UDC 615.711.5

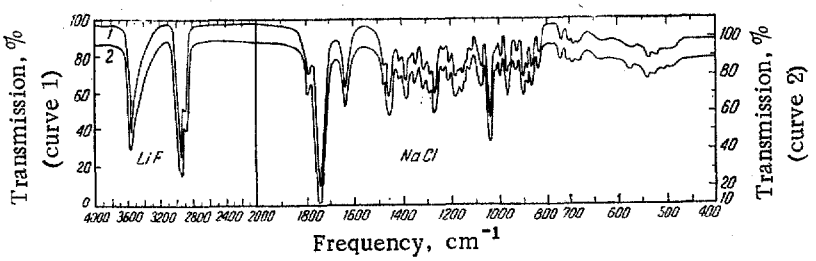
# EVONOLOSIDE FROM THE SEEDS OF EUONYMUS EUROPAEUS. II

S. G. Kislichenko, I. F. Makarevich, and D. G. Kolesnikov

Khimiya Prirodnykh Soedinenii, Vol. 3, No. 4, pp. 241-244, 1967

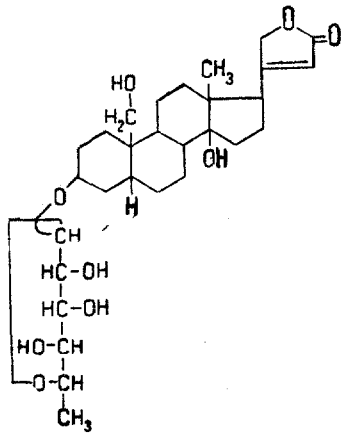
From the seeds of E. europaeus L. (a central Russian strain of the European euonymus) we have obtained [1], besides evomonoside, another cardiac monoglycoside $C_{29}H_{44}O_9$ with mp 158-159° C, $[\alpha]_D$ -20.05°, provisionally given the designation SK-1. Having become convinced that this glycoside is a new one, we have proposed for it the name evonoloside.

The UV spectrum of evonoloside exhibits only one maximum at 219 mμ (log ε 4.14) which, together with qualitative reactions, confirms the presence of a butenolide ring in this glycoside. The glycoside undergoes no change under the action of the enzymes of the snail Helix pomatia and of the fungus Aspergillus oryzae. After acid hydrolysis by the Mannich-Siewert method [2], an aglycone, an anhydroaglycone, and a sugar component were obtained in the pure crystalline state. The aglycone, $C_{23}H_{34}O_5$, was identified by means of its physicochemical properties and its IR spectrum (taken by I. P. Kovalev on a UR-10 spectrometer; see figure) as cannogenol, and the monosaccharide as L-rhamnose.




The aglycone of evonoloside (1) and cannogenol (2) (KBr tablets, 2 mg of substance in 350 mg of KBr).

We assume that the L-rhamnose in evonoloside is present in the pyranose form, since the glycoside is hydrolyzed with difficulty by dilute mineral acids. An analysis of the molecular rotations of the glycoside and the aglycone in accordance with Klyne's rule [3] shows that the rhamnose is attached to the aglycone by a L-glycosidic bond. Consequently, evonoloside is cannogenol 3-α-L-rhamnopyranoside, the structure of which can be shown by the formula




## Experimental

For analysis, the substances were dried over phosphorus pentoxide in a vacuum of $1 \times 10^{-2}$ mm Hg at 118° C for 6 hr. In the identification of the sugar component by paper chromatography, the following solvent systems were used: 1) 1-butanol-acetic acid-water (4:1:5); 2) 1-butanol-methyl ethyl ketone-borate buffer (1:1:2). The borate buffer consisted of equal volumes of 0.1 M aqueous $H_3BO_3$ solution and 0.1 M aqueous $Na_2B_4O_7$ solution. When system 2 was used, the paper (type B of the Volodarskii Leningrad Mill No. 2) was impregnated with the "heavy" phase. The cardenolides were chromatographed in the following systems: 3) tetrahydrofuran-chloroform (1:1)/formamide, and 4) methyl ethyl ketone-m-xylene (1:1)/formamide.

Hydrolysis of evonoloside. A solution of 0.66 g of the glycoside in 40 ml of acetone was treated with 0.4 ml of concentrated hydrochloric acid and the mixture was left at room temperature for six days. Then 40 ml of distilled water was added to the hydrolyzate and the acetone was driven off in vacuum at 40–50°C. The cardenolides were extracted with a mixture of ethanol and chloroform (1:4) until the Raymond test was negative. The ethanol–chloroform extract was washed successively with 4 ml of 2N sodium carbonate solution and water (5 × 5 ml), dried over sodium sulfate, and evaporated in vacuum. As was shown by paper chromatography, the residue (0.45 g) consisted of four cardenolides, two of which were present in very small amount. The cardenolides were separated by chromatography on alumina.

The sugar component (L-rhamnose). The chloroform and alcohol traces were eliminated in vacuum from the acid aqueous solution containing the sugar component. The solution was neutralized with freshly-prepared silver carbonate and filtered, the filtrate was treated with hydrogen sulfide for a short time, filtered again, and evaporated in vacuum. The residue was crystallized from ethanol. The monosaccharide obtained (90 mg) melted at 88–95°C, $[\alpha]_D^{20}$ +5.4 ± 3° (c 0.926; in aqueous solution after 30 min); on paper chromatography, it was located at the level of L-rhamnose. A mixture of the substance with authentic L-rhamnose gave no depression of the melting point (88–96° C). The phenylosazone of the monosaccharide melted at 185–186°C; $[\alpha]_D^{20}$ +85.7 ± 8° (c 0.140; pyridine).

Aglycone (cannogenol). The aglycone was separated from traces of other cardenolides by chromatography on alumina (Brockmann activity grade III) with elution by chloroform containing 5% of ethanol, and was crystallized from acetone. The crystals that deposited melted at 235–238°C, $[\alpha]_D^{23}$ +28.3 ± 3° (c 0.846; methanol). The UV spectrum had one maximum at 219 mμ (log ε 4.15).

Found, %: C 70.61; H 9.20; mol. wt. 387.1 (spectroscopic method). Calculated for $C_{23}H_{34}O_5$, %: C 70.74; H 8.77; mol. wt. 390.52.

The aglycone gave a negative reaction with tetranitromethane. On paper chromatography, it was located at the level of an authentic sample of cannogenol. A mixture of these substances showed no depression of the melting point (235–238°C). In concentrated sulfuric acid, the aglycone, like cannogenol, dissolved to give a lemon yellow coloration changing to yellow after 5 min.

$\Delta^{14}$-Anhydrocannogenol. The substance was eluted from alumina with chloroform and was crystallized from alcohol, mp 262–264°C, $[\alpha]_D^{22}$ −12.14 ± 4° (c 0.346; methanol). The compound obtained gave a positive reaction with tetranitromethane. The Tortelli-Jaffe reaction [4] (for the presence of a tetrasubstituted double bond) was negative. The substance dissolved in concentrated sulfuric acid forming a brown coloration which changed to lemon yellow after 5 min and to yellow after 20 min.

Found, %: C 73.87; H 8.61; mol. wt. 371.79 (Rast). Calculated for $C_{23}H_{32}O_4$, %: C 74.16; H 8.66; mol. wt. 372.49.


## Summary

The chemical structure of a new cardiac glycoside, evonoloside, obtained from the seeds of E. europaeus L. has been established. Evonoloside is cannogenol 3-α-L-rhamnopyranoside.


## REFERENCES


1. S. G. Kislichenko, I. F. Makarevich, and D. G. Kolesnikov, KhPS [Chemistry of Natural Compounds], 440, 1966.
2. C. Mannich and G. Siewert, Ber., 75, 736, 1942.
3. W. Klyne, Biochem., J., 47, no. 4, 1950.
4. M. Tortelli and E. Jaffe, Chemiker-Ztg., 39, 14, 1915.



7 July 1966



Khar'kov Chemical and Pharmaceutical Scientific Research Institute