A DIRECT METHOD OF DETERMINING SALICIN BY PAPER CHROMATOGRAPHY

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The identification of glucosides in plant materials is mainly carried out by isolating crystalline products, which can be determined by physical methods, and by hydrolysis according to the biochemical method of BOURQUELOT[1]. Neither method is especially rapid, though the latter has been considerably improved and simplified by employing the paper strip method of BLISS AND RAMSTAD[2]. CROOK AND STONE[3] identified salicin after enzymic hydrolysis, and the paper-chromatographic examination of cardiac aglycones by BUSH AND TAYLOR[4] included hydrolysis with sulphuric acid. NORKRANS[5] has described a turbidimetric method of determining the activity of β-glucosidase.

During investigations on resistance problems in hybrid aspen, *Populus tremula* × *Populus tremuloides*, and the parent species it was necessary to isolate and identify the glucoside salicin in healthy hybrid aspen and in trees attacked by a destructive fungus *Valsa nivea* Fr. The indirect method according to BLISS AND RAMSTAD, which involves hydrolysis of the salicin with emulsin to yield glucose and benzyl alcohol, was found tedious.

On searching for a more rapid method, the observation made by JOWETT AND POTTER[6] that salicin melted at 200° gave a red colour with sulphuric acid, was adapted and developed as described below.

EXPERIMENTAL

Aqueous solutions of salicin purum of different concentrations were placed on Whatman No. 1 filter paper and allowed to ascend in a 4:1:5 butanol–acetic acid–water solvent for 16 h at room temperature. After drying, the paper was sprayed with 2 *N* sulphuric acid and dried a second time using a thermo-desiccator AEG PL 247346. On heating the paper with the desiccator to 75–80°, the salicin appears as distinct pink spots after 1–2 min. The R_F value from a run in the solvent mentioned above is 0.57. In ethyl acetate–acetic acid–water (60:17:17.5) the R_F value is 0.86.

An attempt was made to obtain information about the quantitative values of the developed spots. The transmission of light was measured with a densitometer EEL X. 110. The optical density of paper and spot was determined at stated distances from the starting point of the chromatogram, and the values obtained were plotted graphically. The filter paper Whatman No. 1 served as a blank. The density values from Fig. 1 were transformed planimetrically to the curve represented in Fig. 2.

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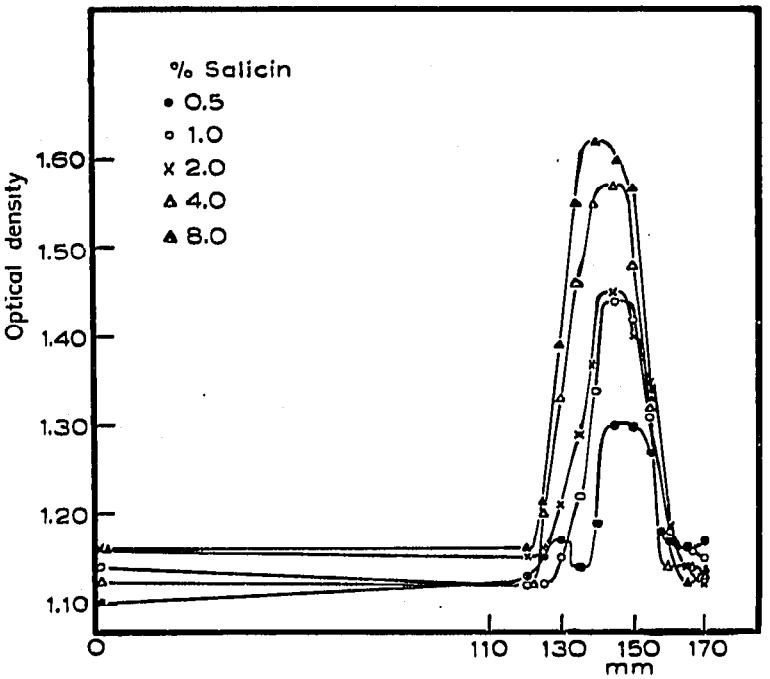

Fig. 1.

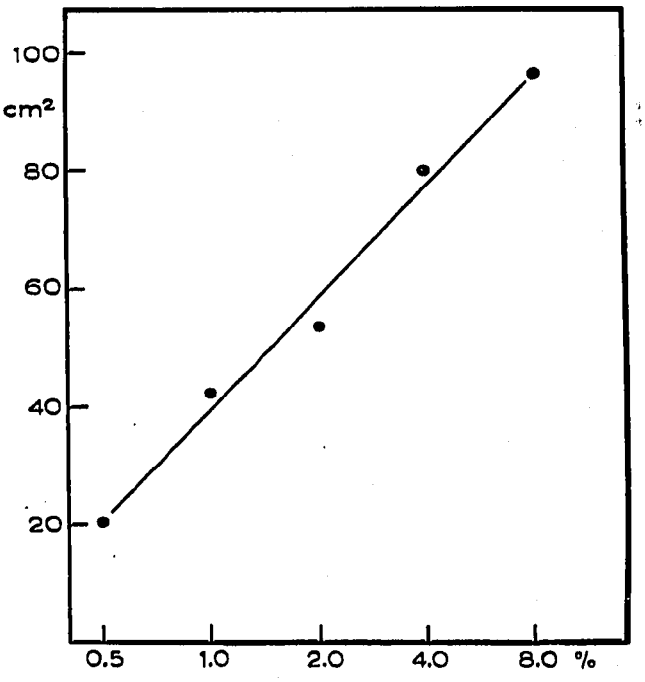

Fig. 2.

Fig. 1. Optical density of salicin at different concentrations.
Fig. 2. The spots of salicin in a chromatogram determined planimetrically.

As can be seen in Fig. 1 there is a rough accordance between the concentration of salicin and the optical density. This accordance is more pronounced when the concentrations are ascertained planimetrically. The method described may be used as a semi-quantitative one, but it must mainly be regarded as qualitative.

One disadvantage in using 2 *N* H_2SO_4 was observed. The ageing paper becomes very brittle and must be handled within a few hours after spraying. For preservation broad strips of tape are suitable.

SUMMARY

A semi-quantitative method of determining salicin by paper chromatography is reported. By using 2 *N* H_2SO_4 as a spraying reagent and heating with a thermo-desiccator salicin appears as distinct pink spots.

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Received November 29th, 1957