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Table 1

1963

SovietRxiv

Abstract

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OLIGOSIDES–A NEW TYPE OF PLANT GLYCOSIDES

Plant glycosides are among the most widespread and, from a practical standpoint, important natural carbohydrate-containing compounds, in which both the aglycone and carbohydrate components vary widely. Despite extensive investigation of this class, the glycosides studied to date have, as a rule, contained one, two, three, and very rarely four monosaccharide residues (mono-, di-, tri-, and tetraosides). At the same time, the presence in plants of oligo- and polysaccharides suggests the existence of glycosides with a longer carbohydrate chain, which may comprise a large number of monosaccharide units. However, although such assumptions have been made earlier (*1, 2*), direct and reliable evidence for the presence of glycosides of this type in the plant kingdom has until now been lacking.

Table 1

Glycoside	Plant	Aglycone	Monosaccharide composition (molar ratio)
Araloside *A* (I)	*Aralia manshurica* Rupr. et Mey	Oleanolic acid	*D*-Gl, *D*-Glur, *L*-Ar (1 : 1 : 1)
Araloside *B* (II)	Same	Same	*D*-Gl, *D*-Glur, *D*-Ar (1 : 1 : 2)
Araloside *C* (III)	Same	“	*D*-Gl, *D*-Glur, *D*-Gal, *D*-Xy (1 : 1 : 1 : 1)
Gypsoside (IV)	*Gypsophila pacifica* Kom.	Gypsogenin	*D*-Gl, *D*-Glur, *D*-Gal, *D*-Fu, *L*-Rha, *L*-Ar, *D*-Xy (1 : 1 : 1 : 1 : 1 : 1 : 3)
Kalopanax-saponin *A*	*Kalopanax septemlobum* Koidz	Hederagenin	*L*-Ar, *L*-Rha, (1 : 1)

Glycoside	Plant	Aglycone	Monosaccharide composition (molar ratio)
Kalopanax-saponin *B*	Same	Same	*D*-Gl, *L*-Ar, *L*-Rha (5 or 6 monosaccharide residues)
Patrinoside *D*	*Patrinia intermedia* Roem. et Schult.	Oleanolic acid	*D*-Gl, *D*-Xy (4 : 2 or 4 : 3)
Clematoside *C*	*Clematis manshurica*	Same	*D*-Gl, Ar, Rha, Xy (not fewer than 10 monosaccharide residues)

Note. Ar—arabinose, Gl—glucose, Glur—glucuronic acid, Gal—galactose, Fu—fucose, Rha—rhamnose, Xy—xylose.

In carrying out a systematic investigation of triterpene saponins, begun in connection with the study of the active principles of certain plants, we found (see Table 1) that a characteristic feature of the chemical structure of glycosides of this class is the presence of a large carbohydrate moiety containing up to 6–9 and even more monosaccharide residues. These results, together with certain literature data (*3*-*8*), indicate the widespread occurrence in nature of glycosides whose composition includes more than four or five monosaccharide units; for these we propose the name "oligosides."

Oligosides, occupying an intermediate position between low-molecular and high-molecular substances, possess a complex of properties that leave their mark on their chemical behavior and require special methods for individualization and structural determination. In this article we summarize the results of our investigations on oligosides belonging to the triterpene saponins*, the characteristic features of which will, in all likelihood, to a significant degree also be inherent in oligosides of other classes.

* The experimental data used in this article are being published by us in *Izvestiya AN SSSR*, Department of Chemical Sciences, in the series "Triterpene Saponins."

The isolation of oligosides requires the maintenance of sufficiently mild conditions under which their destruction is excluded at all stages of purification. In our cases, extraction of the dried plant material was carried out with warm or hot methanol, which completely excluded autolysis. In view of the presence in one and the same plant of oligosides very close in structure, separation of their mixture is a difficult task and not always feasible. We succeeded in carrying out such separation by using partition chromatography on various carriers of

the stationary phase, most often on cellulose. A decisive role at this stage of the work is played by control of the separation of complex mixtures; for this purpose we developed analytical chromatography of triterpene saponins on paper or in a thin fixed layer of silica gel, which differed from those described in the literature (7, 8) by its high sensitivity and good reproducibility of results. As the mobile phase in both cases, the best results were shown by the mixtures: *n*-butanol—ethanol—water (7 : 2 : 5), *n*-butanol—ethanol—conc. NH_4OH (7 : 2 : 5), and *n*-butanol—acetic acid—water (4 : 1 : 1). The sensitivity of detection of oligosides on silica gel with concentrated H_2SO_4 or a saturated solution of $SbCl_3$ containing about 5% $SbCl_5$ was 3-5 γ. In separating low-molecular impurities from oligosides, good results were obtained by purifying the oligosides by dialysis or on Sephadex G-25 in aqueous solutions.

Proof of the individuality of triterpene oligosides and establishment of their empirical formula is a complex problem. As a rule, they are amorphous powders, decomposing above 200°, and they contain a variable amount of solvent that is difficult to remove (water, alcohols). In addition, triterpene oligosides are capable of retaining mineral impurities very firmly. Therefore, in order to obtain analytical data on the basis of which the gross composition could be judged, we converted the oligosides into their complete acetates by the action of acetic anhydride in pyridine (9, 10). Since oligosides and their acetates have molecular weights in the range 1500-2500, the methods for determining molecular weight used both for low-molecular and for high-molecular compounds are poorly suitable for them. Solving this question is not an easy task; the best results were obtained by us in determining the molecular weight of oligosides from the yield of genin upon their quantitative acid hydrolysis (this method proved suitable only in the case of stable genins) or in determining the molecular weight of their acetates by the method of isothermal distillation (11, 12). In both cases the error amounted to a value equal to the molecular weight of one monosaccharide residue. Thus, elemental analysis and the value found for the molecular weight do not permit the empirical formula of oligosides to be established with accuracy; the final clarification of this question is carried out in the process of establishing their structure.

The first stage in establishing the structure of oligosides is their complete hydrolysis. The genins formed in our cases were identified with known triterpenes (see Table 1). Identification of the monosaccharides formed upon hydrolysis was carried out by paper chromatography. Since oligosides often contain several identical monosaccharide residues, it was very important to determine the quantitative ratio of the monosaccharide units of the oligoside. Quantitative chromatography of monosaccharides on paper proved most convenient for this purpose. However, since oligosides often require severe conditions for complete hydrolysis, under which the monosaccharides formed undergo further destruction, the accuracy of determining the amount of monosaccharide residues by this route is not always sufficient, and the data obtained in this way required additional verification and refinement at the subsequent stages of structure determination.

Structural formulas (I)-(IV) shown on the page.

Figure 1: Structural formulas (I)-(IV) shown on the page.

The next stage in establishing the structure is reduced to determining the structure of the carbohydrate portion and proving the site of attachment of the carbohydrate chain or chains to the aglycone. The structure of the carbohydrate portion can be established by methods adopted in polysaccharide chemistry: exhaustive methylation followed by hydrolysis and identification of the methylated monosaccharides, periodate oxidation, partial hydrolysis, and enzymatic cleavage. In addition, since the oligosides of triterpene hydroxy acids often are O-acyl glycosides (see below), evidence for the presence of an acyl-glycosidic bond and its selective cleavage are highly important. We developed methods for the selective cleavage of the O-acyl-glycosidic bond during reduction of methylated glycosides with $LiAlH_4$, by halolysis of acyl glycosides under the action of LiI in collidine; good results were also obtained under alkaline hydrolysis ([13]) and in the cleavage of acyl glycosides with $NaBH_4$ ([14]). To prove the O-acyl-glycosidic bond, the oligoside or its complete acetate was treated with diazomethane, followed by complete acid hydrolysis. The formation of a free triterpene acid unequivocally indicated the presence of an O-acyl-glycosidic bond in the oligoside, whereas identification of the methyl ester of this acid, stable under the conditions of acid hydrolysis, on the contrary indicated its absence.

The methylation of triterpene oligosides presents certain difficulties associated with the presence of highly branched carbohydrate chains (see below). The best results in our case were obtained by methylation with methyl iodide in dimethylformamide in the presence of Ag_2O or BaO ([15], [16]), with two- or threefold repetition of the operation. Thin-layer chromatography of the reaction products on alumina proved to be a convenient means of monitoring the course and completeness of methylation.

The study of triterpene saponins carried out in our laboratory led to the isolation of a number of oligosides of this class, data on which are given in Table 1. The application of the methods mentioned allowed us to establish the structures of aralosides A (I), B (II) ([17]), and C (III), and of gypsoside (IV) ([18]), which proved to be a nonaoside of gypsogenin, unique—

···in their structure, and also to elucidate the basic structural features of individual oligosides.

The structure of these compounds reveals a number of features in the structure of oligosides of the triterpene series. First, they are characterized by a high degree of branching of the carbohydrate part of the molecule, which is especially clearly seen in the example of gypsoside. In the case of such complex aglycones as triterpene hydroxy acids, the carbohydrate chains may be linked to different functional groups of the genin. Second, triterpene saponins that are glycosides (oligosides) of the triterpene series are characterized by the presence

of an O-acylglycosidic bond between the carbohydrate residue and the carboxyl group of the genin, which, owing to steric hindrance, is close in strength to an O-glycosidic bond of the usual type. It should be emphasized that, apart from kalopanax saponin A, the simplest triterpene glycoside, all the triterpene saponins isolated by us contain O-acylglycosidic bonds. Since the genins of triterpene saponins, as a rule, are triterpene hydroxy acids with a hindered carboxyl group, it may be supposed that O-acylglycosides of this type are widely distributed in nature. Moreover, since the stability of O-acylglycosides is determined chiefly by the steric hindrance of the carboxyl group of the genin, O-acylglycosides may also occur among glycosides of other classes in which the genins are sterically hindered acids. This is supported, for example, by the isolation of stevioside (13). Apparently, O-acylglycosides of carboxylic acids with a sterically unhindered carboxyl are also widely represented in the plant world; however, because of their extreme instability they have not yet been isolated. The existence of stable O-acylglycosides of the triterpene series indicates a sufficiently broad distribution not only of acidic but also of neutral triterpene saponins.

In conclusion, it should be noted that the occurrence in nature of a large group of triterpene oligosides indicates a definite, although as yet still unclear, role of substances of this type in the life processes of plants. There can hardly be any doubt that among other groups of plant glycosides, too, oligosides with a large number of carbohydrate units may be discovered. The aglycone of oligosides, linked to the carbohydrate chain, to some extent resembles the prosthetic group of a peptide. In this connection the thought naturally arises of the presence in plants of polysaccharides linked with some aglycone; the search for such compounds and the elucidation of their role are of great fundamental interest.

Institute of the Chemistry of Natural Compounds
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Received
27 III 1963

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Note: Figure translations are in progress. See original paper for figures.