Direct Estimation of Xylose in Hemicelluloses

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The development of a quick direct method for the estimation of xylose in the presence of sugars and other substances is described. It is based on the absorption spectra in 79% sulfuric acid in the ultraviolet range. Within certain limits, the procedure saves time without significant loss of accuracy.

Hemicelluloses are difficult and time-consuming to determine because they are fairly large polymers of mixed composition. As a direct procedure for the estimation of the sugars is not available, it is necessary to remove or to separate the components of the hydrolyzate for quantitative estimation. Xylose often accounts for 60 to 70% or more of forage crop hemicelluloses. The remainder is composed of glucose, galactose, or arabinose, and in some instances other carbohydrates to a lesser extent.

Carbohydrates, when exposed to the action of strong mineral acids, are transformed into products which exhibit strong absorption in the ultraviolet region. These products, together with color-producing reagents, have served as the basis for quantitative procedures. Outstanding among these are the reactions developed or initiated by Dische (*3*) and Dreywood (*4*). Ikawa and Niemann (*5*, *6*), in a study of the absorption spectra of solutions of carbohydrates alone in strong sulfuric acid, found that, under certain conditions, characteristic molecular extinction coefficients could be obtained at 315 mμ. This paper shows the development of a method for the direct estimation of xylose in hemicelluloses, based on the action of strong sulfuric acid and extinction coefficients.

Experimental

All absorbance determinations were made at room temperature with a Beckmann DU spectrophotometer equipped with ultraviolet accessories. Carbohydrates, obtained from Eastman Kodak Co., were used without further purification. The solutions of carbohydrates were treated with sulfuric acid, essentially as outlined by Ikawa and Niemann (*6*). One milliliter of carbohydrate solution was added to ice-chilled, 84% (by weight) sulfuric acid in 15 × 150 mm., glass-stoppered tubes. The tubes were mixed while still in the ice bath; then placed in a boiling water bath for 15 minutes. The sulfuric acid was dispensed from a 50-ml. buret equipped with a Teflon needle valve. The solution was mixed with a 4-mm. glass rod, which had been flattened at one end to a diameter of about 10 mm. The tubes were removed and placed in an ice bath for quick cooling.

Previous work indicated that the absorption curve for xylose was characterized by major peaks at about 256 and 315 mμ, respectively, and a minimum between at 275. The net molecular extinction values represented by $\epsilon_{315} - \epsilon$ 275 for the various substances differed considerably. The net value was practically zero for glucuronic and galacturonic acids, significantly higher for glucose, fructose, and galactose, and highest for xylose and arabinose—xylose was much greater than arabinose. Thus, by subtracting the absorbance at 275 from that at 315 mμ, the effect of associated sugars on xylose may be reduced or eliminated. These values are summarized in Table I. The coefficients are averages of five to six determinations and have an average deviation of 0.17 for the sugars. On this basis, a standard curve for xylose was prepared relating absorbance to concentration in amounts up to 200 mg. per liter. The average deviation for five different levels of xylose and duplicate determinations on three independent samples amounted to ±0.8 mg. per liter.

As arabinose, galactose, and glucose are most likely to be associated with xylose in hemicelluloses, the effect of each on the determination of xylose was determined separately. Arabinose was added in amounts of 5, 10, and 15% of total sugar at levels of 40, 80, 120, 160, and 200 mg. per liter. Galactose and glucose were each added in amounts of 10, 20, and 30% of the total sugar. The average per cent recoveries at different concentrations of other sugars are: arabinose, 100, 102, 104; galactose, 98, 100, 98; and glucose, 99, 99, 104. The percentages are based on the average of duplicate determinations of xylose on three original samples at five levels and at three different concentrations and at the two wave lengths. The average deviation for all three sugars at all levels and concentrations was about ±2.0 mg. per liter; it was less for arabinose and greater for the higher concentration of hexoses.

Table I. Average Molecular Extinction Coefficients for Various Sugars and Uronic Acids

Substance	Wave Lengths 275	315	315–275
	Mol. Extinction Coeff. × 10^3		
Glucose	1.87	3.76	+ 1.89
Galactose	3.44	3.89	+ 0.45
Arabinose	3.09	7.81	+ 4.72
Xylose	3.87	14.82	+10.95
Glucuronic acid	6.50	6.66	+ 0.16
Galacturonic acid	5.44	5.28	− 0.16

The effect of some other constituents which might be associated with hemicelluloses was also ascertained. Thus glucuronic and galacturonic acids would offer no significant contribution. The recovery of xylose at a concentration of 100 mg. per liter was not significantly affected by the presence of protein in the form of casein or alkali lignin when present in concentrations of 100 and 20 mg. per liter, respectively.

The procedure was further tested on purified isolated hemicellulose and on plant extracts containing hemicellulose. The isolated product, in the proper concentration range, was dispersed in water containing a little sodium hydroxide. The values obtained on hemicelluloses from maize cobs and rye straw (*2*) were about 94% of the values obtained by means of phloroglucin. To determine the extent of xylose recovery from natural products, known amounts of xylose were added to relatively impure hemicellulose dispersions obtained from white Dutch clover and Rhode Island Bent. These samples were previously extracted for 3 hours with alcohol and benzene, in the volume ratio of 1 to 2, and hot water. The residues were further extracted with warm 2% sodium hydroxide for 4 hours. The hemicellulose was precipitated with alcohol and partially purified by treatment with liquid bromine in a slightly acidic aqueous medium (*1*). The excess bromine was removed and the solid was dispersed in a suitable volume of water. Alcohol had no effect on subsequent reactions. Solutions of xylose

were added to give net final concentrations of 40 and 150 mg. per liter. In the case of the blank, the solution was diluted with the proper amount of water. The aliquots of the resulting systems were then treated with 84% sulfuric acid in the manner described. At both levels the average percentage recovery amounted to approximately 96%.

Literature Cited

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ANIMAL GROWTH STIMULANTS

The Metabolic Fate of Carbon-14–Labeled Trimethylalkyl Ammonium Stearate

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Trimethylhexadecyl ammonium stearate labeled with carbon-14 in the one position of the hexadecyl chain has been administered to rats orally, as a diet ingredient, and by injection intraperitoneally. This compound is highly insoluble in water and, therefore, appeared unlikely to be absorbed from the intestinal tract or metabolized by the body, when given by injection. However, the compound was absorbed from the intestinal tract of both species to a small extent, and the absorbed fraction was rapidly metabolized and excreted by way of the gastrointestinal tract, urine, and respiratory carbon dioxide. Absorption of the compound appeared to be higher in the chick, but concentration of the label in the tissues was similar in both species.

MANY CHEMICAL COMPOUNDS, which stimulate animal growth in controlled amounts, are toxic in large doses. To evaluate the biological effects of such compounds, feeding as well as metabolism experiments must be conducted to demonstrate the growth-stimulating effect at various levels of intake and to determine the ability of the animal to metabolize and excrete the compound, if it is absorbed, before it accumulates in the tissues to levels which could be toxic either to the animals involved or, indirectly, to man.

In these experiments, trimethylhexadecyl-1-C^{14}-ammonium stearate, which has chick and swine growth-stimulating activity (*1–3*), was administered orally or by injection to rats and chicks and the distribution of the radioactivity in the tissues and excreta was determined.

Materials and Methods

Experiments with Rats. In experiment I (Table I), 500 mg. of C^{14}–labeled trimethylalkyl ammonium stearate [Arquad stearate (Dynafac), Armour and Co.] was thoroughly mixed with each kilogram of a balanced synthetic diet. The diet contained sucrose, casein, and corn oil as sources of carbohydrate, protein, and fat, respectively. Two weanling male albino rats of the Sprague Dawley strain were fed on the above diet for periods of 22 and 25 days. The feces and urine were collected over the whole period and the respiratory carbon dioxide for the last 48 hours. At the end of the feeding period, the rats were killed with ether, the livers and intestines were separated from the carcasses, and the intestinal contents were added to the feces.

The radioactivity was measured by burning each sample with Van Slyke combustion liquid and collecting the carbon dioxide in an ionization chamber. The counts were made on a vibrating reed electrometer.

In experiment II (Tables II and III), the labeled Arquad stearate was injected. Preliminary experiments showed that Arquad stearate was much more readily absorbed when given intraperitoneally than when administered subcutaneously. Two male albino rats weighing 124 and 126 grams were injected intraperitoneally with 7 mg. of Arquad stearate suspended in 1.0 ml. of water. The rats were immediately placed in all-glass metabolism cages for 7 days. The respiratory carbon dioxide from rat I was collected in 12-hour fractions and the urine from rat II in 24-hour fractions. At the end of the seventh day, the rats were killed with ether, and the radioactivity was determined on the feces, urine, respiratory carbon dioxide, carcass, and liver.

In experiment III (Table IV), three male albino rats were fed C^{14}–labeled Arquad stearate at the level of 500 mg. per kg. of a synthetic diet for 7 days. The Arquad stearate was then withheld from the diet. One rat was killed at 0 hour, one after 48 hours, and one after 96 hours from withholding the compound; their carcasses were assayed for radioactivity.

Experiments with Chicks. Two 1-day-old Columbian crossed with New Hampshire chicks were given a nutritionally adequate corn-soybean meal diet for 5 weeks. At this point, 200 mg. of C^{14}–Arquad stearate were mixed with each kilogram of the diet. The chicks were placed in individual wire-bottomed cages in a ventilated hood. Food and water were provided *ad libitum*. The feces and urine of each chick were collected together for the first 4 days on the C^{14}–Arquad stearate diet. On the fifth day, both chicks were operated on to obtain a sample of urine uncontaminated with feces (*4*). This was accomplished on one chick. At the end of the seventh day on the C^{14}–Arquad stearate diet the chicks were sacrificed, feathered, and washed. The abdomen was split open by a midline incision and the liver and the gastrointestinal tract were removed, washed, and frozen. The radioactivity of the carcass and the organs examined was determined as before.

Results and Discussion

Table I shows the distribution of the