

the values above 3. The data in Table II can serve as a useful means for comparative ranking until a better system can be contrived. Thus the extractable chromium content per gram of food items on a fresh weight basis of the edible portion as prepared for analysis was used. The data were arranged in descending order and showed most of the grain and cereal products in the middle group.

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#### LITERATURE CITED

- Dean, J. A., Beverly, M. L., *Anal. Chem.* **30**, 977 (1958).  
 Mertz, W., *Physiol. Rev.* **49**, 163 (1969).  
 Roginski, E. E., Toepfer, E. W., Polansky, M. M., Mertz, W., *Fed. Proc. Abstr.* 695 (1970).  
 Roginski, E. E., Toepfer, E. W., Polansky, M. M., Mertz, W., *Fed. Proc. Abstr.* 238 (1971).  
 Toepfer, E. W., Mertz, W., Roginski, E. E., Polansky, M. M., Abstracts, 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept. 12-17, 1971, AGFD No. 42.  
 Wolf, W. R., Taylor, M. L., Hughes, B. M., Tiernan, T. O., Sievers, R. E., *Anal. Chem.* **44**, 616 (1972).

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## Partition Column Chromatography for Quantitating Effects of Fertilization on Plant Acids

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A method for determining organic acid levels in biological materials by silica gel partition column chromatography (pcc) is described. It uses tetrabromophenolphthalein ethyl ester, which has a high molar absorptency, for quantitating the acids in the column effluent. The concentrations of several organic acids were determined in two species of crested wheatgrass, Nordan (*Agropyron desertorum*) and Fairway (*A. cristatum*), grown under different conditions of K fertilization. For comparison, aconitic, citric, and malic acids were also determined by polarography, spectrophotometry, and fluorometry, respectively. Results obtained by these methods agreed with the data ob-

tained by pcc. The concentration of *trans*-aconitic acid averaged about 96 and 21 mequiv/kg of dry matter in Nordan and Fairway, respectively, when grown in the greenhouse without K. K fertilization (312 kg/ha) approximately doubled the *trans*-aconitic acid concentration in both species. Fairway contained 363 mequiv/kg of malic acid, while Nordan contained 280 mequiv/kg. K fertilization increased these concentrations to 611 and 446 mequiv/kg, respectively. Citric acid was increased by K fertilization, but there was no significant difference between species and in no case was the citric acid concentration greater than 80 mequiv/kg of dry matter.

The influence of fertilization on organic acid concentrations in plants has been a subject of considerable interest in recent years (Grunes *et al.*, 1970). In a field study of southern Indiana, Teel (1966) found that fertilization with  $\text{NH}_4\text{NO}_3$  at the rate of 168 kg N/ha increased the concentration of total organic acids in tall fescue (*Festuca arundinacea*). Such elevated concentrations of organic acids are believed to cause toxic responses in animals (Burau and Stout, 1965; Grunes *et al.*, 1970; Stout *et al.*, 1967). For instance, grass tetany has occurred in animals grazing California spring grasses high in *trans*-aconitic acid (Burau and Stout, 1965; Stout *et al.*, 1967). Stout *et al.* (1967) suggested that this acid was forming complexes with Mg which decreased the availability of  $\text{Mg}^{2+}$  to the animal and that 1% *trans*-aconitic acid (172 mequiv/kg dry matter) was potentially toxic. Additional evidence for the role of organic acids in grass tetany was provided by

Bohman *et al.* (1969), who produced toxic symptoms in cattle by oral administration of KCl with *trans*-aconitic acid or citric acid. In later work, Scotto *et al.* (1971) found that citric acid in the blood of cattle rose as the quantity of KCl, combined with an oral dose of citric acid, was increased.

The above evidence has emphasized the need for analytical methods capable of rapidly quantitating organic acids in biological material. Enzymatic assays (Bergmeyer, 1963), gas chromatography (Alcock, 1969; Clark, 1969; Hautala and Weaver, 1969), thin-layer chromatography (Myers and Huang, 1966), paper chromatography (Barnes *et al.*, 1970; Kennedy and Barker, 1951), polarography (Burau, 1969), and silica gel partition column chromatography (Barnes *et al.*, 1970; Bulen *et al.*, 1952; Dijkshoorn and Lampe, 1962; Kesner and Muntwyler, 1966, 1969a,b; Marvel and Rands, 1950; Neish, 1949; Rosevear *et al.*, 1971) have been used. For our studies we have modified the silica gel partition column chromatography method (pcc) developed by Kesner and Muntwyler (Kesner, 1965; Kesner and Muntwyler, 1966; Kesner and Muntwyler, 1969b). It has also been successfully employed for analyses of organic acids in excreta and tissues of animals. Presented herein are data for organic acid concentrations in crested wheatgrass grown in the greenhouse and in pastures of the western United States. Results obtained with pcc are compared with those obtained by other established methods.

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## MATERIALS AND METHODS

Analytical grade reagents are required.

**Silicic Acid** (Mallinckrodt Chemical Works, St. Louis, Mo. 63169). Between December 1969 and October 1971, different lots of silicic acid (100 mesh, suitable for chromatographic analysis) (Ramsey and Patterson, 1945) were found to vary considerably in particle size and hydration properties, although Kesner and Muntwyler (1969b) apparently found the 100-mesh silicic acid satisfactory without further treatment. We found that lot VHE was satisfactory for nearly 2 years if treated as follows. At least 800 g were loaded upon a 200-mesh screen and shaken mechanically at 180 oscillations/min for 30 min. The material passing through the screen was dried to constant weight at 110° and stored at this temperature. More recently, we have used lot YHN, which is satisfactory if about 900 g are rolled in a ball mill (23 cm diameter) at 8 rpm for 19 hr and dried to constant weight as above. This treatment yields a preparation with 50% of the particles ranging in size from 1 to 5  $\mu$ , 25–30%, from 5 to 10  $\mu$ , and the remainder, 10 to 30  $\mu$ .

**Ethyl alcohol** was obtained by distillation from 95% ethanol.

**Indicator Solution.** Potassium tetrabromophenolphthalein ethyl ester (0.80 g; Eastman No. 7083) is dissolved in 1 l. of redistilled ethanol. The pH of the redistilled ethanol should be 7.0–7.2. A more alkaline solution decreases the sensitivity of the indicator. This indicator solution may be stored at room temperature without deterioration (Hautala and Weaver, 1969; Kesner and Muntwyler, 1966; Kesner and Muntwyler, 1969b).

## APPARATUS

A schematic representation of the apparatus is presented in Figure 1.

One requirement in assembly is that the receptacles containing the incoming and outgoing fluids be above the pumps.

The following equipment was used, the letters referring to Figure 1: (a) Glass and Teflon varigrad (Model 3-6005,

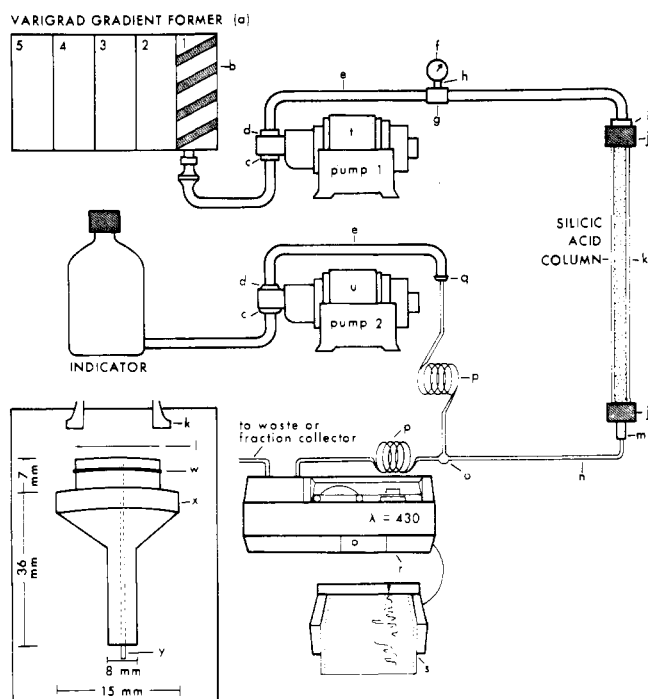
Buchler Instruments, Ft. Lee, N. J.); (b) Heating tape (DET-0.5-6, 1.3 cm  $\times$  1.8 m, 210w, 70v, Glas-Col Apparatus Co., Terre Haute, Ind.), controlled by variable transformer (Staco, Type 3 PM 751, 7.5 A, Scientific Products, Evanston, Ill.); (c) 200-2-2-316 elbow fitting; (d) 200-1-2-316 straight fitting; (e) Teflon tubing (TFE  $\frac{3}{32}$  in. i.d.  $\times$   $\frac{1}{8}$  in. o.d., Chemplast, Inc., 150 Dey Rd., Wayne, N. J. 07470); (f) Pressure gauge (Type 3/6 stainless steel, 0 to 4.14  $\times 10^6$  N/m<sup>2</sup> (0–600 psi), Beckman Instruments, Inc., Palo Alto, Calif. 94304); (g) 200-3-316 tee connector; (h) 3.2 mm ( $\frac{1}{8}$  in.) pipe coupling with 200-1-2-316 straight fitting; (i) Reducing unions, 400-6-2-316 and 500-6-4-316 connected by stainless steel tubing 6.4 mm ( $\frac{1}{4}$  in.) o.d.  $\times$  2.5 cm (1 in.) length; (j) Penton coupling (=687-008) for 5-mm column with =274-761 plain fitting for 5-mm column and =571-158 Tef-seal with O-rings (Fisher and Porter Co., Warminster, Pa.); (k) Column extender, (5 mm i.d.  $\times$  500 mm =274-738, Fisher and Porter Co.); (l) Three circular pieces of =42 Whatman filter paper (5 mm diameter); (m) Teflon fitting (see insert in Figure 1); (n) Chemflur 18 gauge thin-wall spaghetti teflon tubing (Chemplast, Inc., Wayne, N. J.); (o) Three-way connector, TC 18/3 (Small Parts, Inc., 6901 NE Third Ave., Miami, Fla. 33138); (p) Coil of tubing 7.6 m [see (n) above]; (q) Reducing union (400-6-2-316) holding a 6.4-mm diameter teflon rod with 0.2-mm diameter hole in center and 18 gauge stainless steel tube at exit; (r) Beckman DB-G spectrophotometer (Beckman Instruments, Palo Alto, Calif.) with flow cell Model 9120–No. 5 with 10-mm light path and 0.25-ml volume (A. H. Thomas Co., Philadelphia, Pa.); (s) Recorder; (t) Pump (Model 19-60042-001, maximum cap. 240 ml/hr with Viton O-rings; Milton Roy Co., 5000 Park Street N, St. Petersburg, Fla.); (u) Pump (Model 19-60029-001, with Viton O-rings, Milton Roy Co.); (v) Not illustrated are a 24-hr timer which is used to stop equipment at predetermined times and two multi-outlet electrical boxes; (w) Viton O-ring (6 mm i.d.  $\times$  95 mm o.d.); (x) Fitting machined from 1.6-cm teflon rod; (y) Stainless steel tubing (18 gauge), fitted in central channel.

## PROCEDURE

**Solvent System.** The gradient of solvents is freed of air to prevent column disruption and to facilitate accurate metering by pumps. This is accomplished by maintaining Chamber 1 of the gradient former just below the boiling point of chloroform. Complex mixtures of acids are separated by a 5-chamber concave gradient which is formed as the mobile phase solvent is pumped through the column. Formation of the gradient is facilitated by filling Chamber 1 with 49 ml of chloroform plus 1 ml of H<sub>2</sub>O. Chambers 2 and 3 contain 52.2 ml of 7% (v/v) *tert*-amyl alcohol–chloroform; Chamber 4 contains 57.7 ml of 30% (v/v) *tert*-amyl alcohol–chloroform; and Chamber 5 contains 64.4 ml of 50% (v/v) *tert*-amyl alcohol–chloroform. A sixth chamber is filled the same as Chamber 5 to prevent entry of air into the system near the end of a particular chromatographic determination. The weight of fluid in Chambers 2 through 6 equals the weight of chloroform plus H<sub>2</sub>O in Chamber 1. The gradient which results is suitable for a 40-cm column of hydrated silicic acid with an input of 60 ml/hr. Input pressure is 1.38  $\times 10^6$  to 1.72  $\times 10^6$  N/m<sup>2</sup> (200–250 psi) initially, and may increase to 2.07  $\times 10^6$  N/m<sup>2</sup> (300 psi) near the end of the chromatogram.

**Column Preparation.** The silicic acid is hydrated by slowly adding 59 ml of 0.1 N H<sub>2</sub>SO<sub>4</sub> to 92 g of oven-dried silicic acid in a 500-ml screwcap jar while stirring with a heavy glass rod until aggregates disappear. Some 6 mm of glassbeads are added, the cap is replaced tightly, and the jar with contents is rotated at 30 rpm in a horizontal position for several hours.

Three circular pieces of No. 42 Whatman filter paper (5



**Figure 1.** Organic acid analyzer. See under Apparatus for materials used.

mm diameter) are placed on the machined Teflon fitting which is held in place by clamps at the bottom of the column (Figure 1). The 50-cm column is "dry packed" with 6.0 g of hydrated powder poured *via* a funnel, while the column is vibrated with an electric vibrator equipped with a rubber tip. Vibration is continued until the weighed quantity of silicic acid fills the glass column. Before samples for analysis are applied, the silicic acid is washed with H<sub>2</sub>O-saturated 20% (v/v) *tert*-amyl alcohol-chloroform pumped through the column for 8 min, followed by pure H<sub>2</sub>O-saturated chloroform for 15 min, both at 60 ml/hr. This procedure yields a 40-cm column of usable silicic acid.

Chloroform remaining above the silicic acid is removed with a syringe and the inside of the glass column is dried with a gentle stream of air. The plant extract which has been acidified with one or two drops of 6 H<sub>2</sub>SO<sub>4</sub> is mixed with oven-dried silicic acid to form a dry free-flowing mixture. It is then placed on top of the chloroform-wetted column and packed by vibration. At the beginning of the analysis, 6 ml of CCl<sub>4</sub> is pumped through the column, followed by 20 ml of H<sub>2</sub>O-saturated chloroform. Pumping of the gradient is then initiated.

The column effluent is mixed with an excess of indicator solution delivered by the second pump. When acid is present, the indicator salt is converted to its hydrogen form. Absorbance is continuously monitored by a recording photometer at 430 nm. Approximately 5 hr are required to obtain a complete chromatogram. A new column must be packed for each analysis.

**Calibration.** Mixtures of known organic acids are prepared in a 50% (v/v) acetone-water mixture, from stock solutions (0.01 N), by combining 1- to 10-ml aliquots of each acid diluted to a known volume. It is possible to detect 0.01 to 0.3  $\mu$ equiv of an individual acid in a mixture. Quantitation is accomplished by standard triangulation techniques.

**Extract of Organic Acids in Plant Tissues.** Five-hundred milligrams of freeze-dried plant material are weighed directly into a 50-ml centrifuge tube, 20 ml of distilled water are added, and the tube is immersed in boiling water for 30 min with minimum delay to avoid losses from enzyme activity. The light brown liquid is filtered into a 100-ml volumetric flask through Whatman No. 41 filter paper (11 cm), keeping the quantity of solid material transferred to the filter paper at a minimum. Fifteen milliliters of distilled H<sub>2</sub>O are added to the centrifuge tube, which is again placed in boiling water for 15 min. In most cases, the plant sample residue will be held together by precipitated proteins. About one-half of the liquid is transferred to the filter paper used previously. Then the centrifuge tube is shaken and the plant material and the remainder of the liquid are transferred to the same filter paper. Following filtration the filter paper is removed from the funnel, folded, and returned to the centrifuge tube. Fifteen milliliters of distilled H<sub>2</sub>O are added, the tube is immersed in boiling water for 15 min, and the liquid is filtered through a new filter paper into the volumetric flask. Two extractions with 15 ml of boiling H<sub>2</sub>O follow and these are filtered as above. The volume in the volumetric flask is adjusted at room temperature with distilled H<sub>2</sub>O. The extract may be stored at 4° in a tightly capped bottle with 1 ml of chloroform. For analysis, 0.10–0.25 ml of the aqueous phase are applied to the column. Complete extraction of organic acids from plant tissues has been obtained by this procedure. Other biological samples such as liver may be ground in liquid nitrogen and applied directly to the column without extraction. Urine and blood plasma or serum may be chromatographed directly.

#### Determination of Citric, Malic, and Aconitic Acids

**by Other Methods.** Samples of crested wheatgrass, fescue, bromegrass, and perennial ryegrass grown under various conditions were analyzed by pcc. For comparison total aconitic acid was also determined by a modification (Patterson *et al.*, 1972) of the polarographic method of Burau (1969), citric acid by colorimetry (Camp and Farmer, 1967), and malic acid by fluorometry (Lowry *et al.*, 1954).

**Experimental.** Nordan (*Agropyron desertorum*) and Fairway (*Agropyron cristatum*) crested wheatgrass were grown, in the greenhouse, in a mixture of 50% silt loam soil and 50% coarse sand. After a 63-day growth period, the plants were cut above the soil surface. Four days later, two fertilizer treatments were applied. One consisted of NH<sub>4</sub>NO<sub>3</sub> applied at 112 kg N/ha (100 lb N/A); the second was KNO<sub>3</sub> at 312 kg K/ha (278 lb K/A) with N at 112 kg N/ha. There were five replications. The grass was harvested for organic acid analysis after 35 days of regrowth.

**Statistical Analyses.** The data were analyzed by analysis of variance (Steel and Torrie, 1960). Treatment differences were tested using the LSD to test paired means (Steel and Torrie, 1960).

#### RESULTS AND DISCUSSION

The pcc method offers significant advantages for studying organic acid composition of biological samples. It can be automated, requires little sample preparation, and can be used to measure the incorporation of labeled precursors. This procedure has been used successfully to analyze organic acids in silages, rumen contents, blood, liver, kidney, and urine (Prior *et al.*, 1971; Prior and Visek, 1970). In the present system, improvements were introduced by use of: an indicator with increased molar absorptivity; a column with decreased diameter (5 mm) and an improved design of effluent fitting; column effluent lines of 1 mm inside diameter; silicic acid of decreased particle size, ranging from 5 to 30  $\mu$ , H<sub>2</sub>O-saturated organic solvents to prevent column dehydration during the run; and silicic acid washed free of acid contaminants, which gives a flat baseline for the chromatogram tracing. The use of potassium tetrabromophenolphthalein ethyl ester as an indicator increased the sensitivity about two times, as compared to neutral red (3-amino-7-dimethylamine-2-methylphenazine) (Rosevear *et al.*, 1971) and about 30 to 40 times compared to *O*-nitrophenol (Henderson and Jones, 1970). The areas of the chromatographic peaks for most acids are about 100 absorbance seconds per 1  $\times$  10<sup>7</sup> carboxyl group equivalents. Low peak areas were obtained for propionic, acetic,  $\beta$ -OH-butyric, and *cis*-aconitic acids. Based upon our experience, the present method is less tedious than the gas chromatography techniques and the results are more reproducible. Both methods require about the same total time for preparation of each sample and analysis.

There was excellent agreement for data obtained in independent laboratories when pcc was compared to polarographic, colorimetric, or fluorometric methods for total aconitic (*cis* plus *trans*), citric and malic acids, respectively (Figures 2, 3, and 4). The correlation coefficients between data obtained by pcc and corresponding chemical methods were 0.94, 0.96, and 0.97 for total-aconitic acid, malic, and citric acids, respectively (Figures 2, 3, and 4). With pcc, each of 13–15 known acids were analyzed in mixtures at least 10 times and some were analyzed 30 times. The maximum coefficients of variation for any one ranged from 4 to 11%. A sample chromatogram for known acids and one for crested wheatgrass are shown in Figure 5. In Table I, the total organic acid values were calculated by summing the quantities of all the acids detected on the partition column chromatogram. In the sample of Nordan, grown at 312 kg K/ha, this included an estimate of 0.9 mequiv/kg of an unidentified acid which appeared be-

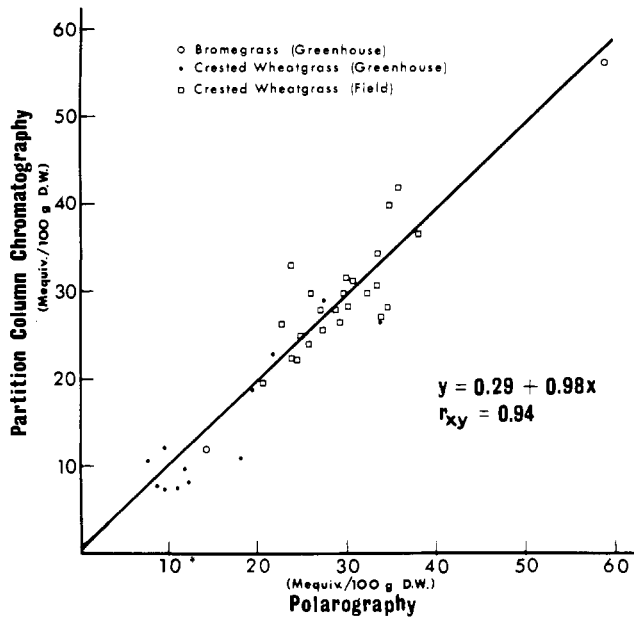


Figure 2. Total of *cis*- plus *trans*-aconitic acid in crested wheatgrass and bromegrass as determined by polarography and partition column chromatography.

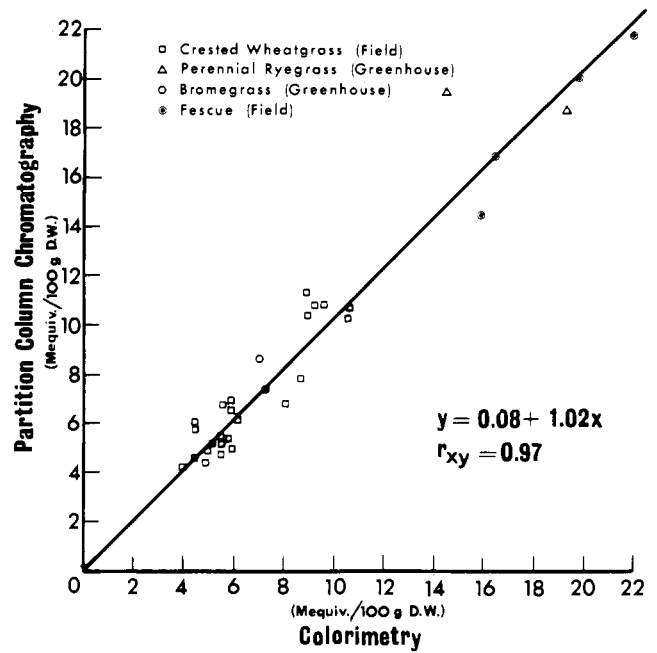


Figure 4. Citric acid concentrations in several grasses as determined by colorimetry and partition column chromatography.

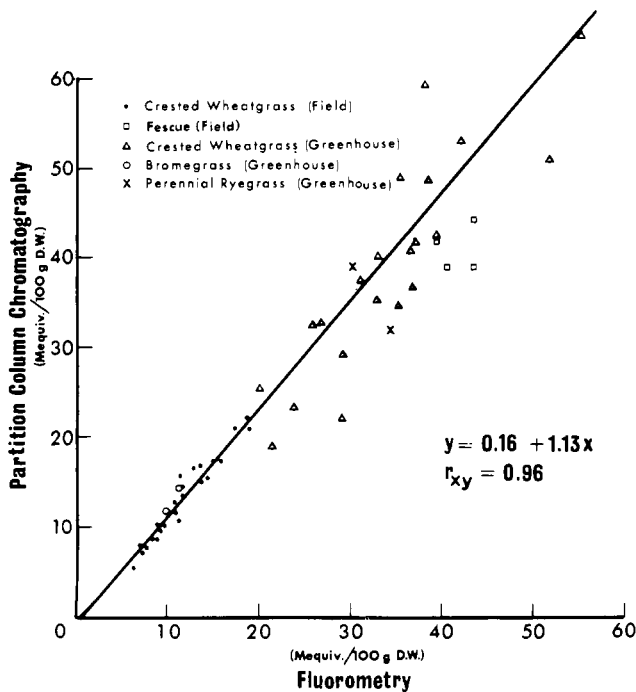


Figure 3. Malic acid concentrations in several grasses as determined by fluorometry and partition column chromatography.

tween *trans*- and *cis*-aconitic acids on the chromatogram. The concentration of *trans*-aconitic acid in Fairway crested wheatgrass was too low for accurate measurement from the pcc chromatogram due to overlap of a small peak of similar retention time. Therefore, the data presented for *trans*-aconitic acid in Table I were obtained polarographically.

In the greenhouse experiment, the yields per pot for Nordan were 4.0 g for no added K and 4.6 g for added K. For Fairway, the yields were 4.4 g for no added K and 5.2 g for added K. For the same treatments, the K concentrations were 1.11 and 2.9 for Nordan and 1.08 and 3.21% for Fairway. Therefore, the plants growing in the no K treatment were low in K, but not extremely deficient.

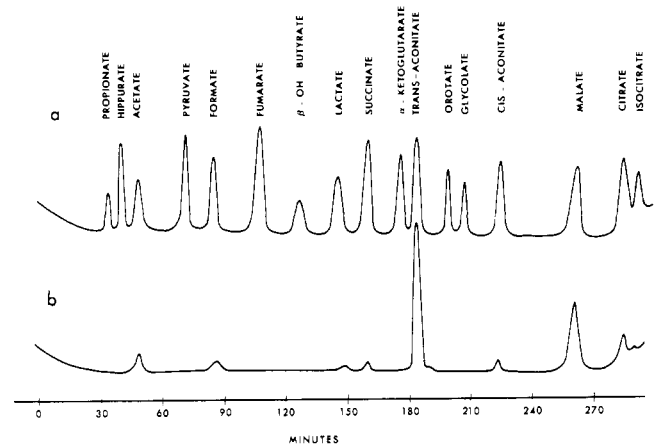


Figure 5. Elution pattern of some organic acids. (a) Standard mixture of acids containing 150 nequiv of each acid; (b) Organic acids in crested wheatgrass grown in a field experiment in Nevada, where ammonium nitrate had been added at the rate of 150 kg N/ha (134 lb N/A).

Most crested wheatgrass seedings in the western United States are *Agropyron desertorum* (Nordan or a similar variety), but there are some seedings of *Agropyron cristatum* (Fairway or a similar variety) in the western United States, and in Canada. Table I shows that K fertilization increased malic acid, citric acid, and total organic acids in both Nordan and Fairway. At the same K level of fertilization, the concentration of total organic acids is similar for Nordan and Fairway. The most striking difference between Nordan and Fairway is that fertilization with K increased *trans*-aconitic acid in Nordan at the expense of malic acid. The individual organic acids in both Nordan and Fairway increased proportionately when fertilized with K. *Trans*-aconitic acid in the K-fertilized Nordan was well above 1% (172 mequiv/kg), suggested as potentially toxic (Stout *et al.*, 1967). No *cis*-aconitic acid was detected in any of the plant samples listed in Table I.

Stability constants for complexing of Mg by citric acid are appreciably higher than those for malic (Sillen and

Table I. Effects of K Fertilization on Organic Acid Concentrations in Two Species of Crested Wheatgrass Grown in the Greenhouse <sup>a</sup>

Organic acid	Nordan ( <i>Agropyron desertorum</i> )		Fairway ( <i>Agropyron cristatum</i> )		Pooled S.E.
	No K	312 kg K/ha	No K	312 kg K/ha	
	mequiv/kg dry matter				
<i>trans</i> -Aconitic	96.0b	203.8c	20.7a	39.0a	19.6
Citric	20.8a	59.5b	31.2a	79.1b	8.0
Malic	280.5a	445.6c	362.6b	611.2d	29.4
Fumaric	N.D.	1.7	N.D.	2.8	
Acetic	17.1	17.6	21.9	22.3	1.5
Formic	2.9	4.4	4.1	8.7	0.7
Total organic acids <sup>b</sup>	417.3a	733.5b	440.5a	763.1b	33.5

<sup>a</sup> Horizontal values followed by different letters differ significantly ( $p < 0.01$ ). N.D. indicates concentration below the level of accurate detection.  
<sup>b</sup> *cis*-Aconitate, isocitrate, and succinate were not detected in these samples.

Martell, 1964). In a solution having an ionic strength and composition similar to ruminant duodenal fluid, citric acid complexed significantly more  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  than malic acid (Molloy and Richards, 1971). *Trans*-aconitic acid was found to be slightly less effective than malic acid in complexing  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Molloy and Richards, 1971). Thus, it is not clear whether the high concentrations of *trans*-aconitic acid in Nordan would complex more Mg than an equivalent amount of malic acid in Fairway.

In K-fertilized Nordan, citric acid was present in appreciably lower concentrations than *trans*-aconitic or malic acids, but citric acid could also participate in complexing Mg in animals grazing both Nordan and Fairway. Citric acid is metabolized more rapidly than *trans*-aconitic acid by rumen microorganisms (Kennedy, 1968). Packett and Fordham (1965) also observed a rapid disappearance of citric acid from the rumen of lambs. However, Wright (1971) indicated that where K is high the ability of cattle to metabolize citric acid in the rumen is decreased appreciably. It is expected that malic acid would also be metabolized in the rumen more rapidly than *trans*-aconitic acid. Thus, it is possible that high concentrations of *trans*-aconitic acid might impair Mg absorption to a greater extent than either citric or malic acids.

Concentrations of acetate were less than 25 mequiv/kg dry matter in both species, and formic and fumaric acids were considerably lower or nondetectable (Table I). Thus, acetic, formic, and fumaric acids from these grasses would not be expected to complex appreciable quantities of Mg.

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#### LITERATURE CITED

- Alcock, N. W., *Methods Enzymol.* **13**, 397 (1969).  
 Barness, L. A., Morrow, G., Nocho, R. E., Maresca, R. A., *Clin. Chem.* **16**, 20 (1970).  
 Bergmeyer, H., "Methods of Enzymatic Analysis," Academic Press, New York, N. Y., 1963.  
 Bohman, V. R., Lesperance, A. L., Harding, G. D., Grunes, D. L., *J. Anim. Sci.* **29**, 99 (1969).  
 Bulen, W. A., Varner, J. E., Burrell, R. C., *Anal. Chem.* **24**, 187 (1952).  
 Burau, R. G., *J. Agr. Food Chem.* **17**, 1332 (1969).  
 Burau, R. G., Stout, P. R., *Science* **150**, 766 (1965).  
 Camp, B. K., Farmer, L., *Clin. Chem.* **13**, 501 (1967).  
 Clark, R. B., *Crop Sci.* **9**, 341 (1969).  
 Dijkshoorn, W., Lampe, J. E. M., 1962 Yearbook I.B.S., Wageningen, The Netherlands, (1962).  
 Grunes, D. L., Stout, P. R., Brownell, J. R., *Advan. Agron.* **22**, 331 (1970).  
 Hautala, E., Weaver, M. L., *Anal. Biochem.* **30**, 32 (1969).  
 Henderson, T. R., Jones, R. K., *Clin. Chem.* **16**, 697 (1970).  
 Kennedy, E. P., Barker, H. A., *Anal. Chem.* **23**, 1033 (1951).  
 Kennedy, G. S., *Aust. J. Biol. Sci.* **21**, 529 (1968).  
 Kesner, L., *J. Biol. Chem.* **240**, 1722 (1965).  
 Kesner, L., Muntwyler, E., *Anal. Chem.* **38**, 1164 (1966).  
 Kesner, L., Muntwyler, E., Abstract of paper presented at 168th National Meeting of the American Chemical Society, New York, N. Y., September 1969a.  
 Kesner, L., Muntwyler, E., *Methods Enzymol.* **13**, 415 (1969b).  
 Lowry, O. H., Roberts, N. R., Wu, M., Hixon, W. S., Crawford, E. J., *J. Biol. Chem.* **207**, 19 (1954).  
 Marvel, C. S., Rands, R. D., Jr., *J. Amer. Chem. Soc.* **72**, 2642 (1950).  
 Molloy, L. R., Richards, E. L., *J. Sci. Food Agr.* **22**, 397 (1971).  
 Myers, W. F., Huang, K., *Anal. Biochem.* **17**, 210 (1966).  
 Neish, A. C., *Can. J. Res. Sect. B* **27**, 6 (1949).  
 Packett, L. V., Fordham, J. R., *J. Anim. Sci.* **24**, 488 (1965).  
 Patterson, R. P., Grunes, D. L., Lathwell, D. J., *Crop Sci.* **12**, 227 (1972).  
 Prior, R. L., Milner, J. A., Visek, W. J., *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **30**, 296 (1971).  
 Prior, R. L., Visek, W. J., *J. Anim. Sci.* **31**, 251 (1970).  
 Ramsey, L. L., Patterson, W. I., *J. Ass. Offic. Agr. Chem.* **28**, 644 (1945).  
 Rosevear, J. W., Pfaff, K. J., Moffitt, E. A., *Clin. Chem.* **17**, 721 (1971).  
 Scotto, K. C., Bohman, V. T., Lesperance, A. L., *J. Anim. Sci.* **32**, 354 (1971).  
 Sillen, L. G., Martell, A. W., "Stability Constants of Metal-Ion Complexes," Chemical Society Special Publication 17, London, 1964, pp 411, 479.  
 Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics," McGraw-Hill, New York, N. Y., 1960.  
 Stout, P. R., Brownell, J. R., Burau, R. G., *Agron. J.* **59**, 21 (1967).  
 Teel, M. R., *Potassium Symp.* **8**, 465 (1966).  
 Wright, D. E., *Appl. Microbiol.* **21**, 165 (1971).

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