QUANTITATIVE PAPER CHROMATOGRAPHIC DETERMINATIONS

I. COUMARINS AND PHENOLIC ACIDS, ESPECIALLY ESCULETIN, DAPHNETIN AND FERULIC ACID

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(Received February 7th, 1961)

Phenolic acids and coumarin derivatives constitute a field of chemical and biochemical research to which increased interest has been paid in the last decades. The widespread occurrence of these compounds in nature has been reported and their possible physiological roles have been stressed. Although several efficient methods have been described for the qualitative identification of coumarins and phenolic acids in various materials, only few quantitative determinations of the compounds mentioned above have been published. Quantitative paper chromatographic methods are needed, and although it is well known that after elution from the paper, phenols can be assessed by means of the Folin-Ciocalteu reagent, too many precautions have to be taken and doubtful results are obtained due to the non-specificity of the reagent. The fact that the Folin-Ciocalteu reagent also reacts with other reducing substances is responsible for the variable blanks, and it has already been mentioned[1] that especially paper irrigated with butanol–acetic acid–water (4:1:5, v/v/v) gives such high and variable blank values. Therefore a more convenient method has been elaborated for esculetin, daphnetin and ferulic acid.

The compounds were dissolved in the minimum amount of ethanol and then further diluted with water to an appropriate concentration. 10 to 100 μg were applied with suitable constriction micropipettes on the reference line of an acetic acid washed and phosphate buffered (pH 7.4) Whatman No. 1 paper. After irrigation of the paper with *sec.*-butanol–water (4:1, v/v) the chromatogram was dried and the spots were located by their fluorescence under U.V. light. In the case of esculetin special precautions have to be taken, since it has been reported that caffeic acid and esculetin are interconvertible[2,3]. After location of the spots, rectangular fragments containing the substance under investigation, were removed from the chromatogram and eluted with water in an assembly as described by ARONOFF[4]. The eluates were collected in 10 ml volumetric flasks. A paper blank was run with each experiment.

After elution, the dihydroxycoumarins and ferulic acid were determined by means of a modified STOUGHTON–PAN method[5,6]. To the eluates were added: 1 ml

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2 *N* H_2SO_4, 0.1 ml $NaNO_2$ 4% (w/v), 1.5 ml concentrated ammonia and enough water to bring to volume. Boiling was omitted, since heating of the reaction mixture resulted in too high blank values. The extinction of the colour produced was measured after 15 minutes with a Beckman DU spectrophotometer in the case of esculetin and daphnetin and with a Coleman Junior spectrophotometer (round cuvettes) in the case of ferulic acid. The absorption maxima of the compounds are given in Table I.

TABLE I

Compound	*Colour of the reaction mixture*	*Absorption max. mμ*
Esculetin	faint yellow	430
Daphnetin	faint brown-violet	350
Ferulic acid	yellow	430

Fig. 1 shows the standard curves. It should be noted that the standard curves obtained *in vitro* and after chromatography were identical. The method is sensitive enough to determine micro amounts of the three compounds.

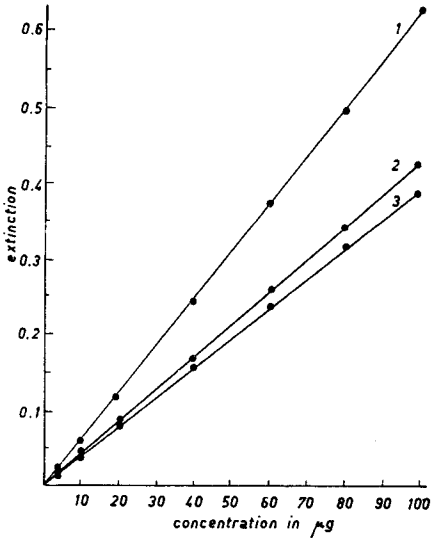

Fig. 1. Standard curves for esculetin (1), ferulic acid (2) and daphnetin (3).

o-Coumaric acid, umbelliferone, vanillic acid and *p*-hydroxybenzoic acid gave no colour under the described conditions.

SUMMARY

A method for the quantitative paper chromatographic determination of esculetin, daphnetin and ferulic acid is described. The compounds were chromatographed on phosphate buffered Whatman No. 1 paper (pH 7.4) with *sec.*-butanol–water (4:1, v/v).

After elution of the spots, the coumarins and the phenolic acid were determined by means of a modified STOUGHTON-PAN method. Standard curves obtained *in vitro* and after elution of the spots from the paper were identical.

REFERENCES

1 R. W. KEITH, D. LE TOURNEAU AND D. MAHLUM, *J. Chromatog.*, 1 (1958) 534.
2 C. F. VAN SUMERE, F. PARMENTIER AND M. VAN POUCKE, *Naturwiss.*, 24 (1959) 668.
3 W. L. BUTLER AND H. W. SIEGELMAN, *Nature*, 183 (1959) 1813.
4 S. ARONOFF, *Techniques of Radiobiochemistry*, Iowa State College Press, Ames, Iowa, 1956, p. 26.
5 R. W. STOUGHTON, *J. Biol. Chem.*, 115 (1936) 293.
6 S. C. PAN, *Anal. Chem.*, 27 (1955) 65.