STEROID GLYCOSIDES

XXI. THE STRUCTURE OF POLYGONATOSIDE E' AND PROTOPOLYGONATOSIDE E' FROM THE LEAVES OF *Polygonatum latifolium*

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We have previously reported the isolation and preliminary study of the chemical structure of steroid glycosides from the roots of *Polygonatum latifolium* Jasq. Desf. [1]. Continuing a study of this plant, we have isolated from the leaves two new glycosides which we have called polygonatoside E' (I) and protopolygonatoside E' (II), and in this paper we give a proof of their structures.

Glycosides (I) and (II) give a positive Sannié reaction (II), which permits them to be assigned to the steroid glycosides. Glycoside (II) is colored pink by the Ehrlich reagent [3], which shows the furostanol nature of its aglycone, while with the same reagent glycoside (I) gives a negative reaction and, consequently, belongs to the spirostanol series.

The IR spectrum of (I) contains adsorption bands characteristic for a spirostanol compound of the 25R series (980, 920 < 900, 850 cm^{-1}), while these bands are absent from the spectrum of compound (II), which has a broad band at 900 cm^{-1} that is characteristic for furostanol glycosides [4]. On silica gel plates in methanolic systems, compound (II) gave two spots — an indication of the fact that it consisted of a mixture of 22-hydroxy- and 22-methoxyfurostanol glycosides (IIa and IIb), i.e., it behaved in the same way as all glycosides of the furostanol series [5]. Glycoside (II) was subjected to enzymatic cleavage with the complex enzyme from *Helix pomatia* at room temperature and was thereby converted into a substance the physical properties of which were identical with those of glycoside (I). In view of the fact that under these conditions the closure of ring F of the aglycone usually takes place, the native genin of compound (II) must be considered to be (25R)-furost-5-ene-3β,22α,26-triol. Since the spirostan (I) was obtained by enzymatic hydrolysis from compound (II), (II) may be considered as a prototype of (I).

When both glycosides were subjected to complete hydrolysis with sulfuric acid, the steroid aglycone diosgenin was isolated and was identified by its melting point $[\alpha]_D$ value, IR spectrum, and R_f values (TLC). The hydrolyzates were shown by paper chromatography and GLC to contain glucose and galactose in a ratio of 3:1 for (I) and 4:1 for (II).

The sequence of attachment of the monosaccharides to the aglycone was determined with the aid of partial hydrolysis. Under these conditions, three progenins (III, IV, and V) were obtained for glycoside (I). Progenin (III) was a monoside the hydrolysis of which gave diosgenin and glucose, progenin (IV) a bioside which was cleaved on hydrolysis into diosgenin, glucose, and galactose, and progenin (V) was a trioside with a carbohydrate chain consisting of glucose and galactose (2:1). For glycoside (II) the same progenins and also glycoside (I) were obtained.

To determine the positions of attachment of the sugar residues to one another, compound (I) and (II) were methylated by Kuhn's method [6] and progenins (IV) and (V) by Hakomori's method [7], followed by the methanolysis of the permethylated products. Methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,4,6-tri-O-methyl-D-glucopyranoside, and methyl 2,3,6-tri-O-methyl-D-galactopyranoside were identified for both glycosides by TLC and GLC in the presence of markers.

For the progenin (IV), methyl 2,3,4,6-tetra-O-methyl-D-galactopyranoside and methyl 2,4,6-tri-O-methyl-D-glucopyranoside were found and for progenin (V), methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,4,6-tri-O-methyl-D-glucopyranoside, and methyl 2,3,6-tri-O-methyl-D-galactopyranoside. In addition, the permethylated glycosides (I) and (II) were

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subjected successively to formolysis, acid hydrolysis, tetrahydroborate reduction, and acetylation [8]. 1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylhexitol, 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylhexitol, and 1,4,5-tri-acetyl-2,6-tri-O-methylhexitol were identified by chromato-mass spectrometry for both glycosides.

To confirm the results of methylation, each of the glycosides was subjected to periodate oxidation followed by hydrolysis of the reaction products. Glucose was found in the hydrolyzates of both glycosides by paper chromatography.

The configurations of the glycosidic centers were determined from the molecular rotation differences between the initial glycosides and their progenins. They are in accordance with Klyne's rule [9]:

Monosaccharide methylglycoside	α, deg	β, deg	Glycoside	$[M]_D^{20}$, deg	Δ, deg	Configuration of the bond
Methyl D-glucopyranoside	−309	−66	Protopolyganatoside E'	−1489		
			Polygonatoside E'	−637	−852	β
Methyl D-glucopyranoside	−309	−66	Polygonatoside E'	−637		
			Progenin (V)	−540	−97	β
Methyl D-glucopyranoside	−309	−66	Progenin (V)	−540		
			Progenin (IV)	−516	−24	β
Methyl D-galactopyranoside	−380	0	Progenin (IV)	−516		
			Progenin (III)	−499	−17	β
Methyl D-glucopyranoside	−309	−66	Progenin (III)	−499		
			Diosgenin	488	−11	β

As proof of the furostanol nature of the aglycone of protopolygonatoside E' and the presence of glucose at the C_{26} atom of the aglycone, oxidative cleavage was carried out by the method of Tschesche et al. [4]. This gave rise to two compounds: the tetraacetylglucoside of methyl δ-hydroxy-γ-methylvalerate (VI), and a glycoside of 3β-hydroxypregna-5,16-dien-20-one (VII). The mass spectrum of (VI) showed peaks characteristic for acetylated glucose, and also the peaks of fragments of valeric acid [4]. Compound (VII) was identified in the form of its 3β-acetoxy derivative [4]. The monosaccharide composition of (V) was identical with that of glycoside (I).

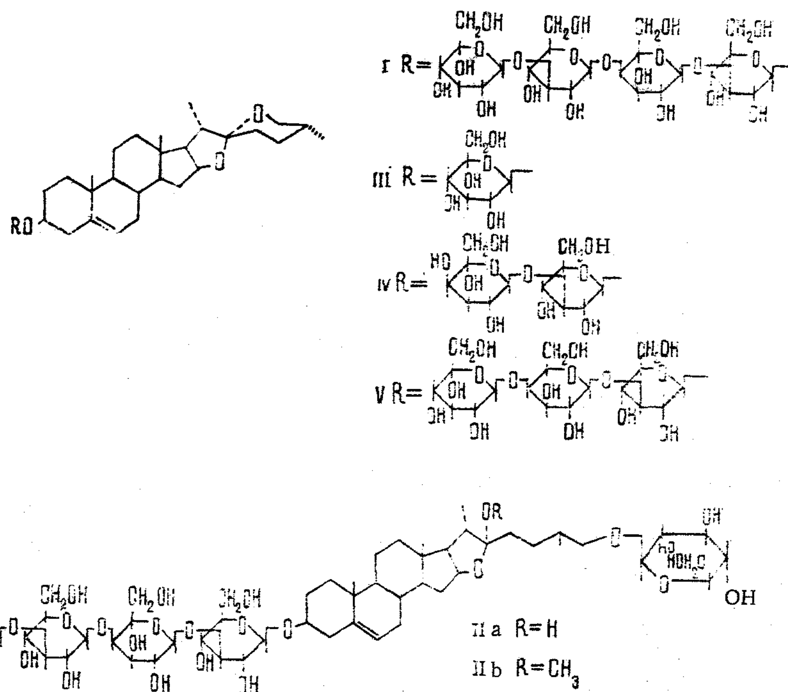
The facts presented permit the structures shown on p. 292 to be ascribed to polygonatoside E' (I), protopolygonatoside E' (II), and their progenins (III-V).

EXPERIMENTAL

The adsorbants used for chromatography were silica gel of type L100/160 μ, LSL_{254} 5/40 μ + 30% of gypsum, and Al_2O_3 (Brockmann activity grade II), and FN-3 chromatographic paper was used for paper chromatography (PC). The following solvent systems were used: 1) chloroform—methanol (9:1); 2) chloroform—methanol—water (65:25:10), lower layer; 3) chloroform—methanol—water (65:30:10), lower layer; 4) benzene—acetone (2:1); 5) benzene—butan-1-ol—pyridine—water (1:5:3:3), upper layer; and 6) benzene—ethanol (9:1). The glycosides were detected on the plates with concentrated H_2SO_4, the Sannié reagent [2], and the Ehrlich reagent [3], and the sugars on paper with aniline phthalate.

Gas—liquid chromatography (GLC) was performed on a "Chrom-4" instrument with a glass column 2.2 m long filled with 5% of Xe-60 on Chromaton N-AW-HMDS with helium as the carrier gas at V_{He} = 45 ml/min. The PMR spectrum were taken on a RS-60 instrument, the IR spectra on a UR-20 spectrometer, and the chromato-mass spectra on a Varian MAT III Gnom instrument using a column containing 30% of SE-30 on Varaport. Enzymatic hydrolysis was performed with the aid of the complex enzyme from *Helix pomatia*.

Isolation of the Individual Glycosides (I) and (II). The dry raw material (1 kg) was extracted three times with 70% MeOH at 70°C. The extract was concentrated, and the total glycosides (15 g) were isolated and purified by chromatography on columns of Al_2O_3 and SiO_2 (successively) and were then rechromatographed repeatedly on a column of SiO_2 with elution



by systems 1, 2, and 3 in succession. The process was monitored by TLC in the same system. Elution by system 2 yielded fractions from which, by precipitation by acetone and recrystallization from ethanol, 2.3 g of polygonatoside E' was isolated with the composition $C_{51}H_{82}O_{23}$, mp 240-244°C, $[\alpha]_D^{20}$ −60° (c 2.0; DMFA). IR spectrum, cm^{-1}: 3400 (broad band), 980, 920 < 890, 850 (spiroketal chain of the 25R series) [10].

System 3 eluted 2.5 g (after precipitation and recrystallization) of protopolygonatoside E', $C_{57}H_{93}O_{29}$, mp 186-190°C, $[\alpha]_D^{20}$ −120° (c 1.0; water). IR spectrum, cm^{-1}: 3400, 900 (broad band).

Hydrolysis of Glycosides (I) and (II). The hydrolysis of 100 mg of each compound was carried out in sealed tubes with 2.5% of H_2SO_4 at 105°C for 10 h. Then the contents of the tubes were diluted with water and extracted with ether. The ethereal extracts yielded an aglycone which was identified as diosgenin (30 mg), mp 206-208°C, $[\alpha]_D^{20}$ −120° (c 1.0; $CHCl_3$) R_f 0.75 in system 1. IR spectrum, cm^{-1}: 3400, 980, 920 < 890, 860. Mass spectrum: M^+ 414. According to the literature: mp 208°C $[\alpha]_D^{20}$ −120° [5].

The aqueous layer was neutralized with ion-exchange resins (Wofatit SKB and KU-2) and was analyzed by paper chromatography in system 5. Glucose and galactose were detected. Gas-liquid chromatography of the acetates of the aldononitriles showed a ratio of these sugars of 3.0:1.1 for (I) and 3.9:0.9 for (II).

Enzymatic Hydrolysis of Protopolygonatoside E'. A solution of 100 mg of glycoside (II) in 10 ml of water was treated with 10 mg of the complex enzyme from *Helix pomatia* and the mixture was left at room temperature. After 2 h, a sample was examined by TLC. After 12 h, the reaction mixture was extracted with n-butanol and the extract was chromatographed on a column of SiO_2 in system 2. This gave 70 mg of glycoside (I) (mp 240-244°C) $[\alpha]_D^{20}$ −60° (c 2.0; DMFA).

Methylation and Methanolysis of Glycosides (I) and (II). The methylation of 500 mg of each glycoside was carried out by Kuhn's method [6]. The methylation of 50 mg of each protogenin was carried out by Hakomori's method. The processes were monitored by TLC in system 6. The permethylate of glycoside (I) (550 mg) had mp 110-114°C, $[\alpha]_D^{20}$ −56° (c 2.5; $CHCl_3$), and the permethylate of glycoside (II), (also 550 mg) had mp 116-121°C, $[\alpha]_D^{20}$ −72° (c 2.51; $CHCl_3$). The permethylated compounds (I, II, III, IV, and V) were subjected to methanolysis with 72% $HClO_4$ in methanol (1:10) in sealed tubes at 105°C for 5 h. After cooling, the re-

action mixtures were diluted with water and neutralized with Wofatit SKB ion-exchange resin. The glycosides mentioned in the discussion were identified by TLC in system 4 and by GLC in the presence of markers.

To record the chromato-mass spectra, the permethylates of (I) and (II) (15 mg each) were subjected to formolysis in 1 ml of 85% HCOOH at 100°C for 2 h. Each mixture was evaporated, treated with 1 ml of 0.3 N HCl, heated at 100°C for 16 h, reevaporated, and diluted with water, the aglycone was filtered off, and the filtrate was neutralized with anion-exchange resin and reduced with $NaBH_4$ (30 mg) followed by reneutralization with KU-2 ion-exchange resin. Then the reaction mixture was evaporated with methanol and the product obtained was acetylated with 1.5 ml of acetic anhydride in 1.3 ml of pyridine at 100°C for 15 min. The mixture was cooled, methanol was added, and it was evaporated off again to eliminate anhydride and pyridine. The resulting mixture was analyzed on a chromatic-mass spectrometer. This showed the presence of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol (m/e 43, 45, 87, 101, 117, 129, 161, 145, 205); 1,4,5-tri-O-acetyl-2,3,6-tri-O-methylhexitol (m/e 43, 45, 87, 99, 101, 113, 117, 129, 131, 161, 173, 189, 233), and 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylhexitol (m/e 43, 45, 87, 99, 101, 115, 117, 129, 145, 187, 217, 233) for compounds (I) and (II).

Partial Hydrolysis of Glycosides (I) and (II). The hydrolysis of 500 mg of each glycoside was carried out with 100 ml of 2% H_2SO_4 on a boiling-water bath for 4 h. The process was monitored by GLC in system 2. Each hydrolyzate was diluted with water and extracted with n-butanol (5 × 40 ml), and the butanolic extracts were washed with water and evaporated to dryness. The dry residue (350 mg) was chromatographed on a column of SiO_2 in systems 1 and 2. In addition to diosgenin, progenins (III-V) were obtained for glycoside (I) and compounds (I) and (III-V) for glycoside (II). Progenin (III) (30 mg) had the composition $C_{33}H_{52}O_8$, mp 264-266°C, $[\alpha]_D^{20}$ −87° (c 0.45; DMFA), and on hydrolysis it gave diosgenin (TLC) and glucose. Progenin (IV) (70 mg), $C_{39}H_{62}O_{13}$, was a bioside with mp 178-181°C, $[\alpha]_D^{20}$ −70° (c 1.6; DMFA), and on hydrolysis it gave diosgenin (TLC), glucose, and galactose (1:1) (PC, GLC).

Progenin (V) (200 mg), $C_{45}H_{72}O_{18}$, was a trioside with mp 230-234°C, $[\alpha]_D^{20}$ −60° (c 2.1; DMFA), and on hydrolysis it gave glucose and galactose (1.9:1) (GLC, PC) and diosgenin (TLC).

Periodate Oxidation. Glycoside (I) or (II) (20 mg) was dissolved in 20 ml of methanol, and 20 ml of 2% $NaIO_4$ solution was added. The mixture was kept at room temperature in the dark for 48 h, and then a few drops of ethylene glycol was added and the product was extracted with n-butanol. The butanolic extracts were evaporated and were hydrolyzed with 2.5% H_2SO_4 at 105°C for 10 h. For both glycosides, only glucose was found in the hydrolyzates by paper chromatography.

Oxidative Cleavage of Compound (II). In the cold, 1 g of glycoside (II) was dissolved in 16 ml of pyridine and 13 ml of acetic anhydride, and the solution was stirred for 1 day. The acetate so obtained was extracted with chloroform, the organic phase was evaporated, and the residue was oxidized with CrO_3 by the method of Tschesche et al. [4]. This gave a butanolic phase A and an aqueous phase B. Phase A was evaporated and the residue was hydrolyzed with a mixture of 4 N HCl and benzene (1:1) at 80°C for 3 h. The product was extracted with benzene and chloroform, and the organic extracts were evaporated, the residue was acetylated, and the product was chromatographed on a column of SiO_2 in system 6. This yielded 200 mg of a substance with mp 201-203°C, $[\alpha]_D^{20}$ −36° (c 0.5; $CHCl_3$). IR spectrum, cm^{-1}: 1735, 16*0, 9*0, 9*0, 890, 820 cm^{-1}. Glucose and galactose were found in the hydrolyzates (PC). Phase B was acidified to pH 3 and extracted with chloroform and butanol, the extracts were evaporated, and the residue was acetylated with acetic anhydride in pyridine, methylated with diazomethane, and chromatographed on a column of SiO_2 in system 4. This gave 150 mg of a viscous gum with $[\alpha]_D^{20}$ −20° (c 1.1; $CHCl_3$). Mass spectrum: m/e 331, 244, 242, 200, 169, 157, 145, 141, 140, 129, 115, 109, 103, 98, 97.

SUMMARY

The chemical structures of two new steroid glycosides from the leaves of *Polygonatum latifolium* have been shown. Polygonatoside E' is 3β-[O-β-D-glucopyranosyl-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-O-β-d-galactopyranosyl-(1 → 3)-β-D-glucopyranosyloxy]-(25R)-spirost-5-ene, and protopolygonatoside E' is 26-β-D-glucopyranosyloxy-3β-[O-β-D-glucopyranosyl-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-galactopyranosyl-(1 → 3)-α-D-glucopyranosyloxy]-(25R)-furost-5-en-22α-ol.

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STEROID SAPONINS AND SAPOGENINS OF *Allium*

XII. TUROSIDE A FROM *Allium turcomanicum*

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Continuing a study of the steroid saponins and sapogenins of *Allium turcomanicum* Rgl. [1, 2], we have investigated a methanolic extract of the bulbs of this plant. From the total extractive substances we isolated a new steroid glycoside which we have call turoside A (I).

A hydrolyzate of glycoside (I) was shown by the GLC method [3, 4] to contain D-xylose, D-glucose, and D-galactose in a ratio of 1:2:1. Neoagigenin (II) was isolated as the aglycone of (I).

The chemical shifts of the C-27 and C-21 methyl groups in the PMR spectrum of (I) (0.94, d, J = 6 Hz, and 1.03, d, J = 6 Hz, respectively), and also the resonance lines of the C-26 proton at 3.24 ppm, from their positions and multiplicities, confirm that glycoside (I) belongs to the 25S series [5].

The partial acid hydrolysis of turoside A yielded three progenins (VI-VIII). Progenin (VI) contained one molecule of galactose in the sugar chain, (VII) contained galactose and glucose in a ratio of 1:1, and (VIII) one molecule of galactose and two molecules of glucose. Consequently, the galactose is bound directly to the aglycone, and, in its turn, the glucose is attached to the galactose.

To determined the positions of the xylose and the second molecule of glucose, we methylated the glycoside (I) [6] and then subjected the resulting permethylate (III) to acid hydrolysis. This yielded dimethoxyneoagigenin (IV) and a mixture of methylated sugars. By separating this mixture on a column of SiO_2 we isolated methylated carbohydrates which were identified by their physicochemical constants, GLC, and TLC as 2,3,4-tri-O-methyl-D-xylopyranose, 2,3,4,6-tetra-O-methyl-D-glucopyranose, 2,3,6-tri-O-methyl-D-galactopyranose, and 4,6-di-O-methyl-D-glucopyranose.

The products obtained showed that the carbohydrate chain of the new glycoside contains branching the center of which is one of the glucose molecules.

When the mixture of methylated sugars was separated, in addition to those we have mentioned, a pentamethylated disaccharide was also isolated which, when subjected to further acid hydrolysis, decomposed into 2,3,6-tri-O-methyl-D-galactopyranose and 4,6-di-O-methylglucopyranose. In view of the fact that in turoside A the galactose is attached directly to the aglycone, it must be assumed that the pentamethylated biose is based on 4-O-D-glucopyranosyl-D-galactose. The PMR spectrum of the biose pentamethylate contained at 4.41 ppm a 1-

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