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PLANT NUTRITION AND FEEDING VALUE

Some Nonfermentable Free Sugars in the Leaf-Petiole Fraction of Alfalfa (*Medicago sativa*)

V. V. RENDIG, E. A. McCOMB, and C. L. HU

Department of Soils and Plant Nutrition, University of California, Davis, Calif.

Sucrose, glucose, and fructose, which are relatively abundant and which interfere with the detection of less abundant sugars, were removed from prepared extracts of alfalfa by yeast fermentation. The nonfermentable free sugars isolated from the leaf-petiole fraction of alfalfa and identified by paper chromatography were D-*glycero*-D-*manno*-octulose, galactose, *manno*-heptulose, arabinose, *altro*-heptulose (sedoheptulose), xylose, and ribose. *manno*-Heptulose was also identified by its x-ray powder diffraction pattern. An additional unknown ketose-reactive material was separated but was not identified. Information concerning the natural occurrence of these sugars provides a basis for examining qualitative and quantitative changes which may occur as a result of altering the nutrition of the plant and which may affect palatability of the hay.

One of the many factors which determine the acceptability of forage by livestock is the sugar content. Plice (*21*) reported several comparisons in which the preference by cattle for different feeds was attributed to differences in their sugar levels. The differences in sugar levels were the result of variations in the conditions of growth of the forage plants or of additions of sweetening solutions of sucrose, molasses, etc., before the feeding. More recently, Bland and Dent (*3*) reported a significant positive correlation between the preference by cattle for several varieties of cocksfoot and their total sugar content. Similar results were obtained in other studies with sheep. Harper and Elvehjem (*9*) reviewed the literature on the influence of dietary carbohydrates on vitamin and amino acid requirements. They concluded that "indirect effects of individual carbohydrates may be of considerable nutritional significance."

The free sugars present in greatest amounts in most forages are sucrose, glucose, and fructose. Small amounts of other free sugars are also present, but prior to the advent of paper chromatography, they could not be readily separated and detected. Some of these, particularly in their phosphorylated form, have been assigned important roles in plant metabolism. Others, such as the oligosaccharides, melibiose, raffinose, and stachyose, have been found in grasses (*20*), but no specific metabolic function has been defined for them.

The purpose of the present study was to identify some of the nonfermentable free sugars which are present in the leaves and petioles of alfalfa. Yeast fermentation of the prepared plant extract removes sucrose, glucose, and fructose which interfere with the detection of some of the less abundant compounds. These studies are part of a project dealing with the effects of mineral nutrition of plants on their composition and feeding value. The authors have reported earlier (*22*) that alfalfa plants grown in solutions containing amounts of sulfur inadequate for maximum growth contain lower amounts of sucrose, glucose, and fructose than do similar plants grown in adequate nutrient solutions. The present findings will allow an extension of this study to include other carbohydrates. Information regarding the natural occurrence of these less common sugars also will provide clues regarding likely biosynthetic pathways to help elucidate, for example, earlier observations regarding the biosynthesis of heptuloses from pentoses introduced into plant tissue (*24*).

Experimental

Young shoots (regrowth about 10 days after cutting) of field alfalfa (Caliverde variety) were collected, and the leaves and petioles stripped from the stems and placed directly into boiling 95% ethyl alcohol. The procedure used for extraction and for subsequent yeast fermentation was that of Williams and Bevenue (*29*). Since, under some conditions, a ketoheptose can be synthesized during fermentation of hexose and ketose monosaccharides with yeast juice (*25*), the possibility that artifacts of yeast metabolism might be synthesized was checked. When yeast was allowed to act on a substrate consisting of 2% sucrose, 1% glucose, 0.66% fructose, and 0.01% xylose and ribose, no new sugars could be detected.

Whatman 3MM chromatographic paper sheets were used for isolation, and Whatman No. 1 paper for chromatographic detection. For one-dimensional chromatography, irrigant A [ethyl acetate–pyridine–water (8:2:1 v./v.)] was employed. For two-dimensional tests, irrigant B [phenol–water (10:2 v./v.)] was used for the second irrigation. Isolates from the alfalfa were cochromatographed with authentic samples of sugars by two-dimensional chromatography. Chromatograms were developed by dipping in orcinol (*2*), aniline (*16*), or silver nitrate (*26*) reagents. The resins used for clarification of the sugar solutions were Amberlite IR 120 (H^+) and Duolite A-4 (OH^-).

The fermented alfalfa extract was subjected to preliminary chromatographic examination, and sugars with R_G($R_{glucose}$)

Table I. Comparison of Chromatographic Data for the Nonfermentable Sugars Isolated from Alfalfa and for Some Known Sugars and Cyclitols

Fractions Isolated from Alfalfa	R_G (in Irrigant A)	Color Developed by: Orcinol	Aniline	Silver nitrate
a	0.80	Crimson		
b	0.89		Brown	
c	1.02	Blue-green		
d	1.18		Pink	
e	1.22	Blue		
f	1.37		Pink	
g	1.42	Yellow		
h	1.48		Pink	
Monosaccharides and Cyclitols				
myo-Inositol	0.29			Black
4-*O*-Methyl-*myo*-inositol	0.70			Black
Sucrose	0.79	Yellow		
D-*glycero*-D-*manno*-Octulose	0.80	Crimson		
Galactose	0.89		Brown	
Glucose	1.00		Brown	
manno-Heptulose	1.02	Blue-green		
5-*O*-Methyl-D-inositol	1.04			Black
Arabinose	1.18		Pink	
Fructose	1.19	Yellow		
altro-Heptulose	1.21	Blue		
Xylose	1.31		Pink	
β-Methyl-D-glucoside	1.43			Black
Ribose	1.47		Pink	

values corresponding to (*a*) an octulose, (*b*) galactose, (*c*) *manno*-heptulose, (*d*) *altro*-heptulose (sedoheptulose), (*e*) arabinose, (*f*) xylose, (*g*) ribose, and (*h*) a ketose-reactive area with an R_G between xylose and ribose were detected.

The concentrated (ca. 10% solids) solution was streaked on 3MM papers and irrigated with irrigant A, and strips were cut from the papers and dipped into the developing reagents. Areas corresponding to the sugars previously detected were marked and the marked bands cut from the papers. These strips were then irrigated with water, and the eluate was collected, passed through resin columns, and concentrated by evaporation under a jet of air on the steam bath. The isolated sugars were further purified by re-separation on heavy paper. Some carbohydrates which interfered with separation, and whose presence in alfalfa has been reported previously (*14, 15, 23*), were allowed to crystallize in the sirup and then were removed by filtration.

Results and Discussion

The chromatographic characteristics of the eight isolated fractions are shown in Table I along with similar data for various monosaccharides and cyclitols. The following conclusions were drawn regarding the compounds present in the eight isolates:

Isolate (*a*)—D-*glycero*-D-*manno*-Octulose. The compound present had an R_G identical to that of D-*glycero*-D-*manno*-octulose by one-dimensional chromatography and gave the typical crimson color (*6*) with orcinol. It cochromatographed with this octulose, and separated from D-*glycero*-D-*ido*-octulose. D-*glycero*-D-*manno*-Octulose has been reported in avocado and Sedum (*7*).

Isolate (*b*) — Galactose. Its behavior in both one- and two-dimensional chromatography was identical to that of authentic galactose. This aldohexose has been reported previously as a component of alfalfa hemicellulose (*10, 18*), but as far as the authors are aware, not as a free sugar in plants. Hemicellulose would not be expected to be soluble in 80% ethyl alcohol used in the extraction, and none was detected chromatographically. Under the conditions used here for fermentation, galactose appeared as a nonfermentable sugar. Galactose has been shown to be fermented, however, by galactose-adapted and lactose-fermenting yeasts (*5, 13*).

Isolate (*c*)—*manno*-Heptulose. The R_G of the compound in the isolate was identical to that of *manno*-heptulose, and on treatment of the chromatogram with the orcinol reagent gave the blue-green color which was observed to be typical for this heptulose. The difference in color is not due to the orcinol alone since this heptulose in the Dische orcinol reaction (*8*) produces a compound having an absorption maximum of 580 mμ, which is the same as that produced by L-*gluco*-heptulose under these conditions (*17*). *manno*-Heptulose also was readily separated from other heptuloses and cochromatographed with an authentic sample. A sufficient amount of the compound crystallized to allow its identity to be confirmed by comparison of its x-ray powder diffraction pattern with that of authentic *manno*-heptulose. This heptulose was isolated from avocado fruit by LaForge in 1917 (*11*) and, more recently, from the leaves of this species (*19*). It has also been identified in fig leaves (*1, 28*). Presumably, *manno*-heptulose was the "ketoheptose" which Williams *et al.* (*30*) detected by the orcinol test in alfalfa, but its identity was not established in their studies.

Isolates (*d*), (*f*), and (*h*)—Arabinose, Xylose, and Ribose, Respectively. The R_G of isolates (*d*) and (*h*) corresponded very closely to those of arabinose and ribose, respectively, by one-dimensional chromatography, and identical behavior was also observed by two-dimensional chromatography. Of the reference compounds tested, the one having the R_G most similar to that of isolate (*f*) and also giving the pink color with aniline was xylose. Further tests using two-dimensional cochromatography supported this conclusion. Lyxose, which has a similar R_G and gives a pink color with aniline, was included in this comparison. Bonner (*4*) includes L-arabinose, D-xylose, and D-ribose in the list of sugars commonly found in plants. Arabinose and xylose have been identified as constituents of the hemicelluloses of cell wall polysaccharides of alfalfa (*10, 18*).

Isolate (*e*)—*altro*-Heptulose. Of the heptuloses which have been reported as naturally occurring, *talo*-heptulose (*7*) most likely could be confused with *altro*-heptulose with the chromatographic techniques used in these studies. From the results obtained by one-dimensional chromatography and cochromatography, the authors concluded that this isolate was primarily *altro*-heptulose. *altro*-Heptulose was found first in *Sedum spectabile* Bor. by LaForge and Hudson (*12*). The extensive literature dealing with *altro*-heptulose has been reviewed by many authors, one of the most recent being Webber (*27*).

Isolate (*g*)—Unidentified. The yellow color produced by treating this compound with orcinol indicates that it is a ketose. It does not give the color test typical of the pentuloses, however. Further work is being done in an attempt to identify this compound.

As mentioned previously in the course of the isolation of the nonfermentable sugars, certain interfering carbohydrates were removed after allowing them to crystallize. Appropriate tests verified that these compounds, 5-*O*-methyl-D-inositol (pinitol) (*23*), 4-*O*-methyl-*myo*-inositol (ononitol) (*14*), and β-methyl-D-glucoside (*15*) are present in alfalfa. Also, the cyclitol, *myo*-inositol, was isolated, crystallized, and identified by R_G and melting point, and by comparison of its x-ray powder diffraction pattern with an authentic sample of *myo*-inositol. While the amount present was not determined quantitatively, *myo*-inositol was obviously present in much lesser amounts than pinitol. Quite likely the abundance of *myo*-inositol will vary, owing to genetic, climatic, and cultural factors.

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FERTILIZER TECHNOLOGY

Granulation Characteristics of a 5–4–12 (5–10–15) Fertilizer Containing Potassium Nitrate

D. R. BOYLAN and D. V. KAMAT
Engineering Experiment Station, Iowa State University, Ames, Iowa

Factors influencing the granulation of a mixed fertilizer containing potassium nitrate have been studied. Kilogram batches of a 5–4–12 (5–10–15) mixed fertilizer containing potassium nitrate were granulated at different feed moisture levels and at inlet gas temperatures ranging from 405° to 575° F. Optimum conditions of moisture in the feed and inlet gas temperature were determined for maximum yield of the granular product and uniform distribution of the three major nutrient elements (N, P, K) in the various product size fractions. Similar experiments were performed with a 2–4–12 (2–10–15) mixed fertilizer containing potassium chloride. This grade represents a simple substitution of KCl for KNO_3 so physical changes due to KNO_3 could be more closely compared. A comparative study has been made of the results obtained with the two mixtures. The experiments were based on a statistical central composite rotable design, and the results were statistically analyzed. An attempt has been made to relate mathematically the yield and total absolute deviation in nutrient analysis between product and feed with moisture in a feed and temperature of granulation.

Potassium nitrate has a high agronomic value as a source of both nitrogen and potash, but high cost has been the chief reason for its limited use as fertilizer. The new production facilities of Southwest Potash Corp. for fertilizer-grade potassium nitrate, 13% N, 36.5% K (44% K_2O), may make this material economically attractive. This has stimulated considerable interest in the properties of potassium nitrate with respect to its behavior in mixed fertilizers during processing and its effect on the physical quality of the resulting products.

Previous Work

During the past few years, much work has been done on the granulation characteristics of mixed fertilizers. Pilot plant studies conducted at TVA (*3*) showed that in production of very low nitrogen grades [e.g., 3–5–9 (3–12–12) or 4–6–13 (4–16–16)] the amount of moisture required for granulation of the charge material was high (14 to 16%), and the products were rather wet. Smith (*4*), in the study of temperature and moisture relationships in granulation, noted that the utilization of ammonia as anhydrous or nitrogen solutions reduced not only the cost of nitrogen but also the free water content. A high salt solution phase contributed to rapid crystallization and therefore aided formulation of granules during agglomeration. These grades contained potassium nitrate as the source of potassium. Recently, Hardesty and his coworkers, working with grades containing potassium nitrate, noted that slightly less moisture was required for agglomeration with potassium nitrate mixtures (*2*), but they produced a "popped-corn" shape of granules which effloresced on drying. Comparison of the yield and homogeneity of the product with mixtures that contained potassium nitrate and potassium chloride was not reported.

Experimental Work

Experiments were performed to study the effect of moisture in the feed and inlet gas temperature on the yield and the distribution of plant nutrients in the various product size fractions. Two