# ISOLATION OF DIOSMIN FROM PLANTS OF THE GENUS *Vicia* AND *Hyssopus officinalis* AND ITS INFLUENCE ON BLOOD COAGULATION

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Modern medicine uses flavonoid compounds for treating many diseases, which exhibit a wide spectrum of biological action. One of the representatives of this class is diosmin (5,7,3′-trihydroxy-4′-methoxyflavone rhamnoglucoside); it is the major active principle of a number of drugs produced in France (Daflone, Vendetrex, etc.) for treatment of phlebitis, hemorrhoid, preulcer state, diabetes, and some gynecological pathologies [1]. Taking into account the high incidence of these diseases in Russia, we initiated a search for new diosmin sources. Since flavonoids are widely distributed in plants, screening was carried out for a number of wild and cultivated plants of North Caucasus and Transcaucasus. It was found that some representatives of the genus Vicia, for example Asian vetch, and Hyssopus officinalis contained diosmin in sufficient concentrations (to 2%) for industrial production. The procedure was developed for isolation of diosmin from the above-ground organs of these plants (see Schemes 1 and 2).

According to these schemes, the procedure for diosmin isolation implies that the air-dried raw material degreased with chloroform is treated with aqueous ethanol or water (and subsequently with 96% ethanol), and then diosmin is extracted from the raw material with dimethyl sulfoxide (DMSO).

Since diosmin, unlike most flavonoid compounds, is very poorly soluble both in polar and nonpolar protic solvents, but freely soluble in some aprotic solvents, for example, dimethyl sulfoxide, this procedure makes it possible to separate diosmin rather easily from accompanying flavonoids.

Since almost all diseases requiring diosmin for treatment are connected with circulatory disturbances in venous vessels, and also due to the absence of literature data on the influence of diosmin on blood coagulation, we carried out a

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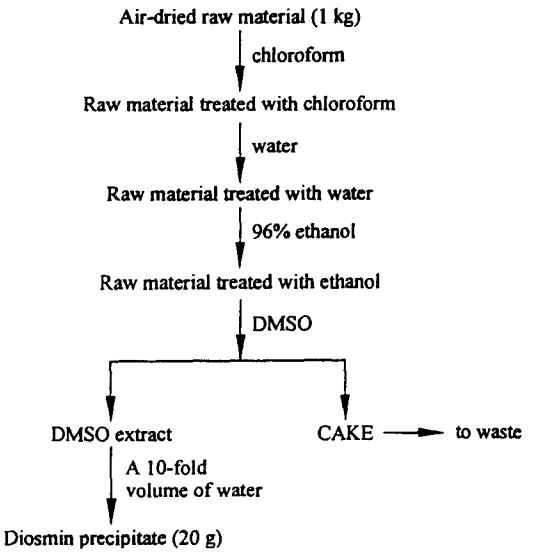
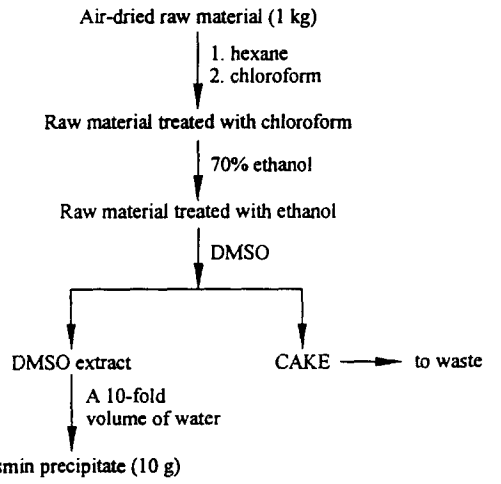



**Scheme 1.** Isolation of diosmin from the above-ground organs of Asian vetch.




**Scheme 2.** Isolation of diosmin from the above-ground organs of *Hyssopus officinalis*.



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pharmacologic study on the influence of different diosmin concentrations on blood coagulation (see Table 1).

The results of investigations show that the diosmin obtained by us causes (in a dose of 2 mg/kg) a decrease in the activity of the blood coagulation system. This is manifested by an increase in the blood coagulation time by 32% due to a more prolonged period for achieving the coagulation end point. Diosmin in a dose of 10 mg/kg increases significantly one of four characteristics, the coagulation time. The compound studied in a dose of 100 mg/kg enhances the activity of the blood coagulation system. This is manifested by 17% reduction of the coagulation time.

The above doses of diosmin ambiguously influence fibrinolysis: in a dose of 2 mg/kg, the drug depresses fibrinolysis (by 62%), in a dose of 10 mg/kg, the drug does not change anything, and in a dose of 100 mg/kg, diosmin stimulates the onset of blood clot retraction (by 29%).

Experimental data [2] indicate that the therapeutic action of diosmin is attributed to the inhibitory effect on capture and metabolism of noradrenaline by the venous walls. This results in an increase in the vascular tonus with favorable consequences, reduction of elasticity and permeability. Our experiments with white rats demonstrated the reduction of activity of the blood coagulation system in low doses of the administered compound. This fact can be one of the positive drug effects in circulatory disturbances in venous vessels.

Diosmin in relatively small doses (2 and 10 mg/kg) enhances the coagulation time and inhibits the onset of fibrinolysis; in high doses (100 mg/kg) diosmin activates blood coagulation and fibrinolysis.

The resulting data show that diosmin is similar to heparin in its action on blood coagulation and fibrinolysis; however, unlike heparin, diosmin, as a flavonoid, has low toxicity and minimum side effect.

The high content of diosmin in the above-mentioned plants enables one to enlarge resources for the production of cardiovascular drugs, the demand for which grows year after year.

## EXPERIMENTAL CHEMICAL PART

The melting point of the isolated compound was determined with a Koffler unit. The UV spectra were recorded on a SF-4A spectrophotometer. The IR spectra were measured on a UR-20 spectrophotometer (KBr tablets). The TLC was performed on Silufol UV-254 plates in a chloroform : ethanol : water mixture, 100 : 50 : 2. Visualization was by iodine vapors. The elemental analysis data corresponded to the calculated values.

### Isolation of Diosmin from the Plant Raw Material

**First procedure.** A sample of 1 kg of air-dried raw material, the above-ground organs of Asian vetch collected on alpine meadows (Armenia), was finely divided to 3 – 4 mm and degreased with chloroform in a Soxhlet apparatus. The raw material was dried at room temperature to complete removal of the solvent, then water was added, and extraction was done at 50°C three times over a period of 30 min (each time). After treatment with water, full extraction was performed with 96% ethanol in a Soxhlet apparatus, then extraction with dimethyl sulfoxide was carried out three times over a period of 30 min (each time) in a water bath (50°C). The extracts obtained were combined and poured into a tenfold amount of water. A crystalline precipitate of diosmin was separated in 48 h.

**Second procedure.** The air-dried raw material, the above-ground organs of Hyssopus officinalis collected and finely divided to 3 – 4 mm, was degreased with petroleum ether (or hexane) and then with chloroform in a Soxhlet apparatus. The dried degreased raw material was fully extracted with 70% alcohol in the flask with a reflux condenser in a water bath. Dimethyl sulfoxide was poured over the cake, and the latter was kept for 48 h at room temperature with periodic stirring. The obtained extract was poured into water (tenfold volume), kept for 48 h, and diosmin was filtered off.

Diosmin isolated according to both procedures was purified by recrystallization from DMSO–water and DMSO–methanol mixtures, and then washed with ethanol. The yield of the compound was determined gravimetrically.

Diosmin, 3′5,7-trihydroxy-4′-methoxyflavone 7-rutinoside, is a white crystalline powder. Empirical formula $C_{28}H_{32}O_{15}$; m.p. 290 – 293°C (DMSO–MeOH); $R_f$ 0.39; the UV spectrum (ethanol), $\lambda_{max}$, nm: $C_2H_5OH$ – 345, 254; the IR spectrum, $cm^{-1}$: 3250 (–OH), 2983 ($-OCH_3$), 1650 (C=O), 1605, 1510, 1450 (C=C arom.).

TABLE 1. Influence of Diosmin on Characteristics of Blood Coagulation and Anticoagulation Systems ($M \pm m$)

| Compound | Onset of coagulation, sec | End of coagulation, sec | Coagulation time, sec | Onset of retraction and fibrinolysis, sec |
|---|---|---|---|---|
| Control, physiological solution | 40 ± 7.0 | 206 ± 14.4 | 166 ± 7.6 | 324 ± 19.3 |
| Diosmin, 2 mg/kg | 36 ± 4.3 | 255 ± 7.6* | 219 ± 3.2* | 524 ± 43.2* |
| Control physiological solution | 62 ± 12.1 | 240 ± 8.1 | 178 ± 3.6 | 300 ± 16.9 |
| Diosmin, 10 mg/kg | 43 ± 6.8 | 277 ± 20.3 | 234 ± 9.7* | 352 ± 47.2 |
| Control physiological solution | 69 ± 16 | 258 ± 27.7 | 189 ± 11.9 | 412 ± 54.4 |
| Diosmin 100 mg/kg | 53 ± 6.1 | 210 ± 8.3 | 157 ± 2.2* | 293 ± 9.5 |

* $p < 0.05$

The isolated diosmin corresponds to the reference sample in physicochemical properties. The data obtained are consistent with literature data [3].

## EXPERIMENTAL BIOLOGICAL PART

Experiments were performed on 36 vigilant white rats (6 groups of 6 animals) weighing 200 – 220 g. The blood was drawn from the sublingual vein in amounts of 3 drops and placed into a coagulograph H-334 cell. The method of electrocoagulography was used to determine the following characteristics: the time of onset of blood coagulation, the time of completion of blood coagulation, the period of blood coagulation, and the time of onset of retraction and fibrinolysis.

Diosmin was administered intraperitoneally in doses of 2, 10, 100 mg / kg of animal body mass 60 min before the blood sampling. A physiological solution was administered in similar doses in control tests.

## REFERENCES

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