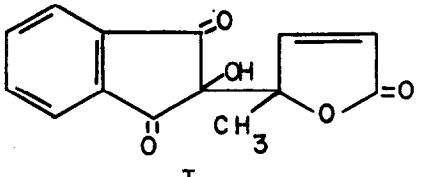
1 Phoenix Precision Instrument Co. Model K5000

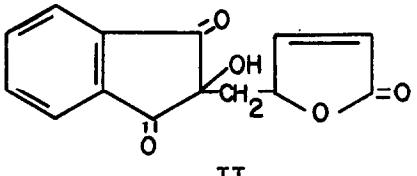
2 R. H. Anderson, General Mills, Inc.

during the acid hydrolysis of the impure protein). In addition to levulinic acid, it was observed that pyruvic acid and α-keto-glutaric acid also react with ninhydrin and appear as early peaks in our chromatograms. Figure I shows a typical elution sequence; pyruvic acid, which is eluted with α-ketoglutaric acid, was not included in this figure.

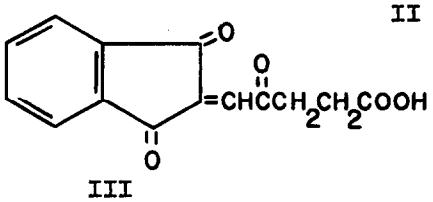
Since, to our knowledge, the reaction of ninhydrin with non-nitrogenous compounds has not been reported, an investigation of the ninhydrin-levulinic acid reaction was undertaken. In a typical experiment, aqueous solutions of levulinic acid (pH 5.2) and ninhydrin (3:1 molar ratio) were heated for 30 minutes at 100°. The wine-red reaction mixture was subjected to continous ether extraction and the concentrated ether extract chromatographed on a Celite-545 [3] column. Elution was effected by a stepwise gradient from Skellysolve B[4] to chloroform. Cooling of the concentrated Skellysolve B:chloroform eluate resulted in the crystallization of colorless needles. The material was recrystallized from absolute ethanol, m.p. 148-149°. Analytical data calculated for $C_{14}H_{10}O_5$: C, 65.11%; H, 3.90%. Found: C, 65.34%; H, 3.96%. The following structures are compatible with the analytical data:



I



II



III

[3] Johns-Manville Co.
[4] Petroleum ether, b.p. 60-72°, Skelly Oil Co.

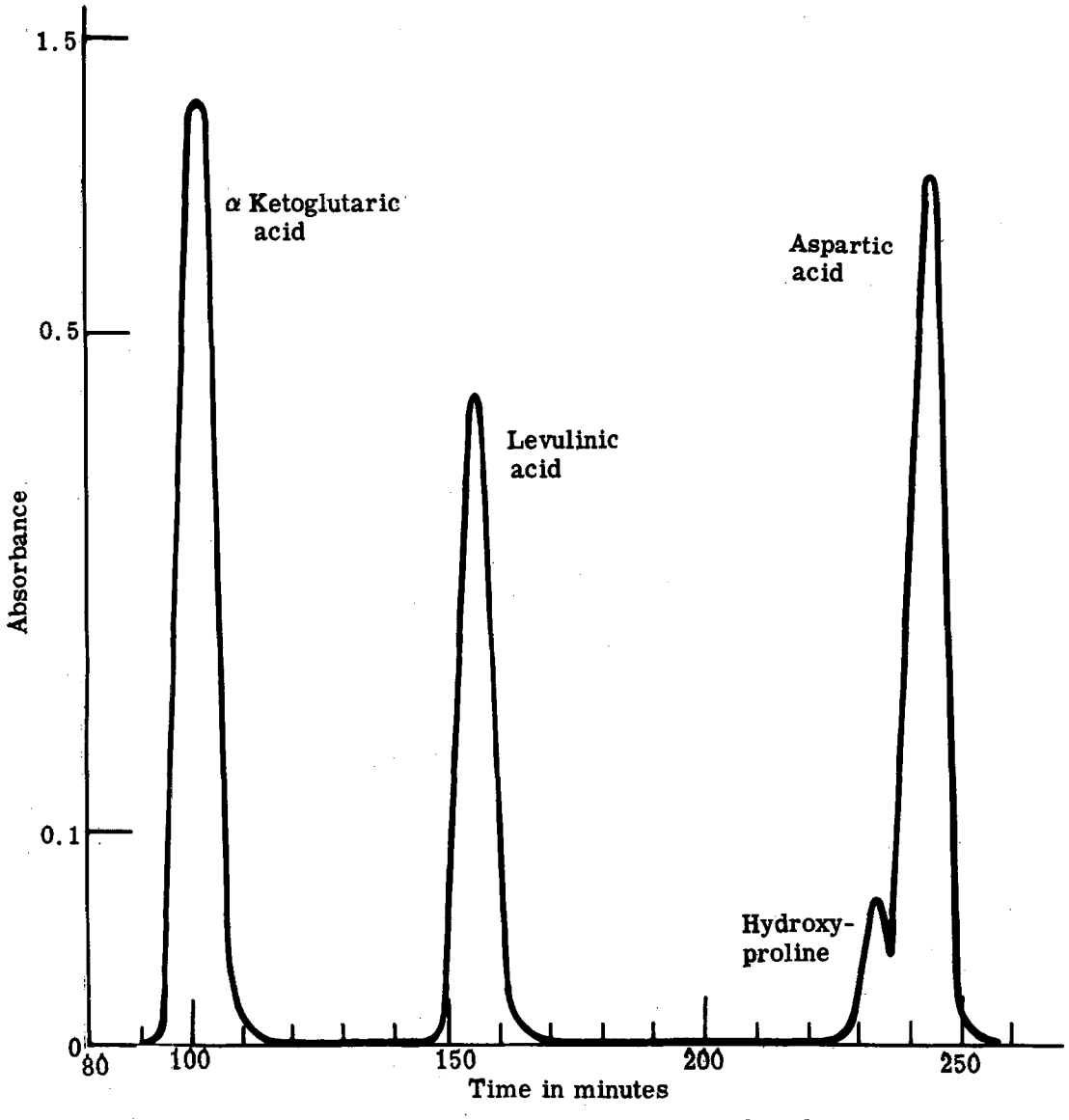


Fig. 1. Elution sequence of levulinic acid and α-ketoglutaric acid as determined with an automatic amino acid analyzer[1]. The 150 cm. column was charged with 100 μmoles of α-ketoglutaric acid, 50 μmoles of levulinic acid and 1 μmole each of hydroxyproline and aspartic acid. The chromatogram was developed with 0.2 N sodium citrate at pH 3.25; column temperature, 50°.

Aspartic acid was detected at 570 mμ; hydroxyproline and the keto acids were detected at 440 mμ.

At this writing our infrared data suggest the presence of a lactone ring and would favor compound (I). Final proof of structure must await the results of derivative study.

In addition to the colorless compound described above, chromatography of the ninhydrin-levulinic acid reaction mixture has led to the isolation of several colored products. The characterization of these compounds is now underway.

REFERENCES

Spackman, D. H., Stein, W. H. and Moore, S., Anal. Chem., 30, 1190 (1958).