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Note

A combination of thin-layer chromatographic systems for analysis of the pigments from *Peniophora sanguinea* (Fr.) Bres.

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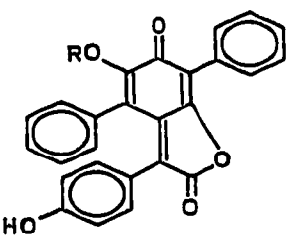
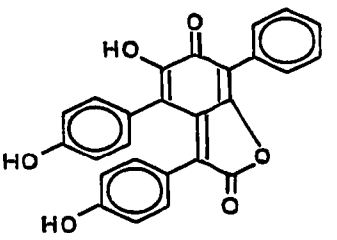
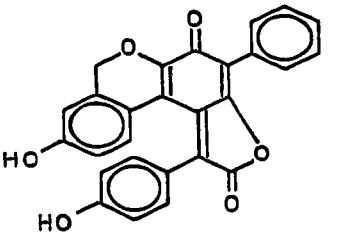
In our studies on the biosynthesis of pigments from the basidiomycete *Peniophora sanguinea* (Fr.) Bres., we required a TLC system for the qualitative and quantitative determination of such pigments. Our search led us to four systems, which (combined) offered the possibility of detecting all the substances in a chloroform–ethyl acetate extract and also of measuring all the known pigments (see Fig. 1).



R=H : Xylerythrine (xyl)
R=CH_3 : 5-O-Methyl-xylerythrine (mxy)



Peniophorine (p)



Peniophorinine (pin)

Fig. 1. Structure of known pigments[1–3,5] from *P. sanguinea* (Fr.) Bres.

MATERIALS AND METHODS

In all cases, we used commercial silica gel plates with a fluorescent indicator (No. 5175, Merck, Darmstadt, G.F.R.).

System I was based on that described in a personal communication from Dr. J. Gripenberg; the chromatograms were developed in the ascending direction with chloroform–methanol–cyclohexane (70:5:20).

In system II, the plates are heated for 1.5 h at 120°, allowed to cool for 5 min, then impregnated with formamide (8% solution in acetone). After impregnation, the extracts are applied and, 30 (max. 45) min later, the plates are developed horizontally in a Camag-Vario-KS chamber (see Camag brochure 251–401) with xylene–methyl ethyl ketone–formamide (11:5:1.1).

System III separates those substances that remain on the start line in system II or that have an R_F value less than about 0.4; the plates are prepared and used as in

system II, but the solvent is dichloromethane–tetrahydrofuran–formamide (6:4:1.1).

In system IV, the solvent is xylene–methyl ethyl ketone–formamide–dioxane (20:6:1:4) and the non-impregnated plates are developed in the Camag chamber.

RESULTS AND DISCUSSION

The results obtained by using these four systems are shown in Fig. 2. The chromatogram for system I shows the best separation of the lipophilic substances (1–4) and good separation of the pigments 5-O-methylxylerythrine[1], xylerythrine[2] and peniophorinine[3]. The components numbered 2 and 3 are coloured, and can also be obtained from fungus cultures by extraction with light petroleum[4]; these substances we have designated up1 and up2*.

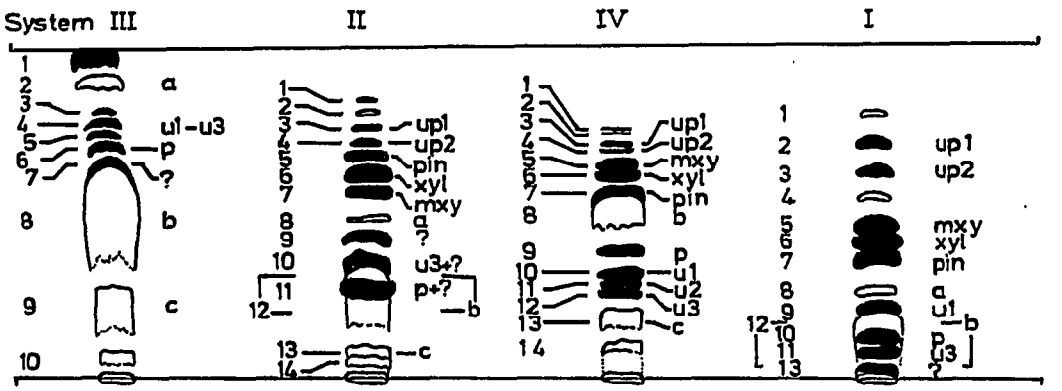


Fig. 2. Chromatograms of the chloroform–ethyl acetate extract[4] from *P. sanguinea* (Fr.) Bres. in the four systems. Coloured components are shown in black. Non-coloured components, *e.g.* the correlative but unknown substances a, b (main component) and c, were visualized under UV (254 nm).

System II is best for the quantitative analysis of xylerythrine, its methyl ether and peniophorinine, but system IV is best for the quantitative analysis of peniophorine[5]. Beyond peniophorine, in system IV, three more polar pigments (u1, u2 and u3) were detected. The quality of each of the systems is judged by the separation of the main component of the extract, the best separation being achieved in system III, which is the system preferred for detecting and determining the polar substances.

ACKNOWLEDGEMENT

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* We obtained up1 (1.2 mg) as yellow needles, the chemical analysis of which will be described in another paper. Substance up2 is red.