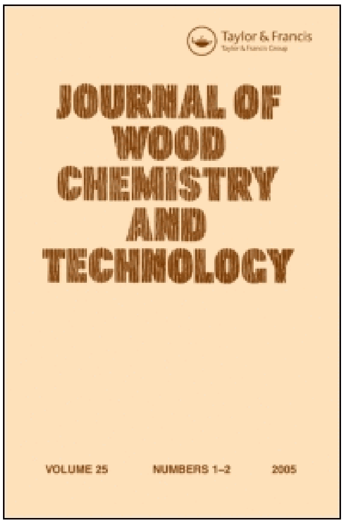

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COMPARISON OF A GRAVIMETRIC CO_2 METHOD FOR URONIC ANHYDRIDE WITH A COLORIMETRIC METHOD

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ABSTRACT

Colorimetry gave consistently lower uronic anhydride values for four species of wood than did measurement by evolved CO_2. These differences were partly related to CO_2 evolution from nonuronide wood extractives, and partly to CO_2 from nonuronide carbohydrates or lignin. After the usual benzene-ethanol extraction to remove interfering extractives, the uronic anhydride contents by gravimetric CO_2 analyses were still 30 to 40% higher than those by colorimetry. Based upon the greater selectivity of the colorimetric method, the authors conclude that colorimetry provides the more accurate uronic anhydride analyses of natural materials.

INTRODUCTION

Many determinations of uronic anhydride in plant materials have been made by measurement of the CO_2 evolved during their digestion with concentrated solutions of hydrochloric or hydriodic acids. The possibility of CO_2 evolution from substances other than uronic acids was stated in the original study by Lefevre and Tollens.[2] It was further discussed by Burkhart et al.,[3] Whistler et al.,[4] and Timell[5] concerning wood analyses, and by others. Enström and Janson[6] reported that the CO_2 evolved by strong acid

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could not be used for uronic acid determinations of wood because of the nonuronide CO_2 formed. Thompson et al.[7] referred to this interference in kraft pulp as "pseudo-uronic" acid.

Recent studies in this Laboratory afforded the opportunity to compare uronic anhydride values obtained by two independent methods. This report compares results from a recently developed colorimetric method[8] with those from a gravimetric measurement of evolved CO_2[9].

EXPERIMENTAL

Materials

Wood was cut from undried logs having 25 to 30 years' growth. The inner, or heartwood, sections were separated and ground to 20-mesh for analysis by the gravimetric CO_2 procedure.[9] They were further ground to 40-mesh for analysis by the colorimetric procedure.[8] Wood species for analyses were quaking aspen (Populus tremuloides), southern red oak (Quercus falcata), slash pine (Pinus elliottii) and Douglas-fir (Pseudotsuga taxifolia). Samples of each were extracted in a Soxhlet extractor with 95% benzene-ethanol (2:1, v/v) for 4 h, and portions were then extracted further by boiling with distilled water for 6 h. Extracted samples were dried to determine weight losses.

D-Galacturonic acid monohydrate was recrystallized from an acetone-water mixture after removal of cations by ion exchange and was dried at 50°C under vacuum (found C 34.01, H 5.9; theory C 33.96, H 5.7). This was the standard for the colorimetric method. Polygalacturonic acid was a commercial sample. Xylan was isolated from aspen wood.

Analyses

The gravimetric procedure for evolved CO_2 published by Browning[9] was closely followed. The evolution of CO_2 was measured every 30 min starting at the moment the reaction temperature reached 135°C (zero time) and continuing for 3 or 6 h.

The colorimetric procedure[8] depended on the formation of 5-formyl-2-furancarboxylic acid from both hexuronic acids and uronides, and the subsequent development of color with 3,5-dimethylphenol. Absorptivity was based upon the 450-400 nm absorbance difference given by the chromophore. The absorptivity due to the glycosidic uronic acid (anhydride) in wood, xylan, and polygalacturonic acid was taken as 1.12 times the measured absorptivity from galacturonic acid (anhydride). A discussion of this relationship and other remarks on the colorimetric method are included in an addendum.

RESULTS AND DISCUSSION

Gravimetric CO_2 measurements contained erratic but relatively small blank values. The mean 3-h reading of seven blanks was 1.0 mg of CO_2 with a standard deviation of 0.45. For comparison, most wood samples of about 2 g produced 20 to 30 mg of CO_2. Thirty data points from 3 h to 6 h for the seven blanks gave an average slope of 0.19 mg CO_2 per hour with a correlation coefficient of only 0.41 because of the scatter. The mean slope had a 3-h value of 0.98 mg CO_2 and a zero time intercept of 0.41 mg CO_2.

After 3 h of reaction, wood samples continued to give a gradual evolution of CO_2 in excess of blank CO_2. This indicated the necessity for a correction because CO_2 evolution from uronides was completed within 3 h. Computation of the gravimetric CO_2 data is illustrated in Figure 1. Results are shown for three materials listed in the order of decreasing uronide content: polygalacturonic acid, aspen xylan, and oak wood. Because uronic anhydride is a minor component of wood, its measurement from evolved CO_2 is made difficult by CO_2 produced from other components. A 3-h reading by itself unavoidably includes this error. Extrapolation of the 3-h to 6-h slope to zero time as in Figure 1 provides correction for blank values and for nonuronide CO_2 evolved uniformly over 6 h, but not for nonuronide CO_2 evolved only between zero time and 3 h.

Results from the colorimetric procedure and from the two methods of calculating the gravimetric measurements are in Table 1.

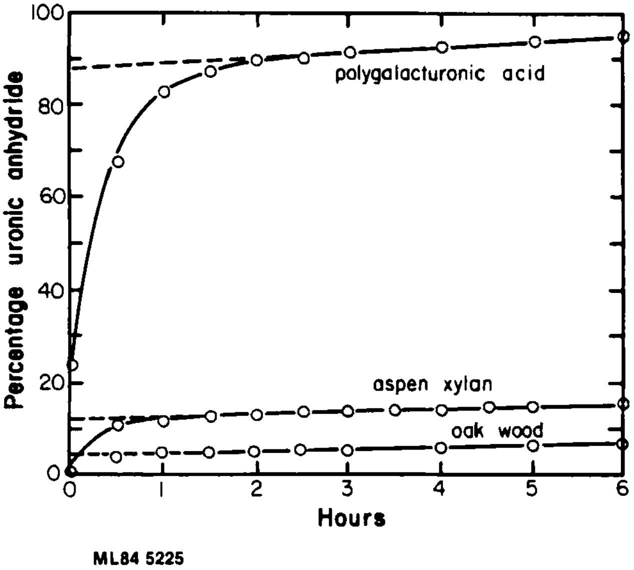

FIGURE 1. The evolution of CO_2 as percentage uronic anhydride for three materials. Zero time is the moment that the reaction mixture reaches 135°C.

Gravimetric values for percentage uronic anhydride were obtained either by extrapolation to zero time as shown in Figure 1 or by subtracting the predetermined mean blank value from the 3-h reading. At the top are the standard galacturonic acid (calculated as galacturonic acid anhydride) together with polygalacturonic acid and aspen xylan, two materials with relatively large uronic anhydride contents. Galacturonic acid runs demonstrated some of the difficulties with measurements of evolved CO_2. There was a slow increase in measured CO_2 after 3 h as with the blanks. The mean of three readings at 3 h, corrected for blank, calculated as 101.1% uronic anhydride recovery (Table 1). The amount over 100% is nearly equivalent to 0.26 mg CO_2, approximately one standard deviation of the mean of three blanks. The slope from 3 to 6 h, calculated from six readings in two runs, is 0.41 mg CO_2 per hour with a correlation coefficient of 0.99 and a zero time intercept equiva-

TABLE 1

Comparison of Uronic Anhydride Analyses Obtained by Colorimetry and by Gravimetric CO_2 Measurements

Materials	Colorimetric analyses	Gravimetric CO_2 analyses	
		Intercept measurement	3-Hour measurement
	- - - - - - - - -	%[a]	- - - - - - - - -
Galacturonic acid	100(std.) (0.8)	$\underline{100.6}$	101.1 (0.2)
Polygalacturonic acid	81.7 (0.5)	87.0	86.9 (0.2)
Aspen xylan	11.1 (0.1)	$\underline{12.0}$	$\underline{12.5}$
	UNEXTRACTED WOOD		
Oak	3.1 (0.06)	$\underline{4.3}$	5.1
Aspen	3.4	4.3	4.7
Pine	2.7 (0.10)	4.5 (0.2)	4.9 (0.1)
Fir	1.8	2.6	3.2
	BENZENE-ETHANOL-EXTRACTED WOOD		
Oak (7.3%)[b]	3.1 (0.05)	$\underline{4.4}$	4.5
Aspen (4.5%)	3.3	4.2	4.6
Pine (8.7%)	2.8 (0.08)	3.6 (0.2)	4.1 (0.2)
Fir (4.8%)	1.8	2.4	3.0
	WATER-EXTRACTED WOOD[c]		
Oak (16.8%)	2.6 (0.05)	3.6	$\underline{3.8}$
Aspen (10.1%)	3.0	3.6	4.1 (0.1)
Pine (19.3%)	2.4 (0.13)	3.1 (0.3)	3.3 (0.4)
Fir (11.3%)	1.5	2.1	2.8

[a] Numbers are averages from two or more samples except for those underlined, which are single determinations. Numbers in parentheses are standard deviations of the means calculated from three or more runs. Galacturonic acid results are based upon the calculated anhydride weight (mol. wt. 176). Percentages after extractions are based upon original wood weight.

[b] In parentheses are total extractive losses as percentages of original unextracted wood weight.

[c] With hot water after benzene-ethanol.

lent to 100.6% uronic anhydride recovery (Table 1). The slope of 0.41 mg CO_2 per hour is also within the variability of the blank runs.

The lower three data groups in Table 1 include analyses of unextracted wood, benzene-ethanol extracted wood, and analyses after further extraction with hot water. Colorimetric analyses were unchanged by benzene-ethanol extraction, indicating that no uronic anhydride was extracted. However, two of the 3-h gravimetric results (for oak and pine) appreciably decreased and must be interpreted as showing that nonuronide sources of CO_2 were extracted. These extractives evolved CO_2 equivalent to 0.6% uronic anhydride in oak wood by the 3-h measurement (the difference between 5.1 and 4.5) and similarly equivalent to 0.8% of the pine. After extraction of the interfering material, the two gravimetric measurements for oak agreed closely, 4.5 and 4.4. Although the first extraction of pine wood removed a CO_2 source, more remained as shown by an appreciable intercept correction from 4.1 to 3.6.

Evolution of CO_2 by nonuronide carbohydrates such as cellulose when heated in 12% HCl was shown by Whistler et al.[4] Their result with purified cotton would amount to about 0.2% as uronic anhydride. We found about 0.5% uronic anhydride equivalent in a crystalline cellulose at the 3-h measurement. The xylan data in Table 1 show uronic anhydride percentages 0.9 to 1.4% higher by CO_2 measurement than by colorimetry. This difference could be due to an apparent 1.4 to 1.3% uronic anhydride found by CO_2 evolution from xylose by Clayton.[10] Using Clayton's report for a xylose correction and 0.5% for a rough estimate of the apparent uronic anhydride in the remaining carbohydrates, the 3-h data for once-extracted wood (Table 1) could be corrected to: oak 4.0, aspen 4.1, pine 3.7, and fir 2.6. These values remain well above the colorimetric values. Results obtained by Bylund and Donetzhuber,[11] who also used an intercept correction for nonuronide CO_2, likewise appear to be above what might have been obtained by colorimetry. All uronic anhydride percentages in the twice-extracted group of Table 1 show decreases after hot water extraction, partly as a consequence of removal of some uronic anhydride.

Colorimetric analyses of unextracted samples were rerun by a second analyst with some slight increases in results. The comparisons are: oak 3.13-3.35, aspen 3.40-3.57, pine 2.66-2.86, and fir 1.84-1.80 (first listed values correspond to those in Table 1).

In summary, the commonly used benzene-ethanol extraction reduced the amount of nonuronide CO_2 interfering with the 3-h gravimetric analyses of wood samples. However, neither by these 3-h readings nor by the intercept method were gravimetric uronic anhydride percentages reduced to those found by colorimetric analyses. Because the benzene-ethanol extraction did not affect the colorimetric analysis, it is likely that the usual benzene-ethanol extractives are noninterfering for this method. Based upon the greater selectivity of the colorimetric method the authors conclude that colorimetry provides the more accurate uronic anhydride analyses of natural materials.

ADDENDUM: NOTES ON THE COLORIMETRIC METHOD

The colorimetric standard previously[8] used was O-(4-O-methyl-α-D-glucopyranosyluronic acid)-(1→2)-O-(β-D-xylopyranosyl)-(1→4)-D-xylose, mol. wt. 472, uronic anhydride 37.29%. This is a xylan fragment containing uronic acid in the same form as most of it is found in wood, and therefore it is a direct standard. It was shown to be satisfactory also for galacturonic acid uronide as in polygalacturonic acid. The 450-400 nm absorptivity difference resulting from this standard is 1.12 times the absorptivity difference resulting from galacturonic acid, based upon the uronic anhydride contents of each. This is due to a 12% greater yield of 5-formyl-2-furancarboxylic acid from polymerically combined uronide reacted as described.[8]

Because of the uniformly lower results by the colorimetric method[8] compared to the CO_2 evolution procedure, two aspects of the colorimetric procedure were reexamined. First, increasing the time for solubilization of the carbohydrates in 72% H_2SO_4 at 50°C had no significant effect from 10 to 30 min for all four woods. Some losses occurred after 40 min. Second, the wood analyses reported here were obtained from samples which were diluted after acid treatment, but were not then made alkaline as before.[8] It was found that alkali did not significantly increase uronic anhydride analyses. The question of whether or not to use alkali depends on the possible occurrence of uronic acid esters, as in pectin, and on the slightly reduced reaction of these esters in the formation of 5-formyl-2-furancarboxylic acid. In the case of wood or pulp, where the amounts of uronide are small, possible corrections for this effect may be lost within experimental variability.

It was not necessary to moisten the air-dry 40 mesh wood samples before adding 72% H_2SO_4. Moistening dry powders, if necessary, should be done with water rather than with alcohol as previously stated[8] because of possible esterification of uronic acids by alcohol. However, the amount of water should be minimized so there is no alteration of acid concentration.

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