Syntheses of Some Derivatives of Glycosyl Pantothenic Acids, Analogues of Growth Factor for MLF Bacteria

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A growth factor (TJF) for a malo-lactic fermentation bacterium (Leuconostoc sp.) has been found to be 4'-O-(β-D-glucopyranosyl)-D-pantothenic acid with structural and synthetical studies. Now other 4'-O-glycosides (β-D-ribofuranosyl, α-D-glucopyranosyl, β-D-galactopyranosyl, β-maltosyl and β-cellobiosyl) and 2',4'-O-di-β-D-glucopyranoside of DL-pantothenic acid, and 4'-O-β-D-glucopyranoside of DL-pantethine were synthesized to examine their biological activities. The improved syntheses of TJF were also examined.

In the preceding paper,1) we reported the structural and synthetical studies on the growth factor (TJF) for a malo-lactic fermentation (MLF) bacterium, strain WNB-75 (Leuconostoc sp.), which had been isolated from wine by one of the authors.2) This TJF had been purified from tomato juice3) and the structure was determined to be 4'-O-(β-D-glucopyranosyl)-D-pantothenic acid4) (I).

It is well known that D-pantothenic acid is metabolized to coenzyme A in microorganisms in which D-pantothenic acid is at first phosphorylated at the C-4' hydroxyl residue.5) It seems reasonable to assume that TJF also is a precursor of coenzyme A, but it is very interesting why TJF is more effective on the growth of some microorganisms in spite of masking of the hydroxyl residue at C-4' position with glucose.

In this paper, we report the syntheses of some other 4'-O-glycosides of DL-pantothenic acid and DL-pantethine, and their biological activities for several microorganisms. Moreover, the improved syntheses of TJF were studied from the view of simplicity, high yield and low cost.

Syntheses of 4'-O-β-D-glycosides of DL-pantothenic acid

Four glycosides [galactopyranosyl (II), maltosyl (III), cellobiosyl (IV), ribofuranosyl (V)] of DL-pantothenic acid were synthesized as shown in Scheme I. Benzyl 2'-O-benzyl-DL-pantothenate6) (VI) was reacted with each acetobromosugar in nitromethane-benzene (2:1) in the presence of mercuric cyanide and freshly activated calcium sulfate under gentle boiling with vigorous stirring. In the case of ribofuranosyl derivative, tri-O-benzoyl-β-D-ribofuranosyl chloride was used in stead of acetobromosugar. Purifications on the silicic acid columns gave expected condensates. These were then hydrogenated over palladium black to remove benzyl groups, and purified on silicic acid columns. The debenzylated compounds were hydrolyzed with barium methoxide or sodium methoxide in methanol to remove acetyl or benzoyl groups. After purifications on Dowex 1→8 ion exchange columns, the lyophilization of the hydrolysates gave hygroscopic matters (II~V).

Synthesis of 4'-O-α-D-glucopyranoside of DL-pantothenic acid

In the case of 4'-O-α-D-glucoside, tetra-O-benzyl-α-D-glucopyranosyl chloride was condensed with VI in benzene—dioxane (2:1) in the presence of mercuric cyanide and calcium sulfate with refluxing. After purifications on silicic acid columns, the benzyl groups were removed over palladium black in acetic acid. Its purification on a column of DEAE Sepha-
SCHEME I. Syntheses of Some 4'-O-β-Glycosylpantothenic Acids.

\[
\begin{align*}
\text{CH}_3 & \quad \text{OBzI} \\
R'X + \text{HOCH}_2-\text{C} \quad \text{CH-CONHCH}_2\text{CH}_2\text{COOBzI} & \quad \text{Hg(CN)}_2 \\
\text{II-a : R'} & = 2,3,4,6\text{-Tetra-O-acetylgalactopyranosyl, X} = \text{Br} \\
\text{III-a : R'} & = \text{Hepta-O-acetylmaltosyl, X} = \text{Br} \\
\text{IV-a : R'} & = \text{Hepta-O-acetylcellobiosyl, X} = \text{Br} \\
\text{V-a : R'} & = 2,3,5\text{-Tri-O-benzoylribofuranosyl, X} = \text{Cl} \\
\beta & = \text{CH}_3 \quad \text{OBzI} \\
R'\text{-OCH}_2-\text{C} \quad \text{CH-CONHCH}_2\text{CH}_2\text{COOBzI} & \quad \text{H}_2/\text{Pd-black} \\
\text{II : R} & = \text{Galactopyranosyl} \\
\text{III : R} & = \text{Maltosyl} \\
\text{IV : R} & = \text{Cellobiosyl} \\
\text{V : R} & = \text{Ribofuranosyl}
\end{align*}
\]

SCHEME II. Improved Syntheses of TJF.
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dex gave 4'-O-(α-D-glucopyranosyl)-DL-pantothenic acid (VII). NMR spectrum of VII shows the presence of one β-anomeric proton at δ 4.82 ppm.

Syntheses of TJF (by improved method) and 2',4'-O-di-(β-D-glucopyranosyl)-D-pantothenic acid (Scheme II)

The authors had reported the synthesis of TJF in the previous paper.1) According to the method, there were some disadvantages such as the difficulties in purifications of the intermediates and the use of expensive D-pantolactone and palladium black. Now we studied the synthesis of TJF starting from calcium D-pantothenate.

D-Pantothenic acid was methylated with diazomethane in methanol, and then the selective glucosidation of the methyl D-pantothenate containing two hydroxyl groups was examined. In the case of reaction with acetobromoglucose and methyl D-pantothenate (1:1) under the same conditions as previously mentioned, methyl 4'-O-(tetra-O-acetyl-β-D-glucopyranosyl)-D-pantothenate (VIII) and small amount of methyl 2',4'-O-di-(tetra-O-acetyl-β-D-glucopyranosyl)-D-pantothenate(IX) were obtained after some purifications of the condensates on silicic acid columns. When the excess amount of acetobromoglucose against methyl D-pantothenate was used, the yield of di-glucoside (IX) was increased. Hydrolysis of each condensate with sodium methoxide and purifications on Dowex 1×8, Sephadex G-25 and DEAE Sephadex A-25 columns afforded 4'-O-(β-D-glucopyranosyl)-D-pantothenic acid (I) and 2',4'-O-di-(β-D-glucopyranosyl)-D-pantothenic acid (X), respectively.

When tetra-O-benzoyl-α-D-glucopyranosyl bromide was used instead of acetobromoglucose with considering steric effect, the glucosidation selectively proceeded at C-4' position of pantothenic acid moiety. Methyl D-pantothenate and tetra-O-benzoyl-α-D-glucopyranosyl bromide were reacted in the presence of mercuric cyanide and calcium sulfate in nitromethane—benzene solution. After the concentration in vacuo, the reaction mixture was dissolved in ether, from which methyl 4'-O-(tetra-O-benzoyl-β-D-glucopyranosyl)-D-pantothenate (XI) was easily precipitated as needle crystals. Hydrolysis of the precipitate (XI) with sodium methoxide in dry methanol and methylene chloride afforded TJF in good yield.

Synthesis of 4'-O-β-D-glucopyranoside of DL-pantethine

4'-O-(β-D-Glucopyranosyl)-DL-pantethine was synthesized as follows (Scheme III): DL-

\[
\text{BzGTu-Br} + \text{HOC}_2\text{H}_2\text{CNCH}_2\text{CH}_2\text{CH}_2\text{CN} \rightarrow \beta-\text{BzGTu-OCH}_2\text{H}_2\text{CNCH}_2\text{CH}_2\text{CN} \quad (XII)
\]

\[
\text{H}_2\text{NCH}_2\text{CH}_2\text{SH} \rightarrow \beta-\text{BzGTu-OCH}_2\text{H}_2\text{CNCH}_2\text{CH}_2\text{SH} \quad (XIII)
\]

\[
\text{NaOCH}_3 \rightarrow \beta-Glu-OCH}_2\text{H}_2\text{CNCH}_2\text{CH}_2\text{CN} \quad (XIV)
\]

When tetra-O-benzoyl-α-D-glucopyranosyl bromide was condensed with tetra-O-benzoyl-α-D-glucopyranosyl bromide in nitromethane—benzene (2:1) in the presence of mercuric cyanide and calcium sulfate with gentle refluxing. The condensate (XII) was purified on silicic acid column and recrystallization from ethyl acetate—ether, and then refluxed with cysteamine in ethanol under N\(_2\) gas to afford 2-[2-[4-O-(2,3,4,6-tetra-O-benzoyl-

Scheme III. Syntheses of 4'-O-(β-D-Glucopyranosyl)-DL-pantetheine and Pantethine.

pantethononitrile\(^7\) was condensed with tetra-O-benzoyl-α-D-glucopyranosyl bromide in nitromethane—benzene (2:1) in the presence of mercuric cyanide and calcium sulfate with gentle refluxing. The condensate (XII) was purified on silicic acid column and recrystallization from ethyl acetate—ether, and then refluxed with cysteamine in ethanol under N\(_2\) gas to afford 2-[2-[4-O-(2,3,4,6-tetra-O-benzoyl-
β-D-glucopyranosyl)pantamido}ethyl]-2-thiazoline (XIII). XIII was debenzoylated with sodium methoxide in methanol, and the product was ion exchanged on Dowex 50 W×8 (H⁺) resin and eluted with NH₄OH. The obtained 2-[4-O-(β-D-glucopyranosyl)pantamido]ethyl]-2-thiazoline (XIV) was hydrolyzed in aqueous acetic acid under N₂ gas to open the thiazoline ring, whereby 4'-O-(β-D-glucopyranosyl)-DL-pantetheine (XV) was obtained. XV was oxidized to 4'-O-(β-D-glucopyranosyl)-DL-pantethine (XVI) with hydrogen peroxide in the presence of ferrous sulfate in dilute ammoniac water. Purification was carried out by passing through the columns of Dowex 50 W×8 (H⁺) and Dowex 1×8 (OH⁻).

**Biological activities of the synthetics**

The growth stimulating activities of these synthetics were measured for some microorganisms, mainly MLF bacteria, according to the methods described in the previous paper. In addition, the biological activities of the authentic precursors of coenzyme A such as D-pantethine and D-pantetheine 4'-phosphate were compared with those of synthetics. The basal media for assays were Thompson's or L. bulgaricus B-1 medium for bacteria and Atkin's media for yeasts. The results (biological activities) were shown by the minimal amount of compound (µg) per ml of medium for adequate growth (Table I).

For the growth of WNB-75 strain, TJF was most effective (100 times) in pantothenic acid derivatives which we investigated. In the other glycosides, 4'-O-α-glucoside, 4'-O-β-galactoside and 4'-O-β-cellobioside showed 5~10 times activity of pantothenic acid. But

<table>
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<th>Compound</th>
<th>Biological activitya</th>
<th>Biological activityb</th>
<th>Biological activityc</th>
<th>Biological activityd</th>
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<td>4.00</td>
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<tr>
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<td>5.00</td>
<td>5.00</td>
<td>—</td>
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</tr>
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</table>

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a) The biological activity is shown by respective minimal amount of compound (µg) per ml of medium for adequate growth.
b) It was gifted by Dr. E. I. Garvie, National Institute for Research in Dairying, England.
c) Leuconostoc citrovorum ML-34, which was gifted by Prof. R. E. Kunkee, University of California, Davis.
d) Lactobacillus brevis IFO 3345.
e) Lactobacillus plantarum IFO 3070.
4′-O-β-maltoside and 4′-O-β-riboside were less effective than pantothenic acid. The growth activities of pantetheine glucoside and pante-thine glucoside (DL form) for WNB-75 strain were approximately half of that of TJF, which suggests that the stimulating effect exists in the β-D-glucopyranosyl linkage. The bioassay test with other MLF bacteria, Leuc. citrovorum ML-34 and Leuc. oenos, showed almost the same results as in the case of WNB-75 strain. In the other bacterial strains and yeast strains we investigated, WNB-35 (Lactobacillus sp.), L. casei IFO 3425, wine yeast Sauternes IFO 2309, Sacch. carlsbergensis etc. could be fairly available TJF for its growth. But the others, L. casei IFO 3914, L. brevis IFO 3345, wine yeast Bordeaux IFO 2294 etc. could scarcely metabolize TJF as the substitute for pantothenic acid.

**EXPERIMENTAL**

4′-O-(β-D-Galactopyranosyl)-DL-pantothenic acid (II). To 2.0g of benzyl 2′-O-benzyl-DL-pantothenate (VI) dissolved in 30 ml of anhydrous benzene-nitromethane (1:2) were added mercuric cyanide (1.9g) and activated calcium sulfate (5.0g) in the form of dried powder, and the mixture was stirred at room temperature for 1 hr. After addition of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl bromide (3.5g), the mixture was gently refluxed with vigorous stirring for 10 hr. The reaction mixture was filtered and throughly washed with benzene. The filtrate combined with washings was concentrated under reduced pressure to a syrup, which was resolved in 100 ml of benzene. The solution was successively washed with 1 M potassium bromide, saturated sodium bicarbonate and water, and then dried with anhydrous potassium carbonate. Concentration of the solution in vacuo gave a yellowish brown syrup, which was subjected to the column chromatography on silicic acid using 2.5% ethanol in chloroform to afford a mixture of two condensates (1.7g, 23%). The mixture was subjected to a column chromatography of silicic acid using 2.5% ethanol in chloroform as a solvent to yield benzyl 2′-O-benzyl-4′-O-(hepta-O-acetyl-β-cellobiosyl)-DL-pantothenate (IV-1-a) at first, and then benzyl 2′-O-benzyl-4′-O-(hepta-O-acetyl-β-cellobiosyl)-L-pantothenate (IV-1-a) as hygroscopic powder, respectively. IV-1: needle, mp 138.5–140°C, [α]D20 +40.3° (c=1.0, CHCl3). Anal. Found: C, 57.81; H, 6.24; N, 1.38%. IV-1-a: needle, mp 137.5–140°C, [α]D20 +1.8° (c=1.0, CHCl3). Anal. Found: C, 57.77; H, 6.24; N, 1.47%. Both of them were lyophilized to afford 700 mg of IV as a hygroscopic matter. Anal. Found: C, 46.48; H, 6.88; N, 2.54. Calcd. for C49H63O22N: C, 46.48; H, 6.88; N, 2.58%.

4′-O-(β-D-Cellobiosyl)-D- and L-pantothenic acid (IV-1, IV-2). Seven g of hepta-O-acetyl-β-cellobiosyl bromide and 4.0 g of VI were reacted in the same condition described above, and the reaction mixture was chromatographed on a column of silicic acid using 2.5% ethanol in chloroform to afford a mixture of two condensates (1.7 g, 23%). The mixture was subjected to a column chromatography of silicic acid using chloroform—ethyl acetate (6:4) as a solvent to yield benzyl 2′-O-benzyl-4′-O-(hepta-O-acetyl-β-cellobiosyl)-D-pantothenate (IV-1-a) at first, and then benzyl 2′-O-benzyl-4′-O-(hepta-O-acetyl-β-cellobiosyl)-L-pantothenate (IV-1-a), each of which was recrystallized from 90% methanol. IV-1-a: needle, mp 138–140°C, [α]D20 +40.3° (c=1.0, CHCl3). Anal. Found: C, 57.91; H, 6.26; N, 1.49. Calcd. for C49H63O22N: C, 57.81; H, 6.24; N, 1.38%. IV-2-a: needle, mp 137.5–140°C, [α]D20 +1.8° (c=1.0, CHCl3). Anal. Found: C, 57.77; H, 6.24; N, 1.47%. Both of them were hydrogenated over palladium black in acetic acid and then hydrolyzed with barium methoxide in anhydrous methanol as the same manner as III. After purification of each on Dowex 1 × 8 (OH−) column, the lyophilizations afforded 4′-O-(β-cellobiosyl)-D-pantothenic acid (IV-1) and 4′-O-(β-cellobiosyl)-L-pantothenic acid (IV-2) as hygroscopic powder, respectively. IV-1:
After desalted by passing through a column of Dowex for 15 hr, and it was purified on a column of DEAE matter (VII). Anal. Found: C, 47.70; H, 7.19; N, 5.80 (H+), the eluate was lyophilized to a hygroscopic Sephadex A-25 using 0.03 M ammonium carbonate.

Over palladium black (200 mg) in 4 ml of acetic acid, the reaction mixture was subjected to silicic acid column chromatography two times using 1% ethanol in chloroform to obtain 1.8 g of benzyl 2'-O-di-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-DL-pantothenate (V-a) (82%). After hydrogenation of V-a over palladium black in acetic acid, 4'-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-DL-pantothenic acid (V-b) was recrystallized from ether-petroleum ether (5.2 g). Anal. Found: C82.33; H, 5.69; N, 2.11%. V-b (3.2 g) was dissolved in 10 ml of methyl chloride—methanol (1:4) and the solution was cooled to 0°C, whereupon 15 ml of 0.5 N sodium methoxide was added to the solution. The mixture was then left to stand at room temperature for 30 hr. After neutralization with dilute HCl and subsequent concentration in vacuo, the residue was made up to 30 ml of an aqueous solution, from which methyl benzoate was removed by extraction with ether. The solution was applied on Dowex 1×8 (OH-) column and it was eluted with 0.5 N acetic acid. Lyophilization of the eluate gave hygroscopic powder of TJF (700 mg, 92%). Anal. Found: C, 47.29; H, 7.28; N, 3.88. Calcd. for C15H27O10N: C, 47.24; H, 7.14; N, 3.67%.

4'-O-(β-D-Ribofuranosyl)-DL-pantothenic acid (I). To a mixture of VI (4.0 g), mercuric cyanide (2.8 g) and calcium sulfate (5 g) in 17 ml of benzene, freshly prepared from 2.7 g of 2,3,4,6-tetra-O-benzyl-D-glucose, was poured to the mixture. The reaction mixture was subjected to silicic acid columns with chloroform-ethanol (96:4) (two times) and benzene-ethyl acetate (1:1) to obtain 1.6 g of methyl 2', 4'-O-di-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-pantothenate (IX). After IX was hydrolyzed with 0.5 N sodium methoxide, it was purified on Dowex 1×8 (OH-) column using acetic acid, Sephadex G-25 column and finally DEAE Sephadex A-25 column using 0.03 M (NH4)2CO3 to afford 220 mg of hygroscopic powder by lyophilization. Anal. Found: C, 46.69; H, 6.91; N, 2.75. Calcd. for C21H37O15N: C, 46.40; H, 6.86; N, 2.58%. NMR (in D2O, δ ppm): 0.90, 0.95, 1.03, 2.58 (2H, t, J=6 cps), 3.15–3.95 (m), 3.67%.

4'-O-(β-D-Glucopyranosyl)-DL-pantothenic acid (VII). The mixture of VI (2.0 g), powdered mercuric cyanide (1.3 g) and calcium sulfate (5 g) in 17 ml of benzene—dioxane (10:7) was refluxed for 30 min with stirring. After cooling, 2,3,4,6-tetra-O-benzyl-D-glucopyranosyl chloride in 4 ml of benzene, freshly prepared from 2.7 g of 2,3,4,6-tetra-O-benzyl-D-glucose, was poured to the mixture and it was refluxed for 20 hr. The reaction mixture was then subjected to silicic acid column chromatography two times using 1% ethanol in chloroform to obtain 260 mg of benzyl 2'-O-benzyl-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-DL-pantothenate (VII-a). NMR (in CDCl3, δ ppm): 0.90 (3H, s), 1.12 (3H, s), 2.44 (2H, t, J=5 cps). VII-a was debenzylated over palladium black (200 mg) in 4 ml of acetic acid for 15 hr, and it was purified on a column of DEAE Sephadex A-25 using 0.03 M ammonium carbonate. After desalted by passing through a column of Dowex 50×8 (H+), the eluate was lyophilized to a hygroscopic matter (VII). Anal. Found: C, 47.70; H, 7.19; N, 3.69. Calcd. for C15H25O9N: C, 47.24; H, 7.14; N, 3.67%.

2', 4'-O-di-(β-D-Glucopyranosyl)-D-pantothenic acid (X). To a mixture of methyl D-pantothenate (2.33 g), powdered mercuric cyanide (6.0 g) and calcium sulfate (10 g) in 40 ml of benzene was dropwisely added a solution of acetobromoglucose (10 g) in 100 ml benzene, and the mixture was gently refluxed for 10 hr with stirring. After treatment of the reaction mixture with usual manner, it was chromatographed three times on silicic acid columns with chloroform—ethanol (96:4) (two times) and benzene—ethyl acetate (1:1) to obtain 1.8 g of methyl 2', 4'-O-di-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-pantothenate (IX). After IX was hydrolyzed with 0.5 N sodium methoxide, it was purified on Dowex 1×8 (OH-) column using acetic acid, Sephadex G-25 column and finally DEAE Sephadex A-25 column using 0.03 M (NH4)2CO3 to afford 220 mg of hygroscopic powder by lyophilization. Anal. Found: C, 46.69; H, 6.91; N, 2.75. Calcd. for C21H37O15N: C, 46.40; H, 6.86; N, 2.58%. NMR (in D2O, δ ppm): 0.90, 0.95, 1.03, 2.58 (2H, t, J=6 cps), 3.15–3.95 (m), 4.00, 4.05, 4.28–4.50 (2H).

4'-O-(β-D-Glucopyranosyl)-DL-pantethine (XVI). To a solution of XI (1.6 g) dissolved in 5 ml of methyl acetate-methanol (96:4), powdered mercuric cyanide (6.0 g) and calcium sulfate (10 g) were stirred and refluxed gently in 30 ml of dried benzene solution, to which 100 ml of benzene solution of 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl bromide (10 g) was added dropwisely during 1.5 hr. The reaction mixture was refluxed for more 2 hr with stirring and after cooling, it was filtered and thoroughly washed with benzene. The filtrate combined with washings was treated in the usual manner and the concentrate was dissolved in 100 ml of ether. Standing of the solution at 0°C overnight precipitated 1.6 g of methyl 2', 4'-O-di-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-pantothenate (IX). After IX was hydrolyzed with 0.5 N sodium methoxide, it was purified on Dowex 1×8 (OH-) column using acetic acid, Sephadex G-25 column and finally DEAE Sephadex A-25 column using 0.03 M (NH4)2CO3 to afford 220 mg of hygroscopic powder by lyophilization. Anal. Found: C, 46.69; H, 6.91; N, 2.75. Calcd. for C21H37O15N: C, 46.40; H, 6.86; N, 2.58%. NMR (in D2O, δ ppm): 0.90, 0.95, 1.03, 2.58 (2H, t, J=6 cps), 3.15–3.95 (m), 4.00, 4.05, 4.28–4.50 (2H).
a mixture of DL-pantothenonitrile (4.0 g), powdered mercuric cyanide (5.0 g) and freshly activated calcium sulfate powder (10 g) dissolved in 45 ml of dried nitromethane—benzene (2:1) was dropwisely added to 2, 3, 4, 6-tetra-O-benzoyl-β-D-glucopyranosyl bromide (11.6 g) in 20 ml of benzene under gentle refluxing with stirring and the mixture was gently refluxed for 15 hr. The reaction mixture was treated in the usual manner as I to a yellowish syrup, which was chromatographed on silicic acid column with 3% ethanol containing chloroform and then crystallized from ethylaceta—ether to give 9.7 g (62%) of 4-O-(2, 3, 4, 6-tetra-O-benzoyl-β-D-glucopyranosyl) DL-pantothenonitrile (XII). Anal. Found: C, 66.10; H, 5.54; N, 3.67. Calcd. for C43H42O12N: C, 66.31; H, 5.44; N, 3.60%.

2.0 g of the nitrile XII and 300 mg of cysteamine were dissolved in 10 ml of ethanol and the solution was refluxed for 15 hr under N2 stream. The resulting solution was evaporated in vacuo and then chromatographed on silicic acid column with chloroform—ethanol (96:4) to obtain 1.4 g (65%) of 2-[2-{4-O-(2, 3, 4, 6-tetra-O-benzoyl-β-D-glucopyranosyl) pantamido} ethyl]-2-thiazoline (XIII). To a solution of XIII (1.4 g) dissolved in 5 ml of methylene chloride was added 21 ml of 0.05 N sodium methoxide under cooling with ice water. The mixture was stood for 24 hr at room temperature in N2 atmosphere and, after neutralization with 0.5 N acetic acid (2.1 ml), was dried up in vacuo. The residue with ether extraction and the residue was applied on Dowex 50×2 (H+) column. The column, after washing with sufficient amount of water, was eluted with 1 N ammoniac water and it was lyophilized to afford 580 mg (82%) of 2-[2-{4-O-(β-D-glucopyranosyl) pantamido} ethyl]-2-thiazoline (XIV) as a hygroscopic powder. \( \lambda_{max}^{H_2O} = 264 \text{ m}\mu \). Anal. Found: C, 48.46; H, 7.34; N, 6.51. Calcd. for C17H30N2O8S: C, 48.33; H, 7.16; N, 6.63%. XIV (350 mg) was dissolved in 10 ml of 0.1 N acetic acid and it was stood under N2 gas for 48 hr at room temperature. The lyophilized afforded 4'-O-(β-D-glucopyranosyl)-DL-pantetheine (XV) as white powder (340 mg, 93%). Anal. Found: C, 46.55; H, 7.46; N, 6.16. Calcd. for C17H32O9N2S: C, 46.35; H, 7.32; N, 6.36%.

One mg of ferrous sulfate, heptahydrate and 0.1 ml of ammoniac water (28%) were added to the solution of XV (300 mg) in 3 ml water, to which hydrogen peroxide (3% aqueous solution) was titrated until the end point of changing the solution color of red purple to pale yellow (about 0.6 ml). The obtained reaction mixture was passed through the columns of Dowex 50W×8 (H+) and Dowex 1×8 (OH-), and the eluate was lyophilized to afford 270 mg of 4'-O-(β-D-glucopyranosyl)-DL-pantetheine (90%). Anal. Found: C, 46.37; H, 7.29; N, 6.45. Calcd. for C34H62O18N4S2: C, 46.46; H, 7.11; N, 6.37%.

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