Phytochemistry, Vol. 24, No. 10, pp. 2431–2433, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

SHORT REPORTS

STRUCTURE OF PANAXAN B, A HYPOGLYCAEMIC GLYCAN OF *PANAX GINSENG* ROOTS*

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(*Received* 22 *January* 1985)

Key Word Index—*Panax ginseng*; Araliaceae; peptidoglycan; glucan; structure; hypoglycaemic activity.

Abstract—A hypoglycaemic principle, panaxan B, obtained from the roots of *Panax ginseng*, was shown to be a peptidoglycan with M_r of about 1 800 000. Physico-chemical and chemical studies indicated panaxan B to be mainly composed of α-1 → 6 linked D-glycopyranose residues with branching at the C-3 position, the ratio of terminals, branching positions and intermediate units being about 1:1:1.8.

INTRODUCTION

The crude drug 'ninjin' (ginseng), the roots of *Panax ginseng* C. A. Meyer, has been utilized as an elixir in Oriental medicine. This crude drug has long been known to possess the ability to lower blood sugar level in normal and experimentally-induced hyperglycaemic animals [1]. Recently, we have identified the hypoglycaemic peptidoglycans, panaxan A, B, C, D and E, in ginseng [1] and we have determined the partial structure of one of the main peptidoglycans, panaxan A [2]. The present paper deals with the structural study of panaxan B, another main peptidoglucan in ginseng.

RESULTS

When panaxan B was subjected to gel chromatography on Sephacryl S-500, a value of *ca* 1 800 000 for the M_r was obtained. Quantitative determination showed that the component sugar of panaxan B was D-glucose (95.9%) and a small amount of peptide moiety was present (0.7%). Its amino acid composition after acid hydrolysis was as follows (mol %): alanine (15.1), glycine (12.6), aspartic acid (11.8), glutamic acid (8.9), threonine (8.7), valine (8.2), serine (7.3), leucine (6.8), lysine (5.6) and other minor amino acids.

Panaxan B exhibited a high positive specific rotation ($[\alpha]_D + 196°$) and its ^{1}H NMR spectrum disclosed two anomeric hydrogen signals at δ4.88 (doublet, *J* 3 Hz) and 5.24 (doublet, *J* 3 Hz), and the ratio of their integrals was about 3:1. Thus α-linkage of all D-glucose units was deduced.

Among nine signals at δ60.6, 65.3, 69.6, 70.2, 71.7, 73.3, 81.5, 97.9 and 99.6 observed in the ^{13}C NMR spectrum of panaxan B, it was evident that those at δ60.6 and 65.3 were attributable to the C-6 carbons, those at δ73.3 and 81.5 ascribable to the C-3 carbons, and those at δ97.9 and 99.6 assignable to the C-1 carbons. Therefore, it was concluded that α-glucose units are linked at the 1, 3 and 6 positions in panaxan B.

Methylation of panaxan B by the Hakomori's method [3] afforded the fully methylated derivative which was hydrolysed with dilute H_2SO_4 in HOAc. The products were converted into alditol acetates [4] and analysed by gas–liquid chromatography-mass spectrometry (GC-MS). As a result, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose and 2,4-di-*O*-methyl-D-glucose were identified and their approximate molar ratio was 1.0:1.8:1.0.

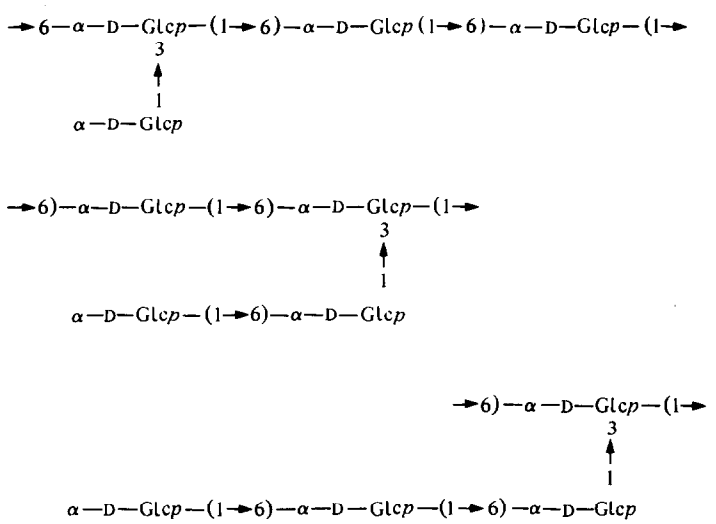
Periodate oxidation of panaxan B consumed 1.47 mol of periodate per mol of component anhydro sugar unit with liberation of 0.83 mol of formic acid. When the periodate-oxidized product was reduced [5], hydrolysed and analysed, the residual glucose was obtained in a yield of 31.0%.

Accumulated data demonstrated that panaxan B is mainly composed of α-1 → 6 linked D-glucopyranose units and possesses branches linked in part at the C-3 position. The ratio of terminals, branching positions and intermediate units should be *ca* 1:1:1.8 on the average. Judging from the above facts, there are more branchings in panaxan B than panaxan A. Thus panaxan B has the following three possible structural fragments (Scheme 1). Detailed study of the full structure is under way.

EXPERIMENTAL

Panaxan B. $[\alpha]_D + 196°$. IR ν_{max}^{KBr} cm^{-1}: 3430, 1012; ^{1}H NMR (D_2O): δ4.88 (*d*, *J* 3 Hz), 5.24 (*d*, *J* 3 Hz) [1]; ^{13}C NMR (D_2O):

*Antidiabetes drugs, Part 12. Also Part 94 in the Tohoku University series on the validity of the Oriental medicines.



Scheme 1. Three possible structural fragments of the polysaccharide moiety of panaxan B. Glc*p* = glucopyranose.

δ60.6, 65.3 (C-6), 69.6 (C-4), 70.2, 71.7 (C-5, 2), 73.3, 81.5 (C-3), 97.9, 99.6 (C-1).

Determination of M_r of panaxan B. Gel chromatography of panaxan B was carried out on a Sephacryl S-500 column (2.6 i.d. × 96 cm) which was eluted with 0.1 M Tris-HCl buffer (pH 7). Fractions (5 ml) were analysed by the phenol–H_2SO_4 method. The M_r was calculated by comparison of the elution vol. of panaxan B with the elution vols of the standard pullulans.

Determination of the components of panaxan B. Panaxan B (0.5 mg) was subjected to hydrolysis with 2 N H_2SO_4 at 100° for 6 hr to afford the product which was reduced, acetylated and analysed for sugar components by GLC performed under conditions A: using a column (0.3 cm i.d. × 200 cm, spiral glass) packed with 3% OV 225 on Gaschrom Q (100–120 mesh) at 220° with He at 50 ml/min [6]. Quantitative determination of glucose was performed by the chromotropic acid–H_2SO_4 method [7].

The determination of peptide content was conducted by the Lowry method [8]. The amino acid composition was determined with an amino acid analyser after hydrolysis with 6 M HCl at 110° for 24 hr.

Methylation analysis of panaxan B. NaH (10 mg) was mixed with DMSO (2 ml) in an ultrasonic bath for 30 min, and the mixture was stirred at 70° for 1 hr and added to panaxan B (5.7 mg) in DMSO (1.8 ml). After stirring at room temp. for 4 hr, MeI (2 ml) was added. Stirring was continued overnight at room temp. All procedures were carried out under N_2. The reaction mixture was poured into H_2O (15 ml) and extracted 5 times with $CHCl_3$ (15 ml each). The combined $CHCl_3$ soln was washed with H_2O and dried over Na_2SO_4. The filtrate was concd to dryness to give the product which was methylated twice more under the same conditions. The final residue was dissolved in $CHCl_3$–MeOH mixture (2:1) and chromatographed over Sephadex LH-20 (1.0 i.d. × 18 cm) which was eluted with the same solvent. The eluates obtained from tubes 5 to 9 (1 ml each) were combined and concd to dryness. The final product (8.3 mg) showed no hydroxyl absorption in its IR spectrum.

The product (1.5 mg) was treated with 10 N H_2SO_4–HOAc (5:95; 0.4 ml) at 80° for 16 hr. After 0.5 N H_2SO_4 (0.4 ml) was added, the soln was heated at 100° for 3 hr and neutralized with Dowex 2 (OH^-). The filtrate and the washings with MeOH were combined and evaporated yielding the residue which was dissolved in H_2O (1.5 ml) in an ultrasonic bath for 5 sec and reduced with $NaBH_4$ (7.5 mg) at 5° for 16 hr. The mixture was treated with Dowex 50W-X8 (H^+) up to pH 5, filtered and concentrated, and boric acid was removed by treatment with MeOH. The product after acetylation with Ac_2O–pyridine (1:1; 2 ml) at 100° for 1 hr

Table 1. Relative retention times on GLC and main fragments in MS of partially methylated alditol acetates

	Relative retention times	Main fragments (*m/z*)
1,5-Ac-2,3,4,6-Me-D-Glucitol	1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,5,6-Ac-2,3,4-Me-D-Glucitol	2.05	43, 87, 99, 101, 117, 129, 161, 189
1,3,5,6-Ac-2,4-Me-D-Glucitol	3.60	43, 87, 117, 129, 189

Abbreviations: Ac = acetyl; Me = methyl (e.g., 1,5-Ac-2,3,4,6-Me- = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-).

was dissolved in $CHCl_3$–MeOH (1:1) and analysed by GC-MS carried out under condition B: using the same column as condition A at 200° with He flow 60 ml/min. The relative retention times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, and their main fragments in the mass spectra are shown in Table 1.

Periodate oxidation followed by Smith degradation of panaxan B. To panaxan B (10.8 mg) in H_2O (2.5 ml), 0.1 M $NaIO_4$ (2.5 ml) was added. The reaction mixture was kept at 4° in the dark. The periodate consumption was determined by a spectrophotometric method [9, 10]. After 5 days ethylene glycol was added, and the HCO_2H liberation was measured by titration with 0.01 M NaOH.

The residue of the reaction mixture was treated with ethylene glycol (0.02 ml) at 4° for 1 hr and reduced with $NaBH_4$ (40 mg) at 4° for 16 hr. The soln was adjusted to pH 5 with HOAc and dialysed against H_2O for 2 days. The non-dialysable fraction was concd and chromatographed over Sephadex G-25 (2.6 i.d. × 95 cm) which was eluted with H_2O. Fractions of 20 ml obtained from tubes 10 and 11 were combined and lyophilized yielding the product which was hydrolysed with 1 N H_2SO_4 containing D-mannitol as an internal standard at 100° for 6 hr. After neutralization with Dowex 2 (OH^-), the hydrolysate was reduced and acetylated as described above, and the resulting alditol acetate mixture was subjected to GC which was conducted under condition A. The retention times of D-glucitol hexa-acetate and D-mannitol hexa-acetate (internal standard) were 19.8 and 16.7 min.

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Phytochemistry, Vol. 24, No. 10, pp. 2433–2435, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

AGMATINE DEIMINASE IN RICE SEEDLINGS

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(*Received* 26 *February* 1985)

Key Word Index—*Oryza sativa*; Gramineae; rice; agmatine deiminase.

Abstract—Agmatine deiminase activity in rice embryos increased gradually upto 24 hr during germination and then decreased. Gibberellic acid and kinetin inhibited the activity when added to the germination medium. The enzyme was purified 717 fold with specific activity 788.5 nkat/mg protein and yield 8.8%. The M_r of the native enzyme was 18.3 × 10^4 and the enzyme was a dimer of two identical subunits. The pH and temperature optimum of the enzyme were 6.0 and 28° respectively. The enzyme followed typical Michaelis–Menten kinetics with a K_m value of 1.5×10^{-2} M. The enzyme activity was inhibited by various divalent cations and spermidine and spermine, but putrescine showed no effect.

INTRODUCTION

In higher plants the polyamines may be formed from arginine [1, 2] or from ornithine [3, 4]. Arginine is first decarboxylated to agmatine [5], which is then hydrolysed in two steps to putrescine. In the initial hydrolytic step agmatine is converted to *N*-carbamylputrescine with the formation of ammonia. This is effected by the enzyme agmatine deiminase (agmatine iminohydrolase; EC 3.5.3.12).

Agmatine deiminase has been reported to be present in tobacco [6, 7], maize and sunflower seedlings [8] and groundnut cotyledons [9]. The enzyme was found to be significantly more active in extracts of potassium-deficient barley leaves [10]. Despite early attempts to purify agmatine deiminase [8, 11], the enzyme was homoge-