# Keioside–A New Flavonoglycoside from Far Eastern Lily of the Valley (*Convallaria keiskei* Miq.)

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## Abstract

## Full Text

# Keioside—A New Flavonoglycoside from Far Eastern Lily of the Valley (*Convallaria keiskei* Miq.)

**N. F. Komissarenko, V. T. Chernobai, D. G. Kolesnikov**

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As reported earlier ($^{1-3}$), a series of cardiac glycosides of the cardenolide group was isolated from Far Eastern lily of the valley.

The present paper sets forth the results of a study of flavonoid compounds isolated from this same plant.

From the above-ground part of Far Eastern lily of the valley, the flavonoids were extracted with 80% ethanol; the alcohol was distilled off from the extract in vacuo, and the aqueous residue was purified with a chloroform–alcohol mixture (9:1). The purified aqueous extract was chromatographed on a polyamide sorbent ($^{4}$). The column was washed first with water and then with aqueous-alcoholic mixtures with an increasing alcohol content from 1 to 30%. The process of separation of the flavonoids was monitored in ultraviolet light. During elution with water, a substance designated C-1 was first washed out, and with aqueous-alcoholic mixtures, substances C-2 and C-3. The isolated compounds give a positive cyanidin reaction.

On the basis of physicochemical properties and the transformations carried out, substances C-2 ($C_{21}H_{20}O_{12}$, m.p. 239–240°) and C-3 ($C_{15}H_{10}O_7$, m.p. 313–316°) were identified as 5,7,3′,4′-tetrahydroxyflavonol-3-$\beta$-*D*-galactoside (hyperoside) and 5,7,3′,4′-tetrahydroxyflavonol (quercetin).

Substance C-1, which we have named keioside (I), proved to be a new flavonoglycoside, m.p. 183–186°, $[\alpha]_D - 21.4 \pm 2^\circ$ ($C = 0.84$; $CH_3OH$).

Found %: C 53.51; 53.97; H 5.22; 5.09
$C_{28}H_{32}O_{16}$ (624.5). Calculated %: C 53.84; H 5.16

With ferric chloride, keioside forms a brown coloration that changes to dirty green.

To elucidate the structure of glycoside (I), acid hydrolysis was carried out with 5% sulfuric acid, as a result of which the sugars *D*-galactose (IV) and *L*-rhamnose (V), as well as the aglycone (II), were obtained. The latter sublimed at 305° and melted within the range 317–139°.

Scheme of transformations of keioside

Figure 1: Scheme of transformations of keioside

Found %: C 61.04; 60.45; H 3.74; 3.93

$C_{16}H_{12}O_7$ (316.3). Calculated %: C 60.75; H 3.82

The IR spectrum of the aglycone has an absorption band in the region of 2980 $cm^{-1}$, characteristic of methoxyl groups. By the Zeisel method ($^5$), one methoxyl was determined.

Scheme of transformations of keioside

The UV spectra (6) of the glycoside (I) and the aglycone (II), recorded in absolute ethanol and with additions of $H_3BO_3$, $CH_3COONa$, $C_2H_5ONa$, $AlCl_3$, and $AlCl_3$ + HCl, make it possible, from the shifts of the maxima of the first and second bands, to assume the presence of phenolic groups in the glycoside at the 5, 7, and 4′-positions, and in the genin at the 3, 5, 7, and 4′-positions. A tetraacetyl derivative of the aglycone (III) was obtained ($C_{24}H_{20}O_{11}$, m.p. 202-204°), which also indicates the presence of four free hydroxyl groups.

The aglycone (II) was demethylated with hydroiodic acid in a medium of liquid phenol and acetic anhydride (7). As a result, substance (VI) was obtained ($C_{15}H_{10}O_7$, m.p. 309-311°), which proved to be identical with quercetin. In order to determine the position of the methoxyl group, we carried out cleavage of the aglycone with a 20% KOH solution at the temperature of a boiling water bath for 2 h (7). Paper chromatography of the cleavage products in the system benzene–ethyl acetate–acetic acid (24.5 : 73.5 : 2)-formamide revealed phloroglucinol (VII) and vanillic acid (VIII). This makes it possible to assert that the methoxyl group is located in the 3′-position and, consequently, the aglycone is identical with isorhamnetin.

To determine the site of attachment of the sugar residues to the aglycone, we used data from spectroscopic analysis in the UV region for (I) and (II), as well as the ability of various flavonoid compounds to fluoresce in UV light (9). As is known, flavonol glycosides and their aglycones with a free hydroxyl group in the third position fluoresce yellow before treatment with alkali. Similar fluorescence was also observed for the aglycone of keioside. In contrast to the aglycone, the glycoside is detected on the paper chromatogram as a dark spot. This indicates that the hydroxyl at position 3 is substituted by a sugar residue.

To determine the order of attachment of the sugar residues in the glycoside under investigation (I), it was hydrolyzed with enzymes (10) from seeds of *Rhamnus catartica* L.; as a result, the aglycone (II) was isolated, and by paper chromatography (Fig. 1, sample 5) a disaccharide identical with robinobiose (IX) (12) was detected.

Thus, keioside is 5,7,5′-trihydroxy-3′-methoxyflavonol-3-$\beta$-D-galactopyranosido-6″-$\beta$-L-rhamnopyranoside and has the most probable formula (I). Until now, only one glycoside—robynin (11), containing robinobiose as the sugar component —has been known.

**Fig. 1.** System: *n*-butanol—acetic acid—water (4 : 1 : 5), 72 h. "Leningradskaya bystraya" paper.
1 —L-rhamnose, 2 —D-glucose, 3 —D-galactose, 4 —sugar portion of keioside after acid hydrolysis, 5 —sugar portion of keioside after enzymatic hydrolysis, 6 —robinobiose from robynin, 7 —rutinose from rutin.

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