CHEMICAL STUDY OF THE SEEDS OF Euonymus czernjaevii

V. A. Lukanov, I. F. Makarevich, S. G. Kislichenko, and A. A. Reznichenko

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Euonymus czernjaevii Klok., family Celastraceae – a small tree or bush – grows in the Caucasus, in the Black Sea region, and in the lower Don regions of the USSR (Eastern Black Sea endemic plant) [1]. It has been introduced into cultivation in the Khar'kov and some other oblasts. We have found no information on the chemical study of this plant in the literature available to us.

We investigated seeds collected in the Khar'kov oblast. By extraction of them with ethanol, purification of the extracts with alumina, and separation by chromatography on cellulose, we isolated in the individual state a cardiac glycoside identified as evonoside. The yield of evonoside was 0.05% of the weight of the raw material. Other cardenolides were found only in trace amounts. It is a fairly rare phenomenon for a plant to contain practically only one cardiac glycoside. In this respect, Euonymus medirossika Klok. differs sharply from the species under study, since it contains more than eight cardiac glycosides and aglycones, six of which have been isolated preparatively [2-4]. These differences in cardenolide composition can apparently be used in the chemotaxonomy of the genus Euonymus.

A native disaccharide was isolated which was identified by its properties, IR spectrum, and hydrolysis products as sucrose. The sucrose content was considerable – more than 5%.

Extraction with petroleum ether gave a fatty oil (28.2% of the weight of the raw material). Its fatty-acid composition was determined by gas–liquid chromatography. The methyl esters of the acids were obtained by the direct transesterification of the triglycerides of the oil with methanol in the presence of caustic potash [5]. The reaction was performed in an inert gas medium preventing the oxidation of the unsaturated compounds. The fatty-acid esters were identified by using authentic samples and tables of relative retention volumes [6].

The quantitative determination was performed by the method of internal normalization. The area under the peak was found from the product of the height of the peak and the width at half-height. In this way, we found the following amounts of acids: palmitic 10.34%, stearic 2.58%, oleic 63.16%, linoleic 18.7%, linolenic 5.25%. The peaks on the chromatogram are 2, 3, 4, 5, and 6, respectively.

EXPERIMENTAL

The following solvent systems were used for paper chromatography: 1) toluene–butan-1-ol (1:1)/water; 2) chloroform–tetrahydrofuran (1:1)/formamide; and 3) butan-1-ol–acetic acid (4:1)/water [7].

Isolation of the Substances from the Plant. The comminuted seeds were treated with petroleum ether by the percolation method. The petroleum ether solutions were evaporated, giving the fatty oil (28.2%).

The defatted raw material was dried in the air and was then extracted with 95% and with 80% ethanol until the bitter taste had disappeared. When the extracts obtained with the 96% ethanol were dried, a crystalline residue of sucrose deposited, which was separated off and was washed with ethanol. The combined solutions were evaporated to an aqueous residue. The latter was treated three times with petroleum ether. The cardenolides were extracted with a mixture of ethanol and chloroform (1:2). To improve their passage

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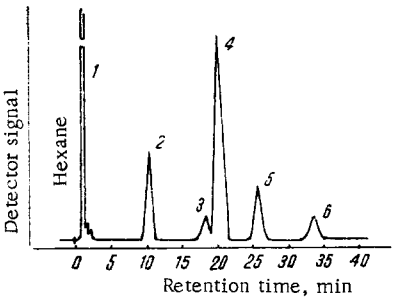

Fig. 1. Gas–liquid chromatogram of the fatty-acid composition of the oil of the euonymus.

into the organic solvent, sodium sulfate was added to the aqueous solution. The ethanolic chloroform extracts were dried with anhydrous sodium sulfate and evaporated. The residue was dissolved in water and the solution was filtered through a layer of alumina (fivefold amount). The cardenolides were extracted from the aqueous solution similarly. The results of a chromatographic analysis (systems 1 and 2) of the extracts showed that they contained cardenolides having the same R_f values as evonoside, and only trace amounts of three other cardenolides. The residue obtained after the evaporation of the ethanolic chloroform solutions was chromatographed on a column of cellulose (300-fold amount) in the toluene–butan-1-ol (1:1)/water system. The fractions were collected by means of an automatic collector. Pure evonoside was isolated chromatographically (0.05% of the weight of the raw material).

Evonoside. The glycoside consisted of a white amorphous powder with $[\alpha]_D$ −34.2 ± 4° (c 0.6; methanol); $R_{evonoside}$ 1.00 (system 1; comparison with a sample kindly given to us by Prof. T. Reichstein). To confirm the identity of the product obtained with evonoside, enzymatic hydrolysis was performed. The glycoside and an equal amount of an enzyme preparation from the pancreatic juice of the grape snail was dissolved in water and left in a thermostat at 42°C for 22 h. The enzymes were precipitated with hot ethanol, and the solution was evaporated. The results of chromatographic analysis (systems 2 and 3) showed that the hydrolyzate consisted of evomonoside and D-glucose.

Sucrose. The disaccharide was recrystallized from 90% ethanol, after which its mp was 189-190°C, $[\alpha]_D$ + 66.5 ± 2° (c 2.0; water). IR spectrum (taken by I. P. Kovalev on a IR = 27G spectrometer in the 4000-400-cm^{-1} region, KBr tablets; 1 mg of substance per 200 mg of KBr) was identical to that of authentic sucrose.

The sucrose obtained was hydrolyzed with 0.5 N sulfuric acid at 100°C for 1.5 h. The acid was neutralized with barium carbonate, and the solution was filtered through a layer of kieselguhr and evaporated. According to paper chromatography (system 3) the residue consisted of a mixture of D-glucose and D-fructose (comparison with authentic samples).

Preparation of the Methyl Esters of the Fatty Acids of the Oil. With stirring and the passage of nitrogen, a solution of potassium hydroxide in absolute methanol was gradually added to the oil which had been dried and heated to 80°C, 10 g of absolute methanol and 0.5 g of KOH being used per 50 g of oil. Heating the reaction mixture to 80-90°C was continued for 3 h. Then the excess of alkali was neutralized with 5% sulfuric acid, and the methanol was driven off by evaporation in vacuum with heating. After the mixture had cooled to 20°C, a layer of glycerol formed on the bottom of the flask. The upper layer consisted of the crude methyl esters.

GLC of the Esters of the Fatty Acids of the Oil. Chromatographic conditions: flame ionization detector (St^{90}), column filling – Celite AW 100-120 mesh (British Standard) impregnated with poly(ethylene adipate) (15%), column 1200 × 4 mm, column temperature 173.5°C, carrier gas argon, rate of flow of the carrier gas 80 ml/min, speed of the paper strip 5 sec/h, weight of a sample 0.4 μl of a solution of the crude methyl esters of the fatty acids in hexane (1:4).

SUMMARY

From the seends of Euonymus czernjaevii Klok. we have isolated the cardiac glycoside evonoside (0.05% of the weight of the raw material), sucrose (about 5%), and a fatty oil (28%). It has been established that the fatty oil consists of triglycerides of the following acids: palmitic (10.34%), stearic (2.58%), oleic (63.16%), linoleic (18.70%), and linolenic (5.25%).

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