SPECTROPHOTOMETRIC DETERMINATION OF THE FUROCOUMARINS OF PSORALEA DRUPACEAE AND FICUS CARICA

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We have previously described a photometric method for the separate determination of psoralen and angelicin in Psoralea drupaceae Bge [1] and of psoralen and bergapten in Ficus carica L. [2].

In this paper we describe a spectrophotometric method we have developed for determining the furocoumarins in these plants. Of the absorption maxima present in the UV spectra of psoralen ($\lambda_{max}^{C_2H_5OH}$ 207, 246, 291, 330 mμ [3, 4]) and angelicin ($\lambda_{max}^{C_2H_5OH}$ 207, 246, 299 mμ [3]), the most suitable for spectrometric investigations is the maximum at 246 mμ (log ε 4.64*), which has the same position and the same intensity in two compounds. Bergapten has a somewhat different spectrum with five absorption bands ($\lambda_{max}^{C_2H_5OH}$ 223, 251, 261, 269, 315 mμ). For analytical determinations it is possible to use the maximum at 223 mμ (log ε 4.43*) or that at 315 mμ (log ε 4.23*) as was done in the determination of the furocoumarins in the seeds of Ammi majus L. [5]. A method for the determination of a mixture of psoralen and bergapten without their separation by measuring the intensities of absorption at 280 and 310 mμ is known [6, 7].

As in the previous photometric determinations [1, 2], in this method the plant extract is chromatographed on paper without any preliminary purification. The desorption of the furocoumarins from the paper is practically total [1]. However, in working with highly diluted solutions, even very small losses on the paper may distort the results and it is desirable to chromatograph solutions of the pure furocoumarins in concentrations close to those in the extracts under investigation on a separate sheet of paper and to use the eluates as standard solutions. Within the concentration range of 0.003–0.0003 mg/ml, the optical density of ethanolic solutions of the furocoumarins under consideration is directly proportional to their concentration, i.e., Beer's law is observed.

The objectivity of the method was tested in experiments with additions. The mean relative error is ±2.0%. The results of the determination of the furocoumarins by the spectrophotometric method in the seeds and roots of P. drupaceae Bge. and also in the leaves of F. carica L. are comparable within the limits of error of the analysis with those obtained by the use of colorimetric methods [1, 2].

EXPERIMENTAL

Preparation of the extract. An accurately-weighed sample (5 g) of the air-dry raw material ground in a coffee mill and passed through a sieve (with apertures 1.0–1.5 mm in diameter) was transferred to a 50-ml measuring flask and covered with 35 ml of ethanol. In the analysis of the Psoralea seeds, 80% ethanol was used and in the analysis of the leaves of F. carica L. and the roots of P. drupaceae Bge., 95% ethanol was used. After being shaken for 3 hr the flasks were filled to the mark with the same ethanol and their contents were well mixed. The extract was filtered through a no. 1 glass filter and subjected to chromatograph separation.

Chromatography. The starting line was marked on a narrow side of type "B" chromatographic paper 7 cm from the upper surface of the sheet. On this line, 3.5 cm from both sides (at a width of the strip of 15 cm) 0.5 ml of an extract of the roots (leaves) or 0.2 ml of an extract of the seeds was deposited in the form of a continuous line by means of a micropipette. The position of deposition was dried slightly and then the whole sheet was sprayed with 15–20 ml of a 20% aqueous solution of ethylene glycol. This method of impregnation, even after the deposition of the extract, proved more convenient than the preliminary preparation of the sheet before the application of the solution under investigation. The paper was dried at room temperature for 1 hr and was then placed in a chromatographic chamber. Petroleum ether (fraction with bp 40–70° C) was used as the mobile phase.

The n-hexane–benzene–methanol (5:4:1) system on paper previously impregnated with 10% formamide solution used in the previous colorimetric determinations [1, 2] is unsuitable in this case because of the considerable

*Our results, obtained on a "Hitachi" EPS-3T spectrophotometer, differ somewhat with respect to intensities from those given in the literature.

adsorption of formamide in UV light. Chromatography (descending method) was carried out until the front had reached a line 2–3 cm from the lower edge of the paper. The chromatogram was removed and dried in the air for 2–3 min and then in the drying chest at 80° C for 30 min. The dried chromatogram was observed in UV light (UI-1 ultrachemiscope) and the positions of the two parallel bands of the furocoumarins were marked. The spot closer to the start corresponds to psoralen (blue fluorescence) and that further away to angelicin or bergapten (fluorescing light blue and light green, respectively). The sections of the paper with the spots of the furocoumarins were cut into small pieces and covered with 30 ml of 95% ethanol in separate flasks. Elution from the paper was carried out at room temperature by shaking for 2 hr on a vibration shaking machine. The eluate was filtered through a no. 1 glass filter with an inserted paper filter.

Preparation of the standard solutions. The standard solutions were prepared in ethanol from repeatedly-recrystallized and chromatographically homogeneous furocoumarins. For this purpose, 4.5 mg (accurately weighed) of each compound was dissolved separately in a 25 ml measuring flask; 0.5-ml samples of these solutions were chromatographed on paper by the method described above. Eluates with a concentration of 0.003 mg/ml were used as standard solutions. The solutions of the individual furocoumarins are stable for a long time on storage in a dark place.

Spectrophotometric determination. The optical densities of the experimental and standard solutions were measured on an SF-4A instrument in 95% ethanol at wavelengths of 246 mμ (psoralen and angelicin) and 223 mμ (bergapten) in a cell with a layer thickness of 1 cm.

The percentage content (x) of the individual furocoumarins in the raw material was calculated from the formula

$$x = \frac{a \cdot b \cdot c_{st} \cdot D_{el}}{10 \cdot m \cdot v \cdot D_{st}},$$

where: a is the total volume of the ethanolic extract of the plant (in the method described, $a = 50$), ml;

b is the volume of ethanol used for the elution of the individual furocoumarins from the paper (in the method given, b = 30), ml;

c_{st} is the concentration of standard solution, mg/ml;

m is the weight of the plant sample, g;

v is the amount of solution deposited on the paper, ml;

D_{el} and D_{st} are the optical densities of the eluate and of the standard solution.

CONCLUSIONS

A spectrophotometric method for the separate quantitative determination of the furocoumarins of Psoralea drupaceae Bge. and Ficus carica L. has been developed.

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