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STRUCTURAL ANALYSIS OF A 4-*O*-METHYL-GLUCURONOARABINOXYLAN WITH IMMUNO-STIMULATING ACTIVITY FROM *ECHINACEA PURPUREA*

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Key Word Index—*Echinacea purpurea*; Asteraceae; hemicellulose; 4-*O*-methyl-glucuronoarabinoxylan; plant polysaccharide; structural analysis; immunological activity.

Abstract—From the hemicellulosic material of *Echinaceae purpurea* a homogeneous 4-*O*-methyl-glucuronoarabinoxylan was isolated by ethanol fractionation, ion-exchange chromatography and gel filtration. Sugar and methylation analysis revealed that the polysaccharide contained a (1 → 4)-linked β-D-xylan backbone with branching points at C-2 and C-3. Further characterization of the structure was performed by periodate oxidation and Smith degradation, methanolysis, uronic acid determination, basic degradation and ^{13}CNMR spectroscopy. The 4-*O*-methyl-glucuronoarabinoxylan showed immuno-stimulating activity in several *in vitro* immunological test systems.

INTRODUCTION

Echinacea purpurea (Asteraceae) is widely distributed in the middle and eastern part of North America. Besides other Echinacea species, the dried roots of *E. purpurea* were used by the Indians of the northern part of America against injuries, insect stings and bites of snakes [1]. Today extracts of the dried roots and the upper plant parts are used for stimulation of non-specific defence mechanisms at infections and chronic inflammations. In order to find a scientific explanation for the medical use, a great number of chemical and pharmacological studies have been undertaken [2]. Up to now no compound could be isolated which might be considered as the active principle of the drug.

As far as the high M_r compounds of the plant are concerned, an acidic polysaccharide containing a fraction with activity against hyaluronidase has been isolated by Bonadeo *et al.* [3] without further characterization. Since in our preliminary studies a polysaccharide fraction obtained from the hemicellulosic material showed remarkable activity in several *in vitro* and *in vivo* immunological test systems [4, 5], we started a thorough investigation of the active crude polysaccharide fraction. We report herein on the structure analysis of one of the isolated pure polysaccharides.

RESULTS AND DISCUSSION

A crude polysaccharide fraction was obtained from ethanol-pretreated herb material by extraction with 0.5 M aqueous sodium hydroxide according to Caldes *et al.* [6], followed by precipitation with ethanol and treatment with trichloroacetic acid. On hydrolysis, the non-dialysable, freeze-dried N-free fraction gave rhamnose, arabinose, xylose, mannose, galactose and glucose in a molar ratio of 0.5:1.0:3.8:0.2:1.4:0.5 (Table 1), and uronic acid as estimated by the carbazole test.

The hemicellulosic fraction was further fractionated by ion-exchange chromatography on DEAE Sepharose C1-6B using a phosphorus buffer system and a NaCl gradient to yield three main fractions, which according to the sugar analysis were suspected to consist of a heteroxylan, an arabinogalactan and an uronic acid, together with four other sugars containing pectic polysaccharide (Table 1) [7]. The heteroxylan was re-chromatographed on DEAE-Sepharose C1-6B and on Biogel P-60. As judged by HPLC this polysaccharide was homogeneous and the mean M_r estimated to be *ca* 35 000. The impurity of pectic material was negligible.

The heteroxylan contained arabinose and xylose in the ratio 1:5.0, as determined by GC of the derived alditol acetates [8]. The uronic acid content was estimated by analysis of the underivatized product of total hydrolysis on an automatic sugar analyser and by $NaBD_4$-reduction of the native polysaccharide according to Taylor and Conrad [9], followed by a quantitative determination of the derived alditol acetates [10] by GC-MS. According to these results the estimated 4-*O*-methyl-glucuronic acid should be present in a ratio of 1.0:5.7 xylose. Hydrolysis of the acidic arabinoxylan under very mild conditions resulted in a specific cleavage of the arabinose units, indicating that they might be present in a furanoid structure and located in the side chains.

Hakomori methylation [11] of the glucuronoarabinoxylan, followed by acid hydrolysis and GC and GC-MS of the derived partially methylated alditol acetates [12] gave the results shown in Table 2. The presence of 2,3,5-tri-*O*-methyl-arabinose indicated the existence of non-reducing terminal arabinofuranose side-units, which might be linked on average to every 12th or 13th xylosyl unit in the main chain. The presence of 2,3-di-*O*-methyl-arabinose in a ratio of 1:5,3 xylosyl residues and 1:2,3 arabinosyl endgroups suggested that in accordance with the results of the mild hydrolysis the side chains of the polysaccharide

Table 1. Sugar composition of hemicellulose fraction, arabinoxylan and degradation products from *E. purpurea*

Fraction	Sugar composition* (molar ratio) Rha	Ara	Xyl	Man	Gal	Glu	Uronic acid†	4-*O*-Me GluA‡
Hemicellulose mixture (0.5 M NaOH)	0.5	1	3.8	0.2	1.4	0.5	++	
Arabinoxylan	Sp	1	5.0	—	0.2	Sp		0.9
Xyl-PH-1	—	—	5.5	—	Sp	—		1
Xyl-PH-2_1	—	1	22.3	—	Sp	Sp		—
Xyl-PH-2_2	—	1	46.8	—	Sp	—		—
Xyl-PH-2_3	—	1	7.3	—	0.1	Sp		—
Xyl-PH-2_4	—	1	4.2	—	0.1	Sp		—
Xyl-PH-2_5	—	—	5.3	—	Sp	—		1
Xyl-PH-2_6	—	—	2.6	—	Sp	—		1
Xyl-PH-2_7	—	—	2.0	—	Sp	—		1

*Neutral sugars were estimated by GC on column A.
†Uronic acid was estimated by the carbazole test.
‡4-*O*-Methyl-glucuronic acid was estimated by a sugar analyser as 4-*O*-methyl-glucuronic acid.

Table 2. Methylation analyses of A: arabinoxylan; B: reduced arabinoxylan; C: Xyl-PH-1; D–H: Xyl-PH-$2_{1-3,5,6}$; I: reduced Xyl-PH-2_5; K: reduced Xyl-PH-2_6

Methylated alditol acetate	Deduced linkage	R_T*	Molar ratio† A	B	C	D	E	F	G	H	I	K
2,3,5-Me_3-Ara	Ara_f-(1-	0.42	1	0.3	—	—	0.04	0.11	—	—	—	—
2,3-Me_2-Ara	→ 5)-Ara_f-(1 →	1.07	2.3	0.8	—	0.2	0.45	0.25	—	—	—	—
2,3,4-Me_3-Xyl	Xyl_p-(1 →	0.53	0.4	0.1	1	1	1	1	1	1	1	1
2,3-Me_2-Xyl	→ 4)-Xyl_p-(1 →	1.19	10.0	10.0	20.7	6.8	0.75	1.1	5.8	3.3	4.9	2.7
2-Me-Xyl	→ 4)-Xyl_p-(1 → 3	2.14	2.3	1.2	—	0.1	—	Sp	—	—	—	—
3-Me-Xyl	→ 4)-Xyl_p-(1 → 2				1.8	—	—	—	1 2	1.0	1.3	1
2,3,4,6-Me_4-Glu	Glc_p-(1 →	1.00	—	0.6	—	—	—	—	—	—	1.1	0.9
2,4,6-Me_3-Glu	→ 3)-Glu_p-(1 →	1.81	—	0.1	—	—	—	—	—	—	—	—

†Estimated by GC of alditol acetates on column A.
*R_t relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol on column A at 170°.

might be composed of 1,5-linked and terminal arabinofuranosyl residues. The presence of 2,3,4-tri-*O*-methyl-xylose in a ratio of 1:30,7 xylosyl residues of the main chains indicates the existence of xylopyranosyl residues also as non-reducing terminal groups in the side chains. The backbone, however, consists of (1 → 4)-linked xylosyl residues, as was evident from the estimated large amount of 2,3-di-*O*-methyl-xylose in the hydrolysate. The side chains should be substituted through positions 2 and 3 of the xylosyl moiety, since the hydrolysate showed on column B a non separable peak which consisted of 2-*O*-methyl and 3-*O*-methyl-xylose. For determining the position and linkage of the 4-*O*-methyl-glucuronic acid the methylated polysaccharide was reduced with $LiAlH_4$ and remethylated, followed by acid hydrolysis and GC and GC-MS of the partially methylated alditol acetates. The identified 2,3,4,6-tetra-*O*-methyl-glucose and small amounts of 2,4,6-tri-*O*-methyl-glucose indicated that the glucuronic acid must be present as non-reducing terminal sugar in a 1 → 2-linkage.

In another experiment we subjected the arabinoxylan to periodate oxidation, followed by Smith degradation [13]. The split products afforded were analysed as alditol acetates by GC, and as the partially methylated alditol acetates by GC and GC-MS. Apart from glycerol, which was the main degradation product of the (1 → 4)-linked xylose backbone, only xylose could be detected as 1,2,4- and 1,3,4-linked xylosyl residues besides small amounts of undegraded 2,3-di-*O*-methyl-xylose. Further degradation was undertaken with the methylated native arabinoxylan. Methanolysis liberated a mixture of products which were remethylated with CD_3I, and again subjected to hydrolysis. Analysis of the partially methylated alditol acetates obtained by GC-MS showed the incorporation of CD_3 into the C-2-position of the 2,3-di-*O*-methyl-xylose. The detected deuterated 2-*O*-methyl-xylose in the hydrolysate (Table 2) indicated that glucuronic acid must be attached to the 2-position of the xylose-moieties of the backbone. This assignment was supported by the detection of the partially methylated aldobiuronic acid 2-*O*-(4-*O*-methyl-

α-D-glucopyranosylurono)-D-xylopyranose. No evidence of a 1 → 3-linked glucuronic acid to the xylose backbone could be found. To ascertain this structural feature the polysaccharide was hydrolysed under very mild conditions yielding a non-dialysable fraction Xyl-PH-1. In a second experiment the native polysaccharide was subjected to partial acid hydrolysis yielding a fraction Xyl-PH-2. The second fraction was further separated into four neutral and three acidic fractions (1–7) on DEAE-Sephadex A-25 using ammonium acetate as eluent. The fractions were investigated by TLC, GC of the obtained alditol acetates (Table 1) and by methylation analyses (Table 2). According to TLC the fractions Xyl-PH-2_{3-4} contained free arabinose and xylose, and a mixture of di-, tri- and tetrasaccharides. The fractions Xyl-PH-2_{1-2} and Xyl-PH-2_{5-7} consisted only of higher oligosaccharides. Fraction 4 consisted primarily of monosaccharides. Fraction 7 showed a similar composition to fraction 6. The methylation analyses of the four neutral fractions yielded 1- and 1,5-linked arabinosyl residues besides 1- and 1,4-linked xylopyranose residues. The small amount of 1,3,4-linked xylose indicated the presence of neutral oligosaccharides branched at C-3 of the xylose main-chain. These oligosaccharides might be built up of xylose in the main chains and arabinose in the side chains. The acidic fractions did not contain arabinose, but as determined by methylation analysis of Xyl-PH-2_5 and Xyl-PH-2_6 1-, 1,4- and 1,2,4-linked xylosyl residues as well as 1-linked glucuronic acid. The Xyl-PH-1 fraction consisted of 1,4- and 1,2,4-linked xylose and glucuronic acid as non-reducing terminal sugar. According to these results, the partially degraded polysaccharide (Xyl-PH-1) and the acidic products of partial acid hydrolysis experiments (Xyl-PH-2_{5-6}) consisted only of (1 → 4)-linked xylosyl chains and glucuronic acid linked through C-2 of the xylose main chains.

In the ^{13}C NMR spectra of the native polysaccharide the chemical shifts at 101.78, 101.42 and 101.05 ppm could be assigned to the anomeric carbon signals of β-(1 → 4)-linked xylopyranose residues. Signals at 109.38 and 107.62 ppm could be deduced from furanoside arabinose residues, whereas the signal at 97.64 ppm should derive from the anomeric carbon of α-linked 4-*O*-methyl-glucuronic acid, the methyl group of which appeared at 59.83 ppm [14–20]. The ^{13}C NMR data of the degradation products Xyl-PH-1 and Xyl-PH-2_6 were in every respect compatible with the results of the degradation products (Table 3).

Based on these results, it can be concluded that the backbone chain of the acidic arabinoxylan of *E. purpurea* is composed of β-(1 → 4)-linked xylopyranosyl residues possessing branching points at positions 2 and 3. The arabinofuranosyl residues are suggested to be attached to the backbone at position 3 as α-(1 → 5)-linked side chains and as non-reducing terminal groups. The β-linked 4-*O*-methyl-glucuronic acid occupies a non-reducing terminal group position 2 of the main chain. The small amounts of 1,3-linked glucuronic acid detected in the methylation analysis of the reduced polysaccharide could be an indication that 4-*O*-methyl-glucuronic acid is linked to further sugar residues.

The heteroxylan of *E. purpurea* described is one of the first polysaccharides of the Asteraceae which has been isolated in a pure form and its basic structure elucidated chemically. It has a similar chemical composition to those reported for some xylose-containing polysaccharides isolated from the hemicellulosic material of hard wood and from the Gramineae [21].

As far as the immunological activity is concerned, we found a strong phagocytosis enhancing effect in the *in vitro* granulocyte test [22] up to 23% at concentrations of 10^{-1} to 10^{-4} mg/ml. The mean yield of chemoluminescence using the same test system [23] was determined as 34% at a concentration of 10^{-1}–10^{-4} mg/ml. In the *in vivo* phagocytosis model, however, no enhancement of the carbon clearance could be observed for the pure polysac-

Table 3. ^{13}C NMR data of carbohydrates isolated from *E. purpurea*

Carbohydrates	Chemical shifts (ppm)						
	C-1	C-2	C-3	C-4	C-5	C-6	–OMe
4-O-Methyl-glucurono-arabinoxylan							
→ 4)-β-Xyl_p-(1 →	101.78	72.82	73.83	76.52	63.09		
→ 2,4)-β-Xyl_p-(1 →	101.42	76.23	73.60	76.67	63.02		
→ 3,4)-β-Xyl_p-(1 →	101.05	71.44	(79.48)*	(75.9)*	62.88		
α-Ara_f-(1 →	109.38	81.45	76.96	84.1	61.44		
→ 5)-α-Ara_f-(1 →	107.62	81.43	76.94	82.43			
4-*O*-Me-β-$GluA_p$-(1 →	97.64	71.44	72.41	82.49	70.03	176.78	59.83
Xyl-PH-1							
→ 4)-β-Xyl_p-(1 →	101.82	72.85	73.95	76.36	62.23		
→ 2,4)-β-Xyl_p-(1 →	101.55	76.35	73.62	76.64			
β-Xyl_p-(1 →	102.07	73.34	74.20	71.39	65.46		
4-*O*-Me-β-$GluA_p$-(1 →	97.99	71.64	72.49	82.25	71.93	175.58	60.09
Xyl-PH-2$_6$							
→ 4)-β-Xyl_p-(1 →	102.12	73.29	74.25	76.97	63.60		
→ 2,4)-β-Xyl_p-(1 →	101.98	76.79	72.71	77.34	63.45		
→ 2)-β-Xyl_p-1 →	102.22	77.34	74.89	70.05	65.78		
→ β-Xyl_p-(1 →	102.38	73.39	76.21	69.83	65.53		
4-*O*-Me-β-$GluA_p$-(1 →	98.19	72.16	72.91	82.54	71.85	176.4	60.12

*Assignment is not definitive.

charide. This result was in contrast to that obtained with the crude hemicellulosic fraction [4].

EXPERIMENTAL

General. All evapns were performed under red. pres. at a temp. below 40°. 1-dm tubes were used for measuring optical rotations. TLC was performed on silica gel $60\,F_{254}$, with $CHCl_3$–MeOH–H_2O (8:5:1) as solvent and detection with diphenylaminophosphoric acid reagent. FD-GC was performed on a glass column (3 m × 2 mm) with 3% OV 225 (A) on Chromosorb W-HP (80/100 mesh) at 215° for alditol acetates. Uronic acid estimation was performed with a sugar analyser on a DA-X4-20, Durrum (0.6 × 27 cm) column. ^{13}C NMR spectra for D_2O solns were recorded using TMS as int. std; chemical shifts are given in δ values. GC-MS was performed on a quartz capillary (25 m × 0.23 mm) coated with OV 225 (B) and on a quartz capillary (10 m × 0.35 mm) coated with SP-1000 (C).

Isolation of polysaccharides. Powdered plant material (1 kg) was extracted with MeOH under reflux in a Soxhlet apparatus for 4 days and the extract discarded. The dried plant residue (945 g) was extracted twice with 0.5 M aq. NaOH (5 ml/g plant material) and allowed to stand overnight. After each extraction the residue was separated from the supernatant soln by filtration. To the combined extracts 4 vol. EtOH were added under stirring and the mixture kept for 24 hr. After decanting of the supernatant soln, the brownish ppt was centrifuged and resuspended at room temp. in H_2O. TCA (15%) was added under stirring at 0° to the soln and it was then kept for 1 hr at room temp. The ppt formed was again separated by centrifugation and 3 vol. EtOH added to the supernatant. After storage for 24 hr and centrifugation, the ppt was resuspended in 2% aq. NaOAC. The soln was centrifuged to remove insoluble material, followed by addition of 1 vol. of EtOH to the supernatant. After standing for 62 hr the supernatant soln was decanted and the ppt collected by centrifugation, dissolved again in H_2O and dialysed against H_2O for 3 days. The crude polysaccharide fraction was obtained after lyophilization (8.93 g).

Fractionation of polysaccharide. Crude polysaccharide fraction (0.5 g) was dissolved in H_2O and dialysed against 0.02 M Pi buffer soln pH 7.5 for 24 hr. The soln was applied to a DEAE Sepharose C1-6B column (2.6 × 50 cm) which had been pre-equilibrated with the same buffer system. Elution started with buffer only (500 ml), followed by a linear gradient of 0.0–1.0 M NaCl (1 l.). The fractionation was monitored by polarimetry. Four fractions were obtained. Fraction II (0.143 g) was rechromatographed on Biogel P-60 using H_2O as eluent.

Homogeneity and M_r. Homogeneity was proven by HPLC on μ-Bondagel E-250, E-500 and E-1000 columns (Water Assoc.) and equilibrated with 0.2 M Pi buffer (pH 7). All samples were prepared as 1% (w/v) solns; 10 μl of the centrifuged soln were injected each time. The eluates were monitored by RI and UV detectors. For M_r estimation, calibration was performed using dextrans of known MW (T 10, T 40, T 70, T 110, Pharmacia Fine Chemicals).

Analysis of sugars. Complete acidic hydrolysis of the polysaccharide was achieved by treatment with TFA at room temp. overnight, followed by heating for 2 hr at 100°, dilution with H_2O up to 80% acid, followed again by heating for 15 min, dilution of the reaction mixture up to 30% content of TFA and heating again for 2 hr [24]. The hydrolysates were then divided into two parts. One part was analysed using a sugar analyser. The monosaccharides of the second part were converted into their corresponding alditol acetates for GC analysis. Neutral fractions obtained by partial hydrolyses were hydrolysed with 0.5 M TFA overnight at 100°. The monosaccharides obtained were converted into their corresponding alditol acetates and analysed by GC [25].

Methylation analyses. Polysaccharide (5 mg) dissolved in dry DMSO (5 ml) was methylated with methylsulphinyl carbanion (2.5 ml) and MeI (2.5 ml) according to the method ref. [11]. After hydrolysis the methylated sugars were converted into their corresponding partially methylated and, with $NaBD_4$, reduced alditol acetates, to be analysed by GC and GC-MS (column B) [12].

Reduction 1 of carboxyl groups [9]. Polysaccharide (10 mg) was dissolved in 20 ml H_2O, 1-cyclohexyl-3-(2-morpholino-ethyl)-carbodiimide metho-*p*-toluenesulphonate (CMC, 200 mg) was added under stirring and the pH maintained at 4.75 by dropwise addition of 0.01 M HCl during 4 hr. 2 M $NaBD_4$ soln (4 ml) was added to the reaction mixture and the pH maintained at 7 by simultaneous addition of 0.1 M HCl. The mixture was stirred for a further 1 hr at room temp., dialysed against running H_2O and lyophilized. The product was hydrolysed with 0.5 M TFA as described above. The monosaccharides obtained were converted into their corresponding alditol acetates, for analysis by GC-MS using column B.

Reduction 2 of carboxyl groups with $LiAlH_4$. Methylated polysaccharide (8 mg) was dissolved in dry THF (15 ml), $LiAlH_4$ (50 mg) was added and the reaction mixture refluxed for 6 hr. After standing at room temp. overnight, excess $LiAlH_4$ was destroyed by addition of H_2O. The reduced, methylated polysaccharide was separated by extraction with CH_2Cl_2 (5 ml × 4) and the combined extracts evapd to dryness. The methylated and reduced polysaccharide was remethylated and the corresponding alditol acetates analysed by GC and GC-MS on column B.

Degradation of polysaccharide to XYL-PH-1. Polysaccharide (90 mg) was treated with 0.05 M TFA at 100° for 1 hr. After evapn of acid, the reaction mixt. was dialysed against H_2O. Both fractions, the dialysable and the non-dialysable part, were analysed as described above.

Partial acidic degradation to XYL-PH-2_{1-7}. Polysaccharide (100 mg) was treated with 0.05 M TFA at 100° for 2 hr. After evapn of acid, the reaction mixture was dissolved in H_2O and applied to a DEAE Sephadex A-25 column (1.6 × 50 cm). After elution with H_2O (300 ml) the acidic fractions were eluted with a linear gradient of 0.0–0.8 M NH_4OAC (500 ml). Elution was monitored by polarometry. Seven fractions were collected, lyophilized and analysed as described above.

Periodate oxidation and Smith degradation. Polysaccharide (20 mg) was oxidized with 0.01 M $NaIO_4$ (40 ml) at 4° in the dark for 7 days. Ethylene glycol (0.8 ml) was then added and the reaction mixture dialysed against H_2O for 48 hr. The non-dialysable fraction was lyophilized, dissolved in H_2O (18 ml) and reduced with $NaBH_4$ (5 mg) for 24 hr at room temp. Excess $NaBH_4$ was destroyed by addition of HOAc and the reaction mixture dialysed again for 24 hr against H_2O. One part of the non-dialysable fraction (2 mg) was lyophylized, hydrolysed with 0.5 M TFA and the monosaccharides obtained analysed as their corresponding alditol acetates by GC. The other part was methylated, hydrolysed and analysed as the partially methylated alditol acetates by GC and GC-MS on column C as described above.

Methanolyses [14]. Methylated polysaccharide (8 mg) was dissolved in dried MeOH (10 ml) and refluxed with 3% HCl overnight. After addition of Ag_2CO_3 the reaction mixture was centrifuged, the soln separated and evapd to dryness. The residue was acetylated with a mixture of Ac_2O–pyridine (1:1) at 100° for 1 hr. The acetylated product was analysed by GC-MS on column B.

Alkaline degradation [26]. To the methylated polysaccharide (5 mg) in a serum vial which was sealed with a rubber cap and

flushed with N_2, 2.5 ml 1 M methylsulphinyl sodium in DMSO was added with the aid of a syringe. The soln was sonicated in an ultrasonic bath for 30 min and kept at room temp. overnight. CD_3I (1 ml) was added dropwise at 0° and the reaction mixture sonicated again for 30 min. The mixture was poured into H_2O (5 ml) and dialysed against H_2O for 24 hr. The non-dialysable fraction was lyophilized, hydrolysed and the sugars analysed as permethylated alditol acetates by GC and GC-MS.

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REFERENCES

1. Schindler, H. (1940) *Pharmaz. Zentralh.* **81**, 589.
2. Wagner, H. and Proksch, A. (1985) in *Economic and Medicinal Plant Research*, Vol. 1, p. 113. Academic Press, London.
3. Bonadeo, J., Botazzi, G. and Lavazza, M. (1971) *Riv. Ital. Essenze-Profumi-Piante Offizin.-Aromi-Saponi-Cosmetici-Aerosol* **53**, 281.
4. Wagner, H., Proksch, A., Riess-Maurer, I., Vollmar, A., Odenthal, S., Stuppner, H., Jurcic, K., Le Turdu, M. and Fang, J. N. (1985) *Arzneim.-Forsch. (Drug Res.)* **35**, 1069.
5. Stimpl, M., Proksch, A., Wagner, H. and Lohmann-Matthes, M.-L. (1984) *Infections Immunity* **46**, 845.
6. Caldes, G., Prescott, B., Thomas, C. A., II and Baker, P. J. (1981) *J. Gen. Appl. Microbiol.* **27**, 157.
7. Proksch, A. and Wagner, H. (in preparation).
8. Albersheimer, P., Nevins, J., English, P. D. and Karr, A. (1967) *Carbohydr. Res.* **5**, 340.
9. Taylor, R. L. and Conrad, H. E. (1972) *Biochemistry* **11**, 1383.
10. Jansson, P. E., Lindberg, B. and Ljunggren, H. (1979) *Carbohydr. Res.* **75**, 207.
11. Hakomori, S.-I. (1964) *J. Biochem. (Tokyo)* **55**, 502.
12. Jansson, P. E., Kenne, L., Liedgren, H., Lindberg, B. and Lönngren, J. (1976) *Chem. Commun. (Stockholm University)* **8**.
13. Abdel-Akher, M., Hamilton, J. K., Montgomery, R. and Smith, F. (1952) *J. Am. Chem. Soc.* **74**, 4970.
14. Aspinall, G. O., Fanous, H. K. and Sen, A. K. (1983) *ACS Symposium Ser. No.* 241, 33.
15. Kováč, P., Hirsch, J., Kováčik, V. and Kociš, P. (1980) *Carbohydr. Res.* **85**, 41.
16. Kováč, P., Hirsch, J., Shaskov, A. S., Usov, A. I. and Yarotsky, S. V. (1980) *Carbohydr. Res.* **85**, 177.
17. Hirsch, J., Kováč, P. and Petráková, E. (1982) *Carbohydr. Res.* **106**, 203.
18. Shimizu, K. (1981) *Carbohydr. Res.* **92**, 65.
19. Shaskov, A. S. and Chizhov, O. S. (1976) *Sov. J. Bioorg. Chem.* **2**, 311.
20. Capek, P., Toman, R., Kordosova, A. and Rosik, J. (1983) *Carbohydr. Res.* **117**, 133.
21. Stephen, A. M. (1983) *The Polysaccharides* (Aspinall, G. O., ed.) Vol. 2, p. 97. Academic Press, New York.
22. Brandt, L. (1967) *Scand. J. Haematol, Suppl.* **2**.
23. Allen, R. C. (1981) in *Bioluminescence and Chemoluminescence: Basic Chemistry and Analytical Applications* (De Luca and Mc Elroy, eds) p. 63. Academic Press, New York.
24. Fengel, D. and Wegener, G. (1979) in *Advances in Chemistry Series* No. 181, 145.
25. Jansson, P. E. and Lindberg, B. (1979) *Carbohydr. Res.* **75**, 207.
26. Lindberg, B., Lönngren, J. and Thompson, J. L. (1973) *Carbohydr. Res.* **28**, 351.