

- Creech, R. G. *Adv. Agron.* 1968, 20, 275.
 Da Silva, W. J.; Teixeira, J. P. F.; Arruda, P.; Lovatto, M. B. *Maydica* 1978, 23, 129.
 Dimler, R. J. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* 1966, 25, 1670.
 FAO/WHO *W.H.O. Tech. Rep. Ser.* 1973, No. 522.
 Jones, R. A.; Larkins, B. A.; Tsai, C. Y. *Plant Physiol.* 1977, 59, 525.
 Landry, J.; Moureaux, T. *Bull. Soc. Chim. Biol.* 1970, 52, 1021.
 Landry, J.; Moureaux, T. *Qual. Plant—Plant Foods Hum. Nutr.* 1976, 25, 343.
 Mertz, E. T. *Agric. Sci. Rev.* 1968, 6, 1.
 Mertz, E. T.; Bates, L. S.; Nelson, O. E. *Science (Washington, D.C.)* 1964, 145, 279.
 Misra, P. S.; Mertz, E. T.; Glover, D. V. *Cereal Chem.* 1975a, 52, 161.
 Misra, P. S.; Mertz, E. T.; Glover, D. V. *Cereal Chem.* 1975b, 52, 734.
 Misra, P. S.; Mertz, E. T.; Glover, D. V. *Cereal Chem.* 1975c, 52, 844.
 Misra, P. S.; Mertz, E. T.; Glover, D. V. *Cereal Chem.* 1976, 53, 699.
 Mitchell, H. H. *J. Biol. Chem.* 1923, 58, 873.
 Mossé, J.; Baudet, J.; Landry, J.; Moureaux, T. *Ann. Physiol. Veg. Univ. Bruxelles* 1966, 8, 331.
 Murphy, J. T.; Dalby, A. *Cereal Chem.* 1971, 48, 336.
 Nelson, O. E.; Mertz, E. T.; Bates, L. S. *Science (Washington, D.C.)* 1965, 150, 1469.
 Paulis, J. W.; Wall, J. S. *Cereal Chem.* 1969, 46, 263.
 Paulis, J. W.; Wall, J. S. *Biochim. Biophys. Acta* 1971, 251, 57.
 Robutti, J. L.; Roseny, R. C.; Deyoe, C. W. *Cereal Chem.* 1974, 51, 163.
 Sgarbieri, V. C.; Da Silva, W. J.; Antunes, P. L.; Amaya, F. J. *J. Agric. Food Chem.* 1977, 25, 1098.
 Sodek, L.; Wilson, C. M. *J. Agric. Food Chem.* 1971, 19, 1144.
 Spies, J. R. *Anal. Chem.* 1967, 39, 1412.

Received for review June 25, 1980. Revised manuscript received October 1, 1981. Accepted October 20, 1982.

Monosaccharide Composition of Alcohol- and Detergent-Insoluble Residues in Maturing Reed Canarygrass Leaves

Allan S. Bittner* and Joseph C. Street

Total cell wall sugars and hemicellulosic sugars of reed canarygrass leaves (*Phalaris arundinaceae* L.) increased with increasing plant maturity. The predominant hemicellulosic polymers appeared to be xylans. Neutral detergent residues contained less noncellulosic sugars than the 80% ethanol insoluble residues. Neutral detergent may have solubilized acidic hemicellulosic polysaccharides in addition to pectic polysaccharides. The acid detergent residues contained considerable amounts of arabinose, xylose, and uronic acids. The high xylose/arabinose ratio present in the acid detergent fiber residues may reflect the presence of linear xylans associated with cellulose in a manner sufficient to render the xylans resistant to dilute acid hydrolysis. As a result, the detergent methods underestimated total hemicellulose in the leaves of maturing reed canarygrass. Hydrolysis of alcohol-insoluble residues with 72% sulfuric acid followed by dilution to 2 N is proposed as a means for estimating noncellulosic polysaccharides.

Most research concerned with the evaluation of forage plants for nutritional purposes has relied on gravimetric methods for the determination of plant cell wall components. The gravimetric detergent methods have been well accepted because of their usefulness for prediction of various parameters, such as dry matter intake, with reasonable accuracy (Van Soest et al., 1978). The detergent methods have been recommended for use in establishing hay standards (Rohweder et al., 1977) and have been used recently for the selection of high-yielding reed canarygrass clones that have low amounts of cell wall constituents (Marum and Hovin, 1979).

The neutral and acid detergent procedures and the crude fiber procedures are similar approaches since, in each case, the plant components are defined in terms of laboratory operations rather than constituent chemicals. Examination of the carbohydrate types present in residues of neutral detergent fiber (NDF) and acid detergent fiber (ADF) has shown that neither method completely partitions cellulose and noncellulosic polysaccharides (Bailey and Ulyatt, 1970; Collings and Yokoyama, 1979; Theander and Aman, 1980; Morrison, 1980). Morrison (1980) reported the presence of hemicellulosic sugars in the ADF residues prepared from a wide variety of plant species. Theander and Aman (1980)

reported the presence of hemicellulosic sugars and crude protein in the ADF residues of several forage species including alkali-treated straw. The NDF residues prepared from the same forages also contained substantial amounts of crude protein in addition to hemicellulose, uronic acids, and Klason lignin. In view of the presence of extraneous substances in the detergent-insoluble residues, the general validity of these methods for the accurate assessment of cell wall constituents is subject to question.

Theander and Aman (1980) defined hemicellulose in terms of monosaccharides other than glucose that were quantitated after acid hydrolysis of plant cell wall preparations. Xylans substituted with arabinose, galactose, and uronic acid are known to be characteristic of the hemicellulosic polysaccharides in grasses (Wilkie, 1979) and have been proposed as the principal hemicellulosic polymers in the cell walls of monocotyledonous plants (Anderson and Stone, 1978). The quantitation of grass hemicellulose and other cell wall polymers in terms of sugar constituents would thus appear to be a more accurate approach to the evaluation of plant composition and the nutritional potential of these forage plants than is provided by the existing empirical methods.

This manuscript reports the results obtained from a detailed study of the acid detergent, neutral detergent, and aqueous ethanol insoluble cell wall polysaccharides in maturing reed canarygrass leaves. This study was performed for the purpose of evaluating methodology designed

Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, Utah 84322.

to determine nutritional properties of forage plants and most specifically grasses.

MATERIALS AND METHODS

Preparation of Plant Samples. Reed canarygrass leaves (*Phalaris arundinaceae* L.) were harvested at six successive stages of maturity from April 22 to June 4. The grass leaves were cut within a 30 × 3 m area in a field on the university dairy farm. Leaf area was measured electronically with a leaf area meter on 20 fresh leaves sampled from each cutting. The remaining samples were immediately frozen with dry ice and then dried in a freeze-dryer. The lyophilized samples were ground in a cold room with a Wiley mill equipped with a 65-mesh screen. The ground samples were stored in a desiccator at -15 °C. Dry weights were determined after placing samples in a 60 °C vacuum oven overnight and cooling in a desiccator (Collings et al., 1978).

Preparation of Cell Walls. Cell walls were prepared as the alcohol-insoluble residue (AIR). Approximately 1 g of ground plant material was placed in a 500-mL round-bottom flask with a magnetic stirring bar and 200 mL of 80% aqueous ethanol. The mixture was refluxed for 1 h with continuous stirring. After 1 h the mixture was immediately filtered with a crucible (coarse porosity) under negative pressure. The flask and crucible were repeatedly washed with hot ethanol solution (30 mL) until the filtrate was colorless. The alcohol-insoluble residue (AIR) was saved for further characterization of sugar content.

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were determined as previously described (Van Soest, 1963; Van Soest and Wine, 1967), and the residues were saved for characterization of carbohydrate content.

GLC Analysis of Neutral Sugars. The residues were hydrolyzed with 12 M sulfuric acid for 30 min at room temperature followed by dilution to 2 N and hydrolysis at 95 °C for 3 h. A 200- μ L aliquot of the hydrolysate was analyzed for neutral sugars as previously described (Bittner et al., 1980). Values obtained for individual sugars were corrected with factors calculated from hydrolysis curves.

Colorimetric Determination of Sugars. Uronic acids were quantitated with *m*-phenylphenol as described by Blumenkrantz and Asboe-Hansen (1973). Total carbohydrate in the acid hydrolysates was estimated colorimetrically with the phenol-sulfuric acid procedure of DuBois et al. (1956). Starch present in the residues was estimated with amyloglucosidase and glucose oxidase by using the method of MacRae (1971).

RESULTS AND DISCUSSION

The neutral and acidic sugar constituents analyzed in the alcohol-insoluble residue (AIR) of reed canarygrass leaves harvested at six stages of growth are described in Table IV. The carbohydrate portion of reed canarygrass cell wall is similar to that of other Poaceae species previously reported (Wilkie, 1979). The increase in xylose relative to arabinose (Table I) and galactose (Table IV) indicates the probable increasing presence of linear xylans in maturing leaves. Morrison (1974) described a similar relationship in the hemicellulose of maturing ryegrass. The coincident increase of xylose and total glucose in the AIR of maturing leaves (Table IV) may also reflect the association of linear xylans with cellulose microfibrils (McNeil et al., 1975).

The rapid change in leaf area that occurred during the first 13 days of growth (Table I) coincided with a relatively small change in the leaf xylose/arabinose ratio. This occurrence reflects rapid plant growth and may also reflect the predominant presence of branched xylans at this stage

Table I. Area and Xylose/Arabinose Ratio of Reed Canarygrass Leaves Harvested at Six Stages of Growth

harvest date	leaf area		<i>F</i> test ^b	AIR
	mean ^a	error		xylose/arabinose ratio
April 22	15.99	0.97		3.4
April 28	23.27	1.44	5.36 ^c	3.2
May 5	34.89	1.47	13.63 ^c	3.8
May 13	38.75	1.93	1.51	3.9
May 22	42.78	2.11	1.64	4.3
June 4	43.95	1.72	0.14	4.8

^a *n* = 20; area in square centimeters. ^b Test for significance. ^c Significant difference observed (*P* less than 0.05).

Table II. Detergent Parameters of Reed Canarygrass Leaves Harvested at Six Stages of Growth^a

harvest date	NDF	ADF	NDF - ADF	ADF lignin ^b	ash
April 22	35.8	19.5	16.3	1.9	2.3
April 28	35.4	19.2	16.2	1.3	1.8
May 5	37.8	22.7	15.1	1.2	2.7
May 13	36.0	22.2	13.8	1.2	2.7
May 22	41.9	26.4	15.5	2.0	3.4
June 4	43.1	27.8	15.3	2.6 ^c	3.4

^a Expressed as percent of whole plant dry matter.

^b Corrected for ash content. ^c Standard error of duplicate greater than 5%.

of maturity. The plateau observed for leaf area during the remaining harvest dates coincides with a substantial increase in the xylose/arabinose ratio. This suggests that the synthesis of linear polymers coincides with formation of secondary walls. A rapid increase in lignin and ash (Table II) was also coincident with the increasing xylose/arabinose ratio during the last two harvest dates.

Plant maturation was also accompanied by an increase in the detergent insoluble residues (Table II). This was anticipated as components of cell wall generally increase with plant maturity. The net decline in estimated hemicellulose with increasing plant maturity was unexpected and led to further investigation of the sugar composition of the detergent-insoluble residues.

Monosaccharide Composition of NDF, ADF, and AIR. The detergent methods were originally designed to partition digestible from nondigestible plant components (Van Soest and Robertson, 1980). The ADF-insoluble residue afforded a relatively simple and rapid estimation of plant fiber components. The mathematical difference between NDF and ADF was proposed as an estimate of plant hemicellulose whereas the ADF residue provided an estimation of cellulose when corrected for lignin and ash.

Noncellulosic sugars are presented in Table III, and individual monosaccharide constituents in AIR, NDF, and ADF are described in Table IV. The noncellulosic sugar content of the NDF residue was less than that of the AIR for all stages of plant maturity. The greatest difference was observed in the younger cuttings and may have been due to the increased solubility of the relatively less lignified cell wall constituents (Table II). The ADF residues also contained substantial amounts of noncellulosic sugars with the greatest abundance of hemicellulosic monosaccharides occurring in the most mature cuttings. The presence of noncellulosic sugars in the ADF residues and the apparent solubility of noncellulosic polysaccharides in the NDF reagent may have contributed, in part, to the underestimation of calculated hemicellulose in Table II. Other factors contributing to the underestimation of hemicellulose by detergent methods have been previously reported (Van Soest and Robertson, 1980).

Table III. Noncellulosic Sugars Present in Acid Hydrolysates of AIR, NDF, and ADF Residues Prepared from Reed Canarygrass Leaves Harvested at Six Stages of Growth^a

harvest date	AIR		NDF		ADF	
	neutral sugars ^b	uronic acids ^c	neutral sugars	uronic acids	neutral sugars	uronic acids
April 22	15.4	3.6	11.9	1.7	3.5	0.5
April 28	16.0	3.5	13.9	1.7	3.6	0.5
May 5	17.0	3.8	15.9	1.8	3.6	0.6
May 13	17.3	3.7	15.0	1.8	3.3	0.6
May 22	18.5	3.2	16.2	1.8	4.9	0.8
June 4	18.6	3.1	16.5	1.8	5.1	0.8

^a Expressed as percent of whole plant dry matter.

^b Sum of arabinose, xylose, and galactose. ^c Determined colorimetrically with method of Blumenkrantz and Asboe-Hansen (1973).

Table IV. Monosaccharide Composition of AIR, NDF, and ADF Residues Prepared from Reed Canarygrass Leaves Harvested at Six Stages of Growth^a

harvest date	residue	noncellulosic monosaccharides				glucose ^b
		arabinose	xylose	galactose	uronic acids	
April 22	AIR	9.4	31.3	4.9	10.7	44.0
	NDF	8.8	31.0	3.3	6.3	50.6
	ADF	3.2	16.9	nd ^c	2.7	77.2
April 28	AIR	9.5	30.5	5.0	9.7	46.0
	NDF	10.1	31.8	3.3	5.5	49.3
	ADF	3.0	17.4	nd	2.6	77.0
May 5	AIR	8.5	31.5	4.6	10.0	46.2
	NDF	9.4	32.4	3.1	5.1	50.0
	ADF	2.6	15.4	nd	3.2	78.8
May 13	AIR	8.1	31.3	4.9	9.4	46.7
	NDF	9.2	31.0	3.8	5.2	50.8
	ADF	2.4	16.3	nd	3.3	78.0
May 22	AIR	8.0	33.8	3.2	7.7	47.2
	NDF	8.4	32.9	3.4	4.9	50.5
	ADF	3.2	17.5	nd	3.2	76.1
June 4	AIR	7.2	34.3	3.6	7.5	47.3
	NDF	7.7	33.2	2.8	4.9	51.4
	ADF	3.0	17.5	nd	3.3	76.1

^a Expressed as percent total residue carbohydrate.

^b Corrected for starch content. ^c None detected.

The NDF residue contained less uronic acids than the AIR for all stages of plant growth. Solubilization of polysaccharides relatively high in uronic acid content by neutral detergent probably resulted in this phenomenon. Grass species previously studied have contained only small amounts of pectic polysaccharides. Burke et al. (1974) reported the occurrence of small amounts of rhamnose in the exudates of ryegrass and oat cell suspension cultures. Further studies of oat coleoptile polysaccharides (Wada and Ray, 1978) confirmed the presence of a rhamnogalacturonan in small quantities. The authors concluded that monocots generally have only small amounts of polygalacturonic acid containing polysaccharides. The presence of only trace amounts of rhamnose in the AIR and NDF fractions suggests that reed canarygrass is not unlike other grasses in this respect. The reduced amount of uronic acids and other noncellulosic sugars (Table III) in the NDF residues suggests that acidic hemicellulosic polysaccharides and small amounts of pectic polysaccharides were solubilized by neutral detergent. The uronic acids present in the ADF residues may be a result of refractory acidic polysaccharides (Van Soest and Robertson, 1980) but more likely originate from uronic acids in hemicellulose that is structurally associated with the ADF cellulose polymers.

Table V. Noncellulosic Glucose in NDF and AIR of Reed Canarygrass Leaves Harvested at Six Stages of Growth^a

harvest date	NDF	AIR
April 22	2.6	7.4
April 28	11.3	16.6
May 5	11.3	9.3
May 13	12.5	16.4
May 22	1.4	6.6
June 4	2.1	2.2

^a Expressed as percent of residue total glucose corrected for starch.

Noncellulosic Glucose. It was assumed that the ADF solution removed most glucose of noncellulosic origin. As a result, glucose originating from the noncellulosic polymers was estimated as the difference between total NDF or AIR glucose and ADF glucose. The AIR total glucose values were corrected for starch. No starch was observed in the NDF or ADF residues. The greatest percentages of noncellulosic glucose were observed in the younger cuttings (Table V). The values in Table V suggest that noncellulosic glucose content is quite variable with respect to maturity. The variability may be a result of experimental error associated with the determination of starch and AIR or NDF glucose.

The presence of noncellulosic glucose may be attributed to the occurrence of β -glucan (Wilkie, 1979) polymers in grass hemicellulose. Burke et al. (1974) could not confirm the presence of xyloglucan in suspension cultured monocots. However, the presence of β -glucans in the water-soluble portion of Me₂SO extracted reed canarygrass cell walls has been previously reported (Bittner, 1980).

Morrison (1980) reported that the ADF and acetic-nitric acid methods overestimated cellulose because noncellulosic polysaccharides remain in the acid-insoluble residue. He, therefore, corrected the values for cellulose by assigning all glucose contained in the residues to cellulose and assigning arabinose, xylose, and mannose to the hemicellulose category. That hemicellulosic "contamination" of the insoluble residues was attributed to covalently bound hemicellulose and lignin still present in the residues after acid hydrolysis. Total hemicellulose was calculated by Morrison (1980) by subtraction of the "corrected" cellulosic value from the neutral detergent residue. The author concluded that hemicellulose was overestimated because of starch remaining in the neutral detergent residue. The presence of uronic acids in hemicellulose was, however, not acknowledged in this study.

According to Theander and Aman (1980), protein is a more likely contaminant of the neutral detergent residue than is starch. It appears that contamination of the neutral detergent residue may arise from starch or protein or both, to a degree somewhat dependent on the species analyzed. For the reasons above, it may be desirable to eliminate neutral detergent from procedures attempting to quantitate all cell wall carbohydrates.

It appears that any procedure that is based on the gravimetric determination of cellulose following dilute acid hydrolysis will overestimate cellulose. This could be due to the failure of the acid to hydrolyze glycosyl linkages (Bauer et al., 1973). This phenomenon could also be due to the hydrogen-bonding affinity of linear xylans for cellulose fibrils. McNeil et al. (1975) observed in barley xylans that the greater the degree of arabinosyl branching, the less the polysaccharide is able to bind to cellulose fibrils. Polymers of a more linear nature have a greater affinity for cellulose fibrils. The extremely high xylose/arabinose ratios occurring in the acid-insoluble residues of reed canary grass leaves (Table IV) and those reported

by Morrison (1980) and Theander and Aman (1980) support this hypothesis. It may be impossible to distinguish chemically between cellulose polymers and linear xylans that remain in the dilute acid insoluble "cellulose" residues. As a result, two-step hydrolysis commencing with 12 M sulfuric acid may be the only rapid means of estimating total hemicellulose in terms of total noncellulosic sugars. From a nutritional standpoint the "hemicellulosic" polymers contained in the insoluble residues may have characteristics similar to cellulose. The need for quantitating hemicellulose for nutritional purposes should proceed with this in mind.

Registry No. Xylan, 9014-63-5; cellulose, 9004-34-6; hemicellulose, 9034-32-6.

LITERATURE CITED

- Anderson, R. L.; Stone, B. A. *Aust. J. Biol. Sci.* **1978**, *31*, 573.
 Bauer, W. D.; Talmadge, K. W.; Kaegstra, K.; Albersheim, P. *Plant Physiol.* **1973**, *51*, 174.
 Bailey, R. W.; Ulyatt, M. J. *N. Z. J. Agric. Res.* **1970**, *13*, 591.
 Bittner, A. S. Ph.D. Dissertation, Utah State University, Logan, UT, 1980.
 Bittner, A. S.; Harris, L. E.; Campbell, W. F. *J. Agric. Food Chem.* **1980**, *28*, 1242.
 Blumenkrantz, N.; Asboe-Hansen, G. *Anal. Chem.* **1973**, *54*, 484.
 Burke, D.; Kaufman, P.; McNeil, M.; Albersheim, P. *Plant Physiol.* **1974**, *54*, 109.
 Collings, G. F.; Yokoyama, M. T. *J. Agric. Food Chem.* **1979**, *27*, 373.
 Collings, G. F.; Yokoyama, M. T.; Bergen, W. G. *J. Dairy Sci.* **1978**, *61*, 1156.
 Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350.
 MacRae, J. C. *Planta* **1971**, *96*, 101.
 Marum, P.; Hovin, A. W. *Crop Sci.* **1979**, *19*, 280.
 McNeil, M.; Albersheim, P.; Taiz, L.; Jones, R. L. *Plant Physiol.* **1975**, *55*, 64.
 Morrison, I. M. *Carbohydr. Res.* **1974**, *36*, 45.
 Morrison, I. M. *J. Sci. Food Agric.* **1980**, *31*, 639.
 Rohweder, D. A.; Barnes, R. F.; Jorgensen, N. *Int. Symp.: Feed Compos., Anim. Nutr. Requir. Comput. Diets, [Proc.], 1st, 1976* **1977**, 242.
 Theander, O.; Aman, P. *J. Sci. Food Chem.* **1980**, *31*, 31.
 Van Soest, P. J. *J. Assoc. Off. Agric. Chem.* **1963**, *46*, 825.
 Van Soest, P. J.; Mertens, D. R.; Deinum, B. *J. Anim. Sci.* **1978**, *3*, 712.
 Van Soest, P. J.; Robertson, J. B. *Int. Dev. Res. Cent., [Tech. Rep.] IORC 1980, IDRC-134*, 49.
 Van Soest, P. J.; Wine, R. H. *J. Assoc. Off. Agric. Chem.* **1967**, *50*, 50.
 Wada, S.; Ray, P. M. *Phytochemistry* **1978**, *17*, 932.
 Wilkie, K. C. B. *Adv. Carbohydr. Chem. Biochem.* **1979**, *36*, 215.

Received for review February 8, 1982. Accepted August 16, 1982. This research was supported, in part, by a grant from the National Cancer Institute (CA25580) entitled "Diet and Colon Cancer in Man: The Effects of Fiber" and by the Utah Agricultural Experiment Station (Project 603).

Conformational Stability and Dissociation of a Peanut Storage Protein (Arachin) Exposed to Organic Solvents

Thomas J. Jacks,* Thomas P. Hensarling, Navin J. Neucere, and Elena E. Graves

Arachin (the major peanut storage protein) was exposed to hexane, acetone, hexane-acetone-water, and acidic hexane (mixtures of hexane and acetic acid), and its antigenicity, electrophoretic mobility, ultraviolet and infrared absorbances, and circular dichroism were examined. Samples exposed to hexane, acetone, and hexane-acetone-water were virtually identical with native arachin in these properties, indicating no effects of these solvents. Exposure to acidic hexane, however, resulted in loss of reactivity to antiarachin and increased electrophoretic mobility in nondenaturing gels but no corresponding change of migrational pattern in dissociating sodium dodecyl sulfate gels and no changes in infrared and circular dichroic spectra. Results were interpreted as irreversible dissociation of arachin by acidic hexane into subunits, each of which maintained the native secondary structure of the multimeric form. Antigenic determinants embraced separate subunits and required quaternary structure for subsequent antibody reactivity.

About one-fourth of the peanut crop produced in the United States and over three-fourths produced elsewhere is processed for oil. Mechanical pressing, solvent extraction, or a combination of the two is employed to obtain the oil. Hexane is generally used for commercial solvent extraction, but other solvents, such as hexane-acetone-water and acidic hexane, offer several advantages over hexane for thorough oil removal (Frampton and Pepperman, 1967; Hensarling et al., 1974; Jacks et al., 1970). After oil removal, the residual oil-free meal had previously received scant consideration as a source of either food-grade or feed-grade protein; however, in view of the current worldwide need for protein, attention has recently focused

on peanut meal as a source of edible protein (Lusas, 1979). Consequently, research concerning the preparation and properties of peanut protein for edible usage has greatly increased in magnitude (Lusas, 1979; Martinez, 1979).

In this communication, we describe the response of the major peanut storage protein (arachin) to exposure to hexane, acetone, hexane-acetone-water, and acidic hexane at the temperatures used commercially for oil extraction (60 °C) and for removal of residual solvent from the oil-free meal (68.5 °C). Structural features of arachin were assessed from examination of antigenicity, electrophoretic mobility, ultraviolet and infrared absorbances, and circular dichroism.

MATERIAL AND METHODS

Arachin was isolated from quiescent peanut seeds (*Arachis hypogaea* L., Virginia 56-R variety) as described previously (Neucere, 1969, 1974). Fifty-milligram samples

Southern Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 19687, New Orleans, Louisiana 70179.