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SEPARATE DETERMINATION OF THE FUROCOUMARINS OF FICUS CARICA

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The basis of all materials with a photosensitizing action used for the treatment of vitiligo consists of furocoumarins, but these do not all possess the capacity for activating the pigment-forming process to the same extent. Musajo and Rodighiero [1], using tests which were simple but duplicated one another, studied the photodynamic activity of a large number of furocoumarins and their derivatives. They found that the strongest activity was possessed by psoralen, followed by xanthotoxin and bergapten (with approximately 30-40% of the activity of psoralen), while the other natural furocoumarins had slight activity or were practically inactive.

In a search for a cheap and accessible source of raw material for the production of a substance with a photosensitizing action, we turned our attention to Ficus carica L. (fig.), family Moraceae; this is a well known fruit plant of the southern republic of the USSR, growing in both the wild and the cultured form. Okahara has

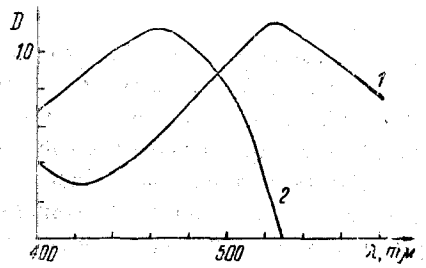

Fig. 1. Absorption spectra of the azo compounds of the fourocoumarins with sulfanilic acid. 1) Psoralen (c 0.015 mg/ml); 2) bergapten (c 0.02 mg/ml).

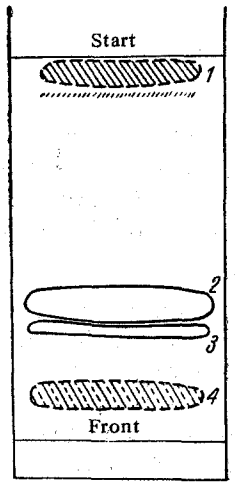

Fig. 2. Chromatogram of an ethanolic extract of the leaves of Ficus carica. 1) Substances reacting with the diazo reagent of a noncoumarin nature; 2) psoralen; 3) bergapten; 4) chlorophyll.

previously shown [2, 3] that fig leaves contain a mixture of psoralen and bergapten which, as is now found [1], is an extremely favorable combination of furocoumarins for the treatment of vitiligo. Subsequently, the same furocoumarins were found in fig leaves by other workers [4-7]. Nevertheless, the data of different authors vary considerably in their quantitative evaluation of the amount of furocoumarins in the plant.

We have previously [8] described a method for the separate determination of the furocoumarins of Psoralea drupaceae Bge. This method can be extended to the furocoumarins of the fig. The difference in the composition of the furocoumarins, i.e., the fact that one plant contains a mixture of psoralen and angelicin and the other psoralen and bergapten, is not of decisive importance. With sulfanilic acid, bergapten gives a light brown azo dye with an absorption maximum at 462 mμ (Fig 1). The optical density of this dye can be measured with the same blue-green filter as that of the azo dye from angelicin.

The methods that we have developed have some advantages. It is not necessary to purify the extract of foreign substances. An alcoholic extract of the plant raw material is deposited on chromatographic paper. The individual furocoumarins eluted from the paper are determined colorimetrically. The foreign compounds that react with the diazo reagent remain in the starting region, and the green coloration of chlorophyll moves just behind the line of the solvent (Fig. 2). The fairly high molar extinction coefficients (11 700 for psoralen and 10 100 for bergapten) make it possible to work with dilute solutions.

By means of the method described below, we have analyzed various garden fig varieties for their furocoumarin content (table). The results of our determination show that, with some variation in the total content of furocoumarins, fig leaves synthesize mainly psoralen. This confirms the prospects of this type of raw material for the production of materials with a high photodynamic activity. It is a characteristic feature that the amount of furocoumarins in the leaves

falling after frost does not decrease. Consequently, they can be used equally as a raw material. The fruit of the fig tree contains only traces of furocoumarins.

Results of a Determination of the Furocoumarins in the Leaves of Ficus carica L. var. domestica (% on the air-dry weight of the plant raw material)

Type of raw material	Time of collection	Psoralen	Bergapten	Total
Local central Asian	June 1964	0.23	0.11	0.34
	July 1964	0.28	0.13	0.41
	August 1964	0.32	0.14	0.46
North American (Kadota)	After frosts, November 1966	0.33	0.10	0.43
	October 1964	0.43	0.11	0.54
Caucasian	September 1966	0.17	0.04	0.21

Experimental

Four grams (accurately weighed) of the air-dry comminuted and sieved (sieve apertures 1.0–1.5 mm in diameter) raw material was covered with 20 ml of ethanol (96% ethanol was used for all the stages) in a 150-ml flask which was shaken for 2 hr on a vibrator. The extract was filtered through a No. 1 glass filter and was used without further purification for the chromatographic separation.

One milliliter of the extract was deposited with a micropipet in a continuous line on a sheet of chromatographic paper 18 cm wide (type B) previously impregnated with a 10% solution of formamide in methanol, leaving 4.5 cm on either side. The paper was dried slightly over an electric hotplate and placed in a chromatographic chamber with the n-hexane–benzene–methanol (5:4:1) solvent system. Chromatography (descending method) was continued until the solvent front reached a line 2–3 cm from the lower edge of the paper. After the chromatogram had been dried, it was observed in a UI-1 ultrachemiscope,* and the sections of the paper corresponding to the furocoumarins were excised, cut up with scissors, and covered with ethanol (the pieces of paper with the psoralen spot with 30 ml, and those with the bergapten with 15 ml). The spots were eluted at room temperature by shaking in a vibrator for 2 hr. The eluate was filtered through a No. 1 glass filter.

For the colorimetric measurements, 5 ml of the eluate was transferred to a 25-ml flask, and 3 ml of a 0.5 N solution of caustic soda and 2 ml of diazo reagent (for preparation, see [8]) were added. Simultaneously, the solution for the two control cells was prepared in another flask by mixing 10 ml of ethanol, 6 ml of 0.05 N caustic soda solution, and 4 ml of diazo reagent. The contents of the flask were gently stirred and poured into the cells (layer thickness 0.5 cm) and the optical density of the azo dye was determined 60 min after the addition of the diazo reagent on the scale of the right drum of a FEK-M photocolorimeter. The density of the azo dye from psoralen was measured with a green filter (λ_{max} 535 mμ) and that from bergapten with a blue-green filter (λ_{max} 485 mμ); the measurement was repeated and the mean value of the optical density D was calculated. The completeness of the desorption of the furocoumarins from the paper was checked with samples of the pure substances [8].

The standard solutions were prepared in alcohol from pure chromatographically homogeneous samples of the furocoumarin. To obtain comparable optical densities, the solutions are desirably prepared at a concentration of 0.3 mg/ml for psoralen and 0.04 mg/ml for bergapten. It was shown by special experiments that Beer's law is obeyed for concentrations of this order and there is therefore no need to construct a calibration curve. The required concentration is calculated by comparing the optical densities of the standard and test solutions. The formulas given in our previous paper [8] can be used to calculate the percentage content of the furocoumarins in the raw material.

Summary

A method for the separate quantitative determination of the furocoumarins of Ficus carica has been developed.

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