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GRAVIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE QUANTITATIVE DETERMINATION OF TRITERPENE GLYCOSIDES IN THE FRUIT OF *Sophora japonica* AND OTHER PLANTS

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For the quantitative determination of triterpene glycosides in the fruit of the Japanese pagoda tree, Sophora japonica, *and of other plants, gravimetric and spectrophotometric methods of analysis with an error of the determination of the total glycosides of about 10% are proposed.*

At the present time, in view of the established immunomodulating activity of the fruit of *Sophora japonica* L. (fam. Fabaceae) [1], its use in clinical practice has increased, which has required the development of methods for the standardization of the raw plant material and preparations for the main group of biologically active substances — triterpene glycosides (TTGs). We have previously proposed a method for the semiquantitative determination of TTGs based on TLC analysis [2]. However, its accuracy is sufficient only for preliminary quick analyses.

In the present paper we propose two variants of the quantitative determination of TTGs in *Sophora* fruit, based on the selective extraction of these substances, followed by the use of the gravimetric method and the spectrophotometry of colored derivatives. Attempts at the direct application of spectrophotometry to total alcoholic extracts of *Sophora* fruit proved unsuccessful in view of the relatively low level of TTGs (not more than 1%) and the high level of phenolic compounds, flavones, and fatty oils [3].

For the use of the above-mentioned methods, we have developed a scheme of selective extraction from the plant raw material of the TTGs free from flavonoids (rutin), oils, and inorganic impurities (salts). The plant raw material (seeds) was ground and was defatted with hexane or heptane, and then the total TTGs and the accompanying phenolic glycosides were extracted with water-saturated butanol. After elimination of the accompanying substances, the total TTGs were obtained in the form of a white, amorphous, nonhygroscopic powder, this being the form for gravimetry.

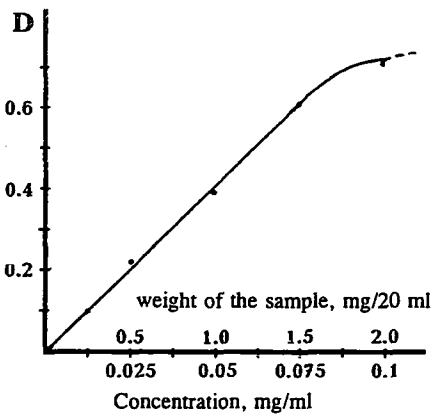

Fig. 1. Calibration curve of the dependence of the optical density *D* on the weight (concentration) of a sample of soyasapogenol-B.

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In view of the fact that *S. japonica* seeds contain mainly glycosides of the triterpene soyasapogenol-B with one, two, or three monosaccharide residues and their specific activity is based on the aglycon moiety, we propose a spectrophotometric method of determining TTGs, calculated as soyasapogenol-B, based on the formation of a colored reaction product with molybdophosphoric acid in a mixture of acetic anhydride and acetic acid. The resulting absorption at λ_{max} 650 nm (blue color of the solution) is due to the aglycon moiety of the glycosides. A calibration curve constructed for various weighed samples of soyasapogenol-B (Fig. 1) is linear up to a concentration of 0.075 mg/ml (1.50 mg in 20 ml of reaction mixture). Formulas for calculating the amount of TTGs in the raw material by this method are given in the Experimental part.

The proposed spectrophotometric method of determining total TTGs can be extended (with other standards) to other plant materials, as well. We have applied this method successfully to the quantitative determination of TTGs in various species and individual organs of one species of plants of the genus *Hedera* L.

EXPERIMENTAL

Absorption spectra were recorded on a Specord UV-VIS instrument. For TLC we used Silufol plates and the solvent mixture chloroform–methanol–25% ammonia (100:45:15). The spots on the chromatograms were detected by spraying with a 20% alcoholic solution of molybdophosphoric acid, followed by heating.

Isolation of the Pure Total Triterpene Glycosides (TTGs) from *Sophora japonica* Seeds. *Sophora japonica* fruit was gathered in accordance with the requirements of pharmaceutical standard FS 42-452-72 in the stage of full ripeness. The seeds containing the TTGs were separated from the pericarp (flesh of the fruit, containing no TTGs) and were ground to a particle size of about 0.25 mm. An accurately weighed sample of the ground seed powder (about 10 g) was defatted three times with 50-ml portions of hexane or heptane, the residue being filtered off with suction on a glass filter. The glycosides were extracted from the defatted material with three 50-ml portions of water-saturated butanol.

The combined butanolic extracts were washed three times with an equal volume of 10% aqueous ammonia (until the aqueous ammoniacal layer was colorless). The presence of TTGs in the butanol layer and the passage of phenolic compounds into the aqueous layer were checked by TLC. The purified butanol layer was separated off and was evaporated to dryness in vacuum. The residue (about 40 mg) was dissolved in 4 ml of methanol, and the TTGs were precipitated with 40 ml of acetone. The precipitate of pure TTGs (about 20 mg) was separated off by centrifugation or by filtration with suction, and was washed with dry acetone and dried in vacuum. The amount of total TTGs in the raw material was calculated from the formula $(m/P)\cdot 100\%$, where m is the weight of the pure TTGs, and P is the weight of the sample of ground seeds.

Spectrophotometric Determination of TTGs in *Sophora* Seeds. To construct a calibration graph, samples of soyasapogenol-B weighing 0.25, 0.50, 1.00, 1.50, and 2.00 mg were each dissolved in 20 ml of a saturated solution of molybdophosphoric acid in a mixture of acetic anhydride and acetic acid (7:3 by volume), and the resulting solution was heated at 115°C for 3 h and was then photometered at 650 nm in a 2-cm cell (see Fig. 1). The dependence of the optical density D on the weight of the sample m so found is described by the equation $D = 0.4m$ or $m = 2.5D$.

To calculate the amount of total TTGs in the raw material as soyasapogenol-B equivalent, the precipitate of purified TTGs was dissolved in 5 ml of methanol, and an aliquot (1 ml) was added to 19 ml of a saturated solution of molybdophosphoric acid in a mixture of acetic anhydride and acetic acid (7:3 by volume), the solution was heated at 115°C for 3 h, and the optical density at 650 nm was determined in a 2-cm cell. As the comparison solution, in both cases, we used a saturated solution of molybdophosphoric acid in a mixture of acetic anhydride and acetic acid (7:3), which was likewise heated at 115°C for 3 h. Then the weight of the sample in the aliquot was $2.5D$, that in the whole precipitate $5\cdot 2.5D$ or $12.5D$ and the TTG content of the seeds $(12.5D/P)\cdot 100\%$, where D is the optical density of the solution and P the weight of the seeds.

Evaluation of the Accuracy of the Gravimetric and Spectrophotometric Methods of Determining the TTG Content of the Raw Material. The accuracy of both methods is determined mainly by random errors, which, according to statistical estimates, usually do not exceed 5%. Estimation of the systematic errors of the proposed methods causes certain difficulties in connection with the absence of high-precision, specific, or direct methods for the analysis of triterpene glycosides. In the gravimetric method the error will be determined by the level of impurities in the purified TTGs, which, according to TLC estimates, does not exceed a few parts per cent. In the spectrophotometric method the systematic error will be determined by the purity of the standard used (soyasapogenol-B) and likewise does not exceed a few parts per cent. Then the total error of determining TTGs will not usually exceed 10%.

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