THE STRUCTURE OF PRIMFLASIDE

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UDC 547.972

By chromatography on a column of polyamide we isolated from the leaves of Primula turkestanica (Rgl.) E. A. White a flavonoid glycoside with the composition $C_{31}H_{36}O_{20} \cdot 2H_2O$ which we called primflaside [1]. The acid hydrolysis of primflaside gave an aglycone which was identified by its physicochemical constants and UV, IR, and NMR spectra as quercetin.

The carbohydrate moiety of the glycoside consists of glucose and arabinose. The IR spectra show that it contains free OH groups in the 3', 4', 5', and 7 positions. Consequently, the carbohydrate moiety of primflaside is attached to the quercetin in position 3. The NMR spectra of silylated primflaside (Fig. 1) show the presence in the substance of three carbohydrate components, which follow from the presence of the signals of three protons of anomeric centers (a doublet at 5.67 ppm, J = 8 Hz, corresponding to β-glucose in position 3 of the flavonol, and doublets at 3.88 and 3.80 ppm, J = 8 Hz, corresponding to two protons of α-arabinose) [2]. The values of the chemical shifts (CSs) of the protons of the anomeric centers of the arabinose residues show that either they are attached to the glucose in the form of a branched chain (variant I) or the carbohydrate moiety has the form of an unbranched chain – β-glucose– α-arabinose–α-arabinose (variant II). If one of the molecules of arabinose were attached to the aglycone directly, the CS of the proton of the anomeric center would have had a value of 5-5.2 ppm [3]. The integral intensity of the signal in the 2.80-3.66 ppm region (16 protons) corresponds to 1 mole of glucose and 2 moles of arabinose. The molecular weight of the substance determined ebullioscopically corresponds to the composition of the glycoside and to the features of the NMR spectrum. The oxidation of primflaside with hydrogen peroxide followed by hydrolysis with a solution of ammonia gave a triose [4], and hydrolysis of the triose gave glucose and arabinose.

Further information on the structure of the carbohydrate moiety of primflaside was provided by its exhaustive methylation followed by methanolysis. By gas-liquid chromatography (GLC) in the presence of markers the methyl glycosides of 2,3,6-tri-O-methyl-D-glucose and of 2,3,4-tri-O-methyl-L-arabinose were identified.

The information given above permits only two variants of the structure of the carbohydrate moiety of primflaside (I and II) to be considered. Variant I does not contradict the IR spectrum, but it is excluded because of the identification of the methyl glycoside of 2,3,6-tri-O-methyl-D-glucose. Variant II also does not contradict the NMR spectrum or the results of exhaustive methylation. On the basis of these facts, primflaside is quercetin 3-O-α-L-arabopyranosyl-(1 → 2)-O-α-L-arabofuranosyl-(1 → 4)-β-D-glucopyranoside.* The structure of its carbohydrate moiety is in harmony with literature information on the GLC of methyl glycosides of 2,3,4-tri-O-methyl-L-arabinose and 3,5-di-O-methyl-L-arabinose [5].

EXPERIMENTAL

Chromatography was performed on Whatman 3MM paper. The following systems of solvents were used: 1) 15% acetic acid; 2) 60% acetic acid; 3) butan-1-ol–acetic acid–water (4 : 1 : 5); 4) ethyl acetate–formic acid–water (10 : 2 : 3); 5) benzene–ethyl acetate–acetic acid/formamide (24 : 5 : 73 : 5 : 2); 6) butan-

*Russian nomenclature does not conform to IUPAC rules. This name is regarded by the translator as the most likely name for the compound.

North-Caucasian Zonal Experimental Station. All-Union Scientific-Research Institute of Medicinal Plants. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 285-289, May-June, 1972. Original article submitted December 6, 1971.

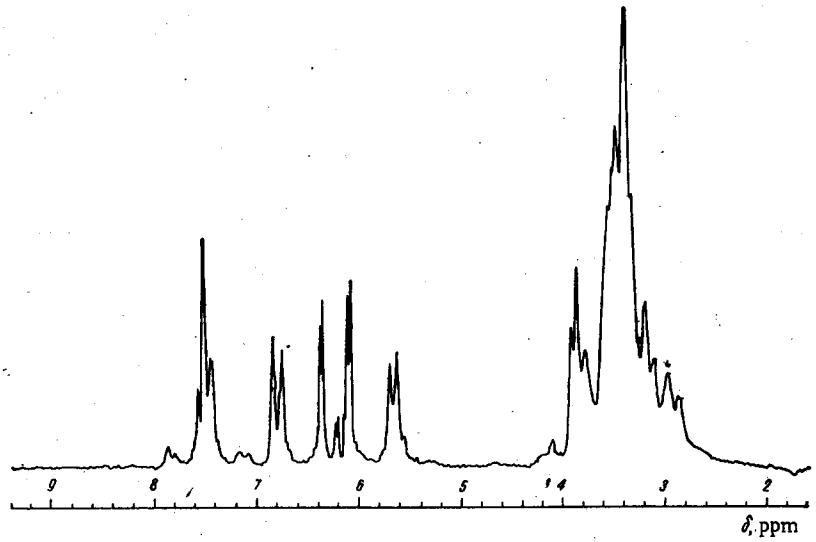

Fig. 1. NMR spectrum of silylated primflaside in carbon tetrachloride.

1-ol–acetone–water (2:7:1); 7) butan-1-ol–pyridine–water (6:4:3); 8) butan-1-ol–ethanol–water (40:11:19); and 9) benzene–butan-1-ol–pyridine–water (1:5:3:3).

The flavonoids were detected by their fluorescence in UV light after the treatment of chromatograms with ammonia vapor, and the sugars by means of the aniline phthalate reagent. The IR spectra of the substances were recorded on a UR-10 spectrophotometer (paraffin oil), the UV spectra on a Hitachi recording spectrophotometer, and the NMR spectra on a Varian HA-100 spectrophotometer.

Gas-liquid chromatography was performed on a Chrom-2 instrument (column containing 15% of tetramethylene succinate on Chromoton N-AW-HWDC with nitrogen as the carrier gas at 185°C). The melting points were determined on a Kofler block. The analyses of all the compounds corresponded to the calculated figures.

Isolation of Primflaside. The leaves of the plant (1.2 kg), which had been comminuted and treated with chloroform (until traces of chlorophyll were absent), were exhaustively extracted with methanol. The concentrated methanolic extract was deposited on a column of polyamide (24 × 7.7 cm) and eluted with water and then with aqueous ethanol with increasing concentrations of ethanol, 250-300-ml fractions being collected. The process of elution was monitored by paper chromatography (PC) in system 1.

Fractions of similar composition (13-31) were combined and evaporated to dryness, the residue was dissolved in the minimum amount of methanol, and the solution was added with stirring to half its volume of acetone. The liquid was filtered, and the filtrate was evaporated in vacuum to incipient precipitation. The precipitate was filtered off and recrystallized from 50% ethanol. This gave 2.04 g of yellow crystals (0.17%) with mp 193-195°C; $[\alpha]_D^{20}$ −100° (c 0.2; dimethylformamide); R_f 0.52, 0.50, and 0.54 (in systems 1, 3, and 4, respectively). UV spectrum, nm: $\lambda_{max}^{C_2H_5OH}$ 259, 363; $\lambda_{max}^{CH_3COONa}$ 262, 373; $\lambda_{max}^{CH_3COONa+H_3BO_3}$ 267, 387; $\lambda_{max}^{AlCl_3}$ 272, 405; $\lambda_{max}^{C_2H_5ONa}$ 262, 405.

Found: mol. wt. 765 (ebullioscopically in absolute ethanol, Hitachi-115 instrument). $C_{31}H_{26}O_{20} \cdot 2H_2O$. Calculated: mol. wt. 764.

Acid Hydrolysis. Primflaside (0.1005 g) was hydrolyzed with 5% H_2SO_4 (10 ml) for 75 min. The time of hydrolysis was determined by the PC method in system 1.

The aglycone was filtered off, washed with water to neutrality, and dried on a previously weighed filter to constant weight. Yield 42.7%; mp 305-310°C; R_f 0.28, 0.20 (systems 2 and 5, respectively).

Found: mol. wt. 302 (mass spectrometrically). $C_{15}H_{10}O_7$. Calculated: mol. wt. 302.

Acetylation of the Aglycone of Primflaside. The aglycone of primflaside (0.05 g) was dissolved in 4.5 ml of freshly distilled pyridine and 2 ml of acetic anhydride, and acetylation was performed for 48 h with periodic stirring, and the mixture was then poured into ice water. The white precipitate that deposited was filtered off and recrystallized from 96% ethanol. Mp 195-197°C.

The constants of the aglycone corresponded to those of quercetin, which was confirmed by a direct comparison with an authentic sample.

Identification of the Sugars. The filtrate was neutralized with barium carbonate, filtered, evaporated to a volume of 0.5 ml, and chromatographed in systems 8 and 9. Application of the aniline phthalate reagent showed the presence of glucose and arabinose with R_f 0.21 and 0.30 (system 8) and 0.54 and 0.66 (system 9, at a R_f value for rhamnose of 1).

Preparation of the Triose. A solution of 0.02 g of primflaside in 2 ml of 0.1 N ammonia was treated with 0.6 ml of perhydrol, and the mixture was left with periodic stirring for 4 h. Then a palladium catalyst was added, and the mixture was left for 18 h for the destruction of the excess of hydrogen peroxide. The palladium catalyst was filtered off, and the filtrate was treated with 1 ml of conc. ammonia, boiled for 5 min, evaporated to a volume of 0.5 ml, and chromatographed in systems 6-8. The application of the aniline phthalate reagent showed the presence of a triose with R_f 0.09, 0.36, and 0.09 (with an R_f value of rhamnose in system 7 of 1).

The triose remaining after chromatography was hydrolyzed with 3% H_2SO_4 for 15 min and the hydrolyzate was chromatographed in systems 8 and 9. The application of the aniline phthalate reagent showed the presence of spots corresponding to glucose and arabinose.

Silylation of Primflaside. To a solution of 0.05 g of primflaside in 3 ml of pyridine were gradually added 0.5 ml of freshly distilled hexamethyldisilazane and 0.5 ml of chlorotrimethylsilane. The solvent and the excess of the reagents were eliminated under vacuum. The residue was extracted with carbon tetrachloride, and the resulting solution was used for recording the NMR spectrum.

Preparation of 3,5-Dihydroxy-3',4',7-trimethoxyquercetin. A solution of 0.2 g of primflaside in 4 ml of methanol was treated with 2 ml of diazomethane solution. After a day, another 2 ml of diazomethane was added, and the mixture was left for a further day. Then the methanol was distilled off under vacuum, 2 ml of 3 N H_2SO_4 was added, and hydrolysis was performed with heating in the water bath for 3 h. The end of the hydrolysis was determined by chromatography in system 1.

The mixture was evaporated to small volume and left in the refrigerator until the methylated aglycone had precipitated, and this was filtered off. On chromatography in system 1, in addition to 3,5-dihydroxy-3',4',7-trimethoxyquercetin (R_f 0.54) 3-hydroxy-3',4',5,7-tetramethoxyquercetin was detected as an impurity (R_f 0.64). The first substance was purified on a column of polyamide (17 × 2 cm) by elution with water and then with aqueous ethanol of increasing concentration. The NMR spectrum was recorded in deuteroacetone. It had signals at 3.88 ppm with an intensity of 9H corresponding to three OCH_3 groups, a doublet at 6.28 and 6.44 ppm (1H each), J = 2.5 Hz, due to the H-6 and H-8 protons, a doublet at 6.08 ppm (1H), J = 9Hz, due to the proton in position 5', a signal at 7.85 ppm (2H), corresponding to the H-2' and H-6' protons, and a singlet at 12.14 ppm corresponding to the proton of the hydroxy group in position 5.

Preparation and Identification of the Methyl Glycosides of the Methylated Sugars. A solution of 0.1 g of primflaside in 3 ml of dimethyl sulfoxide was treated with 0.2 g of sodium hydride and 3 ml of methyl iodide. The reaction mixture was stirred at room temperature for 14 h and was then concentrated in vacuum, poured into 15 ml of chloroform, and extracted with water (8 × 15 ml) until the reaction of the wash waters with $Na_2S_2O_3$ was negative. The chloroform solution was evaporated, and the residue was treated with 3 ml of methanolic hydrogen chloride and heated with stirring for 4.5 h. The liquid was diluted with 3 ml of water, filtered, and evaporated under vacuum to eliminate the methanol, and the methylated sugars were extracted with chloroform (3 × 5 ml). The chloroform extracts were evaporated to a volume of 0.5 ml and were investigated by the GLC method. The methyl glycosides of 2,3,6-tri-O-methyl-D-glucose and of 2,3,4-tri-O-methyl-L-arabinose were detected.

SUMMARY

The structure of primflaside, a new flavonol trioside isolated from the leaves of Primula turkestanica has been established as quercetin 3-O-α-L-arabopyranosyl-(1 → 2)-O-α-L-arabofuranosyl-(1 → 4)-β-D-glucopyranoside.*

LITERATURE CITED

1. A. M. Zakharov, V. I. Glyzin, and A. I. Ban'kovskii, Khim. Prirodn. Soedin., 472 (1970).
2. W. Olechnowicz-Stepien et al., Herba Polonica, 1968, No. 3, 179.
3. V. I. Glyzin and A. I. Ban'kovskii, Khim. Prirodn. Soedin., 662 (1971).
4. A. S. Sadykov, B. Makhsudova, and Z. P. Pakudina, Khim. Prirodn. Soedin., 11 (1967).
5. G. Aspinall, J. Chem. Soc., 1963, 1676.

* This name is regarded by the translator as the most likely name for the compound.