TRITERPENE GLYCOSIDES OF *Cephalaria gigantea*.

III. THE STRUCTURE OF GIGANTEASIDES E AND H

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The structures of giganteasides E and H, isolated from the roots of *Cephalaria gigantea*, have been established as a tetraoside and pentaoside of hederagenin, respectively. The O-glycosidic chains of the two glycosides are identical: L-rhamnopyranosyl-(1 $\overset{\alpha}{\rightarrow}$ 2)-L-rhamnopyranosyl-(1 $\overset{\alpha}{\rightarrow}$ 2)-D-xylopyranosyl-(1 $\overset{\beta}{\rightarrow}$ 2)-L-arabinopyranosyl-1 $\overset{\alpha}{\rightarrow}$. The acyl glycosidic moiety of the giganteasides H is β-D-glucose.

We have previously isolated from the roots of *Cephalaria gigantea* (Led.) E. Bobr. triterpene glycosides, which we called giganteasides [1]. The determination of the structures of giganteasides D and G has been reported previously [2]. The first of them is L-Rhap-(1 $\overset{\alpha}{\rightarrow}$ 2)-D-Xylp-(1 $\overset{\beta}{\rightarrow}$ 3)-oleanolic acid, and the second is L-Rhap-(1 $\overset{\alpha}{\rightarrow}$ 2)-D-Xylp-(1 $\overset{\beta}{\rightarrow}$ 3)-(oleanolic acid)-28←D-Glcp.

In the present paper we give the results of a chemical study of giganteasides E and H.

It was established with the aid of acid hydrolysis that giganteasides E and H are hederagenin derivatives. Rhamnose, xylose, and arabinose were identified in the carbohydrate moiety of giganteaside E and the same sugars with the addition of glucose in giganteaside H. On reduction and acetylation of a hydrolysate of giganteaside E, the acetates of rhamnitol, xylitol, and arabitol were identified by the GLC method in a ratio of 2 : 1 : 1, and the similar treatment of giganteaside H led to the acetates of rhamnitol, xylitol, arabitol, and sorbitol in a ratio of 2 : 1 : 1 : 1.

The IR spectrum of compound H had an absorption band of the ester bonds at 1740 cm^{-1} which was shifted in the spectrum of compound E to the 1700 cm^{-1} region, as is characteristic for an unsubstituted carboxy group (Table 1). The presence of a presumed ester group in giganteaside H was confirmed with the aid of alkaline hydrolysis, as a result of which glucose was split off and identified. The modified compound obtained by this treatment had a band at 1700 cm^{-1} in the IR spectrum and according to TLC it was identical with the biogenically related less polar glycoside E. The facts given show unambiguously that in the initial compound the glucose residue was bound to the aglycone by an ester bond, while the monosaccharides rhamnose, xylose, and arabinose were attached to the aglycone by glycosidic bonds in both the compounds under investigation.

The nature of the substitution of the monosaccharide residues in giganteasides E and H was established by the methylation of the initial compounds using Hakomori's method [3]. By the hydrolysis of the methylated glycosides, reduction of the hydrolysates, and subsequent

TABLE 1. Physicochemical Constants and Compositions of Giganteasides E and H

Giganteaside	mp, deg C	$[\alpha]_D^{20}$, deg	ν, cm^{-1}	Aglycone	Monosaccharides and their ratio
E	228–232	0	1700 (COOH)	Hederagenin	Rhamnose:xylose:arabinose, 2:1:1
H	214–218	−10	1740 (COOR)	"	Rhamnose:xylose:arabinose: glucose, 2:1:1:1.

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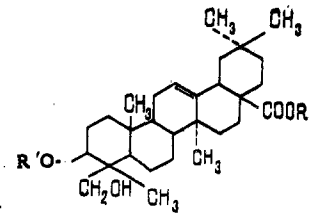
acetylation the acetates of partially methylated polyols were obtained, and these were characterized by chromato-mass spectrometry [4]. In the case of giganteaside E, 1,5-di-O-acetyl-2,3,4-tri-O-methylrhamnitol (I), 1,2,5-tri-O-acetyl-3,4 di-O-methylrhamnitol (II), 1,2,5-tri-O-acetyl-3,4,-O-methylxylitol (III), and 1,2,5-tri-O-acetyl-3,4,-di-O-methylarabitol (IV) were identified. In the case of giganteaside H, 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylsorbitol (V) was identified in addition to compounds (I-IV). This shows that in the initial compound H, the glucose residue was attached to the aglycone by an acyl-glycosidic bond.

To established the sequence of attachment of the monosaccharide units in the glycosides E and H, the methylated glycosides were subjected to stepwise methanolysis. In both cases methyl 2,3,4-tri-O-methylrhamnopyranoside (VI), methyl 3,4-di-O-methylrhamnopyranoside (VII), and methyl 3,4,-di-O-methylxylopyranoside (VIII), split off successively, were identified, while complete methanolysis of the progenins formed in this process permitted the identification of methyl 3,4-di-O-methylarabopyranoside (IX) and hederagenin in the first case and the same products with the addition of methyl 2,3,4,6-tetra-O-methylglucopyranoside (X) in the second.

The configurations of the glycosidic bonds were determined by comparing the ^{13}C NMR spectra of compounds E and H with the corresponding spectra of giganteasides D and G [2] and of model compounds [5, 6].

The size of the chemical shift of the C-1 atom of the xylopyranose residue unambiguously showed the β configuration of the glycosidic center [5, 6]. A comparison of the spectra of compounds E and H with those of methyl α- and β-L-rhamno- and arabinopyranosides permitted the corresponding residues in the giganteasides to be assigned the α configuration. The size of the chemical shift of the C-1 atom of the glucopyranose residues in giganteaside H permits it to be ascribed the β configuration of the glycosidic center unambiguously [5, 6].

On the basis of the results obtained and comparison with known glycosides [7], structures (1) and (2), respectively, can be ascribed to giganteasides E and H.



R' = L-Rhap 1→2-L-Rhap 1→2-D-Xylp 1→2-L-Arap 1→

(1) R = H

(2) R = β-D-Glc

EXPERIMENTAL

For the chromatography of the compounds studied and their derivatives we used type M ["slow"] paper of the Leningrad mill and FN-13 paper, plates with a fixed layer of type KSK silica gel, and, for column chromatography, silica gel of types L 40/100 and L 100/160 and the following solvent systems: 1) chloroform–methanol–water (26 : 14 : 3); 2) butanol–ethanol–water (10 : 2 : 5); 3) ethyl acetate–methanol–water (10 : 2 : 5); 4) pyridine–ethyl acetate–water (2 : 8 : 1); and 5) chloroform–methanol (20 : 1). The triterpene glycosides and their aglycones were detected by spraying the chromatograms with a 25% solution of tungstophosphoric acid in ethanol and the monosaccharides with o-toluidine salicylate. GLC analysis was performed on a LKhM-8 MD instrument using a column containing 3% of ECNSS on Chromosorb W. Mass spectra were recorded on a Varian MAT Gnom-111 instrument using a column containing 3% of ECNSS; ^{13}C NMR spectra on a Bruker WP-60 instrument (in DMSO); and IR spectra on a UR-20 spectrophotometer (in paraffin oil).

Acid Hydrolysis. A 20-mg sample of glycoside E or H was hydrolyzed with 2 N HCl at 100°C for 3 h. The resulting aglycone was filtered off, washed, and identified in comparison with a standard sample of hederagenin (system 5). The hydrolysate was neutralized and evaporated to dryness, and the residue was chromatographed on paper in system 4 in parallel with authentic samples of monosaccharides. In the case of compound E, rhamnose, xylose, and arabinose were identified, and in the case of compound H the same sugars together with glucose.

Preparation of the Polyol Acetates. A 10-mg sample of giganteaside E or H was hydrolyzed with 1 N HCl at 100°C for 3 h, and the reaction mixture was evaporated to dryness. The residue was dissolved in 2 ml of 50% methanol and was reduced to the polyols with $NaBH_4$ in 50% aqueous methanol for 12 h and was then treated with a cation-exchange resin (KU-2, H^+ form), which was filtered off, and the filtrate was evaporated several times with methanol to eliminate the boric acid that had been formed. The carefully dried substance was acetylated in a mixture of 2 ml of pyridine and 2 ml of acetic anhydride for 12 h and the reaction mixture was evaporated to dryness with methanol (to eliminate the Ac_2O) and with toluene (to eliminate the pyridine). The product was extracted with chloroform, and the extract was evaporated. The polyol acetates so obtained were identified by the GLC method.

Alkaline Hydrolysis. A solution of 20 mg of giganteaside E or H in 5 ml of 1% KOH was heated at 100°C for 2 h. The reaction mixture was neutralized with KU-2 cation-exchange resin (H^+ form), filtered, evaporated, and analyzed by TLC in systems 1-4.

Hakomori Methylation [3]. A 100-mg sample of giganteaside E or H was methylated by Hakomori's method. The methylated glycosides were hydrolyzed (HCOOH, 100°C, 1 h; 1 N HCl, 100°C, 3 h) and the reaction products were evaporated, reduced, and acetylated as described above. The resulting acetates of partially methylated polyols were identified by chromato-mass spectrometry.

Partial Methanolysis. Giganteasides E and H methylated by Hakomori's method were subjected to mild methanolysis (1% HCl/MeOH, 100°C). The resulting methyl derivatives were identified by chromato-mass spectrometry. After the lapse of 30 min, the rhamnopyranosides (VI) and (VII) and after 1.5 h the xylopyranoside (VIII) have been split off from giganteasides E and H.

When the modified giganteasides E and H were subjected to complete methanolysis (100°C, 4 h) and the methanolysates had been neutralized with aqueous ammonia, evaporated, and extracted with chloroform, the arabopyranoside (IX) was identified in the first case and the glucopyranoside (X), in addition, in the second case.

SUMMARY

1. The structures of giganteasides E and H, isolated from the roots of *Cephalaria gigantea*, have been established as a tetraoside and a pentaoside of hederagenin, respectively. The O-glycosidic chains of the two glycosides are identical: L-rhamnopyranosyl-(1 $\overset{\alpha}{\rightarrow}$ 2)-L-rhamnopyranosyl-(1 $\overset{\alpha}{\rightarrow}$ 2)-β-D-xylopyranosyl-(1 $\overset{\beta}{\rightarrow}$ 2)-L-arabinopyranosyl-1-$\overset{\alpha}{\rightarrow}$. The acyl moiety of the giganteaside H is β-D-glucose.

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