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TRITERPENE GLYCOSIDES OF *Hedera colchica*.

STRUCTURE OF HEDERACOLCHISIDES E AND F

G. E. Dekanosidze, O. D. Dzhikiya,
M. M. Vugal'ter, and É. P. Kemertelidze

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The leaves of Colchis ivy (family Araliaceae) have yielded polar glycosides — hederacolchisides E and F — and their structures have been established. It has been shown on the basis of the results of methylation and of acid and alkaline hydrolysis that these glycosides are hexaosides of oleanolic acid and of hederagenin.

In the leaves of *Hedera colchica* C. Koch (Colchis ivy), family Araliaceae, we have detected six glycosides — hederacolchisides A, B, C, D, E, and F [1]. Hederacolchiside D has been identified as kalopanax saponin B [2], and a preliminary structure has been proposed for hederacolchiside E [3, 4]. In the present paper we give information enabling the structure of hederacolchiside E to be refined and the structure of the most polar glycoside F to be established.

The complete acid hydrolysis of the glycosides gave the aglycones: oleanolic acid for hederacolchiside E and hederagenin for hederacolchiside F. In the carbohydrate moieties of both glycosides rhamnose, arabinose, and glucose in a ratio of 2:1:3 were identified by the GLC of the acetates of the corresponding polyols. The nature of the substitution of the monosaccharide residues was established by the Hakomori methylation [5] of the glycosides followed by methanolysis and identification of the methyl glycosides obtained. In both cases, methyl 2,3,4,6-tetra-O-methylglycopyranoside (1), methyl 2,3,4-tri-O-methylglucopyranoside (2), methyl 2,3,6-tri-O-methylpyranoside (3), methyl 2,3,4-tri-O-methylrhamnopyranoside (4), and methyl 3-O-methylarabinopyranoside (5) were identified by the GLC method. These results were confirmed by the identification of the acetates of the partially methylated polyols obtained as the result of the acid hydrolysis of the metholated glycosides followed by reduction and acetylation. The following polyols were identified by chromato-mass spectrometry (CMS): 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylsorbitol (6), 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylsorbitol (7), 1,4,5-tri-O-acetyl-2,3,6-O-methylsorbitol (8), 1,5-di-O-acetyl-2,3,4-tri-O-methylrhamnitol (9), and 1,2,4,5-tetra-O-acetyl-3-O-methylarabitol (10) [6]. Thus, hederacolchisides E and F contained rhamnose and glucose as terminal monosaccharide residues. The arabinose residue formed a point of branching and was substituted in positions 2 and 4, while the glucopyranose residue in the chain was substituted in positions 4 and 6.

In order to determine the localization of the carbohydrate chains we performed the alkaline hydrolysis of the compounds under investigation. In the oligosaccharide fractions

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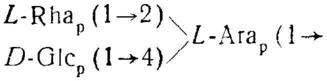
of each glycoside, by the GLC method we identified glucose and rhamnose (in the form of the corresponding polyol acetates) in a ratio of 2:1, and in the glycosides deprived of their acyloside moieties we found glucose, arabinose, and rhamnose in a ratio of 1:1:1. Consequently, hederacolchisides E and F are bisdesmosides, as was also confirmed by an analysis of the IR spectra, which each had an adsorption band in the 1740 cm^{-1} region.

In order to establish the structures of the carbohydrate chains in the glycosides, the oligosaccharide fraction obtained as the result of alkaline hydrolysis was reduced with sodium tetrahydroborate and methylated by Makomori's method and was then subjected to methanolysis. In the reaction products, by GLC, we identified fully methylated methyl rhamnopyranoside, representing the terminal monosaccharide residue, and a lateral methyl glucopyranoside with a free hydroxyl at C-4. Consequently, the oligosaccharide under investigation was a linear trisaccharide with the following structure:

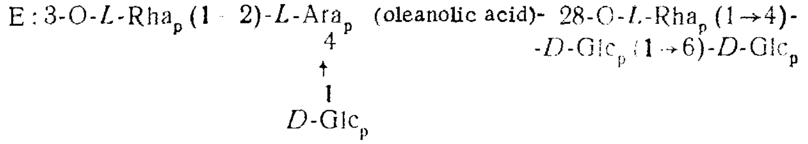
$$L\text{-Rha}_p(1\rightarrow 4)\text{-}D\text{-Glc}_p(1\rightarrow 6)\text{-}D\text{-Glc}_p(1\rightarrow$$

The monodesmosides obtained after the alkaline hydrolysis of the native compounds were subjected to partial acid hydrolysis. After this, rhamnose and traces of glucose were detected, the sugars being separated by preparative TLC [7]. As a result of methylation followed by methanolysis, fully methylated methyl glucopyranoside and a methyl arabinopyranoside with a free hydroxyl at C-4 were detected. Consequently when the nature of the substitution of the arabinopyranose residue was taken into account the carbohydrate chain under investigation could be represented by a branched trisaccharide structure:

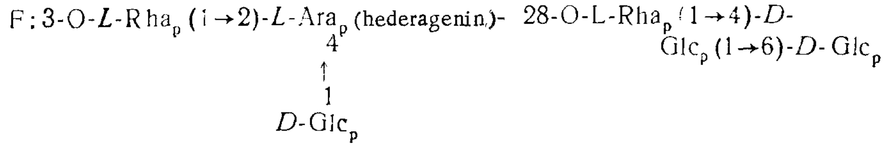


$$\left.\begin{array}{l} L\text{-Rha}_p(1\rightarrow 2) \\ D\text{-Glc}_p(1\rightarrow 4) \end{array}\right\rangle L\text{-Ara}_p(1\rightarrow$$

Summing the facts given above and previous results [1-4] it was possible to conclude that the most polar glucosides of the leaves of *Hedera colchica* — hederacolchisides E and F — consisted of hexaosides with the following structures:



$$E: 3\text{-}O\text{-}L\text{-Rha}_p(1\rightarrow 2)\text{-}\begin{array}[t]{c} L\text{-Ara}_p \\ 4 \\ \uparrow \\ 1 \\ D\text{-Glc}_p \end{array}(\text{oleanolic acid})\text{-}\ 28\text{-}O\text{-}L\text{-Rha}_p(1\rightarrow 4)\text{-}D\text{-Glc}_p(1\rightarrow 6)\text{-}D\text{-Glc}_p$$



$$F: 3\text{-}O\text{-}L\text{-Rha}_p(1\rightarrow 2)\text{-}\begin{array}[t]{c} L\text{-Ara}_p \\ 4 \\ \uparrow \\ 1 \\ D\text{-Glc}_p \end{array}(\text{hederagenin})\text{-}\ 28\text{-}O\text{-L-Rha}_p(1\rightarrow 4)\text{-}D\text{-Glc}_p(1\rightarrow 6)\text{-}D\text{-Glc}_p$$

EXPERIMENTAL

GLC analysis was performed on a Chrom-5 instrument using a column containing 5% of XE-60 on Chromaton N-AW-HMDS, with a FID. Mass spectra were recorded on a Varian MAT-111 instrument after a column containing 3% of ECNSS, and IR spectra on a UR-20 instrument (in paraffin oil).

Acid Hydrolysis. A 20-mg sample of hederacolchiside E or F was hydrolyzed with 2 N HCl at 100°C for 3 h. The aglycone was filtered off, washed several times with distilled water, and identified by comparison with authentic samples of oleanic acid and hederagenin, respectively [7]. The hydrolysate was evaporated to dryness several times with the addition of distilled water until the last traces of HCl had disappeared, after which rhamnose, arabinose, and glucose were identified by the TLC method [7]. The monosaccharides were dissolved in 2 ml of 50% aqueous ethanol and reduced with sodium tetrahydroborate at room temperature for 12 h, after which the reaction mixture was treated with cation-exchange resin (KU-2, H$^+$ form), filtered, and evaporated. The carefully dried residue was acetylated in a mixture of pyridine and acetic anhydride (2 ml each) at room temperature for 12 h, and the reaction mixture was evaporated to dryness and extracted with chloroform. Rhamnitol, arabitol, and sorbitol acetates were identified in a ratio of 2:1:3 by GLC for both glycosides.

Determination of the Positions of the Glycosidic Bonds in Hederacolchisides E and F. Glycosides E and F (50 mg each) were methylated by Hakomori's method [5]. The methylated glycosides were subjected to methanolysis (5% HCl/MeOH, 100°C, 4 h) and then, in each case, the mixture was neutralized with aqueous ammonia solution and evaporated to dryness, and the residue was extracted with chloroform. Methyl glycopyranosides (1-5) were identified in each residue by the GLC method. Each mixture of methylated glycosides (20 mg) was hydrolyzed with 1 N HCl (100°C, 3 h), reduced with sodium tetrahydroborate, and acetylated as described above. The acetates of the partially acetylated polyols (6-10) were identified by the CMS method [6].

Alkaline Hydrolysis. A 100-g sample of hederacolchiside E or F was subjected to alkaline hydrolysis (5% KOH, 100°C, 1.5 h), then the reaction mixture was treated with KU-2 cation-exchange resin (H^+), which was filtered off again and washed several times with distilled water. The filtrate was evaporated to dryness, and then 10 mg of the residue was hydrolyzed with 2 N HCl (100°C, 3 h), and 10 mg of the precipitate, which contained the progenin, was hydrolyzed under the same conditions. Using the TLC method as described above, glucose and rhamnose were identified in the first hydrolysate and glucose, arabinose, and rhamnose in the second. As a result of the reduction of the hydrolysates with sodium tetrahydroborate followed by acetylation and the application of the GLC method, the acetates of the sugars mentioned were identified in a ratio of 2:1 for the first hydrolysate and 1:1:1 for the second.

The fractions containing the progenin was subjected to mild hydrolysis (0.5 N HCl, 100°C, 0.3 h) and the reaction mixture was evaporated to dryness several times with distilled water to eliminate the last traces of HCl. The rhamnose split off was separated by preparative TLC [7].

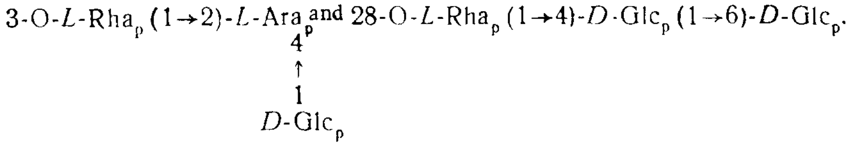
The carefully dried residue was methylated by Hakomori's method and subjected to methanolysis (5% HCl/MeOH, 100°C, 4 h). The solution was neutralized with aqueous ammonia, evaporated, and extracted with chloroform. Methyl glucopyranoside (1) and methyl 2,3-di-O-methylarabinopyranoside were identified in the residue by the GLC method in comparison with authentic samples.

The oligosaccharide fraction was reduced with sodium tetrahydroborate at room temperature for 12 h, and, after being treated with KU-2 resin (H^+), which was filtered off again, it was evaporated. The carefully dried residue was methylated by Hakomori's method and subjected to methanolysis as described above. The methyl glycopyranosides (3) and (4) were identified in the methanolysate by the GLC method in comparison with authentic samples.

SUMMARY

1. The structures of hederacolchisides E and F isolated from the leaves of *Hedera colchica* have been established; they are hexaosides of oleanolic acid and of hederagenin, respectively.

2. Hederacolchisides E and F are bisglycosides with carbohydrate chains of identical structures:



$$3\text{-O-}L\text{-Rha}_p(1\rightarrow2)\text{-}L\text{-}\underset{\substack{4\\\uparrow\\1\\D\text{-Glc}_p}}{\text{Ara}_p}\ \text{and}\ 28\text{-O-}L\text{-Rha}_p(1\rightarrow4)\text{-}D\text{-Glc}_p(1\rightarrow6)\text{-}D\text{-Glc}_p.$$

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