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EXTRACTIVE SUBSTANCES OF LARIX DAHURICA

II. Quantitative Contents of Quercetin and Dihydroquercetin

N. A. Tyukavkina, K. I. Lapteva, V. A. Larina, and N. G. Devyatko

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Dihydroquercetin and quercetin are the predominant components of the flavonoid mixture isolated from the extractive substances of the heartwood of Larix dahurica (Dahurian larch) [1]. To determine their amounts quantitatively, we have used the photocolorimetric method, with thin-layer chromatography to separate the mixture into individual components and subsequent elution of the spots. Previously, the mixture was separated by chromatography on paper with subsequent elution of the spots by alcoholic solvents [2] or by eluting the components from a column filled with Kapron powder [3].

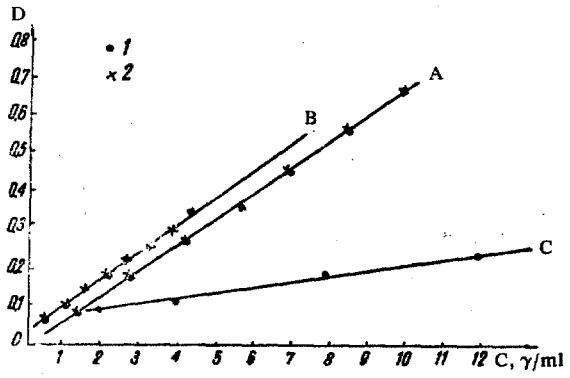

Fig. 1. Calibration curves for quercetin and dihydroquercetin. A) Quercetin in methanol–dimethylformamide (DMFA) (1:1) solution, 364 mμ; B) quercetin in methanol–DMFA (1:1) solution with the addition of $AlCl_3$, 430 mμ; dihydroquercetin in methanol–DMFA (1:1) solution, 315 mμ. 1) Solutions of the flavonoids eluted from Kapron powder; 2) standard solutions.

As compared with paper chromatography, thin-layer chromatography enables separation and elution to be considerably quickened (to 1.5–2 hr instead of 18–20 hr) and the irreversible sorption of the flavonoids by the paper [4] to be avoided.

The flavonoids of the Dahurian larch are well separated on a thin non-fixed layer of Kapron powder in the ethanol–chloroform (35:65) system. Of the large number of solvents tested as eluting agents, the most suitable is 1:1 (vol/vol) mixture of the polar compounds methanol and dimethylformamide. Glycosidated flavonoids are eluted from Kapron powder somewhat more readily (with hot methanol) [5], while the aglycones are not desorbed at all by methanol without the addition of dimethylformamide. Figure 1 shows, for example, that the calibration curve constructed with a standard solution of quercetin accurately coincides with the curve constructed for the same solutions after chromatography and elution of the spots from the Kapron (cf. Fig. 1A, 1B). The same result is obtained when a calibration curve is constructed for dihydroquercetin. After the elution of the flavonoids, the Kapron is practically regenerated.

Figure 1, curves A, B, and C show that the Bouguer-Beer law is obeyed over a wide range of concentrations, i.e., from 2 to 10 γ/ml. Any of the known complex-forming agents may be used in the process of colorimetry. We chose aluminum chloride. However, dimethylformamide does not play only the part of a solvent. A solution of quercetin in methanol acquires a more intense coloration when dimethylformamide is added, the optical density of the solution falling somewhat in the first 3–5 min and then remaining constant for several hours. This enabled us to make calibration curves without the addition of aluminum chloride to the solutions, as well (cf. Fig. 1A, 1C).

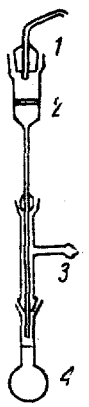

Fig. 2. Elution of the flavonoids formed on Kapron powder. 1) Ground stopper with a tube for sucking up the powder; 2) funnel with a No. 4 glass filter; 3) connecting-piece with a vacuum-sidearm; 4) 50-ml measuring flask.

By using the results obtained for quercetin and dihydroquercetin separately, we determined the amounts in a mixture of larch flavonoids. It was found that the flavonoids of the heart of Dahurian larch contained 69% of dihydroquercetin and 11% of quercetin (of the total flavonoids).

Experimental

The optical densities were measured in a FEK-56 photoelectric colorimeter (1.0-cm cell) at 364 mμ for quercetin and 315 mμ for dihydroquercetin in a mixture of methanol and dimethylformamide (DMFA), and at 430 and 315 mμ, respectively, with the addition of aluminum chloride to the solution.

Chromatography. The separation of the mixture of flavonoids for the treatment of an individual component in model experiments was carried out on a nonfixed layer of Kapron powder (layer thickness 1.0 cm) deposited on a glass plate (35 × 6 cm) by the method described by Davidek and Procházka [6]. The particle size of the Kapron was 0.2 mm and the solvent system was ethanol–chloroform (35:65). The spots were detected by their fluorescence in UV light. The rise of the solvent to a height of 35 cm took 1.5–2 hr. The substance was deposited at the start in the form of an ethanolic solution by means of a measuring capillary.

Elution of the flavonoid spots. Under UV illumination, the area of a spot occupied by a component was outlined, and the Kapron powder containing it was transferred quantitatively to funnel 2 (Fig. 2) with a No. 4 glass filter. The powder was sucked through tube 1 through the application of a slight vacuum at the end of the funnel. Then the funnel was connected to the 50-ml measuring flask 4 by means of the connecting-piece 3. With the side-arm of 3 shut, 20 ml of a mixture of methanol and DMFA (1:1) was poured into the funnel and after 30 min steeping with periodic stirring the solvent was run into the flask (tube 3 was removed). This operation was repeated three times with 10 ml of the mixture each time. The last portion of solvent was kept for 15 min and run off, and complete filtration was achieved by applying a slight vacuum through the side-arm of 3 for a few minutes. The solution was made up to the mark by the addition of a few drops of the solvent.

Colorimetric measurements. A. Calibration curves were constructed of standard solutions of quercetin and dihydroquercetin. The initial solutions were prepared in ethanol in a 1.0-ml pycnometer with 0.0136 g of quercetin dihydrate (concentration 3.95×10^{-2} M) and 0.747 g of dihydroquercetin (2.17×10^{-1} M). The solution obtained were used for colorimetric measurement, both with the addition of $AlCl_3$ (cf. Fig. 1B) and without it (cf. Fig. 1A, 1C). Solutions with lower concentrations which were also subjected to colorimetry were obtained by diluting the initial solution. It was found that in the case of quercetin the Bouguer-Beer law was satisfied over the range from 2 to 10 γ, while for dihydroquercetin this range was considerably wider, from 2 to 20 γ (in Fig. 1C we have shown it only up to 12 γ).

B. The calibration curves were recorded for the flavonoids eluted from a layer of Kapron. For this purpose the spot of quercetin (or dihydroquercetin) obtained after the chromatography of a definitely known amount of the component was eluted by the method described above into a 50-ml measuring flask and from this solution were taken 20-ml portions which, in turn, were diluted to 50 ml with a 0.01 M solution of $AlCl_3$ [4]. After 10 min, the solution was examined in a photoelectric colorimeter for quercetin (430 mμ filter, Fig. 1B) and for dihydroquercetin (315 mμ filter).

C. A 1.0-cm cell was filled with a solution of the eluted flavonoids and colorimetry was carried out for quercetin (364 mμ filter, Fig. 1A) and for dihydroquercetin (315 mμ filter, Fig. 1C).

Determination of quercetin and dihydroquercetin. The flavonoid fractions were separated by treatment of an acetone extract of the wood of Larix dahurica successively with ether and benzene [1]. The material investigated in this work was the total flavonoids of the benzene-insoluble fraction. They were chromatographed under the conditions described above. For this purpose, an ethanolic solution of the mixture of flavonoids (0.4063 g of material) was prepared in a 10-ml pycnometer. At each of ten points on the chromatogram, 0.003 ml of the solution prepared was deposited by a measuring capillary. Consequently, the total amount of solution used was 0.03 ml, containing 0.00122 g of flavonoids. The eluted solutions from the ten quercetin spots had a density D = 0.15, which corresponded to 2.4 γ/ml on Fig. 1, curve A. The density of the dihydroquercetin solution was 0.32, which corresponded to a concentration of 15.3 γ/ml on Fig. 1, curve C. Calculation then showed that the total flavonoids studied contained 11% of quercetin and 69% of dihydroquercetin (on the total flavonoids).

After completing our work and writing the present paper, we became aware of a communication from Drawert, Heiman, and Ziegler [7] in which a spectrophotometric method for the quantitative determination of the glycosidated flavonoids likewise using thin-layer chromatography is described.

Summary

The quantitative determination of quercetin and dihydroquercetin in the total flavonoids of Latrix dahurica has been effected by the use of thin-layer chromatography on Kapron for separating the mixture with subsequent elution of the spots and colorimetry of the eluates obtained. The methanol–dimethylformamide (1:1) system was used as the eluate. It has been found that the flavonoids of the heartwood of Latrix dahurica contain a predominating amount, 69% of dihydroquercetin, the amount of quercetin being 11%.

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Kirkutsk State University; Institute of Petroleum and Coal Chemicals Synthesis at the University