## Letter to the Editor

Dear Sir,

We wish to comment on the paper *Artifacts in Amino Acid Analysis. Ninhydrin-positive Products of Carbohydrate Hydrolysis*, by I. E. P. TAYLOR (*J. Chromatogr.*, 50 (1970) 331.

As early as 1953, SCHRAM *et al.*[1] described a red peak which eluted early in their amino acid analyses. They noted that foods containing starch, cellulose, glucose or lactose gave this anomalous peak with ninhydrin. It was shown that this peak (which they intimated might be levulinic acid) could be produced from carbohydrates in the absence of amino acids under conditions of acid hydrolysis. Subsequently, ZACHARIUS AND TALLEY[2] isolated a compound from acid hydrolysates of the non-protein nitrogen fraction of potato tubers and from bulk protein of kidney bean seeds that gave a similar red peak, which was unequivocally identified as levulinic acid. SCHILLING *et al.*[3] also identified the levulinic acid peak in amino acid chromatograms of crude protein hydrolysates.

Studying other anomalous peaks on amino acid analyzer chromatograms, ZACHARIUS AND PORTER[4] isolated from an unhydrolyzed extract of tart cherries material responsible for a high 440-nm peak with ninhydrin which they identified as fructose and glucose. The "positive" behavior of a large number of carbohydrates and related non-nitrogenous compounds with ninhydrin has been described[4–6].

More recently, products of the degradation of carbohydrates have been implicated to explain some anomalous peaks on the amino acid chromatograms of wood proteins[7]. A warning was extended on the potential hazard of interpreting peaks resulting from ninhydrin and high levels of carbohydrates and their hydrolytic products as products of imino acids[2,4,5].

In his paper, TAYLOR has indicated that some of his artifact peaks, which arose from the strong acid hydrolysis of pea seed coats, could also be demonstrated with sucrose under similar hydrolytic conditions. Such artifacts had also been demonstrated with nitrogen-free glucose and starch[2,8] using acid conditions similar[8] to those of TAYLOR. Although based on only the limited data provided, we conclude from our earlier work that TAYLOR's artifact peak No. 2 is almost certainly levulinic acid. The almost equal 440-nm and 570-nm absorbance of the ninhydrin reaction product is a characteristic of levulinic acid and some of its synthetic derivatives. While TAYLOR's artifact peak No. 1 might possibly be attributed to fructose, it and the remaining artifacts are more likely related to the isomeric angelicalactones, particularly products of the more unstable $\beta,\gamma$-angelicalactone[2]. The angelicalactones can be produced from levulinic acid.

*Eastern Marketing and Nutrition Research Division,*
*Agricultural Research Service, U.S. Department of Agriculture,*
*Philadelphia, Pa. 19118 (U.S.A.)*

R. M. ZACHARIUS
E. A. TALLEY
W. L. PORTER

1 E. SCHRAM, J. P. DUSTIN, S. MOORE AND E. J. BIGWOOD, *Anal. Chim. Acta*, 9 (1953) 149.
2 R. M. ZACHARIUS AND E. A. TALLEY, *J. Chromatogr.*, 7 (1962) 51.

3 E. D. SCHILLING, P. I. BURCHILL, J. R. COFFMAN AND R. A. CLAYTON, *Biochem. Biophys. Res. Commun.*, 5 (1961) 217.
4 R. M. ZACHARIUS AND W. L. PORTER, *J. Chromatogr.*, 30 (1967) 190.
5 E. D. SCHILLING, P. I. BURCHILL AND R. A. CLAYTON, *Anal. Biochem.*, 5 (1963) 1.
6 C. L. HU, V. V. RENDIG AND E. A. MCCOMB, *J. Chromatogr.*, 19 (1965) 622.
7 G. SCURFIELD AND P. W. NICHOLLS, *J. Exp. Bot.*, 21 (1970) 857.
8 J. P. DUSTIN, C. CZAJKOWSKA, S. MOORE AND E. J. BIGWOOD, *Anal. Chim. Acta*, 9 (1953) 256.