QUANTITATIVE PAPER CHROMATOGRAPHIC DETERMINATIONS

II. PHENOLIC ACIDS, ESPECIALLY VANILLIC ACID AND *p*-HYDROXYBENZOIC ACID

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In our note on the quantitative paper chromatographic determination of esculetin, daphnetin and ferulic acid[1], we mentioned the fact that these three compounds could easily be determined after chromatography and elution from the paper, by means of a modified STOUGHTON–PAN method[2,3]. However, as reported, several other important phenolic acids and coumarins did not give a colour reaction with the STOUGHTON–PAN reagents under the conditions that we found favourable for quantitative paper chromatographic determinations. In order also to be able to assess the non-reacting phenolic acids, *e.g.* vanillic acid and *p*-hydroxybenzoic acid, we have developed a different method in which the compounds under investigation are measured colorimetrically after reaction with the EMERSON reagent (4-aminoantipyrine).

FUJITA AND FURUYA[4,5] employed 4-aminoantipyrine for the quantitative determination of coumarin and several of its derivatives and therefore it seemed possible to us that the same reagent could be useful in the elaboration of quantitative paper chromatographic methods for phenolic acids. In fact, EMERSON[6] and EMERSON AND KELLY[7] have found that phenols, oxidized with $K_3Fe(CN)_6$ in alkaline medium, give a colour reaction with 4-aminoantipyrine (exception should be made for phenols with certain *para*-substituents[6,8]).

The following method has proved satisfactory. The phenolic acids 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. Test spots were applied on either side of the starting line. After irrigation of the paper with *sec.*-butanol–water (4:1, v/v), the chromatogram was dried and the side strips were cut from the chromatogram. The spots on the side strips were revealed by spraying with diazotized *p*-nitroaniline, whereupon the side strips were again placed alongside the chromatogram in their original position.

Rectangular areas, in which the actual spots would be located on the chromatogram, were removed and eluted into 10 ml volumetric flasks by capillary washing in

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an assembly as described by ARONOFF[9]. A paper blank was always treated in the same way. The phenolic acids were then determined by means of the EMERSON reagent. To the eluates, 0.3 ml 0.1 *N* NaOH, 0.2 ml 4-aminoantipyrine 1% and 0.1 ml $K_3Fe(CN)_6$ 5%, were added. The red reaction mixture was shaken and further diluted with distilled water to 10 ml. The optical density was measured with a Coleman Junior spectrophotometer (square cuvettes) at 510 mμ in the case of *p*-hydroxybenzoic acid and at 500 mμ for vanillic acid. The standard curves (Fig. 1) *in vitro* and those obtained after chromatography were slightly different, the latter showing an error of about 2% in comparison with the standard curves *in vitro*. Further work with this method, in order to apply it to other compounds, is still in progress.

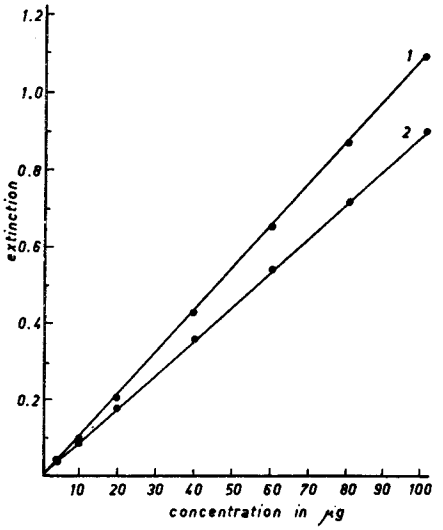

Fig. 1. Standard curves for *p*-hydroxybenzoic acid (1) and vanillic acid (2).

SUMMARY

A method for the quantitative paper chromatographic determination of vanillic acid and *p*-hydroxybenzoic 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 phenolic acids were determined by means of the EMERSON reagent (4-aminoantipyrine). Standard curves obtained *in vitro* and after elution of the spots from the paper differed slightly (2%).

REFERENCES

1 C. F. VAN SUMERE, H. TEUCHY AND F. PARMENTIER, *J. Chromatog.*, 6 (1961) 481.
2 R. W. STOUGHTON, *J. Biol. Chem.*, 115 (1936) 293.
3 S. C. PAN, *Anal. Chem.*, 27 (1955) 65.
4 M. FUJITA AND T. FURUYA, *Chem. and Pharm. Bull. (Tokyo)*, 6 (1958) 517.
5 T. FURUYA, *Chem. and Pharm. Bull. (Tokyo)*, 6 (1958) 696, 701, 706.
6 E. EMERSON, *J. Org. Chem.*, 18 (1943) 417.
7 E. EMERSON AND K. KELLY, *J. Org. Chem.*, 13 (1938) 532.
8 S. GOTTLIEB AND P. B. MARSCH, *Ind. & Eng. Chem., Anal. Ed.*, 18 (1946) 16.
9 S. ARONOFF, *Techniques of Radiobiochemistry*, Iowa State College Press, Ames, Iowa, 1956, p. 26.