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Note

Forensic examination of sawdusts by thin-layer chromatography and flying spot densitometry

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The forensic scientist, when analysing sawdusts, is normally concerned not with the identity of the constituent woods making up the dusts sample but with the overall identity of two or more dust samples from a suspect and, for example, safe ballast found at the scene of the crime. These samples may be mixtures of as many as thirty different wood dusts. At present, the routine method for comparison relies on microscopy. The dust is subjected to an initial sorting process based on colour and particle size and shape. The constituent woods are then identified by reference to a multiple-entry perforated card key of the type devised by the Forest Products Research Laboratories[1,2]. These keys require the microscopical examination of wood sections and this can be particularly difficult and time consuming when dust analysis is to be undertaken.

A number of workers have attempted to simplify wood analysis by the use of chemical tests[3–5], X-ray microscopy[6], and IR spectroscopy[7], but these methods have little value in the analysis of mixtures.

Chromatographic analysis is obviously more applicable to this problem and many wood extractives have been examined by chromatographic methods[8–11]. In most cases this work was carried out for taxonomic purposes. Worthy of special mention is the work of Bevan *et al.*[12], who examined the extractives of timbers derived from the family *Meliaceae* and concluded that, at least within that family, chromatographic analysis could be used to provide an identification system.

This paper describes the thin-layer chromatographic (TLC) examination of some common woods that may be found admixed in dusts. The use of TLC and, particularly, thin-layer densitometry is demonstrated in the forensic situation.

EXPERIMENTAL

The following woods were examined: Softwoods—Archangel white*, cedar *(Thuja plicata)*, Czechoslovakian white*, hemlock *(Tsuga heterophylla)*, Parana pine *(Araucaria angustifolia)*, Russian red*, and spruce *(Picea abies)*. Hardwoods—

* These samples are all commercial species of *Pinus*. It was impossible to fully identify these samples. All other samples were provided by the Police Forensic Laboratory, Llanishen, Glamorganshire, and were further authenticated in our laboratory.

TABLE I

HARDWOODS—*R* VALUES AND COLOURS

(A) Eluent, light petroleum (b.p. 40–60°)–ethyl acetate (4:1); anisaldehyde spray; $R_{sitosterol}$ values. (B) Eluent, light petroleum (b.p. 40–60°)–ethyl acetate (4:1); 2,4-DNP spray; $R_{\beta\text{-amyrenone}}$ values. (C) Eluent, ethyl acetate; anisaldehyde spray; $R_{sitosterol}$ values. (D) Eluent ethyl acetate; 2,4-DNP spray; $R_{\beta\text{-amyrenone}}$ values. Colour abbreviations: bl = blue; br = brown; gr = green; gy = grey; ma = mauve; or = orange; pi = pink; pu = purple; re = red; ye = yellow.

Wood	*A*	*B*	*C*	*D*
Afrormosia	0.14 ye	0.48 pu	0.32	—
	0.41 or	0.66 pu		
	0.49 pu	0.82 ye		
	0.88 or	1.06 ma		
	0.97 pu	1.09 or		
	1.31 pu	1.15 ma		
	1.97 pu	1.21 pu		
	2.82 pu	1.31 ma		
Balsa	1.00 pu	0.43 bl	0.18	—
		1.09 pu		
		1.43 pi		
Iroko	0.14 or	0.48 pu	0.09	0.88
	0.45 br	0.83 pu	0.25	
	1.03 pu	0.94 pu		
	2.70 pu	1.06 or		
		1.33 ma		
Japanese oak	0.33 pu	0.53 pu	0.21	0.73
	1.02 pu	1.10 pu		
	2.70 pu	1.30 pu		
		1.44 pu		
Quassia	0.83 pu	0.53 gy	0.22	0.83
	1.07 pu	0.64 ma	0.34	
		1.07 pu	0.51	
		1.29 ma	0.61	
Sapele	0.10 ye	0.59 pu	0.70	1.14
	0.29 pu	0.86 ye		
	1.05 pu	1.06 pu		
	1.30 pu	1.12 ma		
	1.83 pu	1.20 ma		
	2.60 ma			
Teak	0.09 gy	0.20 gr	0.16	0.17
	0.30 br	0.30 gr	0.29	0.79
	0.61 bl	0.56 ma	0.40	0.85
	0.75 pu	0.76 gr	0.62	0.96
	0.96 pu	0.97 br	0.70	1.20
	1.37 ma	1.08 pu	0.86	
	1.60 ye	1.14 gr		
	1.96 ye	1.27 ma		
	2.15 ma	1.43 re		
	2.68 re			
	2.82 ma			
Utile	0.10 ye	0.19 gy	—	—
	0.29 pu	0.52 pu		
	1.05 pu	0.78 pu		
	1.30 pu	1.04 pu		
	1.83 pu	1.12 ma		
	2.68 pa	1.21 ma		
	2.80 ma	1.34 pu		

TABLE II

SOFTWOODS — *R* VALUES AND COLOURS

For explanation of A–D and colour abbreviations, see Table I.

Wood	*A*	*B*	*C*	*D*
Archangel white	0.20 gy 0.49 ma 0.76 gy 0.95 ma 2.37 ma 2.46 ma	0.23 pi	0.07 0.22	—
Cedar	0.15 ma 0.33 ye 0.61 ye 0.85 ma 1.02 bl 1.14 ma	0.24 ye 0.37 ye 0.55 ma 0.77 bl 0.93 ye 1.05 pu 1.27 ma 1.47 ma	0.11 0.33 0.91	0.15 0.96
Czechoslovakian white	0.24 ma 0.94 ma 1.13 ma 1.41 ma	0.52 pu 0.75 pu 1.07 pu 1.11 pu 1.30 pu 1.43 pu	—	0.98
Hemlock	1.03 ma 2.54 ma	0.52 pu 0.81 pu 1.08 pu 1.28 pu	—	—
Parana pine	0.46 pu 0.98 pu	0.63 bl 1.08 ma 1.27 ma	0.21 0.30	—
Russian red	0.17 br 0.26 br 0.35 br 0.46 ma 0.71 ma 1.06 br 1.27 re 2.60 ma	0.55 ma 0.68 ma 0.89 ye 1.03 ma 1.25 ma 1.38 re	0.13 0.24 0.51 0.74	0.64 0.96
Spruce	0.29 pi 0.57 pu 1.09 br 1.20 ma 1.33 ma 1.43 ma 1.52 br 1.92 pu 2.31 br 2.47 ma 2.60 pu	0.31 ma 0.90 ma 1.07 ma 1.19 ma 1.43 ma	0.05 0.41 1.07	0.99

afrormosia *(Afrormosia elata)*, balsa *(Ochroma lagopus)*, Iroko *(Chlorophora regia)*, Japanese oak *(Quercus mongolica)*, quassia *(Picroena excelsa)*, sapele *(Entandrophragma cylindricum)*, utile *(Entandrophrama utile)*, and teak *(Tectona grandis)*.

Extraction

Individual wood dusts and mixtures (500 mg) were extracted in chloroform (5.0 ml) at room temperature.

Thin-layer chromatography

Silica gel/CT Type Code S13 TLC (H. Reeve Angel, Clifton, N.J., U.S.A.) 20-cm × 20-cm plates, 0.25 mm thick, were heated at 110° for 30 min. 20–30 μl of extract were applied. The eluents used were: (1) light petroleum (b.p. 40–60°)–ethyl acetate (4:1) and (2) ethyl acetate. Anisaldehyde and 2,4-dinitrophenylhydrazine (2,4-DNP) were used as spray reagents. The anisaldehyde reagent was prepared by adding 1 ml of concentrated sulphuric acid to a solution of 0.5 ml anisaldehyde in

TABLE III

UV FLUORESCENT COMPONENTS—*R* VALUES AND COLOURS

Eluent, light petroleum (b.p. 40–60°)—ethyl acetate (4:1). For colour abbreviations, see Table I.

Wood	$R_{sitosterol}$
Afrormosia	0.77 ye
	0.86 pu
	1.06 gr
	1.14 re
Balsa	streak pu
Iroko	1.06 pu*
Japanese oak	0.77 pu
Quassia	0.22 pu
	0.64 pu
	0.78 ye
	1.00 pu
	1.24 bl
Sapele	1.10 gr
Teak	0.24 pu
	0.65 pu
	0.84 re
	0.91 re
	1.04 re
	1.19 re
Utile	1.10 gr
Cedar	0.22 pu
	0.30 pu
	0.94 ye
Spruce	1.21 ye

* Appears yellow in visible light after irradiation.

50 ml glacial acetic acid, the 2,4-DNP reagent by adding 10 ml concentrated hydrochloric acid to 1.0 g 2,4-DNP in 100 ml ethanol. After spraying with anisaldehyde reagent the plates were heated at 110° for 10 min. β-Amyrenone (0.5% in chloroform) and sitosterol (0.5% in chloroform) were used as standards.

Thin-layer densitometry

Thin-layer densitometry was carried out using a Vitatron flying spot densitometer, operating in the log (−) mode with an aperture of 0.25 mm and a filter set at 477 nm.

RESULTS AND DISCUSSION

Anisaldehyde was selected as a general spray reagent as it reacts with most isoprenoidal compounds and these are the major compounds expected in a chloroform extractive of a wood. It was particularly useful in that it produced a wide range of colours with the different components, although these colours were variable from plate to plate. The use of 2,4-DNP as a spray reagent for carbonyl compounds was of less value, but again each wood extract presented its own chromatoprint. It was essential to use *R* standard values and the results displayed in Tables I and II are obtained as average values of a minimum of three determinations. A good degree of reproducibility was found.

All the hardwoods examined showed fluorescent compounds when viewed

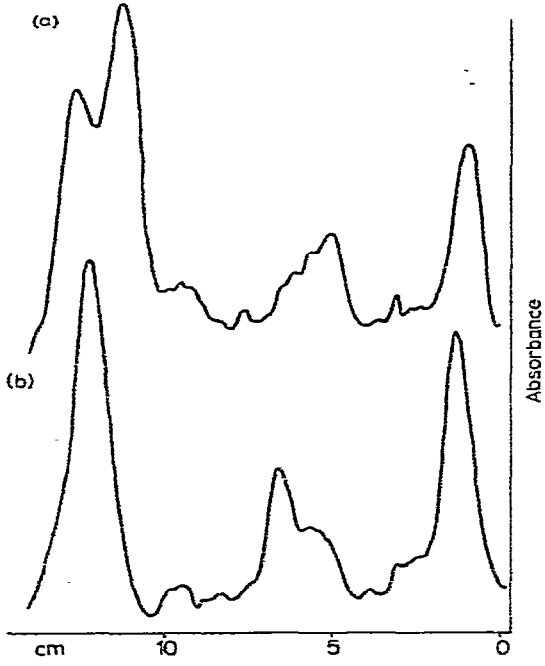

Fig. 1. Densitometric record of (a) utile and (b) sapele. Eluent, ethyl acetate. Spray reagent, anisaldehyde. Absorbance measured at 477 nm.

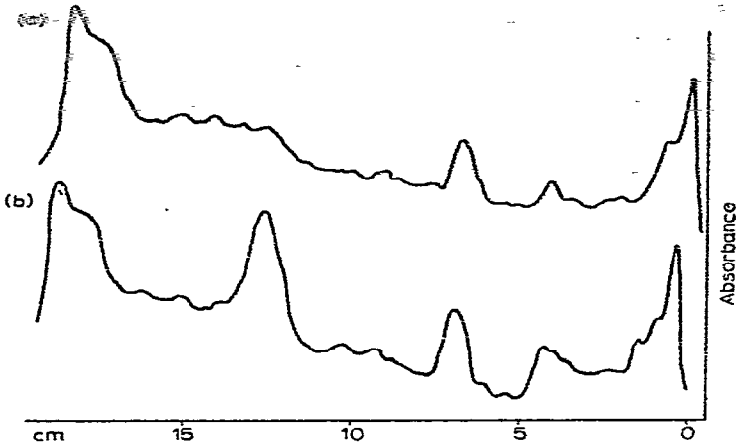

Fig. 2. Densitometric record of sawdust mixtures. (a) Mixture 1 —equal parts of Russian red, Archangel white, hemlock, and Czechoslovakian white; (b) mixture 2—mixture 1 plus 20% spruce. Eluent, light petroleum (b.p. 40–60°)–ethyl acetate (4:1); spray reagent, anisaldehyde. Absorbance measured at 477 nm.

under ultraviolet light at 254 nm whereas only spruce and cedar, amongst the softwoods, showed any fluorescence (Table III). The development of a coloured spot after UV irradiation of iroko extract is attributable to a stilbene derivative[13]. The use of a thin-layer densitometer is particularly valuable in that it allows the simple reproduction of results, provides a permanent record and, which is particularly important in forensic work, removes the subjectivity inherent in a visual inspection of complex chromatograms. Of particular interest are the densitometric traces of utile *(Entandrophragma utile)* and sapele *(E. cylindricum)* (Fig. 1). These are easily distinguishable by chromatographic inspection although as dusts they are indistinguishable by microscopical examination. Numerous samples of sawdusts have been examined by this method and where the dusts are completely different this can be seen very easily by visual inspection of the chromatograms. More difficult is the examination of essentially similar mixtures. Where the difference lies in the presence of one wood having particularly intense and characteristic peaks as, for example, teak in a mixture of softwoods, it is possible to demonstrate its presence in amounts of less than 1.0%. A more typical situation is demonstrated in Fig. 2.

One of the advantages of this system in forensic analysis is that the microscopical features of the dusts are not affected by the mild extraction method and they can still be subject to conventional analysis should the chromatographic analysis indicate its desirability. Although extractions were carried out initially on 500-mg samples, it was found that suitable chromatograms could be obtained with 2.0 mg of pure wood dust, but the problem of obtaining a representative sample of a dust mixture makes this of little value for forensic work.

The method described thus allows the production of densitometric "fingerprints" which makes the rapid differentiation possible between sawdust mixtures, even when these differ in only one component.

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