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Note

Thin-layer chromatographic detection of the lignan lactones of *Cleistanthus collinus* (Roxb.)

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Cleistanthus collinus (Roxb.) Benth & Hook f. (family Euphorbiaceae) is a highly poisonous plant[1] that grows in dry forests in several parts of India. The use of its leaves for suicidal and homicidal purposes[2] has aroused interest in its chemical constituents and their detection in biological materials[3,4]. Recently, Govindachari *et al.*[5] isolated from the leaves the lignan lactones diphyllin, cleistanthin and collinusin (Fig. 1). Cleistanthin, a highly toxic glycoside, is the major constituent of the leaves.

The identification of these lignan lactones in biological materials is of value to the forensic toxicologist and to the clinical chemist in the diagnosis of poisoning by *Cleistanthus collinus*. A rapid method for the specific detection of these compounds is at present not available. In this paper we report a thin-layer chromatographic (TLC) method for the separation and detection of these lignan lactones in submicrogram amounts on thin layers of silica gel using non-aqueous solvent systems for development. The intense blue to green fluorescence displayed by these compounds under ultraviolet light offers a highly sensitive method for their detection on developed chromatograms. Further detection is made possible by the use of chromotropic acid as spray reagent.

EXPERIMENTAL

Materials and methods

Silica gel G (Merck, Darmstadt, G.F.R.) was layered on glass plates to a thickness of 0.25 mm by the standard technique, activated for 30 min at 110° and stored in a desiccator prior to use. The plates were scored into several lanes so as to ensure greater reproducibility of R_F values.

Standard solutions in acetone of authentic samples of diphyllin, cleistanthin and collinusin (1 mg/ml) were used as references; a mixed standard, containing 1 mg/ml of each constituent, was also used.

The solvent systems employed are shown in Table I. All solvents were of analytical reagent grade and used as supplied. The chromotropic acid spray reagent was prepared as described by Beroza[6].

For the isolation of the lignan lactones from visceral material, the protein-free aqueous filtrate obtained by a modified Stas–Otto process using acetone as protein

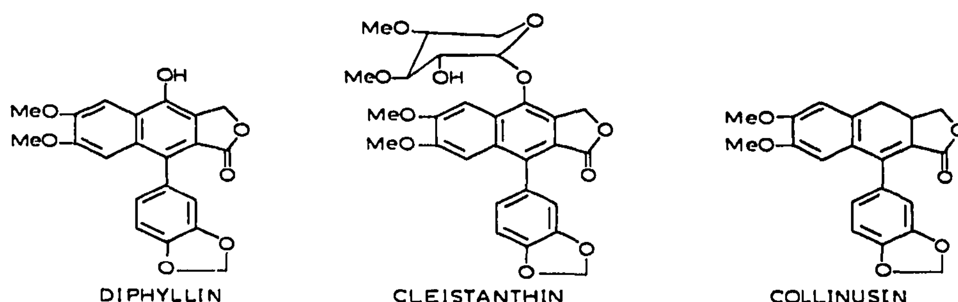

Fig. 1. Structural formulae of diphyllin, cleistanthin and collinusin. Me = Methyl group.

precipitant was adjusted to pH 3 with acetic acid and extracted with benzene–chloroform (1:1). The extract was concentrated to dryness and a solution of the residue in acetone used for TLC.

Chromatography

Suitable aliquots (1–5 μl) of the individual lignan lactones, their mixture and visceral extract were applied to the thin-layer plates for separation. Development was carried out in the solvent systems shown in Table I by the ascending technique, the solvent migration being 10 cm. After development, the chromatograms were air-dried and examined under short-wave ultraviolet light (254 nm) in order to observe the fluorescence colours of the spots. The plates were then sprayed with the chromotropic acid reagent and heated for 15 min at 110°.

In order to determine the lower limit of detection, various amounts of the lignan lactones were spotted and developed in the solvent system chloroform–*n*-heptane–ethanol (50:50:5) before examination under ultraviolet light or spraying with the chromotropic acid spray reagent.

RESULTS AND DISCUSSION

Table I gives the R_F values of the lignan lactones of *Cleistanthus collinus* in six different solvent systems and the time for the solvent front to advance 10 cm in each instance. Although all of the solvent systems effected satisfactory separations of

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF THE LIGNAN LACTONES OF *Cleistanthus collinus* ON SEVERAL SOLVENT SYSTEMS

Solvent system	*Time (min)**	R_F *value*		
		Diphyllin	*Cleistanthin*	*Collinusin*
Chloroform–*n*-heptane–ethanol (50:50:5)	25	0.27	0.37	0.55
Benzene–ethanol (85:5)	25	0.35	0.38	0.74
Xylene–methanol (90:10)	15	0.22	0.28	0.46
Chloroform–ethyl acetate (90:30)	20	0.39	0.23	0.75
Benzene–ethyl acetate (80:20)	20	0.29	0.14	0.59
Benzene–acetone (90:10)	15	0.28	0.22	0.66

* Time for the solvent front to move 10 cm.

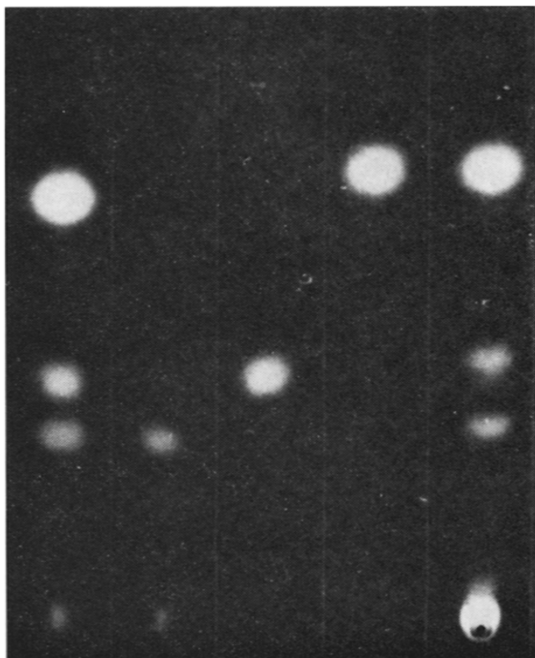

Fig. 2. Thin-layer chromatogram developed in chloroform–*n*-heptane–ethanol (50:50:5) and photographed in short-wave UV light (254 nm) without the use of a spray reagent. Spotting (left to right): mixture of three lignan lactones; diphyllin; cleistanthin; collinusin; and extract from the gastrointestinal contents in a case of poisoning by *Cleistanthus collinus*.

the three compounds, the system chloroform–*n*-heptane–ethanol (50:50:5) was found to be the best, yielding compact, round spots with good separation.

The lignan lactones exhibit bright fluorescence (Fig. 2) in short-wave ultraviolet light (254 nm) and can be distinguished by their fluorescence colours (diphyllin, blue; cleistanthin, greyish blue; collinusin, yellowish green). The fluorescence detection is highly sensitive, the lower limit being 0.01 μg for cleistanthin and 0.1 μg for diphyllin and collinusin. The fluorescence colours are stable for several days, except for diphyllin, whose blue fluorescence gradually diminishes in intensity with time.

The chromotropic acid spray reagent, already found to be of value in the detection of the methylenedioxy group on thin-layer chromatograms[7], produced purple spots with all the three lignan lactones. With 1–5 μg of the applied material, clearly defined spots on a nearly colourless background were obtained. The minimum detectable amount is of the order of 0.5 μg, at which level all the three compounds are detected as distinct purple spots.

It may be noted that diphyllin, which follows cleistanthin in the first three solvent systems, precedes it in the other three solvent systems. This reversal in the order of R_F values may be employed as a further aid in identification.

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