Stenhagen, E.; Abrahamsson, S.; Mc Lafferty, F. W. "Registry of Mass Spectral Data"; Wiley: New York, 1974; Vol. 2.
Swords, G.; Hunter, G. L. K. *J. Agric. Food Chem.* **1978**, *26*, 734.
Tressl, R.; Friese, L.; Fendesack, F.; Köppler, H. *J. Agric. Food Chem.* **1978a**, *26*, 1422.
Tressl, R.; Friese, L.; Fendesack, F.; Köppler, H. *J. Agric. Food Chem.* **1978b**, *26*, 1426.
Vlahov, R.; Holub, M.; Ognjanov, I.; Herout, V. *Collect. Czech. Chem. Commun.* **1966**, *32*, 808.
Yoshihara, K.; Ohta, Y.; Sakai, T.; Hirose, Y. *Tetrahedron Lett.* **1969**, *27*, 2236.

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Fractionation of Bright Tobacco

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Polymeric cell wall components were determined for ground, uncased bright tobacco lamina by using a systematic fractionation scheme. Starch was determined by an enzymatic procedure utilizing a thermophilic amylase. Analysis for pectin utilized an acid-catalyzed decarboxylation method. Lignin was determined by both the Klason lignin procedure and ^{13}C NMR integration of aromatic carbons. Estimation of protein was based on the results of Kjeldahl digestion. Hemicellulose and cellulose were determined by a capillary gas chromatography procedure for quantitation of neutral sugars. The results from this fractionation procedure were compared with those from a serial extraction procedure. Values for all components except cellulose were found to vary considerably between the two procedures. Errors in the serial extraction procedure were attributed to the fact that many distinct types of biopolymers have overlapping ranges of solubility. The cell walls of cured bright lamina were found to resemble the primary cell walls of dicots.

Tobacco has been one of the most thoroughly analyzed of all plant materials. However, tobacco cell wall biopolymers have received relatively little attention. In general, these compounds have been individually isolated from tobacco for analysis and characterization. Further, the analyses of these biopolymers have frequently utilized nonspecific gravimetric procedures which introduced potential errors since many distinct types of cell wall components have overlapping ranges of solubility. Thus, Bourne et al. (1967) obtained pectin from bright tobacco by ammonium oxalate extraction and Christy and Samfield (1960) isolated cellulose from various tobacco types by a 17-step procedure. Typically hemicellulose has not been isolated; instead, analyses have been performed for pentosans (Phillips and Bacot, 1953) which are found in pectin as well as hemicellulose. Over the past few years Katō and co-workers have characterized several biopolymers isolated from various tobacco materials (Eda et al., 1976, 1977; Eda and Katō, 1978, 1980; Mori et al., 1979, 1980; Mori and Katō, 1981). To date, no overall fractionation scheme to separate systematically the cell wall biopolymers prior to analysis has been presented.

Other plant materials, especially forage crops, have been analyzed by using more comprehensive fractionation schemes. Van Soest and colleagues have pioneered the use of detergent extraction procedures (Van Soest, 1963; Van Soest and Wine, 1967, 1968; Goering and Van Soest, 1970). More recently, procedures for the chemical characterization of water-soluble and water-insoluble dietary fibers have been developed (Theander and Åman, 1979; Salomonsson et al., 1980).

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The present research was undertaken to develop methods for the systematic fractionation and characterization of cell wall biopolymers in tobacco based on procedures that had been used with other plant materials.

EXPERIMENTAL SECTION

Fractionation Procedure. The tobacco used for this work was heavy, or bodied, flue-cured bright tobacco lamina from the upper mid-stalk position. This uncased tobacco was ground to pass a 20-mesh screen prior to extraction.

The fractionation procedure is schematically presented in Figure 1. In the first step the tobacco sample (100.0 g) was extracted with 80% ethanol in a Soxhlet apparatus for 18 h. The ethanol extraction solution was concentrated in vacuo to a constant weight in a tared round-bottom flask. The residue from the extraction was then dried overnight in a vacuum oven at 55 °C. For the removal of starch, the ethanol-extracted residue was added to 1200 mL of deionized water which had been preheated to 85 °C. The mixture was maintained at that temperature, with occasional swirling of the flask, for 30 min. Then 7.2 mL of Termamyl 60-L enzyme used as received from Novo Laboratories, Inc., was added, and the resultant mixture was heated for another 45 min at 85 °C with occasional swirling. Next, this mixture was brought to reflux and maintained at that temperature for 15 min. Finally, the mixture was filtered through a Büchner funnel with a sintered glass filter disk (ASTM 40-60). The filtrate was stored in a refrigerator. The residue was dried overnight in a vacuum oven at 55 °C.

The dried Termamyl-treated residue was refluxed under nitrogen for 3 h in a solution containing 2.5 L of 0.10 M aqueous KOH, 2.5 g of $NaBH_4$, and 1 mL of 1-octanol. After being refluxed, the mixture was allowed to cool to

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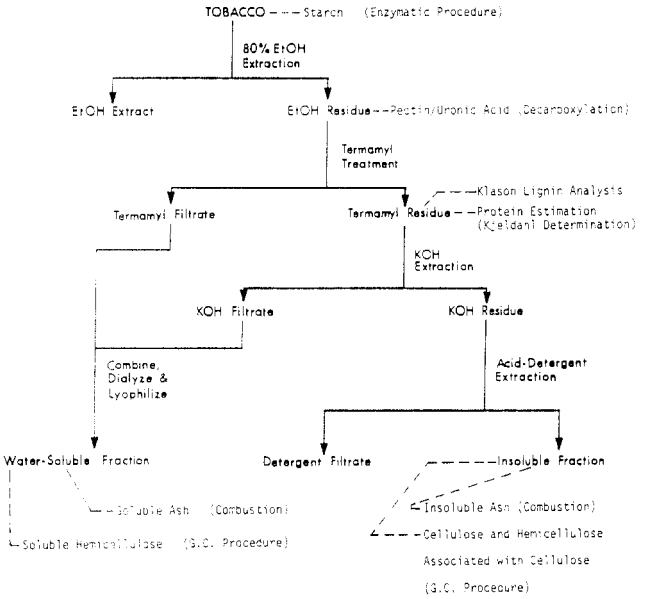

Figure 1. Schematic representation of the fractionation procedure.

room temperature, acidified by the addition of glacial acetic acid, and filtered through a Büchner funnel with a sintered glass filter disk (ASTM 40-60). The residue was washed with water and then with acetone and dried overnight in a vacuum oven at 55 °C.

The filtrates from the Termamyl treatment and from the alkaline extraction were combined and dialyzed against running distilled water for 65 h in dialysis tubing with a nominal molecular weight cutoff of 12 000. The dialyzed mixture was then lyophilized to yield the water-soluble fraction.

The Van Soest conditions for acid detergent extraction (Goering and Van Soest, 1970) were applied to the residue from the alkaline extraction. The KOH residue was refluxed for 1 h in a solution containing 1.1 L of 1.0 N H_2SO_4 and 22.0 g of hexadecyltrimethylammonium bromide (technical grade). After being refluxed, the mixture was filtered while still hot through a Büchner funnel with a sintered glass filter disk (ASTM 40-60). The filtrate was discarded. The residue was washed with hot water and then acetone and subsequently dried overnight in a vacuum oven at 55 °C. This residue was the insoluble fraction.

Analytical Procedures. Numerous analyses were performed on the various fractions obtained from the flue-cured bright tobacco. Uronic acid content was determined by the acid-catalyzed decarboxylation procedure of Bylund and Donetzhuber (1968) as modified by Theander and Åman (1979). Starch was determined by the glucose oxidase colorimetric method developed by Oakley (1983). The analysis of neutral sugar constituents and Klason lignin was performed by a modified procedure based on the method used by Theander and Åman (1979). Complete details on the modified procedure will appear in a subsequent publication (Ryan, 1982). Total nitrogen was determined by the usual Kjeldahl procedure, and ash was determined by combustion at 550 °C.

Serial Extraction Procedure. An empirical serial extraction procedure was used which was very similar to several of the ASTM standard test methods (D1102-56, D1103-60, D1105-56, D1106-56, and D1107-56) for the various components of wood. In this procedure the tobacco was serially extracted with a mixture of toluene and ethyl alcohol, hot water, an ammonium oxalate solution, a sodium chlorite solution, and a potassium hydroxide solution. After each extraction step the percentage of weight loss was determined. Finally, the residue was ignited and the weight loss was calculated as percent cellulose. The unburned residue was reported as percent ash.

Table I. Fraction Weights Obtained by the Fractionation Procedure

fraction	dry weight, g
bright lamina	89.6
EtOH extract	50.4
EtOH residue	41.1
termamyl residue	25.3
KOH residue	10.9
water-soluble fraction	13.8
insoluble fraction	7.6

Table II. Starch and Uronic Acid Contents of the Ethanol-Extracted Residue

component	dry weight, g
starch	2.9
uronic acid	8.63

RESULTS AND DISCUSSION

Determination of Biopolymer Cell Wall Composition from the Fractionation Procedure. Starting from a sample of flue-cured bright tobacco lamina, six fractions were separated by the scheme shown in Figure 1. The weights of all these materials, corrected to a dry weight basis (DWB), are reported in Table I. A material balance for tobacco was obtained by analysis of the fractions for selected tobacco components.

The initial 80% EtOH extraction of the bright lamina removed 56.3% of the solids. The EtOH extract was thought to contain reducing sugars, acids, alkaloids, polyphenols, lipids, waxes, resins, some pigments, and a portion of the water-soluble salts. The small discrepancy between the weight of the bright lamina and a summation of the weights for the EtOH extract and the EtOH residue (see Table I) may reflect the inherent variation in the moisture determinations.

The ethanol-extracted residue was analyzed for starch and uronic acid. The results are reported in Table II.

The residue from the 80% ethanol extraction in the Soxhlet apparatus contained less than 0.01% reducing sugars as determined by the method of Davis (1976). Analyses of the starting material and the ethanol-extracted residue by the procedure of Gaines (1970) revealed that little, if any, starch was removed by this extraction. The 41.1 g of residue from the ethanol extraction was found to contain 2.9 g of starch by the glucose oxidase colorimetric method. This value is equivalent to a starch level of 3.2% in the original tobacco. The ethanol extraction also removed chlorogenic acid, the only known non uronic acid component which gives a positive interference by the decarboxylation procedure (Bokelman, 1982). Hence, it was felt that the uronic acid content of the ethanol-extracted residue was indicative of the total pectin content of the tobacco. On the basis of the assumption that cured leaf pectin contains ~90% D-galacturonic acid (Bourne et al., 1967), the 8.63 g of uronic acid found in the ethanol residue represented a 10.7% pectin content in the original tobacco material.

The ethanol-extracted residue was then treated with Termamyl 60-L enzyme to convert the starch into water-soluble oligosaccharide fragments which were removed by subsequent dialysis. The use of this thermophilic amylase at relatively high temperature should have deactivated any contaminating enzymes, such as proteases, which may have been present.

An estimation of the protein content of this tobacco sample was made by using the procedure of Phillips and

Bacot (1953). The total nitrogen in the Termamyl residue was determined by the Kjeldahl procedure, and the nitrogen value obtained was multiplied by the conventional factor 6.25 to obtain the protein value (Anson, 1947). The 25.3 g of residue from the Termamyl treatment contained 0.98 g of total nitrogen, which is equivalent to 6.1 g of protein. It was thought that the protein content of the Termamyl residue represented the structural protein content of the initial material.

The material remaining after the Termamyl treatment had a Klason residue value of 11.0%. This Klason residue was further analyzed for total nitrogen and ash. The 4.45% total nitrogen value was equivalent to 27.8% protein. Correcting the weight of the Klason residue for protein and ash gave a weight of 1.53 g. This weight which represents 1.7% of the initial material was thought to be a maximum value for the lignin content of the bright tobacco.

The residue from the Termamyl treatment was extracted with dilute alkali to solubilize hemicellulose, protein, and pectin. The extraction was done under a nitrogen atmosphere to prevent the formation of oxidation products. Sodium borohydride was added to the solution to minimize elimination reactions. The 1-octanol was an effective antifoaming agent.

As shown in Figure 1, the final treatment consisted of an acid detergent extraction of the KOH residue. Prior work had shown that detergent extraction solubilized pectin and hemicellulose more effectively than alkaline extraction; however, the detergent could not be completely removed from the structural components in the filtrate. Therefore, the detergent extraction was performed on the residue from the alkaline extraction as a final purification of the insoluble residue. The filtrate was discarded.

As shown in Figure 1, the tobacco cell wall components were now separated into two classes: a water-soluble fraction and an insoluble fraction. These two fractions were analyzed for structural components.

The water-soluble fraction had a uronic acid content of 22.5%. On the basis of the assumption that cured leaf pectin contains ~90% D-galacturonic acid (Bourne et al., 1967), the water-soluble fraction was calculated to have a pectin content of 25.0%. The total neutral sugar content of this fraction was 22.3%. Of the neutral sugars present, 2.5% were assumed to be associated with pectin and the remaining 19.8% were thought to represent soluble hemicellulose present in this fraction. From this value, the soluble hemicellulose content was calculated to be 2.5 g which represented 2.8% of the initial material.

Integration of a 25-MHz ^{13}C NMR spectrum (XL-100) of the water-soluble fraction in D_2O indicated that the aromatic resonances from 123 to 136 ppm equaled 3% of the total carbon present in the fraction. It was assumed that these aromatic components were derived from lignin, e.g., by the hydrolysis of *p*-coumaric acid and ferulic ester groups (Sarkanen and Ludwig, 1971). Since phenylpropane groups are the basic units present in lignin, the NMR value of 3% aromatic carbon corresponds to 4.5% lignin. Thus, it may be estimated that the water-soluble fraction had a soluble lignin content of 0.6 g, which represents 0.7% of the initial tobacco. It is thought that some of the soluble lignin may have been lost during dialysis, so that value cited may understate the actual soluble lignin content.

The total ash content of the water-soluble fraction was 18.1%, which represented 2.5 g of the weight of this fraction. This value for "soluble" ash corresponded to 2.8% of the starting material. This fraction also had a 5.5% Klason residue value. It was thought that this residue represented material that was not hydrolyzed by sulfuric acid due to mechanical problems in the procedure. This value represented 0.8 g of the weight of this fraction, which corresponded to 0.8% of the initial material.

The insoluble fraction had an 87.8% neutral sugar content. The neutral sugar distribution for this fraction was 6.3% xylose, 5.4% mannose, and 88.3% glucose (Ryan, 1982). The absence of fucose in the neutral sugar distribution was thought to indicate that there were no xyloglucans present (McNeil et al., 1979). This finding with bright lamina was consistent with previous tobacco tissue culture research which demonstrated that tobacco did not contain xyloglucans (Bokelman, 1980). Thus, it was assumed that all the glucose was derived from cellulose. On the basis of this assumption, a cellulose content of 5.3 g was calculated. This value represents 5.9% of the total solids in the starting material. The xylose and mannose were thought to represent a hemicellulose relatively tightly bound to the surface of the cellulose. The amount of hemicellulose associated with the cellulose was calculated to be 0.7 g, which was equivalent to 0.8% of the initial material.

A Klason residue value of 20.0% was obtained from the neutral sugar analysis of the insoluble fraction. The 1.5 g of this residue was corrected for the 0.9 g of insoluble ash, yielding an insoluble lignin value of 0.6 g which represents 0.7% of the total solids in the initial material. The major component in the insoluble ash was determined by atomic absorption spectroscopy to be silica.

In order to complete a mass balance for this fractionation procedure, the weights of two additional fractions must be calculated. These two fractions are (1) "aqueous solubles lost during dialysis" and (2) acid detergent solubles.

The weight of the "aqueous solubles lost during dialysis" fraction was calculated by subtracting the weight of the KOH residue plus the weight of the water-soluble fraction corrected for the Termamyl enzyme content from the weight of the EtOH residue. Additional corrections were made for the degraded starch, soluble pectin, and solubilized protein lost during dialysis in order that these three components would not be counted twice in the mass balance.

The Termamyl treatment used 7.2 mL of Termamyl 60-L enzyme with a density of 1.17 g/mL and a nitrogen content of 1.04%. The enzyme solution therefore contains 0.5 g of Termamyl enzyme. Since this soluble enzyme has a molecular weight of ~100 000, none of it would have been lost during dialysis. The weight of the water-soluble fraction corrected for the contribution from Termamyl enzyme is 13.3 g. In addition, the Termamyl enzyme degraded all of the starch in the EtOH residue to oligosaccharides that would have been lost during dialysis. Therefore, 2.9 g of starch was lost.

It was previously calculated from the uronic acid analysis that the pectin content of the 13.8 g of water-soluble fraction was 25.0% or 3.5 g. Since the total pectin content of the bright lamina was 9.6 g, then 9.6 g − 3.5 g = 6.1 g of soluble pectin was lost during dialysis. The KOH residue had a total nitrogen content of 0.08 g. The water-soluble fraction had a total nitrogen content of 0.48 g of which 0.09 g came from Termamyl enzyme. Hence, the total non-Termamyl total nitrogen content of the KOH residue plus the water-soluble fraction was 0.47 g, which is equivalent to a protein content of 2.9 g. Since the total protein content of the initial material was 6.1 g, then 3.2 g of solubilized structural protein was lost during dialysis.

Table III. Biopolymer Cell Wall Composition of Bright Tobacco Obtained by the Fractionation Procedure

tobacco component		dry weight, g	% of total solids
ethanol solubles		50.4	56.3
aqueous solubles lost during dialysis		4.7	5.2
acid detergent solubles		2.9	3.2
pectin		9.6	10.7
starch		2.9	3.2
protein		6.1	6.8
hemicellulose:	total	3.2	3.6
	soluble	2.5	
	associated with cellulose	0.7	
lignin:	total	1.2	1.3
	soluble	0.6	
	insoluble	0.6	
cellulose		5.3	5.9
ash:	total	3.4	3.8
	soluble	2.5	
	insoluble	0.9	
total		89.7	100.0

A synopsis of the calculation of the weight of aqueous solubles lost during dialysis is as follows:

EtOH residue	41.1 g
−KOH residue	10.9 g
−water-soluble fraction corrected for Termamyl	13.3 g
−solubilized starch	2.9 g
−solubilized pectin	6.1 g
−solubilized protein	3.2 g
=aqueous solubles lost during dialysis	=4.7 g

It was thought that this fraction consisted primarily of water-soluble salts. However, some soluble lignin and small amounts of low molecular weight or degraded hemicellulose may contribute to this total.

The acid detergent soluble fraction must also be calculated. This fraction consisted of those tobacco components that were extracted by the Van Soest acid detergent procedure. It is thought that this fraction contained hemicellulose, protein, and possibly some small amount of lignin. The KOH residue weighed 10.9 g and had a total nitrogen content of 0.08 g. The insoluble fraction from the acid detergent extraction weighed 7.6 g and had a total nitrogen content of 0.02 g. Subtracting the weight of the insoluble fraction from the weight of the KOH residue and correcting for the protein content gave a value of 2.9 g as the weight of the acid detergent soluble fraction.

A summary of the weights of the tobacco components separated by this fractionation procedure is presented in Table III.

Comparison of Biopolymer Cell Wall Compositions Determined from the Fractionation Procedure and from the Serial Extraction Procedure. The bright tobacco used in Figure 1 was also characterized by the empirical serial extraction procedure formerly used at our company to characterize "structural carbohydrates" (see Table IV). This procedure does not yield values for starch or protein.

The value for toluene–alcohol solubles (47.4%) in Table IV is a little less than the value for ethanol solubles (48.9%) in Table III. This difference may reflect less removal of reducing sugars by the toluene–alcohol extraction procedure.

The values for cellulose listed in the two tables are also in reasonable agreement. However, most of the other values vary considerably between the two tables. This fact is not surprising since the characterization of components for Table IV is based almost exclusively on the concept of selective extraction and it is well-known that many distinct types of biopolymers have overlapping ranges of solubility.

Table IV. Biopolymer Cell Wall Composition of Bright Tobacco Obtained by the Serial Extraction Procedure

tobacco component	% of total solids
toluene–alcohol solubles	47.4
aqueous solubles	19.8
ammonium oxalate solubles ("pectin")	6.9
sodium chlorite solubles ("lignin")	7.8
hemicellulose	13.0
cellulose	5.0
ash	0.2
total	100.1

The aqueous solubles value (19.8%) in Table IV is much higher than the "aqueous solubles lost during dialysis" value (5.2%) in Table III. This difference probably can be attributed to the inclusion of starch, reducing sugars, some pectin, and some ash in the value for water-solubles.

The value for ammonium oxalate solubles (6.9%) in Table IV is significantly less than the pectin value (10.7%) in Table III. This discrepancy may indicate that either some of the pectin has been included in the value for water-solubles or ammonium oxalate does not extract all the available pectin.

A very striking difference between these tables can be seen by comparing the 7.8% value for sodium chlorite solubles in Table IV with the 1.3% lignin value in Table III. It may be that both figures are in error and that the true lignin content is somewhere between these two numbers. Some of the soluble lignin may have been lost during dialysis for the fractionation procedure given in Figure 1. However, according to Phillips and Bacot (1953) the lignin content for flue-cured tobacco should not exceed a range of 2.05–4.76%.

The hemicellulose value listed in Table IV (13.0%) is much greater than the hemicellulose value given in Table III (3.6%). It may be that the major constituent of the acid detergent solubles listed in Table III is hemicellulose. If so, then the total hemicellulose content from Table III would approach 6.8%. The difference between 13.0% and 6.8% can probably be attributed to the extraction of protein by the 24% KOH used in the hemicellulose solubilization step for Table IV (Lamport, 1965).

The final major difference between these two procedures was in ash content. The lower ash value in Table IV was probably a reflection of the greater number of extraction steps incorporated in the serial extraction procedure.

Comparison of the Biopolymer Cell Wall Composition of Bright Tobacco Determined from the Fractionation Procedure with the Biopolymer Composition of the Primary Cell Walls of Dicots. It has been predicted (Albersheim, 1980) that the cell walls of tobacco lamina should resemble the cell walls of suspension-cultured sycamore cells, since both tobacco and sycamore are dicotyledons. The walls of suspension-cultured sycamore cells, which grow as small cell aggregates, have been extensively studied as a model of primary cell walls (Talmadge et al., 1973; Bauer et al., 1973; Keegstra et al., 1973; Wilder and Albersheim, 1973; McNeil et al., 1979). Thus, it was informative to compare the composition of cell wall components from the bright tobacco used in this study with that of suspension-cultured sycamore cells (McNeil et al., 1979) as shown in Table V.

The values for the various tobacco cell wall components listed in Table V were taken directly from Table III. The

Table V. Biopolymer Cell Wall Compositions of Bright Tobacco and Suspension-Cultured Sycamore Cells

cell wall component	% of tobacco total solids		% of tobacco cell walls		% of sycamore cell walls
pectin		10.7		34.0	34
hemicellulose	3.6	6.8	11.4	21.6	24
acid detergent solubles	3.2		10.2		
cellulose		5.9		18.7	23
protein		6.8		21.6	19
lignin		1.3		4.1	0
total		31.5		100.0	100

only exception was the value for hemicellulose. As discussed in the preceding section, it was thought that a combination of the values for hemicellulose and acid detergent solubles would produce a more accurate representation of the total hemicellulose content. A summation of the values for pectin, hemicellulose, acid detergent solubles, cellulose, protein, and lignin accounted for 31.5% of the total tobacco solids. The values of these tobacco cell wall components were normalized to facilitate comparison with a model system, the primary cell wall of suspension-cultured sycamore cells.

On first examination, the similarity of biopolymer cell wall compositions of flue-cured bright tobacco lamina and suspension-cultured sycamore cells is obvious. However, certain factors suggest that the cells of this bright tobacco had started to form secondary walls. Lignin, which is not found in primary cell walls, is present in the tobacco cell walls. Also, the significantly higher content of mannose in the tobacco cell walls (Ryan, 1982) than in the suspension-cultured sycamore cell walls (Talmadge et al., 1973) indicates the presence of polysaccharides usually associated with secondary cell walls.

These results suggest that tobacco lamina initially consists of primary cell walls but that as the leaves mature a cessation of growth may be accompanied by a small amount of secondary wall formation.

CONCLUSIONS

A general procedure for the separation of tobacco into several fractions has been developed. Numerous polymeric tobacco cell wall components were determined by analysis of these fractions. Further work on the structural biopolymers of tobacco, based on the fractions generated by this procedure, is in progress.

ACKNOWLEDGMENT

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Registry No. Pectin, 9000-69-5; cellulose, 9004-34-6; hemicellulose, 9034-32-6; lignin, 9005-53-2; starch, 9005-25-8.

LITERATURE CITED

Albersheim, P., Department of Chemistry, University of Colorado, Boulder, CO, personal communication, 1980.
Anson, M. L. *Adv. Protein Chem.* **1947**, *3*, 130.
Bauer, W. D.; Talmadge, K. W.; Keegstra, K.; Albersheim, P. *Plant Physiol.* **1973**, *51*, 174–187.
Bokelman, G. H., Research Center, Philip Morris U.S.A., Richmond, VA, unpublished research, 1980.
Bokelman, G. H., Research Center, Philip Morris U.S.A., Richmond, VA, unpublished research, 1982.
Bourne, E. J.; Pridham, J. B.; Worth, H. G. J. *Phytochemistry* **1967**, *6*, 423–431.
Bylund, M.; Donetzhuber, A. *Sven. Papperstidn.* **1968**, *15*, 505–508.
Christy, M. G.; Samfield, M. *Tob. Sci.* **1960**, *4*, 33–37.
Davis, R. E. *Tob. Sci.* **1976**, *20*, 146–151.
Eda, S.; Katō, K. *Agric. Biol. Chem.* **1978**, *42*, 351–357.
Eda, S.; Katō, K. *Agric. Biol. Chem.* **1980**, *44*, 2793–2801.
Eda, S.; Ohnishi, A.; Katō, K. *Agric. Biol. Chem.* **1976**, *40*, 359–364.
Eda, S.; Watanabe, F.; Katō, K. *Agric. Biol. Chem.* **1977**, *41*, 429–434.
Gaines, T. P. *Tob. Sci.* **1970**, *14*, 164–166.
Goering, H. K.; Van Soest, P. J. *U.S., Dep. Agric., Agric. Handb.* **1970**, *No. 379*.
Keegstra, K.; Talmadge, K. W.; Bauer, W. D.; Albersheim, P. *Plant Physiol.* **1973**, *51*, 188–196.
Lamport, D. T. A. *Adv. Bot. Res.* **1965**, *2*, 151–218.
McNeil, M.; Darvill, A. G.; Albersheim, P. *Fortschr. Chem. Org. Naturst.* **1979**, *37*, 191–249.
Mori, M.; Eda, S.; Katō, K. *Agric. Biol. Chem.* **1979**, *43*, 145–149.
Mori, M.; Eda, S.; Katō, K. *Carbohydr. Res.* **1980**, *84*, 125–135.
Mori, M.; Katō, K. *Carbohydr. Res.* **1981**, *91*, 49–58.
Oakley, E. T. *J. Agric. Food Chem.* **1983**, following paper in this issue.
Phillips, M.; Bacot, A. M. *J. Assoc. Off. Agric. Chem.* **1953**, *36*, 504–524.
Ryan, W. S., Jr., submitted for publication in *Beitr. Tabakforsch.*, 1982.
Salomonsson, A.-C.; Theander, O.; Åman, P. *Swed. J. Agric. Res.* **1980**, *10*, 11–16.
Sarkanen, K. V.; Ludwig, C. H. "Lignins-Occurrence, Formation, Structure and Reactions"; Wiley-Interscience: New York, 1971; Chapter 5, p 188.
Talmadge, K. W.; Keegstra, K.; Bauer, W. D.; Albersheim, P. *Plant Physiol.* **1973**, *51*, 158–173.
Theander, O.; Åman, P. *Swed. J. Agric. Res.* **1979**, *9*, 97–106.
Van Soest, P. J. *J. Assoc. Off. Agric. Chem.* **1963**, *46*, 829–835.
Van Soest, P. J.; Wine, R. H. *J. Assoc. Off. Anal. Chem.* **1967**, *50*, 50–55.
Van Soest, P. J.; Wine, R. H. *J. Assoc. Off. Anal. Chem.* **1968**, *51*, 780–785.
Wilder, B. M.; Albersheim, P. *Plant Physiol.* **1973**, *51*, 889–893.

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