Characterization of a Fucoarabinogalactan, the Main Polysaccharide from the Gum Exudate of *Croton urucurana*

Begoña Milo, Ester Risco, Roser Vila, José Iglesias, and Salvador Cañigueral*

Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Avinguda Diagonal, 643, E-08028 Barcelona, Spain

Received April 17, 2001

A fucoarabinogalactan (CU-1), the main component of the gum exudate of the medicinal plant *Croton urucurana*, has been isolated by precipitation and subsequent dialysis and finally purified by ion-exchange chromatography. The estimated average molecular weight of CU-1 by gel permeation chromatography was ~2.48 × 10^6 Da. CU-1 was found to contain 1.0% proteins and 93.7% total sugars, mainly fucose, arabinose, and galactose (molar ratio: 7.8, 8.1, 19.0), and minor quantities of mannose, xylose, glucose, and uronic acids (molar ratio: 2.2, 1.0, 0.3, 3.0). Among the uronic acids, glucuronic acid was identified and the presence of mannuronic acid could be also presumed. Methylation analysis of this polysaccharide revealed high proportions of 1,3-linked, 1,2,3-linked, and 1,2,3,6-linked galactose, 1-linked fucose, and 1-linked arabinose. This suggests that CU-1 is constituted by a principal skeleton of (1→3)-linked galactopyranose units, with some of these galactose units branched at the 2-position or at both the 2- and 6-positions, and with mainly terminal fucopyranosyl and arabinofuranosyl residues in the side chains. This is the first report on the polysaccharide constitution of a gum exudate from a *Croton* species.

Croton urucurana Baill. (Euphorbiaceae), known as "Urukurã"[1] and "Uruchnum",[2] is a common species in Paraguay, Northern Argentina, Southern Brazil, and Uruguay. It grows in sandy and wet soils that may flood during the rainy season and on damp areas near river banks. Three different products from this species are used primarily in folk medicine: the red latex, the bark, and the gum exudate.[1] Incision in the bark of the trunk and branches produces an immediate excision of a blood red latex. Once the "bleeding" has stopped, the gum then exudes over the same lesion and may be collected solidified a few days later.

No chemical studies on the gum of *C. urucurana* have appeared in the literature, and only three reports[3−5] have been published on the chemical composition of the stem bark of this species, which described the presence of acetyl aleuritolic acid, catechin, gallocatechin, sonderianin, *β*-sitosterol and its glucoside, stigmasterol, and campesterol,[3,4] as well as two novel clerodane diterpenes.[5] The oligomeric proanthocyanidin SP-303 has been described in the red latex.[2] The antidiarrheal activity of this latex has been recently described.[6]

The gum exudate is used in folk medicine by both oral and topical administration. In the first case, it is used against dysentery and to treat cancer and other illness. Topically, it is applied in wounds and ulcers directly over the affected zone. The aim of the investigation was to isolate and characterize the main polysaccharide from the gum of *C. urucurana*.

Results and Discussion

The crude extract (CUPD) of the gum exudate of *C. urucurana* was isolated from its aqueous solution by ethanol precipitation and dialysis. Fucose, arabinose, and galactose were the prominent sugar constituents (molar ratio: 7.9, 10.0, 20.0). CUPD was fractionated by ion-exchange chromatography on a DEAE-Sepharose fast-flow column, giving the two weak acidic fractions CU-1 (86.0%) and CU-2 (14.0%), which eluted with 0.3−0.6 and 0.9−1.0 M KCl, respectively. The elution profile of the fractions is shown in Figure 1. CU-1 was homogeneous by gel permeation chromatography on a Sepharose CL-4B column. CU-1 and CU-2 showed a similar sugar composition by acetylation and GC analysis, containing galactose, arabinose, and fucose as the main neutral sugars.

The estimated equivalent dextran molecular weight of CU-1 was ~2.48 × 10^6 Da. The IR spectrum of CU-1 showed a characteristic wide band at 3424 cm^{-1} corresponding to hydroxyl groups of the polysaccharide. A band at 1638 cm^{-1}, belonging to carbonyl groups of uronic acids, was also observed. CU-1 showed a $[\alpha]^{20}_D$ of −63.6° (*c* 0.1, H_2O). CU-1 gave a highly viscous aqueous solution, with an intrinsic viscosity of 5.347 dL/g at 30 °C, which is in agreement with the high molecular weight of the polysaccharide. The intrinsic viscosity of the solution of the crude extract (CUPD) was about twice as high as that of CU-1.

By acetylation and GC analysis[7] (neutral sugars) and the *meta*-hydroxybiphenyl method[8] (uronic acids), CU-1 was found to be a fucoarabinogalactan, containing 93.7% total sugars, mainly galactose, arabinose, and fucose (molar ratio: 19.0, 8.1, 7.8), and minor quantities of mannose, xylose, glucose, and uronic acids (molar ratio: 2.2, 1.0, 0.3, 3.0). An unidentified compound (NI) was also detected in a molar percentage of 0.9% (Table 1).

Comparison between the results of the analysis by acetylation and GC of CU-1 and of carboxyl-reduced CU-1 (CU-1R) showed an increase in the content of mannose, glucose, and the unidentified compound (Table 2), which suggested the presence of glucuronic acid and mannuronic acid. Nevertheless, analysis by methanolysis and trimethylsilylation showed only the presence of glucuronic acid (Table 1).

The content of N of CU-1 was 0.16%, as determined by elemental analysis. Aminosugars (galactosamine and glucosamine) were not detected in CU-1 by TLC or by acetylation and GC analysis, suggesting that the presence of N comes from a protein fraction of CU-1, being estimated at a level of 1.0% (N × 6.25). Table 3 shows the relative

* To whom correspondence should be addressed. Tel: +34-934024531. Fax: +34-934035982. E-mail: caniguer@farmacia.far.ub.es.

10.1021/np010188f CCC: $22.00
Published on Web 07/12/2002

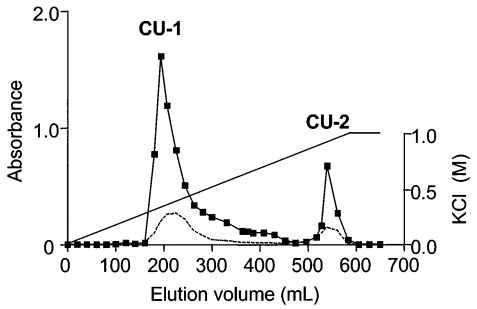

Figure 1. Fractionation of the crude extract (CUPD) of the gum exudate of *Croton urucurana* on a DEAE-Sepharose fast flow column. The carbohydrate profile was determined by anthrone (-■-) and *m*-hydroxybiphenyl (- - -) methods for total sugars and uronic acids, respectively.

Table 1. Sugar Composition of the Crude Extract (CUPD) of the Gum Exudate of *C. urucurana* and Its Main Polysaccharide CU-1, Determined by Different Methods

	molar %			
	CUPD		CU-1	
monosaccharide	alditol acetate	TMS derivative	alditol acetate	TMS derivative
neutral sugars				
fucose	17.3	18.4	18.6	19.7
arabinose	22.1	20.9	19.4	21.0
xylose	2.2	3.0	2.4	3.0
mannose	5.7	3.8	5.2	3.6
galactose	44.0	50.0	45.6	49.0
glucose	0.6	0.2	0.6	0.3
unidentified (NI)[a]	2.0		0.9	
uronic acids[b]	6.1		7.3	
glucuronic acid[c]		3.7		3.4
total sugars[d]	83.4		93.7	

[a] Calculated according to a hypothetical molecular weight of 180. [b] Determined by the *meta*-hydroxybiphenyl method. [c] Determined by trimethylsilylation and GLC analysis. [d] Percentage (w/w) in relation to CUPD or CU-1.

Table 2. Monosaccharide Composition of CU-1 and Its Carboxyl-Reduced Derivative

	%[a]	
monosaccharide	CU-1	CU-1R[b]
fucose	18.4	15.1
arabinose	18.5	15.6
xylose	1.9	2.1
mannose	4.7	7.2
galactose	49.0	44.3
glucose	0.3	3.4
NI[c]	1.3	7.2
uronic acids[d]	5.9	

[a] Molar percentage in relation to total sugars. [b] CU-1R: carboxyl-reduced CU-1. [c] NI: unidentified compound. [d] Determined by the *meta*-hydroxybiphenyl method.

amino acid composition of CU-1; glycine, alanine, and proline accounted for ca. 44% of the total amino acid content.

The interglycosidic linkages were determined by GC−MS of the partially *O*-methylated alditol acetate derivatives (PMAA) with or without carboxyl-deuteroreduction with $LiAlD_4$ of the permethylated polysaccharide. Results are shown in Tables 4 and 6, respectively. The absence of undermethylation in the permethylated CU-1 was confirmed by the practical disappearance of the 3424 cm^{-1} band in the IR spectrum.

Methylation analysis of CU-1 without deuteroreduction revealed a high content of 1,3-linked, 1,2,3-linked, and 1,2,3,6-linked galactose, as well as 1-linked fucose and

Table 3. Amino Acid Composition of CU-1, Obtained from the Gum Exudate of *C. urucurana*

amino acid	molar %[a]	amino acid	molar %[a]
aspartic acid	9.6	methionine	0.8
threonine	3.4	isoleucine	2.9
serine	4.9	leucine	4.6
glutamic acid	9.5	tyrosine	1.7
proline	10.5	phenylalanine	2.7
glycine	19.4	lysine	5.9
alanine	14.4	histidine	1.4
valine	5.4	arginine	2.8
cysteine	0.1		

[a] Calculated in relation to total amino acid content.

Table 4. Glycosyl-Linkage Composition of the Main Polysaccharide (CU-1) of the Gum Exudate of *C. urucurana*

glycosyl residue[a]	deduced linkage[a]	molar %[b]
fucosyl	Fuc-(1→	16.1
arabinosyl	Ara_f-(1→	15.0
	→2)-Ara_f-(1→	1.0
	→3)-Ara_f-(1→	5.4
	→5)-Ara_f-(1→	2.1
xylosyl	Xyl-(1→	0.9
galactosyl	Gal_p-(1→	2.5
	→3)-Gal_p-(1→	15.6
	→6)-Gal_p-(1→	2.7
	→2,3)-Gal_p-(1→	9.0
	→3,6)-Gal_p-(1→	2.0
	→2,6)-Gal_p-(1→	0.8
	→2,3,6)-Gal_p-(1→	11.1
mannosyl	→6)-Man_p-(1→[c]	3.5
	→2,3)-Man_p-(1→[c]	3.4
glucosyl	→3)-Glc_p-(1→	1.9
	→4)-Glc_p-(1→	1.9
glucuronosyl	→4)-$GlcA_p$-(1→	1.4

[a] Determined by GC−MS analysis of PMAA (partially *O*-methylated alditol acetate derivatives). After permethylation, CU-1 was carboxyl deuteroreduced prior to its conversion to PMAA. [b] Determined by GLC-FID analysis of PMAA and corrected using the molar response factors given by Sweet et al.[14] [c] Tentatively identified.

Table 5. Monosaccharide Composition of CU-1 before and after Partial Hydrolysis, under Mild Conditions

	composition (%)[a]	
sugar	before hydrolysis	after hydrolysis
Fuc	18.3	3.9
Ara	18.9	1.2
Xyl	2.3	4.4
Man	4.4	11.1
Gal	54.0	76.3
Glc	0.7	0.6
N.I.	1.4	2.5

[a] Molar percentage in relation to total neutral sugars.

1-linked arabinose. The analysis allowed the detection of minor amounts of terminal xylose, 1,3-linked glucose, and 1,2-linked, 1,3-linked, and 1,5-linked arabinose; 1,6-linked and 1,2,3-linked mannose were tentatively identified.

CU-1 was partially hydrolyzed in very mild conditions (0.05 M TFA, 100 °C, 90 min), dialyzed, and analyzed by acetylation. The results of this analysis revealed a specific cleavage of the arabinose units, with almost the total removal of this sugar and the fucose units (Table 5). This suggests that fucose and arabinose are located mainly in the side chains of CU-1. The results of the methylation analysis of degraded CU-1 showed that the loss of the above-mentioned monosaccharides detected by acetylation corresponded to 1-fucose, almost all of the 1-arabinose, and all of the 1,3-linked, 1,5-linked, and 1,2-linked arabinose. Also, a decrease of 1,2,3-linked and 1,2,3,6-linked galactose

Table 6. Glycosyl-Linkage Composition of CU-1 before and after Partial Hydrolysis, under Mild Conditions

CU-1		% molar[b]	
glycosyl residue[a]	deduced linkage[a]	CU-1	hydrolyzed CU-1
fucosyl	terminal	16.5	7.4
arabinosyl	terminal *f*	16.4	2.4
	→2)-Ara*f*-(1→	0.8	
	→3)-Ara*f*-(1→	5.4	
	→5)-Ara*f*-(1→	1.5	
xylosyl	terminal	1.9	3.1
galactosyl	terminal	3.8	10.8
	→3)-Gal*p*-(1→	15.1	25.6
	→6)-Gal*p*-(1→	2.3	8.7
	→2,3)-Gal*p*-(1→	7.7	4.4
	→3,6)-Gal*p*-(1→	1.8	13.3
	→2,6)-Gal*p*-(1→	0.7	
	→2,3,6)-Gal*p*-(1→	13.6	5.0
manosyl	→6)-Man*p*-(1→[c]	2.5	3.8
	→2,3)-Man*p*-(1→[c]	3.2	9.5
glucosyl	→3)-Glc*p*-(1→	1.0	
	→4)-Glc*p*-(1→	0.5	

[a] Determined by GC−MS analysis of PMAA (partially *O*-methylated alditol acetate derivatives). [b] Determined by GLC-FID analysis of PMAA and corrected using the molar response factors given by Sweet et al.[14] [c] Tentatively identified.

and an increase of 1-linked, 1,3-linked, 1,3,6-linked, and 1,6-linked galactose were observed (Table 6). These data confirmed that almost all the arabinose and fucose was in the side chains. Moreover, all the fucose residues were in terminal positions, whereas the arabinose residues appeared in terminal positions and were 1,3-, 1,5-, and 1,2-linked.

GC−MS analysis of deuteroreduced CU-1 (Table 4) showed only one deuterated compound, corresponding to 1,4-glucuronic acid.

In conclusion, the gum exudate of *C. urucurana* is mainly constituted by CU-1, a very viscous and high molecular weight fucoarabinogalactan, which has a principal skeleton of (1→3)-linked galactose, with a high degree of branching, mainly at the C-2, and C-2 and C-6 positions. It also contains high percentages of arabinose and fucose, principally as terminal units, located in the side chains. Small proportions of 1,4-linked glucuronic acid and protein were also detected in CU-1. Finally, the results of the acetylation analysis of CU-1 and CU-1R also suggested the presence of minor amounts of mannuronic acid, but this compound was not detected by trimethylsilylation analysis of CU-1 or by methylation analysis of carboxyl-deuteroreduced CU-1.

To the best of our knowledge, this is the first report on the polysaccharide constitution of a gum exudate from a *Croton* species.

Experimental Section

General Experimental Procedures. Total carbohydrates were determined by the anthrone method.[9] Uronic acids were measured by the *meta*-hydroxybiphenyl method using a glucuronic acid and galacturonic acid mixture (1:1) as standard.[8] Total protein was estimated by nitrogen elemental analysis (N × 6.25). Thin-layer chromatography (TLC) was performed over silica gel 60 F_{254} aluminum sheets (SDS, Peypin, France), eluting with $CHCl_3$−MeOH−H_2O (15:10:2) and using diphenylamine/aniline/H_3PO_4 as spray reagent for detection.[10] Analysis by gas−liquid chromatography (GLC) was performed on a Hewlett-Packard model 6890 gas chromatograph equipped with a FID detector and a split injector (ratio 60:1). Helium was used as carrier gas (flow: 1 mL min^{-1}).

Neutral sugar composition was determined by GLC after hydrolysis and acetylation. The samples were hydrolyzed with 2 N trifluoroacetic acid (TFA) at 121 °C for 1 h.[11] The hydrolyzed sample was acetylated by the method described by Blakeney et al.,[7] and the alditol acetates obtained were analyzed by GLC using a fused silica capillary column (30 m × 0.32 mm i.d.) with cyanopropylphenylsiloxane (BP-225, SGE, Victoria, Australia) as stationary phase. Temperature settings were as follows: injector 250 °C, detector 250 °C, oven 220 °C (isotherm). Myo-inositol was used as internal standard.

Neutral sugars were also determined simultaneously with uronic acids by GLC after methanolysis and trimethylsilylation. Methanolysis was performed with 0.5 M hydrochloric acid in methanol at 80 °C for 16 h, and methylglycosides were derivatized using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) as catalyst. GLC analysis of trimethylsilyl (TMS) derivatives was performed with a fused silica capillary column (25 m × 0.25 mm i.d.) with methylsilicone SE-30 (SPB-1, Supelco, Bellefonte, PA) as stationary phase. The oven temperature was programmed from 150 to 220 °C (rate 2 °C min^{-1}, injector 250 °C, and detector 250 °C). Ribose was used as internal standard.

Infrared spectra were recorded on a Perkin-Elmer FTIR spectrophotometer model 1600. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter for solutions in H_2O at 20 °C and 589.6 nm.

Plant Material. The gum exudate of *Croton urucurana* Baill. (Euphorbiaceae) was collected near Encarnación (Departamento de Itapuá, Paraguay) in January 1995. A voucher specimen has been deposited in the BCF Herbarium (Faculty of Pharmacy, University of Barcelona) under the number 50918.

Sample Pretreatment. The gum in the raw form showed a semisolid appearance. The raw material was dispersed in purified water (100 g gum/1 L H_2O) and then filtered through a glass wool filter to remove impurities. Polysaccharides were precipitated from the gum dispersion by addition of five volumes of 96% v/v ethanol and left to stand overnight chilled at 4 °C. The resultant precipitate was collected by centrifugation, washed with 96% v/v ethanol, and resuspended in water. The dispersion was subsequently dialyzed at a cutoff of 8000−15 000 Da against purified water and freeze-dried to give a dry crude extract (CUPD) with a yield of 5%.

Ion-Exchange Chromatography (IEC). The freeze-dried gum material CUPD (80 mg) was applied to a DEAE-Sepharose fast-flow column (40 × 2.6 cm) with chloride as counterion. Elution (flow rate 0.4 mL min^{-1}) was performed with water (300 mL) followed by a gradient from 0 to 1 M KCl. Fractions of 8 mL were collected, in which polysaccharides were detected by the anthrone assay. Separation was repeated with new portions of CUPD, since the quantity of the sample to be applied was limited because of the high viscosity of CUPD solutions.

Gel Permeation Chromatography (GPC). For the purification of CU-1 and CU-2 after IEC, a Shepharose CL-4B (Pharmacia) column (2.6 × 100 cm) eluted with 0.2 M NaCl at 0.4 mL min^{-1} was used. To estimate the equivalent dextran molecular weight, the fractions were chromatographed on a Superose 6 HR 10/30 (Pharmacia) column at room temperature. The mobile phase was water at a flow rate of 0.3 mL min^{-1}. The chromatographic system consisted of a pump (Pharmacia LKB-HPLC 2248 model), a 100 mL sample loop, and a Sedex 45 evaporative light-scattering detector (SEDERE, Vitry/Seine, France). The evaporation tube temperature on the detector was 53 °C and the pressure of the nebulization gas (N_2), 2.0 bar. The column was calibrated using dextrans with an average molecular weight of 11 000, 70 000, 503 000, and 2 000 000 (Sigma Chemical Co., St. Louis, MO) and 3 515 000 Da (Fluka, Buchs, Switzerland).

Methylation Analysis. Glycosidic linkages were determined by methylation analysis, which was performed following Hakomori's method[12] as modified by Harris.[13] The methylated samples were hydrolyzed, and the resulting products were acetylated. Partially methylated alditol acetates (PMAA) were analyzed by GC−MS with a Hewlett-Packard model 5890 series II gas chromatograph coupled to a 5971 A mass selective detector, equipped with a 30 m × 0.25 mm i.d. fused-silica

column coated with a 0.25 mm film thickness of DB-1701 (14% cyanopropylphenyl−86% dimethylsiloxane) (J&W Scientific, Folsom, CA). Operating parameters were as follows: carrier gas helium, flow rate 1 mL min^{-1}, injector temperature 250 °C, detector temperature 290 °C, oven temperature programmed from 170 to 210 °C (flow rate 1 °C min^{-1}), split ratio 1:60. Identification was made by comparison of the mass spectra with the corresponding standards. Quantification of PMAA was performed by GC using the same conditions described above for GC−MS; peak areas were corrected using the molar response factors given by Sweet et al.[14]

To determine the linkages of glycosyluronic acid residues, per-*O*-methylated polysaccharides were reduced with lithium aluminum borodeuteride in anhydrous tetrahydrofuran for 4 h under reflux.[15] Then, samples were re-methylated, hydrolyzed, with 2 N trifluoroacetic acid, and then acetylated following the above procedure. Derivatives were analyzed by GC−MS and GLC as described above.

Weak Acid Hydrolysis. The polysaccharide sample was hydrolyzed under mild conditions with 0.05 M TFA at 100 °C for 90 min.[16] Sugar composition was determined by acetylation as described above.

Reduction of Uronic Acids. The carboxyl group of the uronic acids were reduced with $NaBH_4$ directly in the presence of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC).[17] Then, samples were hydrolyzed, with 2 N trifluoroacetic acid, and then acetylated following the above procedure. Derivatives were analyzed by GLC as described above.

Amino Acid Analysis. Amino acid composition was determined after hydrolysis with 6 M HCl (16 h, 121 °C). Acid was removed by evaporation, and amino acids were analyzed with a Pharmacia LKB Biotechnology model Alpha Plus (series II) automatic analyzer.

Rheological Properties. Intrinsic viscosity [η] was determined at 30 °C in a Cannon-Fenske viscosimeter.

Acknowledgment. The authors are grateful to CEIA (Brussels, Belgium) and to Laboratorios Roda (Valencia, Spain) for financial support through the Fundació Bosch i Gimpera (University of Barcelona). B.M. was supported by a grant from the University of Barcelona.

References and Notes

(1) González Torres, D. M. *Catálogo de Plantas Medicinales (Alimenticias y Útiles) Usadas en Paraguay*; Asunción: Paraguay, 1970; p 397.
(2) Ubillas, R.; Jolad, S.; Bruening, R.; Kernan, M.; King, S.; Sesin, D.; Barrett, M.; Stoddart, C.; Flaster, T.; Kuo, J.; Ayala, F.; Meza, E.; Catañel, M.; McMeekin, D.; Rozhon, E.; Tempesta, M.; Barnard, D.; Huffman, J.; Smee, D.; Sidwell, R.; Soike, K.; Brazier, A.; Safrin, S.; Orlando, R.; Kenny, P. *Phytomedicine* **1994**, *1*, 77−106.
(3) Peres, M. T. L. P.; delle Monache, F.; Bella Cruz, A.; Pizzolatti, M. G.; Yunes, R. A. *J. Ethnopharmacol.* **1997**, *56*, 223−226.
(4) Peres, M. T. L. P.; delle Monache, F.; Pizzolatti, M. G.; Santos, A. R. S.; Beirith, A. Calixto, J. B.; Yunes, R. A. *Phytother. Res.* **1998**, *12*, 209−211.
(5) Peres, M. T. L. P.; Pizzolatti, M. G.; Yunes, R. A.; delle Monache, F. *Phytochemistry* **1998**, *49*, 171−174.
(6) Gurgel, L. A.; Silva, R. M.; Santos, F. A.; Martins, D. T. O.; Mattos, P. O.; Rao, V. S. N. *Phytother. Res.* **2001**, *15*, 319−32.
(7) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. *Carbohydr. Res.* **1983**, *113*, 291−299.
(8) Blumenkrantz, N.; Asboe-Hansen, G. *Anal. Biochem.* **1973**, *54*, 484−489.
(9) Morris, D. L. *Science* **1948**, *107*, 254−255.
(10) Chaplin, M. F. In *Carbohydrate Analysis. A Practical Approach*; Chaplin, M. F., Kennedy, J. F., Eds.; IRL Press: Oxford, 1986; p 34.
(11) Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. *Carbohydr. Res.* **1967**, *5*, 340−345.
(12) Hakomori, S. *J. Biochem. (Tokyo)* **1964**, *55*, 205−208.
(13) Harris, P. J.; Henry, R. J.; Blakeney, A. B.; Stone, B. A. *Carbohydr. Res.* **1984**, *127*, 59−73.
(14) Sweet, D. P.; Shapiro, R. H.; Albersheim, P. *Carbohydr. Res.* **1975**, *40*, 217−225.
(15) Lindberg, B. *Methods Enzymol.* **1972**, *28*, 178−195.
(16) Proksch, A.; Wagner, H. *Phytochemistry* **1987**, *26*, 1989−1993.
(17) Taylor, R. T.; Conrad, H. E. *Biochemistry* **1972**, *11*, 1383−1388.

NP010188F