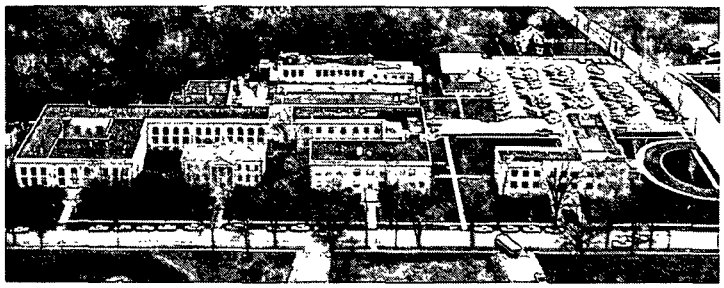

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THE STRUCTURE OF A "XYLAN" FROM KENAF (*HIBISCUS CANNABINUS*)

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The Structure of a "Xylan" from Kenaf (Hibiscus cannabinus)

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Summary

Xylan-rich extracts have been isolated from kenaf holocellulose using 5 and 24% potassium hydroxide. Fractionation showed the latter to be a mixture of polysaccharides which were characterized by yield, methoxyl and carboxyl content, sugar composition, optical rotation and ^{13}C NMR (in DMSO). Hydrolysis indicated the presence of a major polymer rich in xylan as well as impurities composed of galactan, mannan and nonstarch glucan. Both the constancy of the rhamnose, arabinose and xylose ratios of the hydrolyzates of the fractions and the presence of two monomethyl xyloses and tetramethyl arabinose and rhamnose in the hydrolyzates of methylated kenaf xylan, suggest that this xylan contains terminal anhydrorhamnose and anhydroarabinose units in its structure. Thus kenaf xylan, like many tropical hardwood xylans, differs from the published composition of xylans of northern arborescent deciduous plants.

The variation of the xylose to 4-O-methyl-D-glucuronic acid ratios observed in these fractions demonstrates that the uronic acid component of the xylan is not uniformly distributed among different molecules, but varies from acid-rich to acid-poor. The absence of galacturonic and glucuronic acids in the hydrolyzates of these fractions does not preclude their presence in other fractions from kenaf.

The similarity of the optical rotations and ^{13}C NMR spectra of these polymers to the xylans from elm and birch suggests that the xylose units are linked by β-glycosidic bonds while the uronic acid is linked by α bonds to the main chain. Qualitative methylation results confirmed the existence of 1-4 linked

xylopyranosyl units in the molecule and the presence of terminal branches of arabinose and rhamnose units.

Introduction

A search for nonwoody fiber sources for the paper industry has been conducted by the United States Department of Agriculture (1). Promising results have been obtained from kenaf (Hibiscus cannabinus) - an annual dicotyledon of the Malyaceae family which grows to a height of 6 to 20 feet and has a diameter of 1 to 2 inches. Kenaf is composed of two main fiber types - bast fibers and inner woody core fibers (2).

The whole stem of kenaf is usually pulped to make paper with strength properties intermediate between those of hardwood and softwood pulps (3). The chemical components of kenaf (unlike its paper properties) are inadequately studied and little is known of its polysaccharide content.

The purpose of this investigation is to determine if the xylan from kenaf (an annual dicotyledon) differs from the xylans of perennial arborescent dicotyledons.

Experimental

Sections of kenaf stem (1 foot in length and 0.75 inch in diameter) obtained from the United States Department of Agriculture, Peoria, Illinois, were Wiley milled to pass a 40 mesh screen. Extractives were removed with 50/50 ethanol/chloroform and the powder was air dried.

Delignification was achieved using an acid chlorite procedure at room temperature to minimize carbohydrate degradation (4). The liquor to wood ratio was

10 to 1 using 1.2% oxidant at pH 4. After 2-1/2 weeks, an additional charge of chlorite (5%) was added and the delignification was continued another 2-1/2 weeks until Klason lignin could no longer be detected. The holocellulose was washed with water until free of chloride ion.

An elm holocellulose was prepared in a similar manner from wood meal, while a sample of milled birch wood was obtained to provide another reference hemicellulose (7).

Extraction

Preliminary experiments were conducted using 4 and 10% sodium hydroxide as extraction media as suggested in a review by Ward (5). A small scale extraction based on that of Wise (6) was also carried out and the experience gained was used to formulate the following large scale extraction.

The never-dried holocellulose (90% moisture) was extracted with 5% KOH for 24 hours at 20°C in a stoppered container. After filtering and washing the residue three times with water, it was extracted with 24% aqueous KOH for 24 hours. After filtration, the extract was acidified with acetic acid. The resulting cloudy suspension did not separate from the aqueous phase even after dialysis against daily changes of water for 5 days. Separation was achieved by centrifugation at 10,000 rpm and 3°C.

The centrifuge was washed three times with methanol and solvent exchanged to water. Amberlite IR 120 (H) resin was added to remove potassium ions and the suspension was freeze-dried - [the acid insoluble xylan (I) in this report].

The aqueous phase from centrifugation was concentrated to one-fourth its original volume and was added to three volumes of methanol. Since a small amount of potassium acetate was still thought to be present, an aqueous slurry of the precipitate was shaken with Amberlite IR 120 (H). This fraction was also isolated by freeze-drying - [the acid soluble xylan (II) in this report].

Fractionation of the Extracts

Extracts I and II dissolved readily in 1N NaOH to give clear 1% solutions. Saturated aqueous $Ba(OH)_2$ (about 0.5N) was added dropwise until further addition gave no additional precipitate. The soluble and insoluble components from I and II were acidified with acetic acid and dialyzed free of inorganic components. An excess of methanol was added to precipitate the soluble component, which was then washed three times with methanol (with centrifugation), three times with ether and air dried.

Extracts I and II were shaken with 45% aqueous $CaCl_2$ to give 1% suspensions. The acid soluble Extract II dissolved; the insoluble Extract II did not dissolve completely and its insoluble component was separated by centrifugation. One-tenth N KI-I_2 was added dropwise to both soluble fractions until further addition did not produce additional precipitate. Following centrifugation, sufficient KBH_4 was added to the four iodine complexes (2 soluble and 2 insoluble) to destroy I_2 and reduce oxidized polysaccharide. All five fractions were dialyzed free of most inorganic components and the hemicelluloses were precipitated by the addition of excess methanol. These fractions were also washed three times with methanol, three times with ether (with centrifugation) and dried.

Sugar analyses were conducted by the GLC analysis of the hydrolyzate by an alditol acetate technique (8) on a Hewlett Packard HP-5890 Gas Chromatograph. Conventional paper chromatography was used to separate oligosaccharides and oligouronides derived from the xylans by partial acid hydrolysis (9). Methoxyl analyses were determined at the Mikroanalytisches Laboratorium of the University of Vienna. Molecular weight estimations were made on the carbanilated xylans from birch and kenaf using an HPLC technique developed by Schroeder and Haigh (10) employing Waters gel permeation chromatographic equipment and Styragel columns. The carboxylic acid content of the polysaccharides was determined by a conventional titration technique (11).

The ^{13}C NMR spectra of cation free xylans were measured on 25% solutions in DMSO. The spectra were obtained using a JEOL FX-100 FT-NMR spectrometer at 25 MHz while the spectrum was held at 90°C. Lignin-free preparations did not darken under these conditions.

The technique of Hakamori (12) was used to methylate kenaf xylan. The reduced alditol acetates of the acid hydrolyzates of kenaf were compared with the corresponding products from a methylated xylan described in an earlier publication (13). The GLC-MS technique described by Bjorndahl, et al. (14) was used for this evaluation, which was carried out using an HP-5985 Mass Spectrometer coupled to an HO-5840 Gas Chromatograph.

Results and Discussion

The data in Table 1 demonstrate that KOH is a more desirable extractant for kenaf xylan than NaOH. The KOH sequence used here was based on a classical technique described by Wise (5). Unlike his results, no heavy precipitate was obtained when the 24% KOH extract was acidified. The relatively stable xylan

suspension (I) was broken only by centrifugation and the soluble component (II) was precipitated with 3 volumes of alcohol. Both fractions were isolated by freeze drying.

Table 1 here

The 4% KOH extract of kenaf was particularly rich in acidic material and might represent a xylan contaminated with unique pectinlike materials often found in plants of the Malvaceae family (15). This extract was not investigated further.

In general, galactose and glucose containing substances and, to a lesser extent, uronic acid rich polymers, were concentrated into the more soluble fraction (II) of the 24% KOH extract. The xylan of this fraction contains a greater proportion of uronic acid while that fraction recovered by centrifugation contains less. The hydrolyzates of xylans extracted from birch wood and elm holocellulose have sugar ratios similar to that of kenaf fraction (I).

Some physical properties of the 24% KOH extract are compared in Table 2 with those found in the literature (16) and with controls run in this laboratory. Although the optical rotations and molecular weights of kenaf xylan are similar to the controls (17), the positive effect of temperature on its optical rotation in contrast to the negative effect observed here for elm and birch (not reported) suggests the crude kenaf xylan is a mixture of polymers.

Table 2 here

Fractionation of Kenaf Xylans

Alkaline earth hydroxides form complexes with polysaccharides (18), whose stability and solubility depend upon a variety of factors (19,20). Precipitation with excess $Ba(OH)_2$ is frequently used to separate soluble, frequently branched components from less soluble, linear ones. The results of such a test are shown in Table 3.

Table 3 here

These results indicate those fractions richer in anhydrosugar units other than xylose are more soluble in $Ba(OH)_2$ than those containing lesser amounts and might arise from incomplete precipitation or chemical bonding to the major component. The small magnitude of all miscellaneous units in the $Ba(OH)_2$ insoluble fractions (except xylose and uronic acid) makes precise analysis difficult. Nevertheless, the extracts are mixtures of polymers, and fractionation with $Ba(OH)_2$ partially resolves them.

Alkaline earth halides dissolve linear β(1-4) linked polysaccharides if the chain length is not too great (20). I_2 or Br_2 added to such polysaccharide solutions results in strongly colored complexes, some of which precipitate readily (20,21). Previous investigators using the $CaCl_2$-I_2 technique to fractionate deciduous xylans have found them to be freely soluble in saturated $CaCl_2$ (21-23). This was not observed here, for 15% of the acid insoluble fraction of kenaf xylan was insoluble in the solvent. Tests showed that only 24% of esparto xylan and 72% of elm xylan would dissolve under these conditions. It is likely that the xylans investigated in the literature were xylan fractions of lower degree of polymerization than those studied here.

Table 4 here

The fractions soluble in the presence of I_2 have greater quantities of miscellaneous sugars (apart from xylose and uronic acid) compared to the corresponding iodine insoluble and calcium insoluble fractions. The results are similar to the behavior of kenaf xylan in $Ba(OH)_2$, and since the mechanism of precipitation is probably different in these two cases the likelihood of chemical bonding is increased. The similar magnitude of the arabinose and rhamnose contents in both series of fractions suggests that they are components of kenaf xylan.

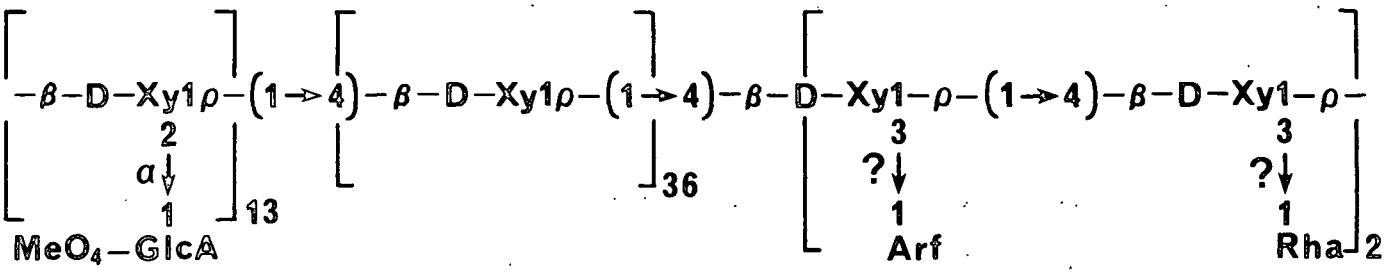
Methylation of the $CaCl_2$ insoluble xylan was accomplished by the Hakamoi technique (12). The product was hydrolyzed, reduced, acetylated and compared with the corresponding products from an authentic methylated xylan (13) using GLC and GLC-MS techniques (14). The results showed the major presence of the alditol acetate of 2,3-di-O-methyl xylose, and traces of alditol acetates derived from 3-mono-O-methyl xylose, 2-mono-O-methyl xylose, 2,3,4-tri-O-methyl xylose, 2,3,5-tri-O-methyl arabinose, and 2,3,4-tri-O-methyl rhamnose.

Partial acid hydrolysis and qualitative separation on paper by established techniques (9) showed only the expected oligosaccharides and the expected ubiquitous family of aldobiouronic, aldotriouronic, etc., acids to be present. No indication could be found for the presence of a 1-3 linked series of oligouronides found elsewhere (24). Thus it is likely that 2-O-methyl xylose represents the branch to which most of the arabinofuranosyl and rhamnopyranosyl units are attached to the xylan chain. In addition, it was not possible to detect galacturonic, glucuronic acid or oligouronides containing those acids and xylose in any kenaf xylan hydrolyzates examined here.

The cation-free kenaf xylan fractions as well as equally pure birch, elm, ponderosa pine, and esparto xylans dissolved easily in anhydrous DMSO to give clear 25% solutions from which ^{13}C NMR spectra were obtained. The spectra of kenaf (Fig. 1) and elm (not shown) were identical; the spectra of birch and ponderosa pine (Fig. 2) exhibit an unexpected additional carbonyl band at about 171 ppm. In the case of birch and pine, the existence of this band may reflect the presence of galacturonic acid, since such xylans were isolated from lignified wood meals by others (25,26). However, Lee and coworkers (27) have shown that the quantity of galacturonic acid in their fractions is frequently too small for detection by ^{13}C NMR. Lactone formation offers another possibility for the band, since these xylans were cation free. The inability to detect galacturonic acid and glucuronic acid in the kenaf hydrolyzates does not preclude their presence in other xylan components of kenaf. Esparto xylan (Fig. 3) contains no uronic acid (28) and therefore does not exhibit a carbonyl band but does give a somewhat more complex pattern identical to that described by Brillouet et al. (29), suggesting greater branching.

Fig. 1-3 here

An empirical representation of a structure for the kenaf xylan studied here can be developed if it is assumed that the uronic acid units are linked by $\alpha(1\text{-}2)$ glycosidic bonds and that the conjectured terminal arabinose and rhamnose units are linked by (1-3) bonds of unknown configuration to anhydroxylose units of the main chain. Since very small amounts of rhamnose and arabinose are encountered in the hydrolyzates of xylans from birch, aspen, elm, and others, it is likely they are components of some of the xylan fractions of those woods also.



Conclusions

A 24% KOH extract from kenaf contains a mixture of related xylans which differ in their content of 4-O-methyl glucuronic acid and, because of the presence of terminal rhamnose and arabinose units, resemble the xylans isolated from tropical hardwoods. Although it was not possible to detect galacturonic acid or glucuronic acid in the partial acid hydrolyzates of the xylan subfraction studied here, the result does not preclude the possibility that these acids are components of other kenaf xylan fractions that were not studied. Analytical techniques cannot determine whether the subfractions differ greatly in rhamnose and arabinose contents. Apart from these differences, the optical rotation, molecular weight, methylation products and solubility characteristics of this xylan fraction resemble those of other deciduous xylans.

Acknowledgments

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Figure Captions

Fig. 1. ^{13}C NMR spectra of kenaf xylan concentration 25% (w/v) in DMSO-d_6 (39.6 ppm) at 90°C.

Fig. 2. ^{13}C NMR spectra of ponderosa pine glucouronoarabinoxylan concentration 25% (w/v) in DMSO-d_6 (39.6 ppm) at 90°C.

Fig. 3. ^{13}C NMR spectra of esparto xylan concentration 25% (w/v) in DMSO-d_6 (39.6 ppm) at 90°C.

Table 1. A comparison of molar ratios of xylans obtained by different extraction techniques.

Source	Molar Ratio of						Uronic Acid	Yield,[a] %
	Gal	Glc	Man	Ar	Xyl	Rha		
Kenaf holocellulose								
4% NaOH	6.7	13.3	4.2	0.2	10	na	na	na
10% NaOH	3.4	6.0	4.5	0.2	10	na	na	na
4% KOH	1.1	1.1	0.3	0.3	10	na	note[b]	9.0
24% KOH								
Acid insoluble (I)	0.2	0.07	0.02	0.2	10	0.07	1.4[c,d]	4.2
Acid soluble (II)	0.3	1.3	0.3	0.3	10	0.08	2.1[d,e]	5.8
Birch wood								
10% NaOH	0.2	0.4	0.07	0.2	10	na	1.2[f]	na
Elm holocellulose								
10% NaOH	0.4	0.9	0.2	0.3	10	na	1.8[f]	na

[a]Percent of holocellulose.
[b]10/2 for methoxyl and 10/10 for carboxyl estimation,
[c]$[\alpha]_D$, (Cl,NaOH) = -70°.
[d]Based on methoxyl and carboxyl.
[e]$[\alpha]_D$, (Cl, NaOH) = -36.1°.
[f]Based on methoxyl only.
na = not analyzed.

Table 2. Physical properties of various xylans.

Source	$[\alpha]_D$[a]	DP_N	DP_W	M_W/M_N
Kenaf (24% KOH)	-70.0	132[b]	317[b]	2.4
Elm (10% NaOH)	-72.9	132[b]	195[b]	1.5
Elm (18)	-68.8	185[c]	440[d]	1.4
Birch (18)	-76.5	215[c]	500[d]	2.3

[a]1% in NaOH.
[b]Determined by HPLC analysis of the xylan carbanilate derivative.
[c]Determined by osmometry.
[d]Determined by light scattering.

Table 3. The composition of kenaf xylan fractionated with barium hydroxide.

	Xylan I		Xylan II	
	Insoluble	Soluble	Insoluble	Soluble
Yield, %[a]	67	14	9	52
	Molar Ratio of Sugars in Hydrolyzate			
Galactose	0.16	1.3	0.08	0.35
Glucose	0.14	6.0	0.11	0.66
Mannose	0.05	1.8	0.05	0.12
Arabinose	0.20	0.37	0.19	0.24
Xylose	10	10	10	10
Rhamnose	0.17	0.19	0.13	0.09
Uronic acid[b]	1.1	2.5	1.5	2.1
Accountability, %[c]	93	--	--	95

[a]Based on starting material.
[b]Based on methoxyl content.
[c]Based on summation of analyses.

Table 4. The composition of xylan fractions obtained by calcium chloride-iodine complexing.

Fraction	Xylan I	Xylan I	Xylan I	Xylan II	Xylan II
Solution Behavior	$CaCl_2$ Ins.	I_2 Ins.	I_2 Ins.	I_2 Sol.	I_2 Sol.
Yield, %	15	22	30	27	35
	Molar Ratio of Sugars after Hydrolysis				
Galactose	0.3	0.06	0.40	0.30	0.62
Glucose	0.10	0.09	1.09	0.04	1.5
Mannose	0.07	0	0.24	0.05	0.22
Arabinose	0.17	0.11	0.17	0.21	0.24
Xylose	10	10	10	10	10
Rhamnose	0.07	0.07	0.06	0.07	0.09
Uronic acid	1.5	1.4	2.0	2.7	n.a.
Account-ability, %	100	104	88	102	n.a.

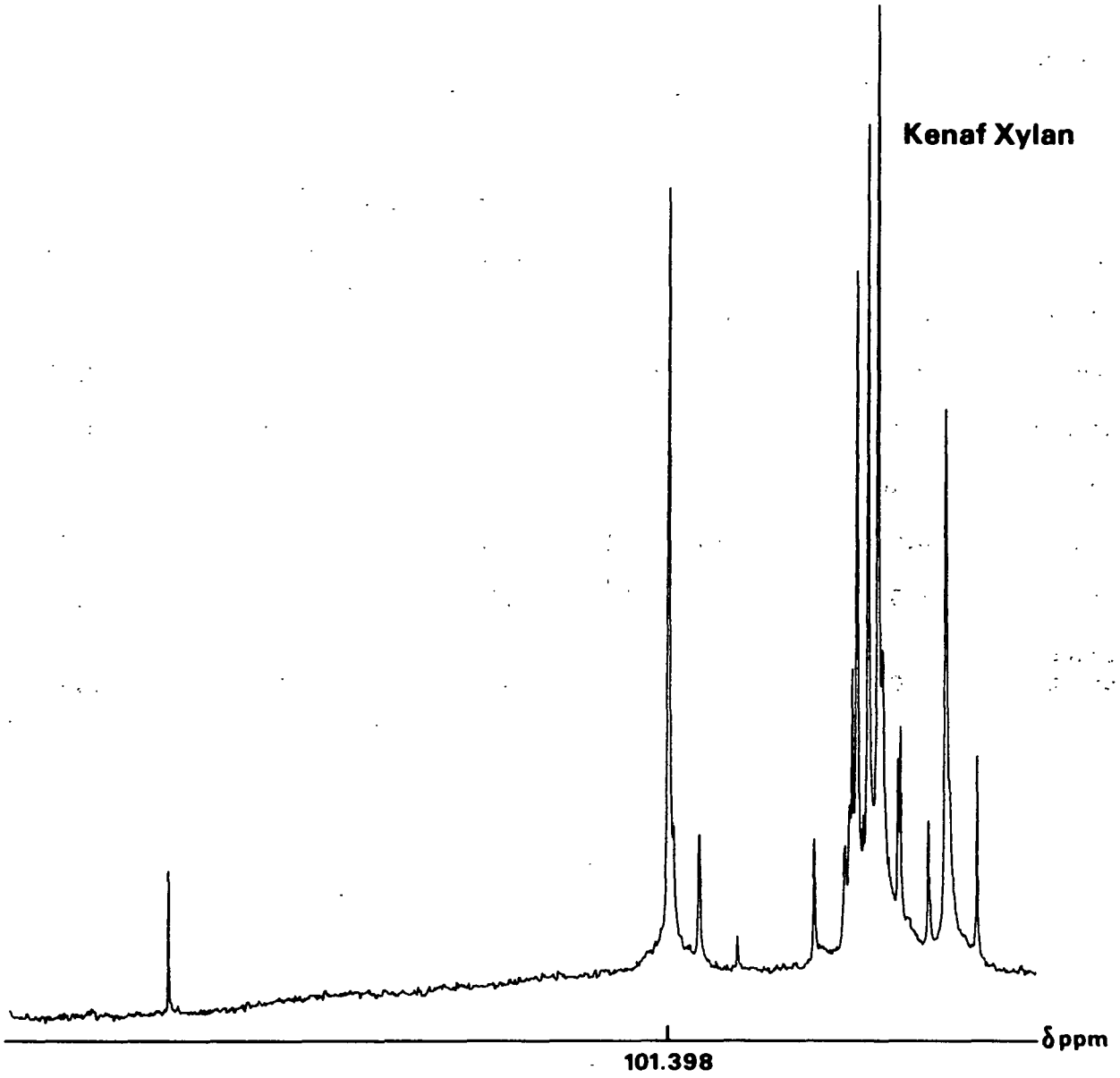

Fig. 1. ^{13}C NMR spectra of kenaf xylan concentration 25% (w/v) in DMSO-d_6 (39.6 ppm) at 90°C.

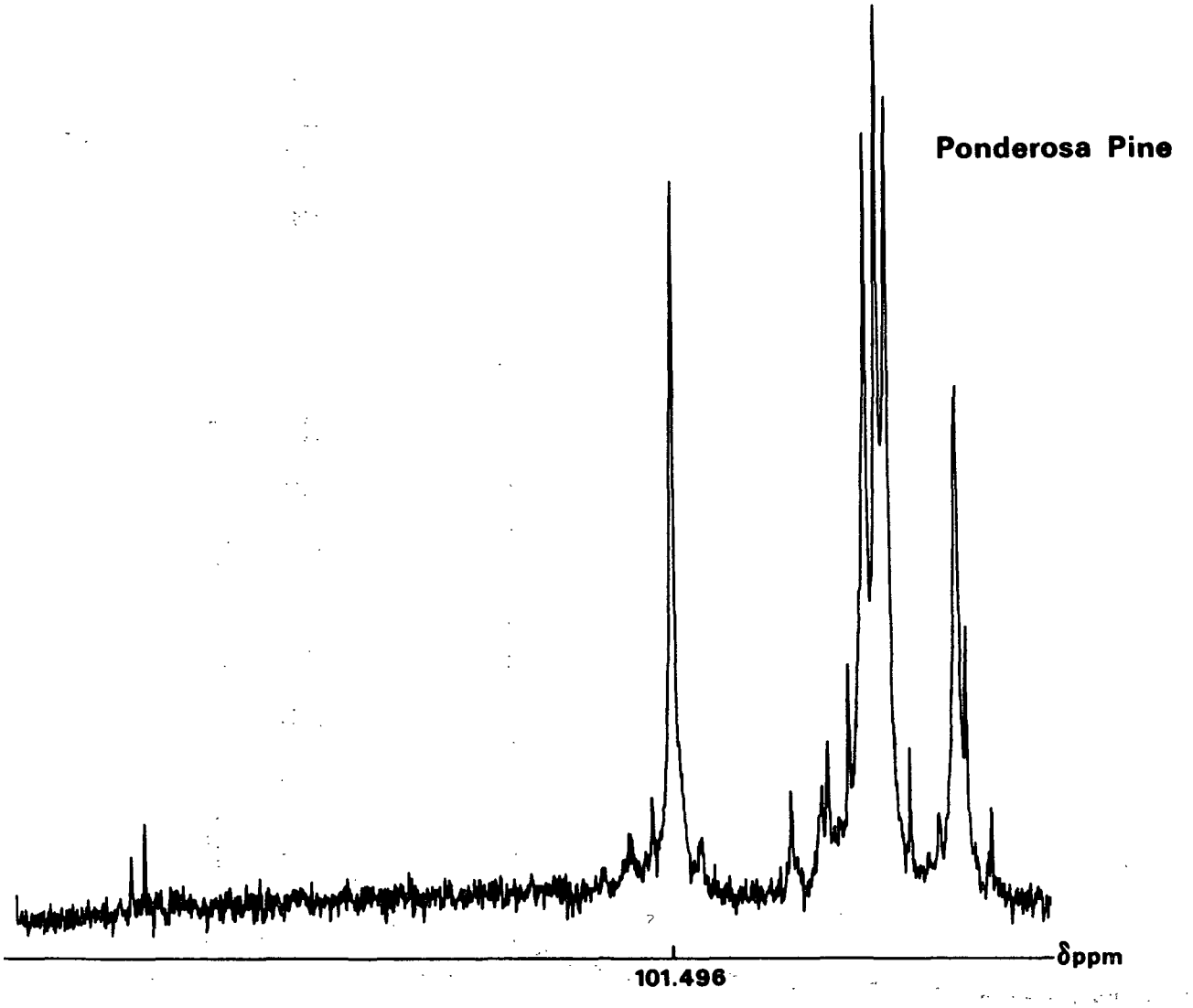

Fig. 2. ^{13}C NMR spectra of ponderosa pine glucouronoarabinoxylan concentration 25% (w/v) in DMSO-d_6 (39.6 ppm) at 90°C.

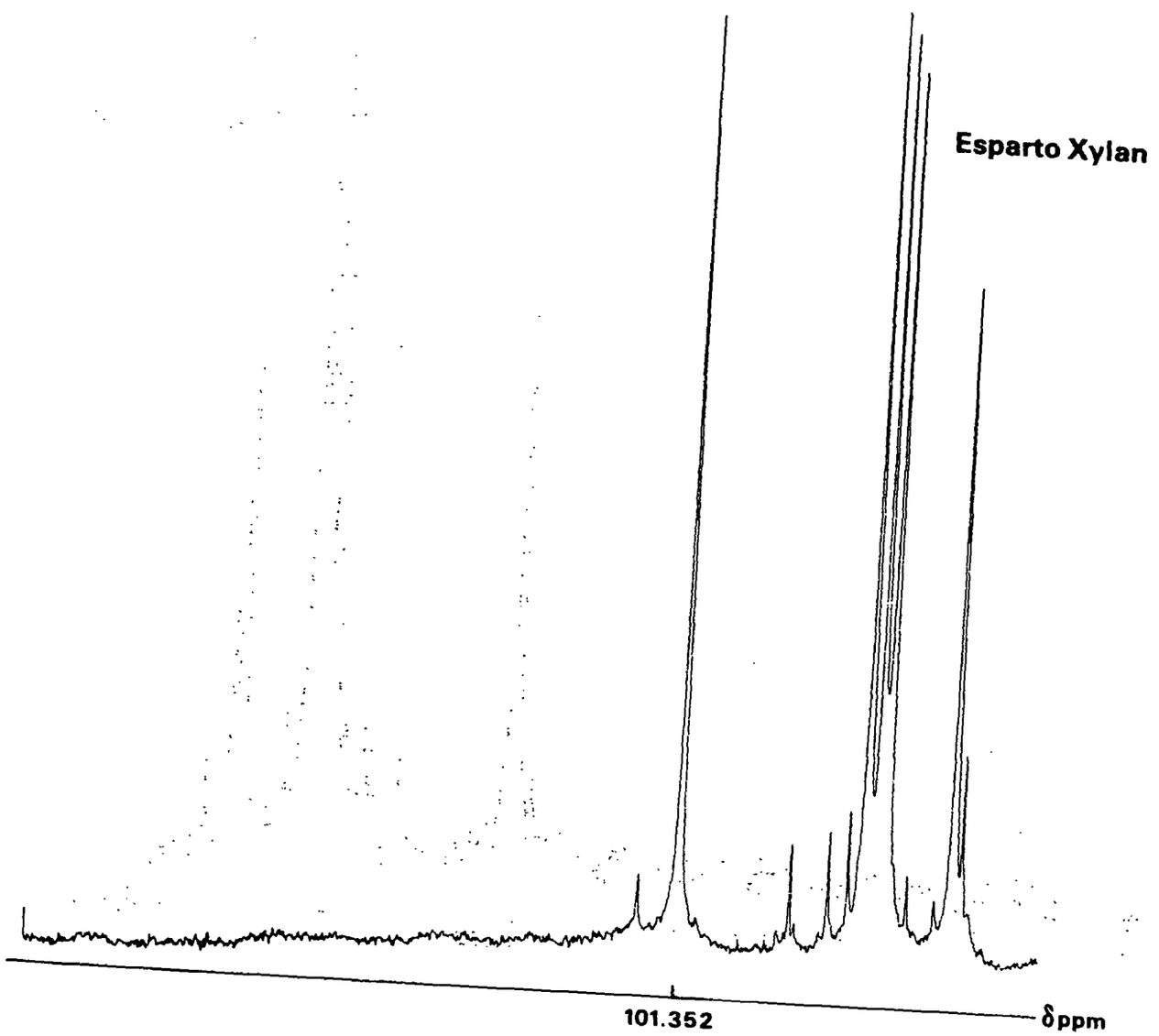

Fig. 3. ^{13}C NMR spectra of esparto xylan concentration 25% (w/v) in DMSO-d_6 (39.6 ppm) at 90°C.