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Selective Isolation of β-Glucan from Corn Pericarp Hemicelluloses by Affinity Chromatography on Cellulose Column

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16 **Abstract**

17 A combination of anion-exchange chromatography and affinity chromatography on a
18 cellulose column was found to be effective for the isolation of β-(1,3;1,4)-glucan (BG)
19 from corn pericarp hemicelluloses (CPHs). CPHs containing 6.6% BG were extracted
20 from corn pericarp with 6 M urea-2 wt% NaOH solution and initially fractionated into
21 neutral and acidic parts by anion exchange chromatography to remove acidic
22 arabinoxylan consisting of arabinose (35.6%) and xylose (50.9%). The neutral fraction
23 (yield; 10.1% on the basis of CPHs) consisting of 1.0% arabinose, 10.1% xylose and
24 80.3% glucose containing 28.4% BG was then applied to a cellulose column of Watman
25 CF-11. BG could be recovered from the adsorbed fraction on the cellulose column by
26 elution with 2% NaOH in a yield of 2.6% on the basis of CPHs with a purity of 84.7%.
27 The chemical structure of the isolated corn pericarp BG was confirmed by ^{13}C-NMR
28 spectroscopic, methylation and lichenase treatment analyses. The results indicate that
29 the ratios of (1,4)/(1,3) linkage and cellotriosyl/cellotetraosyl segments of the BG were
30 2.60 and 2.5, respectively.

31 *Keywords:* β-Glucan; Corn pericarp hemicelluloses; Cellulose column; Affinity
32 chromatography

33

34 **1. Introduction**

35 Corn pericarp is a by-product of industrial corn starch production and its annual
36 global generation is estimated to be over 4 million tons (Yoshida, Dwianto, Honda,
37 Uyama, & Azuma, 2014). Although it is frequently used as an ingredient in animal feed
38 with the addition of corn protein (Shukla, & Cheryan, 2001), finding out more valuable
39 applications is expected. Because hemicelluloses are its major constituents amounting to
40 about 75%, its functional use largely depends on their extended characterization. Use of
41 corn pericarp hemicelluloses (CPHs) as an emulsifier is a candidate for this line of
42 investigation (Yadav, Johnston, & Hicks, 2007; Yadav, Parris, Johnston, & Hicks,
43 2008).

44 Recently we found that corn pericarp contains 3.2% of β-(1,3;1,4)-glucan (BG)
45 (Yoshida, Sakamoto, & Azuma, 2012). BG is commonly present in cereal grains and is
46 included in many kinds of commercially available cereal based foods as a nutritionally
47 important ingredient, because it improves food qualities such as mouthfeel and texture
48 (Lazaridou, & Biliaderis, 2007). In addition, BG provides some specific health benefits,
49 such as attenuating blood postprandial glycemic and insulinemic responses, lowering
50 blood total cholesterol and low-density lipoprotein (LDL) cholesterol, and improving
51 high-density lipoprotein (HDL) cholesterol and blood lipid profiles (Braaten *et al.*, 1994;
52 Daou, & Zhang, 2012; Brennan, & Cleary, 2005).

53 BG is a linear homopolysaccharide comprised of two types of D-glucopyranosyl
54 residues linked by a mixture of β-(1-3) and β-(1-4) linkages, with blocks of (1-4)-linked
55 residues (oligomeric cellulose-like segments) separated by (1-3)-linkages. Its structural

56 features, such as linkage ratio, number of units of cellulose-like segments and
57 distribution of the cellulose-like segments, are known to be important determinants for
58 its physical properties and functionalities, including its use as a food additive
59 (Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004).

60 Previously we demonstrated the effectiveness of a NaOH-urea solvent system for
61 extraction of hemicelluloses from corn pericarp, including BG (Yoshida, Sakamoto, &
62 Azuma, 2012). Although biorefinement of corn pericarp targeted to produce BG is
63 desirable as it is an innovative utilization of corn starch residues, rather tedious steps for
64 the removal of large amounts of other polysaccharides are usually required for the
65 isolation of BG from monocotyledonous crops (Ahmad, Anjum, Zahoor, Nawaz, &
66 Ahmed, 2009; Ahmad, Anjum, Zahoor, Nawaz, & Din, 2007; Beer, Arrigoin, & Amadò,
67 1996; Bhatty, 1993; Burkus, & Temelli, 1998; Lazaridou, Biliaderis, Micha-Screttas, &
68 Steele, 2004; Wood, Weisz, Fedec, & Burrows, 1989). Two important steps so far
69 noticed were removal of contaminating starch and arabinoxylan. Repeated treatments
70 with thermo-stable starch-degrading enzymes were usually necessary to enrich BG for
71 removal of starch. Solubility difference in aqueous media was frequently used to
72 remove arabinoxylan (Izydorczyk, & Biliaderis, 1995; Izydorczyk, Biliaderis, Macri, &
73 MacGregor, 1997; Izydorczyk, & MacGregor, 2000). However, in the case of CPHs,
74 removal of starch was not prerequisite because of the low content (about 1%) and our
75 trials of fractional precipitation of BG from a mixture with arabinoylan by using ethanol
76 and ammonium sulfate were unsuccessful.

77 In this study, we developed a convenient isolation method specific for corn pericarp

78 BG by using affinity chromatography on a cellulose column, and present the chemical
79 properties of the isolated BG were also investigated.
80

81 **2. Materials and Methods**

82 *2. 1. Materials*

83 Kernels of sweet corn cultivated and steamed for food in Hokkaido, Japan, were
84 purchased from Kewpie Co., Japan. Corn pericarp was manually peeled from the upper
85 portion of each kernel (9.4 ± 0.7% on basis of dried kernel, n = 10) and treated with hot
86 water (121°C) for 1 h. Corn pericarp hemicelluloses (CPHs) consisting of 40.5% of
87 xylose, 29.2% of arabinose, 26.2% of glucose and 4.1% of galactose were prepared by
88 extraction of corn pericarp with 2 wt% NaOH-6 M urea in a yield of 74.8% on the basis
89 of dried corn pericarp as described previously (Yoshida, Sakamoto, & Azuma, 2012).
90 β-Glucan (BG) from barley (>95%) was purchased from Sigma (St. Louis, Missouri,
91 USA). Amounts of BG and starch were determined by using the mixed-linkage β-glucan
92 and total starch content assay kits (Megazyme International Ireland Ltd., Wicklow,
93 Ireland), respectively. Whatman CF-11 cellulose powder (Whatman™, a part of GE
94 Healthcare Life Science, Ltd., Buckinghamshire, UK) was used for affinity
95 chromatography after pre-washing with 5% NaOH and neutralization with acetic acid.
96

97 *2. 2. Isolation of β-(1,3;1,4)-glucan*

98 Anion exchange chromatography was first applied for partial purification of BG. Hot
99 water-soluble CPHs (94.2 ± 0.5% on the basis of CPHs, n = 4) obtained by extraction of

100 corn pericarp with a 100 fold excess of water at 80°C for 2-3 h were applied to a column
101 (15 × 150 mm) of TOYOPEARL DEAE-650M (Tosoh Co., Tokyo, Japan) equilibrated
102 with 5 mM sodium phosphate buffer (pH 6.8) and eluted with the same solution to
103 recover neutral BG. Acidic arabinoxylan was next recovered by elution with the same
104 buffer containing 1.2 M NaCl. Elution was monitored by the phenol-sulfuric acid
105 method. Both polysaccharide fractions were separately pooled, dialyzed against water
106 and freezed-dried. The BG-rich fraction (Neutral fraction; 8.7 ± 2.4% on the basis of
107 CPHs, *n* = 3, Table 1) was dissolved in 5 mM sodium acetate buffer (SAB), pH 5.0 and
108 applied to a cellulose column (15 × 150 mm). After equilibrating for 30 min at room
109 temperature, the column was washed with the same buffer to remove unadsorbed
110 material, eluted with distilled water, and finally adsorbed BG was recovered by elution
111 with 2% NaOH (2% NaOH fraction; yield 3.3 ± 1.3% on the basis of CPHs, *n* = 3,
112 Table 1). All carbohydrate containing fractions were pooled, neutralized, dialyzed
113 against water and freeze-dried.
114
115 *2. 3. Chemical analysis*
116 CPHs and all of the materials recovered by anion exchange and affinity
117 chromatographic techniques were hydrolyzed according to the Saeman method (Saeman,
118 Bubl, & Harris, 1945), and their monosaccharide compositions were determined by
119 high-performance anion exchange chromatography (HPAEC) on a Dionex DX-500
120 system (Sunnyvale, CA, USA) equipped with a pulsed amperometric detector (ED-40)
121 as described in our previous report (Yoshida, Tusbaki, Teramoto, & Azuma, 2010).

122 The liquid state ^{13}C-NMR spectrum of the isolated BG was recorded in D_2O on a
123 Bruker DPX-400 instrument (Billerica, Bruker, MA, USA) operating at 400 MHz and
124 the chemical shifts in ppm were normalized as downfield values from that of internal
125 standard, TSP (sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propionate).

126 Permethylation of polysaccharides was carried out according to the Hakomori
127 method (Hakamori, 1964). The permethylated polysaccharides were subjected to two-
128 step hydrolysis with 90% formic acid for 2 h at 100°C and 0.5 N sulfuric acid for 12 h
129 at 100°C. After neutralization with barium carbonate, the hydrolyzate was reduced with
130 sodium borohydride and acetylated with a mixture of acetic anhydride and pyridine (1:1,
131 v/v) for 1 h at 100°C. The resulting mixture of partially methylated alditol acetates was
132 analyzed by GC/MS with a Shimadzu Parvum 2 (70 eV) using a column of Shimadzu
133 CBP-1 (0.25 μm, 0.25 mm × 25 m) and a linear temperature gradient from 140°C to
134 220°C at 2°C/min.

135 The distribution of (1,4)-linked-glucopyranosyl segments in BG was determined by
136 lichenase treatment and high-performance liquid chromatography (HPLC). BG samples
137 were dissolved in sodium phosphate buffer (20 mM, pH6.5) and incubated with
138 lichenase [(1,3;1,4)-beta-glucan-4-glucanohydrolase, 1000 U/mL, included in the
139 Megazyme kit for measurement of BG content] for 2 h at 50°C. After centrifugation, the
140 supernatant was purified by passage through a joint column of cation (Dowex 50x, 8 H^+
141 form) and anion (Dowex 1x8, acetate form) exchange resins. The passed solution and
142 washed solution with pure water were freeze-dried (recovery, 62.7%). The distribution
143 of segments was analyzed by HPLC on a column of MCI GEL CK04SS (7.5 × 200 mm,

144 Mitsubishi Chemical Industry Co., Tokyo, Japan) at 80°C with refractive index detector

145 (RI-8, Tosoh Co., Tokyo, Japan). The eluent was deionized water and flow rate was 0.3

146 mL/min. Elution was monitored using Chrom NAV Station, Jasco, Co., Tokyo, Japan).

147

148 **3. Results and discussion**

149 CPHs (BG content 6.6%) were separated into neutral and acidic fractions by anion
150 exchange chromatography on a DEAE-column as shown in Fig. 1 (A). The neutral
151 fraction was further separated into three fractions by affinity chromatography on a
152 cellulose column as shown Fig. 1 (B). Yields of the separated fractions are listed in
153 Table 1. The relative monosaccharide compositions of CPHs and the fractions separated
154 by anion exchange and affinity chromatography are listed in Table 1. The results
155 indicate that the neutral fraction contained BG (28.4%) together with a small amount of
156 xylan (10.1%). On the other hand, the acidic fraction was predominantly arabinoxylan
157 consisting of xylose (50.9%) and arabinose (35.6%), with glucose as a minor constituent
158 (3.5%). These results indicate that anion exchange chromatography was effective for
159 partial purification of BG in corn pericarp. Previously, Gruppen *et al.* (1992) have
160 reported that anion exchange chromatography is an efficient tool for the fractionation of
161 arabinoxylans present in wheat flour. On the other hand, our results indicate its
162 suitability for the removal of the acidic arabinoxylan present in abundance in CPHs.

163 In the present study, affinity chromatography on a cellulose column was found to be
164 more effective for selective purification of BG. The glucose contents of the fractions
165 eluted with SAB, distilled water and 2% NaOH were 29.5, 50.6 and 91.5%, respectively
166 (Table 1). The starch content of the SAB, water and 2% NaOH fractions were 4.3, 5.7
167 and 3.5%, respectively. BG was recovered in 84.7% purity from the column by elution
168 with 2% NaOH (Table 1) on the basis of hot water (100°C)-soluble materials. When the
169 BG content in the NaOH fraction was initially examined by using its whole amount, BG

170 content was calculated as 67.0 ± 3.6%. This value, however, seemed to be invalid,
171 because this fraction was mainly consisted of glucose (91.5%) with minor contaminants
172 of starch (3.5%) and contained a large amount of insolubilized materials (20.8%)
173 mainly composed of glucose (78.6%). Insolubilization of BG after purification was
174 pointed out by Lazaridou and Biliaderis (2007). Therefore, in the present study, the
175 purity of the NaOH fraction was estimated by calculation on the basis of soluble
176 materials.

177 When the water-soluble portion of CPHs was directly applied to the cellulose column,
178 the adsorbed fraction was found to be contaminated with a larger amount of xylose
179 (23.2%). This result shows the necessity of anion-exchange chromatography prior to
180 cellulose affinity chromatography.

181 Fig. 2 shows the ^{13}C-NMR spectrum of the materials separated into the 2% NaOH
182 fraction. Each signal was assigned according to the previous report (Bock, Duus,
183 Norman, & Pedersen, 1991; Cui, Wood, Blackwell, & Nikiforuk, 2000; Roubroeks,
184 Andersson, & Aman, 2000) and the peak assignments are listed in Table 2. The
185 spectrum was identical to that of pure β-glucan (Cui, Wood, Blackwell, & Nikiforuk,
186 2000; Roubroeks, Andersson, & Aman, 2000). The spectrum of materials isolated in the
187 2% NaOH fraction showed characteristic intense peaks at 81.3 and 86.6 ppm, assigned
188 to the signals of C-3 of (1,4)-linked- and (1,3)-linked-D-glucopyranosyl residues of
189 β-glucan, respectively. Although very weak signals assignable to arabinoxylan
190 (Roubroeks, Andersson, & Aman, 2000) were detected, no intense peaks other than BG
191 could be detected. These results also indicate that the material in the 2% NaOH fraction

192 was high purity BG. The present study shows for the first time that BG can be
193 effectively isolated from other hemicellulosic polysaccharides present in corn pericarp
194 by using a combination of anion exchange and cellulose affinity column
195 chromatography.

196 The glucosidic linkage analysis of the purified BG was investigated by methylation
197 analysis. The corn pericarp BG consisted of 2,3,6-Me-Glc*p*, 2,4,6-Me-Glc*p* and
198 2,3,4,6-Me-Glc*p* in a molar ratio of 58.1 (1,4-linked Glc*p*), 22.3 (1,3-linked Glc*p*) and
199 0.5% (terminal Glc*p*), respectively (Table 3). The ratio of (1,4)-glucose linkages to
200 (1,3)-linkages for the corn pericarp β-glucan was calculated as 2.60, which was slightly
201 higher than that for barley (2.37) but within the ranges previously reported; 2.3-2.8 for
202 oat, 1.9-2.8 for barley and 2.3 for rye (Lazaridou, & Biliaderis, 2007). The high affinity
203 of the corn pericarp BG to cellulose suggests the existence of strong interactions
204 between BG and cellulose in corn pericarp.

205 The distribution of (1,4)-linked glucopyranosyl segments in corn pericarp BG was
206 examined by fragmentation analyses with lichenase which splits (1,3)-β-D-glycosidic
207 linkages in BG (Table 4). After lichenase treatment, cello-octomer and shorter
208 oligomers were detected in the corn pericarp BG. The ratio of cellotriosyl/cellotetraosyl
209 units for corn pericarp BG was 2.5, which was slightly higher than that for barley (2.2).
210 Previously, the same ratios in the native cereal β-glucan structures were reported to be
211 within the range of 1.5-2.3 for oat, 1.8-3.5 for barley, 1.9-3.8 for rye and 3.0-4.5 for
212 wheat (Lazaridou, & Biliaderis, 2007). Ebringerová *et al.* (2005) described in their
213 review as ‘In comparison to the water-extractable β-glucan-rich fractions, the

214 alkali-extractable ones were characterized by high ratios of cellotriosyl/cellotetraosyl
215 units and large amounts of long, contiguously linked (1→4)-linkage segments. Such
216 polymers exhibit a tendency for interchain aggregation through strong hydrogen
217 bonding along the cellulose-like regions and hence lower solubility'. The present results
218 might fit their descriptions and be suffice to show that the affinity of BG for cellulose
219 was the basis for its isolation.

220

221 **3. Conclusion**

222 The effectiveness of the combination of anion-exchange and cellulose affinity
223 chromatographic techniques for the isolation of BG from CPHs was established for the
224 first time. By using the present system, the BG present at 6.6% in CPHs was purified to
225 84.7%. The methylation and fragmentation analyses showed that the BG isolated from
226 the CPHs has (1,4)/(1,3) linkage and cellotriosyl/cellotetraosyl segment ratios of 2.60
227 and 2.5, respectively. The chemical structure of the BG was also confirmed by
228 ^{13}C-NMR spectroscopic analysis.

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314

315

316 **Figure captions**

317 Fig. 1. Anion exchange chromatographic (A) and affinity chromatographic (B) profiles. (A) The

318 neutral and acidic fractions shown as separate bars at the top of the figure were pooled. (B)

319 Neutral fraction was applied to the cellulose column. The three fractions eluted with SAB,

320 distilled water and 2% NaOH were recovered.

321 Fig. 2. ^{13}C-NMR spectrum of materials isolated in 2% NaOH fraction using a cellulose column

322

323

324 Table 1. Yields of the separated fractions and sugar compositions of corn pericarp

325 hemicelluloses (CPHs) and separated fractions.

Sample	Yield[a] (%)	Relative monosaccharide composition (%, w/w)					β-Glucan content[b] (%)	Starch content[b] (%)
		Ara	Gal	Glc	Xyl	Man		
CPHs	-	29.2	4.1	26.2	40.5	tr[c]	6.6	1.4
Anion exchange chromatography								
Neutral fraction	10.1 ± 0.4	1.0	2.0	80.3	10.1	6.7	28.4	5.0
Acidic fraction	42.8 ± 4.8	35.6	8.2	3.5	50.9	1.9	0.6	0.6
Affinity chromatography on cellulose column								
SAB fraction	7.1 ± 3.2	23.4	7.6	29.5	35.8	3.7	1.3	4.3
Water fraction	1.0 ± 0.7	14.3	7.1	50.6	23.4	4.6	1.4	5.7
2% NaOH fraction	2.6 ± 0.5	1.3	0.9	91.5	6.3	-	84.7[d]	3.5

326

327 [a]Values are expressed as a percentage on the basis of the raw material corn pericarp

328 hemicelluloses. Values are expressed as mean ± SD (*n* = 3).

329 [b]Values represent the average of duplication.

330 [c]tr represents trace.

331 [d]Value is expressed as a percentage on the basis of the hot water (100°C)-soluble materials.

332

333 Table 2. Chemical shifts (ppm) of the ^{13}C responses of the glucose residues of β-glucan isolated

334 in the NaOH fraction in D_2O

Linkage type	Chemical shift (ppm)					
	C1	C2	C3	C4	C5	C6
→4)-β-Glc*p* (1→3)	105.41	76.08	81.29	76.92	77.68	62.81
→3)-β-Glc*p* (1→4)	105.22	75.84	86.60	70.81	78.45	63.41
→4)-β-Glc*p* (1→4)	105.22	76.08	81.29	76.92	77.68	62.81

335

336 Table 3. Methylation analysis of the materials isolated in the 2% NaOH fraction using a

337 cellulose column and of barley β-glucan.

Methylation position	Linkage type	Molar ratio (%)	
		NaOH fraction	Barley β-glucan
2,3,6-Me_3-Glc*p*	1,4-	71.8	69.4
2,4,6-Me_3-Glc*p*	1,3-	27.6	29.3
2,3,4,6-Me_4-Glc*p*	Terminal	0.6	1.0
3,6-Me_2-Glc*p*	1,2,4-	-	0.3

338

339 Values are expressed as a relative percentage of the total partially methylated glucose residues.

340

341 Table 4. Fragmentation analysis with lichenase of the materials isolated in the 2% NaOH

342 fraction after affinity chromatography on a cellulose column and of barley β-glucan.

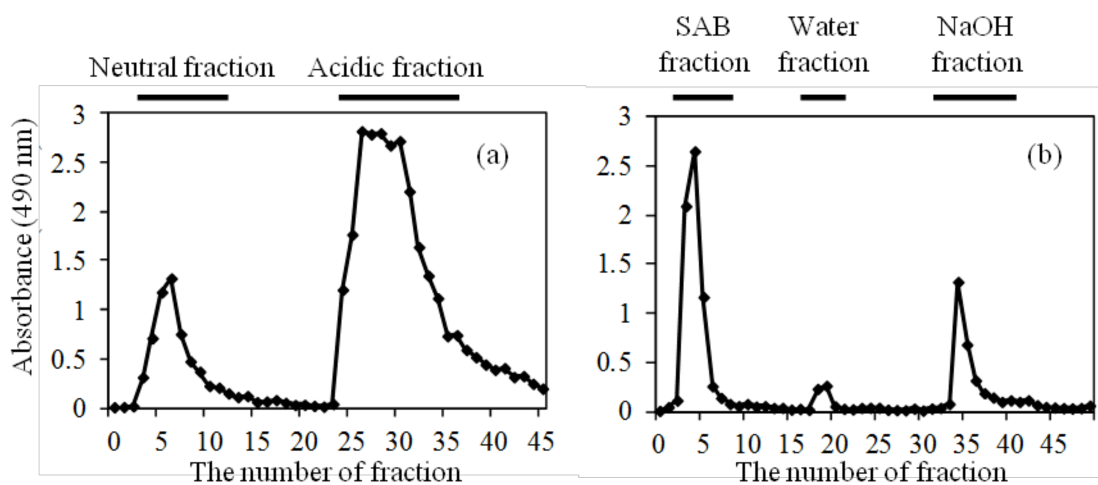
Oligmer	DP	Molar ratio (%)	
		NaOH fraction	Barley
cellobiose	2	0.3	1.3
cello-trimer	3	67.5	63.8
cello-tetramer	4	26.7	28.7
cello-pentamer	5	3.4	4.4
cello-hexamer	6	1.7	1.1
cello-heptamer	7	0.2	0.4
cello-octamer	8	0.1	0.3
cello-trimer + cello-tetramer	3 + 4	94.2	92.5
cello-trimer/cello-tetramer	3/4	2.5	2.2

343

344 DP represents degree of polymerization.

345

346



347 Fig. 1. Anion exchange chromatographic (A) and affinity chromatographic (B) profiles. (A) The

348 neutral and acidic fractions shown as separate bars at the top of the figure were pooled.

349 Fractions 5-15 and 25-36 separated by anion exchange chromatography were designated as

350 neutral and acidic fractions, respectively. The eluent for the anion exchange chromatography

351 was changed at fraction 21 from 5 mM sodium phosphate buffer (pH 6.8) to the same buffer

352 containing 1.2 M NaCl. (B) The neutral fraction was applied to the cellulose column. Three

353 fractions eluted with SAB, distilled water and 2% NaOH were recovered. Fractions 4-11, 19-21

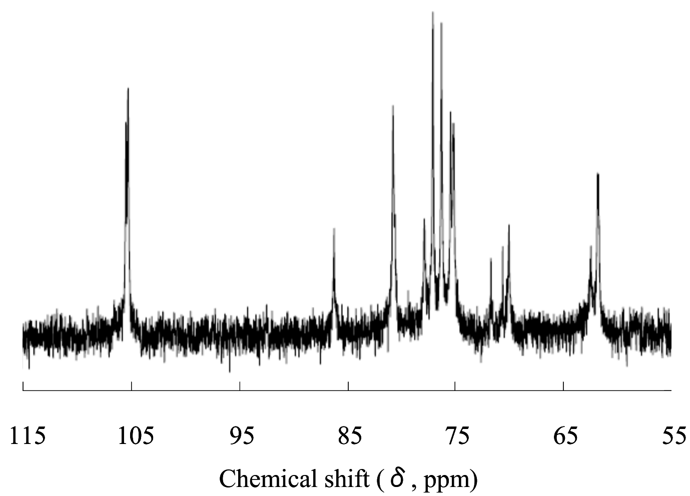
354 and 35-44 separated by affinity chromatography on a cellulose column were designated as SAB,

355 water and NaOH fractions, respectively. The eluent for the affinity chromatography was

356 changed at fraction 17 from SAB to water, and at fraction 33 to 2% NaOH. The volume of each

357 fraction was 3 mL.

358



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360 Fig. 2. ^{13}C-NMR spectrum of materials isolated in 2% NaOH fraction using a cellulose column