New coumarin glycosides -- haploperosides C, D, and E -- have been isolated from the epigeal part of the *Haplophyllum perforatum* (MB) Kar et Kir. On the basis of chemical transformations and spectral characteristics, haploperoside D has the structure of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-2H-benzopyran-2-one, and haploperoside C that of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 6)-(2-O-acetyl-β-D-glucopyranosyloxy)]-2H-benzopyran-2-one. The structure of haploperoside E has been established as 7-[O-α-L-rhamnopyranosyl-(1 → 2)-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyloxy]-2H-benzopyran-2-one. The structures of haploperosides A and B have been refined. An assignment has been made of the carbon signals in the $^{13}$C NMR spectra of haploperosides A, D, C, and E.

We have previously reported the isolation of two coumarin glycosides from *Haplophyllum perforatum* [1-3]. Continuing this investigation we have isolated another three glycosides, which we have called haploperosides C, D, and E. In the present paper we give proofs of their structures.

The UV spectrum of haploperoside D (I) is characteristic for 6,7-di-O-substituted coumarins and is similar to the spectra of scopoletin and of haploperoside A [1]. It was established by the GLC method that the molecule of (I) contained D-glucose and L-rhamnose residues in a ratio of 1:1. The acid hydrolysis of (I) gave, in addition to the monosaccharides mentioned above, an aglycone identified as scopoletin. The acetylation of haploperoside D led to a hexaacetate with the composition C$_{34}$H$_{40}$O$_{19}$, M$^+$ 752. Consequently, haploperoside D is a scopoletin bioside.

The mass spectrum of the acetate of (I) contained strong peaks of ions with m/z 273, 213, and 153, showing that in the haploperoside D molecule the L-rhamnose is the terminal sugar residue [4]. This was confirmed by the production, on partial hydrolysis, of a monoglucoside which was identified as scopoletin 7-O-β-D-glucopyranoside (scopolin) [1]. To determine the structure of the carbohydrate chain we performed the Hakomori methylation of glycoside (I) [5]. In a hydrolysate of the methylation product we identified by GLC
2,3,4-tri-O-methyl-L-rhamnose and 3,4,6-tri-O-methyl-D-glucose [6]. Thus, the terminal L-
rhamnose sugar residue was attached to the D-glucose residue by a 1 \( \rightarrow \) 2 bond.

In the PMR spectrum of haploperoside D, the anomic protons of the glucose and the rhamnose resonated at 5.54 and 4.59 ppm in the form of doublets with spin–spin coupling constants \( ^3J = 8 \) and 2 Hz, respectively. This shows that Cl conformation of the glucopyranose ring and the IC conformation of the rhamopyranose ring and, consequently, the \( \beta \)-configuration of the glycosidic center of the D-glucose residue and the \( \alpha \)-configuration of the L-rhamnose residue [7, 8].

Thus, haploperoside D has the structure of 6-methoxy-7-\([O-\alpha-L-rhamnopyranosyl-(1 \xrightarrow{} 2)-\beta-B-D-glucopyranosyloxy]\)-2H-benzopyran-2-one (I).

We had previously proposed a similar structure for haploperoside A [1], and this made it necessary to reconsider the structure of the latter. Haploperoside A and D possibly differ by the position of attachment of the rhamnose residue to the glucose residue. For an unambiguous answer to this question, haploperoside A (II) was also subjected to Hakomori methylation. From a hydrolysate of the methylation products we isolated and identified by the TLC and GLC methods 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose [6]. Consequently, in the (II) molecule the L-rhamnose residue by a 1 \( \rightarrow \) 6 bond and haploperoside A has the structure of 6-methoxy-7-\([O-\alpha-L-rhamnopyranosyl-(1 \xrightarrow{} 6)-\beta-D-glucopyranosyloxy]\)-2H-benzopyran-2-one (II).

We have previously effected the passage from haploperoside B to (II) [2] and therefore haploperoside B has the structure of 7-\([O-(4''-O-acetyl-\alpha-L-rhamnopyranosyl)-(1 \xrightarrow{} 6)-\beta-D-glucopyranosyloxy]\)-6-methoxy-2H-benzopyran-2-one (III). The position of the acetyl group at C-4'' of the L-rhamnose residue by a 1 \( \rightarrow \) 6 bond had been established previously by the INDO method [3].

The structures of glycosides (I) and (II) were also confirmed by a study of the \( ^{13} \)C NMR spectra obtained under the conditions of complete and incomplete (off-resonance) decoupling from protons. In the spectra of haploperosides A and D in the 102.8-160.5 ppm region nine signals appeared in each case from sp\(^2\)-hybridized carbon atoms of coumarin nucleus and 12 signals in each case from sp\(^3\)-hybridized carbon atoms of the sugar moiety (\( \delta 17.7-100.2\) ppm). In the assignment of the signals of the carbon atoms in the \( ^{13} \)C NMR spectra of (I) and (II) we made use of literature information on the CSs of the carbon atoms for 7-\( \beta \)-D-glucopyranosyl-

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On the basis of an analysis of its UV and PMR spectra, haploperoside C (IV) was also assigned to the coumarin glycosides. In fact, on acid hydrolysis (IV) was split with the formation of scopoletin, D-glucose, and L-rhamnose. The presence of the absorption band of an ester carbonyl at 1749 cm\(^{-1}\) in the IR spectrum and of a three-proton singlet at 1.98 ppm in the PMR spectrum indicated that haploperoside C was an acylated coumarin glycoside and contained one acetate group.

The alkaline hydrolysis of (IV) with a 0.5% solution of caustic soda led to the formation of the coumarin glycosides. In fact, on acid hydrolysis (IV) was split with the formation of scopoletin, D-glucose, and L-rhamnose. The presence of the absorption band of an ester carbonyl at 1749 cm\(^{-1}\) in the IR spectrum and of a three-proton singlet at 1.98 ppm in the PMR spectrum indicated that haploperoside C was an acylated coumarin glycoside and contained one acetate group.

In the PMR spectrum of glycoside (IV), the proton geminal to the ester function resonated at 5.62 ppm in the form of a triplet with \( ^{3}J_1 = ^{3}J_2 = 9 \) Hz. In the same spectrum, the signals of the anomic protons of the D-glucose and L-rhamnose residue appeared at 5.31 ppm (d, \( ^{3}J = 9 \) Hz) and 5.18 ppm (br.s), respectively. The position of the acetyl group cannot be determined from the multiplicity and spin–spin coupling constants of the geminal proton, although it is possible to exclude the C-2'' and C-3'' positions of the L-rhamnose residue. On the superposition of an additional radiofrequency field with \( v = 531 \) Hz (H-1'), the triplet at 5.62 ppm was converted into a doublet with \( ^{3}J = 9 \) Hz. Conversely, irradiation with a frequency \( v = 562 \) Hz (H-2') led to the conversion of the doublet signal of the anomic proton of the glucose residue.