Colorimetric Determination of Aconitic Acid in *Avena sativa* (Oat)

The colorimetric method for aconitic acid in sorgo juice was extended to include the oat plant. Acid levels varied between 0.21% and 1.18%. The oat plant aconitic acid level was studied extensively due to a high rate of hypomagnesasmia in lactating ruminants while grazing. Aconitic acid is considered by many to be responsible for this disease. The aconitates are extracted from the dried ground plant material with water. After lowering the extract pH to 1.3, the acid is extracted with 2-butanone. The addition of acetic anhydride and pyridine produces a purple color which is read on a spectrophotometer.

During our investigation of aconitic acid (1,2,3-propenetricarboxylic acid) in sorgo juice, a colorimetric method was developed (Poe and Barrentine, 1968) which subsequently was applied to the analysis of the acid in the oat plant. The importance of aconitic acid in forage materials was initiated by its potential effect on ruminants as a possible cause of grass tetany as shown by Camp *et al.* (1968) and Bohman *et al.* (1969). These researchers experimentally induced hypomagnesemia in ruminants by feeding KCl plus either *trans*-aconitic or citric acid. *Trans*-aconitate is known to be a metabolic inhibitor of aconitase, thereby blocking the conversion of citric to isocitric acid in the Kreb cycle. Citrates and aconitates both form chelates with magnesium and calcium. These complexes could possibly be unavailable for use by the animal.

A rapid and sensitive technique for aconitic acid in plant material seems desirable in view of the widespread distribution of this acid and its possible relationship to animal health. This paper describes an extension of the colorimetric method to include aconitic acid assay in the oat plant.

EXPERIMENTAL

Apparatus. Ion exchange column chromatography using columns of Dowex 1X8, 100- to 200-mesh, was used to separate aconitic acid from other organic acids. Positive identification was obtained on Mallinckrodt's ChromAR sheets using ethanol, ammonium hydroxide, and water as the mobile phase. The chromatograms were viewed under shortwave ultraviolet light. The pyridine-acetic anhydride-aconitic acid complex was read on a Coleman Model 14 spectrophotometer at 550 mμ.

Extraction and Determination of Aconitic Acid. Plant material was dried 8 hr at 100° C and ground to pass a 40-mesh sieve. One gram was shaken 30 min with 50 ml of water and filtered with suction using Celite filter aid. The pH of the extract was lowered to 1.3 with sulfuric acid solution to free the acids and extracted with 2-butanone. The color development and standard curve are identical to that described by Poe and Barrentine (1968). For the recovery, aconitic acid was added to 1 g of dried oat plant and extracted as shown above.

Column and Thin-Layer Chromatographic Identification. One gram of dried forage sample was extracted with 25 ml of water and filtered. An aliquot was added to a 10 × 280 mm column of Dowex 1X8, 100- to 200-mesh ion exchange resin in the acetate form. The acids were eluted with 100 ml of 3N acetic acid, 100 ml of 6N acetic acid, and 200 ml of 6N formic acid. Forty 10-ml fractions were collected, evaporated to dryness on a steam bath using a stream of air, and titrated to the phenol red endpoint with 0.02N NaOH. Aconitic acid appeared in fractions 24 through 28, the same fractions in which standard *trans*- and *cis*-aconitic acid solutions appeared.

Prior to titration, aliquots of the aconitic acid fractions were spotted on Mallinckrodt's ChromAR sheets and developed in ethanol-ammonium hydroxide-water (75:12½:12½). When viewed under shortwave ultraviolet light, the spots were compared with standards of *trans*- and *cis*-aconitic acid. R_f values were 0.32 for *trans*- and 0.20 for *cis*-aconitic acid.

RESULTS AND DISCUSSION

The data for the recovery of *trans*-aconitic acid in the oat plant is shown in Table I. No acid could be detected in the oats if the pH of the extract was not lowered, indicating no free acid. When the pH of the solution was lowered to 1.3

Table I. Recovery of Aconitic Acid from Oat Plant

Aconitic Acid, mg/gm Tissue		Recovery
Added	Found	%
0	3.2	...
2	5.7	108.6
4	7.1	98.6
6	8.7	94.6
8	11.0	98.2
10	12.3	93.2
12	13.3	87.5
16	19.3	100.3

prior to extraction, the same quantity of aconitic acid was extracted. Average percent recovery was 97.3, indicating the completeness of one extraction.

The colorimetric method does not differentiate between *cis*- and *trans*-aconitates. However, no *cis*-aconitic acid could be seen on the thin layer sheets. This is in agreement with Stout *et al.* (1967) who reports the level of *cis*-aconitates to be 0.01% or less.

To study the effect of drying on loss of aconitic acid, freshly cut oat forage was analyzed before and after drying. No significant decrease in aconitic acid was observed.

Ion exchange column chromatography has long been used successfully for the separation and identification of organic acids in plant materials. An examination of Table II would indicate that the proposed method apparently gives satisfactory results as compared to the ion exchange method.

Table II. Comparison of Ion Exchange Column Chromatography and Colorimetric Method for Total Aconitic Acid[a]

	Total Aconitic Acid, mg/gm	
Sample No.	Ion Exchange	Colorimetric
1	5.8	5.5
2	6.7	6.0
3	12.8	11.8
4	2.1	2.4
5	5.8	6.1
6	5.3	5.2
7	4.0	4.0
8	2.0	2.1
9	6.5	6.4
10	3.8	4.7

[a] Samples 1–5: oat plant; 6–10: Sorgo juice.

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Received for review November 28, 1969. Accepted March 16, 1970